52nd ANNUAL CONFERENCE
Beyond Possible: Remarkable Transformation of Reproductive Biology
Abstracts
2019 Focus Sessions

S1.1 - The maternal lactocrine continuum programming uterine capacity. Frank Bartol, Jeffrey Vallet, Carol Bagnell

Lactocrine signaling describes a mechanism by which milk-borne bioactive factors (MbFs) are communicated from mother to offspring by consequence of nursing. Lactocrine communication is an element of the maternal environmental continuum of factors that define pre- and postnatal developmental conditions and, therefore, the trajectory of mammalian development and offspring phenotype. Lactocrine-active factors can include MbFs of maternal origin, as well as factors of environmental origin to which the lactating female is exposed. Ideally, lactocrine signals communicated to nursing offspring insure a smooth transition and adaptation to extrauterine life. Disruption of lactocrine communication occurs when the quality or quantity of colostrum (first milk) is compromised. This can have lasting consequences for the health and fitness of nursing young. In the pig (Sus domesticus), imposition of a lactocrine-null state from birth (postnatal day = PND 0) by feeding milk replacer altered global patterns of uterine gene expression by PND 2, and inhibited endometrial development by PND 14. Similarly, lactocrine deficiency, reflecting minimal colostrum consumption from birth, retarded endometrial development in neonates, altered endometrial gene expression on pregnancy day (PxD) 13, and reduced live litter size in adult, neonatally lactocrine-deficient gilts. Elements of the lactocrine-sensitive, adult endometrial transcriptome at PxD 13 included factors affecting conceptus-endometrial interactions. A window for lactocrine programming of porcine endometrial function and uterine capacity was defined during the first 24 h of postnatal life. Lactocrine effects on the neonatal uterine miRNA-mRNA interactome at PND 2 implicated miRNAs as elements of the maternal lactocrine programming mechanism. In addition to molecular signals, maternally-derived somatic cells delivered to nursing young may affect the trajectory of postnatal development. Lactocrine targets in other species include pituitary mammotropes, immune system, and brain. Observations reinforce the importance of defining lactocrine mechanisms driving maternal programming of postnatal development and offspring phenotype. [Support: USDA-NIFA-2013-67016-20523]

S1.2 - Maternal programming of fetal development via endocrine disruptors. Almudena Veiga-Lopez

The burden of endocrine disruptor chemicals (EDCs) has risen in the past decades. Bisphenol A, S, and F (BPA, BPS, and BPF) are among the most common bisphenolic EDCs use in the manufacture of consumer products. Because exposure to bisphenols during pregnancy occurs in >90% of pregnancies, there is a growing interest to understand the risk that these chemicals may pose to human health. The goal of our research is to investigate the effect that bisphenol chemicals may have on the developing fetus, as well as the placenta. Because BPA has been demonstrated to lead to insulin resistance and obesity, we were particularly interested in
evaluating if adipogenesis was altered as early as the fetal stage and if so, the mechanism by which BPA could program an adipogenic phenotype. Our work using sheep as an animal model shows that BPA, but not BPS, increases the adipogenic ability in fetal-derived preadipocytes and that this effect is accompanied by alterations in the uncoupled protein response in female fetuses. Because chemical exposure effects on the fetus are highly driven by effective crossing of the placental barrier, we also focused our attention in delineating pregnancy bisphenol toxicokinetics and their ability to persist in fetal circulation. We have demonstrated significant differences in bisphenol kinetics between mother and fetus as well as among bisphenols. We have also explored the potential effects of bisphenols on placental function and demonstrated deleterious effects of the emerging chemical BPS on placental endocrine capacity and maintenance of the trophoblast layer. Taken together, these studies show novel effects from bisphenol chemicals on fetal and placental development suggesting that pregnancy is a vulnerable window for chemical exposures.

**S1.3 - Ovarian Metabolism of an Environmentally Relevant Phthalate Mixture.** Genoa Warner, Zhong Li, Madeline Houde, Cassandra Atkinson, Daryl Meling, Catheryne Chiang, Jodi Flaws

Phthalates are synthetic chemicals with widespread human exposure that are commonly used as additives in consumer products. Evidence suggests that metabolites mediate the toxic effects of phthalates and that intravenous medical exposure can lead to significant concentrations of unmetabolized phthalates reaching organs such as the ovary. Thus, the ovary may be bioactivating phthalates in-house, increasing the local concentration of toxic metabolites in one of the most sensitive organs for endocrine disruption. This study investigated the ability of neonatal and adult mouse ovaries to metabolize an environmentally relevant phthalate mixture. Whole neonatal ovaries (postnatal day 4) and adult antral follicles from CD-1 mice were cultured in media treated with dimethyl sulfoxide (DMSO; vehicle control) or phthalate mixture (0.1–100 µg/mL) composed of 35% diethyl phthalate (DEP), 21% di(2-ethylhexyl) phthalate (DEHP), 15% dibutyl phthalate (DBP), 15% diisononyl phthalate (DiNP), 8% diisobutyl phthalate (DiBP), and 5% benzylbutyl phthalate (BBzP), which is representative of exposure of pregnant women in Illinois. After four days of culture, media were subjected to liquid chromatography mass spectrometry (LCMS) to measure the amounts of monoester metabolites. Metabolites for all phthalates except DiNP were detected in the media for both culture types. The long-chain phthalates BBzP, DEHP, and DiNP were metabolized less than the short-chain phthalates DEP, DBP, and DiBP, with neonatal ovaries showing preferential metabolism of short-chain phthalates. Ovaries and follicles were collected to measure gene and protein expression of enzymes required for phthalate metabolism, lipoprotein lipase (LPL) and aldehyde dehydrogenase family 1, subfamily A1 (ALDH1A1). Neonatal ovaries predominantly expressed LPL, whereas adult follicles expressed high levels of ALDH1A1. These data demonstrate that neonatal and adult ovaries are capable of metabolizing low doses of phthalates and suggest that metabolic capacity differs for follicles at different stages of development. Supported by NIH R56ES025147, NIH R01ES028661, and NIH T32ES007326.
**S1.4 - Worldwide pollution by persistent organic pollutants impacts the sperm DNA methylation of Inuit men in a dose-dependent manner.** Marie-Charlotte Dumargne, Xiaojian Shao, Mathieu Dalvai, Gunnar Toft, Jens Peter Bonde, Marie-Michelle Simon, Tony Kwan, Vanessa Dumeaux, Donovan Chan, Guillaume Bourque, Tomi Pastinen, Sarah Kimmins, Jacquetta Trasler, Janice Bailey

Persistent organic pollutants (POPs), either used as pesticides (e.g. DDT and its principal metabolite DDE) or industrial by-products (e.g. polychlorinated biphenyls), bio-accumulate in organisms. At the top of the Arctic food chain, Inuit populations have been exposed to POPs for generations in orders of magnitude higher than the safety threshold. We hypothesize that the sperm DNA methylome from Inuit men may be altered in response to exposure to POPs. We collected blood and sperm from 47 Inuit men from Greenland (with a serum DDE ranging from 39.4 ng/g to 5000 ng/g lipids). The sperm DNA methylome was examined using a custom human sperm-specific 5mC-Capture-sequencing approach. The quantitative association between sperm DNA methylation and an increasing environmental exposure to DDE was assessed using a binomial regression, adjusted for potential confounders (smoking, age and body mass index). We identified 8506 dose-dependent differentially methylated regions (DMRs), of which the majority were hypermethylated (74%). Genomic annotation indicated a positive enrichment at CpG islands, exons, CpG shores, sub-telomeres, transfer-RNAs and promoters (permutation test, p<0.001). Gene ontology analysis revealed enrichment for genes with roles in biological processes such as axon guidance, neurotransmitter secretion, cardiac and osteoblast development, retinoic acid receptor signaling, and histone lysine methylation (p<0.01). We report a dose-dependent association between an environmental exposure to DDE and the sperm DNA methylome. The extensive epigenetic changes detected in the most variable regions strongly suggest an alteration by environmental toxicants. This study is the first to accurately assess the genome-wide DNA methylation of sensitive sites in sperm from Inuit men and pave the way to the identification of environmental stressors which could inform on the sperm epigenetic integrity and may be predictive of fertility status and/or embryonic outcomes.

**S2.2 - Engineering Reproduction.** Jennifer Woodruff

Infertility is a major health concern, affecting more than 50 million couples. In the Western world most infertile couples resort to assisted reproduction technology (ART) to try to conceive a child. Despite undeniable successes, near half the couples who seek medical assistance for infertility fail to achieve a pregnancy. ART, as it is currently proposed, is mainly palliative, with little effort made to understand and specifically address the dysfunctions responsible for couple infertility. In these couples, a defective spermatogenesis is observed in half of the male partners. With over one thousand genes expressed almost exclusively in the testis, we believe that gene defects constitute the main cause for male infertility, especially for the most severe sperm defects (subjects with azoospermia or near 100% abnormal sperm).

Children conceived by ART have been shown to have an increased incidence of de novo chromosomal aberrations, genetic imprinting defects and birth defects. For these reasons, we believe that it would be imprudent to continue to support the entirely empirical ART approaches...
while neglecting parallel efforts to discover the causes of infertility and their consequences for the quality of gametes bearing the human genome to future generations.

Genetic testing for infertile men is currently largely inefficient. Here I will show that the use of whole exome sequencing (WES) is an effective strategy to obtain a genetic diagnosis for severe sperm defects such as non-obstructive azoospermia, multiple morphological anomalies of the flagella (MMAF) or globozoospermia. The next step is to understand the pathophysiology associated with a particular genetic defect and to improve our understanding of the basic mechanism underlying an efficient spermatogenesis. To that effect we used two different models: knock out mice created with the CRISPR/Cas9 mice technique and Trypanosoma brucei (T. brucei) which is one of the best models for sperm flagella studies as it has a 9+2 axonemal structure surrounded by para-axonemal fibers that are specific to the flagella and are absent from the other motile cilia. I will present some examples illustrating how these two models allowed us to understand the role of several proteins identified in infertile men and necessary for normal sperm production and function.

**S2.3 - Follicle Stimulating Hormone Stimulation Restores Ovarian Microenvironment of Beef Heifers with Androgen Excess to Reduce Inflammation.** Shelby Springman, Sarah Nafziger, Mohamed Abedel-Majed, Alex Snider, Kerri Bochantin, Jeff Bergman, Renee McFee, John Davis, Jennifer Wood, Andrea Cupp

Our laboratory has been investigating a naturally-occurring cow model with excess androstenedione (A4, High A4) in follicular fluid and secreted from the ovarian cortex. High A4 cows exhibit irregular estrous cycles, increased ovarian inflammation, follicle arrest and are sub-fertile. Furthermore, High A4 cows have similar traits as women with polycystic ovary syndrome (PCOS). Because women with PCOS often have cyclicity problems at puberty, we monitored pubertal attainment in heifers and identified 4 pubertal classifications: Typical, Early, Start-Stop and Non-Cycling. Non-cycling heifers and Start-Stop heifers have problems with cyclicity, secrete A4 from their ovarian cortex and appear to be predisposed to become High A4 cows. Follicle stimulating hormone (FSH) treatment often induces ovulation in women with PCOS. Thus, we hypothesized that FSH-stimulation (245 total IU) in Non-cycling heifers (n=3) would restore the ovarian microenvironment to control (or Typical; two injections prostaglandin 12 days apart; n=3) conditions. Ovarian cortical pieces were collected at ovariectomy, cultured for 7 days and media was collected daily. Unstimulated Non-cycling heifers had increased A4, progesterone, androsterone, and 11-deoxycorticosterone (P ≤ 0.04) compared to Unstimulated Typical heifers (Controls). There was also a tendency (P ≤ 0.09) for increased 17-OH progesterone and 11-deoxycortisol and fibrosis in Non-Cycling heifers. Cytokines CCL5, decorin, and IL-10 were also greater (P ≤ 0.05) in Unstimulated Non-cycling heifers with a tendency (P ≤ 0.09) for increased IFNβ, IL-1β, IL-17, CCL4, and IL-36Ra. However, FSH-stimulation in Non-cycling heifers tended to reduce fibrosis, decorin, IFNβ, IL-1β, and CCL5 (P ≤ 0.03) to the level of the Unstimulated Typical (Control) heifers. In contrast, TNFα was increased (P = 0.05) in FSH-stimulated Non-Cycling heifers compared to other groups. Taken together, these results indicate that FSH-stimulation may restore the ovarian microenvironment of Non-cycling (High A4) heifers and reduce ovarian inflammation which may be a cause of follicle arrest.
**S2.4 - Impaired follicle development in female mice lacking receptor tyrosine kinase 4 (Erbb4) in ovarian granulosa cells.** Florence Naillat, Ville Veikkolainen, Milena Doroszko, Nsrein Ali, Ilkka Miinalainen, Claes Ohlsson, Matti Poutanen, Nafis Rahman, Klaus Elenius, Seppo Vainio

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders leading to infertility in women. Recent genome-wide association studies on PCOS cohorts revealed a single nucleotide polymorphism (SNP) in the *ERBB4* receptor tyrosine kinase 4 gene. *Erbb4* is implicated in the development of the testes, kidneys, mammary glands and heart, but its role in ovary development or during folliculogenesis remains poorly understood. To characterise *Erbb4* function in the ovaries, we conditionally deleted *Erbb4* in murine granulosa cells under the control of *Mis* promoter. The *Erbb4Flox/Flox; MisCre* mice presented several reproductive features similar to those observed in the human PCOS disorder. The mice had a reduced amount of Erbb4 in granulosa cells from primary to antral follicles, slight ovary weight, asynchronous oestrous cycle leading to few ovulations and subfertility, and impaired oocyte development. These phenotypes were associated with an upregulation of serum luteinizing hormone, higher androgen levels, increased production of ovarian and serum anti-Müllerian hormone. To further understand the ovarian phenotype, selected ovarian, granulosa cells and cell adhesion markers were analysed. The analysis showed defective intercellular junctions between the granulosa cells and the oocytes with increased of *Gdf9* and *KitL* mRNA expression. In conclusion, our results indicate a functional role for Erbb4 in the ovary, especially during folliculogenesis.

**S3.1 - Apoptosis in the fetal mouse testis eliminates developmentally defective germ cell clones.** Daniel Nguyen

Many germ cells are eliminated during development, long before differentiating to egg or sperm, but it is not clear why. Here, we examined how germ cell composition in the mouse fetal testis is altered by scheduled apoptosis during sex differentiation. Multicolored-lineage tracing revealed that apoptosis affects clonally-related germ cells, suggesting that this fate decision occurs autonomously based on shared intrinsic properties. We identified extensive transcriptional heterogeneity among fetal germ cells including an apoptosis-susceptible subpopulation delineated by high *Trp53* and deviant differentiation. Alternatively, the germ cell subpopulation most likely to survive was advanced in differentiation. These results indicate that germ cell developmental fate is based upon discrete and cell-heritable fitnesses and imply that a dichotomy between sex-differentiation and apoptosis coordinates the removal of developmentally incompetent cells to improve gamete quality. Evidence that germ cell subpopulations are in different epigenetic states suggests that errors in epigenetic reprogramming form the basis of aberrant differentiation and apoptotic selection.

**S3.2 - Induced pluripotent stem cells (iPSCs) in Mitochondrial Medicine.** Alessandro Prigione
Mitochondrial diseases are rare genetic diseases caused by mutations in oxidative phosphorylation (OXPHOS) genes encoded by either the mitochondrial DNA (mtDNA) or nuclear DNA (nDNA). The diseases lead to early-onset neurological impairment, given the dependency of neural cells on OXPHOS metabolism. A major hurdle for the study of mitochondrial diseases is the paucity of mechanistic model systems.

In this talk I will focus primarily on Leigh syndrome (LS), which is the most severe mitochondrial disease affecting children with a frequency of 1/40,000 newborns. We generated induced pluripotent stem cells (iPSCs) from LS patients carrying mtDNA or nDNA mutations. We show that neural progenitor cells (NPCs) and post-mitotic neurons differentiated from LS-iPSCs exhibit mutation-specific defects that are rescued when we repaired the nDNA mutations using CRISPR/Cas9. We then used the cellular phenotypes to identify potential treatment strategies. We suggest NPCs as an effective model system to study LS and to carry out phenotypic compound screenings. Our data pave the way to the identification of disease-modifying therapies for currently incurable mitochondrial disorders.

**S3.3 - Differentiation of Primate Primordial Germ Cell-Like Cells Using Mouse Xenogeneic Reconstituted Testis.** Enrique Sosa, Esmeralda Villavicencio, Ernesto Rojas, Amander Clark

Xenotransplantation and homologous transplantation of rhesus macaque primordial germ cell-like cells (rPGCLCs), generated from induced pluripotent stem cells (iPSCs), into the adult testicular niche leads to the in vivo advancement of rPGCLC differentiation. Recently, human oogonia-like cells were generated in vitro using hPGCLCs when reconstituted with female somatic cells from dissociated embryonic mouse ovaries (xrOvaries) suggesting that an embryonic niche may be required to support coordinated PGCLC differentiation. To address this, we evaluated rhesus and human PGCLC differentiation in xrTestis self-assembled from single cell suspensions of E12.5 embryonic mouse testicular cells. These single cell suspensions were aggregated as floating cultures in low adhesion 96-well plates before transferring to transwell membranes to create self-assembling rTestes. Using immunofluorescence (IF) staining, we found Sox9-positive (+) sertoli cells cluster and polarize in the rTestes, forming tubule-like structures, as early as day (D) 14 after transfer to the membrane. By D21 of transfer, these tubule-like structures become more numerous and morphologically complex. At both D14 and D21 we discovered that the extracellular matrix protein Laminin formed a basement membrane enclosing the sertoli cells as epithelial tubes. To evaluate whether this self-assembling embryonic testicular niche supports rPGCLC differentiation, we combined FACS-sorted GFP+/EpCAM+/ITAG6+ D4 PGCLCs (human or rhesus) with SSEA1 MACS-depleted embryonic mouse testicular cells (lacking endogenous mouse PGCs) to generate rhesus-mouse and human-mouse xenogeneic reconstituted testis (xrTestis). Using this approach, we observed donor-specific incorporation and survival of GFP+ PGCLCs in developing xrTestis. Taken together our findings suggest that the xrTestis may be a powerful model to study testicular niche development and physiology in vitro, as well as provide a new research tool for studying human prenatal germ cell differentiation towards in vitro gametogenesis.
**S3.4 - Developmental Kinetics and Transcriptome Dynamics of Stem Cell Specification in the Spermatogenic Lineage.** Nathan Law, Melissa Oatley, Jon Oatley

Continuity, robustness, and regeneration of cell lineages rely on stem cell pools that are established during development. For the mammalian spermatogenic lineage, the timing and mechanisms by which the foundational spermatogonial stem cell (SSC) population arises within the male germline during development are undefined. Here, we mapped the underlying dynamics of this process *in vivo* using a novel multi-transgenic reporter mouse model, *in silico* with single-cell RNA sequencing, and functionally with transplantation analyses to define the SSC trajectory. We discovered that SSC fate is pre-programmed within a subset of prospermatogonia during late fetal development. Transcriptome profiling revealed a continuum within the male germline throughout late fetal and neonatal development that defines SSCs and a prospermatogonial subset that is destined to become them. Additionally, whole-tissue 3D imaging revealed that spatial clustering is associated with SSC pool establishment. Lastly, transplantation analyses demonstrated that a defined subset of prospermatogonia are functionally fated to become SSCs in late fetal development. Collectively, our findings present a model that stem cell fate is pre-programmed in the spermatogenic lineage.

**S4.1 - The evolution of cooperative sperm in Peromyscus mice.** Heidi Fisher

The competition that ensues as sperm migrate to towards the egg is fierce, but never more so than when sperm of rival males compete. In a rare number of systems, sperm form cooperative groups to outswim sperm of rival males, and in the deer mouse, *Peromyscus maniculatus*, sperm can recognize the most closely related cells and selectively group with them. Here we examine the cellular and molecular mechanisms that regulate sperm aggregation and swimming performance. First, we investigate the relationship between sperm ultrastructure and the collective sperm behavior observed across *Peromyscus* mice using both theoretical models and empirical data from divergent species. We examine shared morphological traits associated with similar aggregate geometries and motility performance. Moreover, we explore a sub-population of one species that produce sperm that lack apical hooks, a nearly ubiquitous feature on rodent sperm heads, to understand how this trait relates to sperm performance and fertilization. These results reveal how sperm morphology may be an informative measure for predicting sperm motility and reproductive success in a competitive context. Second, we focus on the molecular regulation of sperm aggregation. We examine proteomic data from mature sperm and transcriptomic data from testes of three *Peromyscus* species, two that produce sperm that aggregate, and one that produces non-aggregating sperm, to characterize the patterns of protein and gene expression across the species. We also show that cellular changes involved in sperm capacitation are associated with sperm aggregate disassociation in *Peromyscus*, and use this to further enrich our analysis for possible causal aggregation proteins. Using theoretical models, we predict optimal localization of causal adhesive molecules, and possible involvement of the apical hook, to further examine how sperm form associations to collectively improve their swimming performance and opportunity to achieve fertilization.
**S4.2 - The two centrioles of the zygote are inherited from the sperm in human and other non-murine mammals.** Tomer Avidor-Reiss

One of the major unresolved questions during fertilization in humans and most other mammals is the precise way in which the centrioles are inherited and how they function in the spermatozoon. A key reason for this is the lack of accurate tracking of the centriole, since many centriolar proteins are transient due to a process referred to as Centrosome Reduction. As a result, the current dogma holds that centrosome reduction leads to structural degeneration and functional inactivation of the centriole that is located at the base of the flagellum (the distal centriole), causing the spermatozoa to have only one intact, functional centriole (the proximal centriole). The idea that the spermatozoon has one centriole and this centriole is not connected to the flagellum adds to the difficulty in understanding centriole inheritance and sperm flagellum movement.

Recently, our studies in insects and mammals suggested that sperm have an atypical centriole that is remodeled during sperm formation. These findings suggest a new hypothesis, Centriole Remodeling, and enables, for the first time, the study of the role of the centriole in sperm movement and in the zygote. The structure of the remodeled distal centriole consists of splayed microtubules that are flanked by bars made of centriole luminal proteins at the base of the axoneme, with only a subset of the centriolar proteins typically found in a centriole.

Currently, we study how and why the sperm centrioles are remodeled, using a comparative approach *in vivo* and *in vitro*, in combination with microscopy, biochemistry, bioinformatics, and genetics. We aim to shed light on the clinical implication of the sperm centrioles and their evolution.

**S4.3 - Mitochondrial porin, VDAC2, has unexpected roles in sperm flagella formation.** Jessica Dunleavy, Denis Korneev, Donna Merriner, Anne O'Connor, Grant Dewson, Moira O'Bryan

VDAC2 is a voltage-gated porin in the outer mitochondrial membrane. It has well-established roles in apoptosis and cellular metabolism, however there is increasing evidence that VDAC2 is important for male fertility. Previous studies have localized VDAC2 to the flagella outer dense fibers in bovine sperm and correlated decreased VDAC2 levels with human male infertility. Herein, we sought to characterize the precise roles of VDAC2 in spermatogenesis.

Using a *Vdac2* knockout mouse model, we reveal that VDAC2 is not only essential for male fertility, but that spermatogenesis is uniquely sensitive to even partial VDAC2 reduction. Indeed, mice heterozygous for the *Vdac2* deletion allele (*Vdac2*+/−) are completely male sterile. Sperm production rates and testis weights are normal in these mice, suggesting disruption of VDAC2 apoptotic function is not driving the male sterility phenotype. Instead, our analyses indicate it is due to an inability to produce functionally motile sperm. Both the percentage of motile sperm and the percentage of progressively motile sperm were significantly reduced, by 62% and 93% respectively. Intriguingly, our analysis revealed these motility defects are structural in origin. Most notably trafficking and incorporation of mitochondria into the sperm flagella mid-piece is
severely compromised. Consistent with these data, we also show that VDAC2 is highly testis enriched and while it is present in all ages of the postnatal mouse testis it is particularly upregulated after the onset of spermiogenesis. Collectively, our data unequivocally establishes VDAC2 as an essential component of the spermiogenesis machinery and supports a model wherein VDAC2 facilitates the loading of mitochondria into the sperm flagellum. Notably, given that VDAC2 has previously been shown to interact with the microtubule motor protein, KLC3, these data raise the distinct possibility that VDAC2 is required to interact with motor proteins to facilitate mitochondrial trafficking into the sperm mid-piece.

S4.4 - FAM170A loss causes subfertility and defective sperm motility in mice. Darius Devlin, Kaori Nozawa, Masahito Ikawa, Martin Matzuk

Family with sequence similarity 170 members A and B (FAM170A and FAM170B) are two testis-specific, acrosome-localized paralogous proteins conserved throughout mammals. While in vitro experiments in previous literature suggested FAM170B plays a role in mouse sperm acrosome reaction, the role of FAM170A in the testis has not been explored. In this study, we have used CRISPR/Cas9 to generate null alleles for each gene and homozygous null male mice were mated to wildtype (WT) females for six months to assess fertility. Fam170b KO males were found to produce normal litter sizes, had no significant difference in sperm counts, and sperm morphology appeared normal. In contrast, mating experiments revealed significantly reduced litter sizes from Fam170a KO males compared to controls. Though both Fam170a KO and control males had normal testis weights, CASA experiments revealed a significant reduction in Fam170a KO sperm count. Although overall percent motility was similar to control males, KO males had dramatically reduced progressive motility. In addition, light microscopy of Fam170a KO sperm revealed abnormal sperm head morphology and a bent neck. FAM170A has an important role in male fertility, as loss of the protein leads to subfertility; however, it is unclear whether the FAM170B paralog gives partial rescue of fertility in these KO males. We have begun assessing the fertility of Fam170a/Fam170b double KO males to determine if there is any redundancy. This work will further our molecular understanding of sperm function and could help improve male infertility diagnoses. Research was supported by Eunice Kennedy Shriver National Institute of Child Health & Human Development grants P01 HD087157 and R01 HD088412, the Bill & Melinda Gates Foundation grant OPP1160866, and training fellowships from the Interdepartmental Program in Translational Biology and Molecular Medicine grant T32 GM088129 and the Training Interdisciplinary Pharmacology Scientists (TIPS) Program grant T32 GM120011.


Disorders of sex development (DSDs) are conditions affecting the development of the gonads or genitalia. Variants in two key genes directing the early stages of gonadal development — testis-
determining gene *SRY* and its direct target *SOX9* — are an established cause of 46,XY DSD, but the genetic basis of many DSDs remains unknown. *SRY*-mediated *SOX9* upregulation in the early gonad is crucial for testis development, yet the elements regulating *SOX9* have not been identified in humans.

We screened a cohort of 44 DSD patients for Copy Number Variations (CNVs) in the upstream regulatory region of *SOX9*, using CGH-array technology. This led to the identification of four DSD patients with duplications or deletions upstream of *SOX9* that allowed the precise mapping of the minimal critical regions required for sex-reversal. Bioinformatic analysis of the overlapping CNVs identified three putative enhancers for *SOX9*, and luciferase tiling assays confirmed their potential as novel *SOX9* human testis enhancers. In cell-based reporter assays, these enhancers responded to different combinations of testis-specific regulators including *SRY*, NR5A1 (SF1) and *SOX9* itself, and were repressed by a female sex-determining factor FOXL2.

The three enhancers play different roles in *SOX9* initiation, upregulation and maintenance. Importantly, all three enhancers showed synergistic activity and together co-operate to drive *SOX9* up-regulation over two-fold in the male gonad. One enhancer is human-specific, the second has conserved function in humans and mice resulting in complete sex reversal when deleted, and mice null for the third enhancer showed reduced Sox9 transcription without sex reversal.

This is the first study to identify *SOX9* core enhancers that, when duplicated or deleted, result in 46,XX or 46,XY sex reversal, respectively. Together, these three enhancers provide a hitherto missing link of how *SRY* activates *SOX9* in humans, and establish *SOX9* enhancer mutations as a significant cause of DSD.

**S5.2 - The Battle of the Sexes: Setting the gonad on the male trajectory.** Blanche Capel, Sara Garcia-Moreno, Christopher Futtner, Isabella Salamone, Danielle Maatouk

XX and XY fetal gonads are initially bipotential, poised between the ovary and testis fate. Initially, a testis and ovary network are operating simultaneously in XY gonads. Transcriptome analysis and other experiments suggest that commitment to testis fate requires both the activation of testis genes and the repression of genes associated with ovary fate. We performed a quantitative genome-wide profile of the repressive histone mark H3K27me3 and its active counterpart H3K4me3, in isolated XY and XX gonadal supporting cells before and after sex determination. We show that testis and ovary sex-determining genes are bivalent before sex determination. After sex determination in XY gonads, genes associated with testis development lose H3K27me3 marks. Surprisingly, genes associated with the alternate ovary pathway remain bivalent, possibly contributing to the ability of these cells to transdifferentiate in adult life. We are investigating the role of epigenetic modifiers including histone demethylases and CBX2, a key member of the polycomb complex, in mediating these events. We show that CBX2 directly binds the downstream Wnt signaler *Lef1*, an ovary-promoting gene that remains bivalent in Sertoli cells. Our results suggest that stabilization of the testis fate requires CBX2-mediated repression of bivalent ovary-determining genes, which would otherwise block testis development.
**S5.3 - Estrogen regulates key developmental pathways to determine somatic cell fate in the gonad.** Deidre Mattiske, Andrew Pask

An increase in the incidence of disorders of sex development (DSDs) has been linked to increased exposure to environmental endocrine disruptors (EEDs). Many EEDs affect estrogen signalling and therefore have the potential to disrupt sexual differentiation and the maintenance of somatic cell fate within the gonad. Determining the mechanisms of estrogen regulation of gonad somatic identity is essential to understanding gonadal development and maintenance, as well as the consequences of exposure to estrogenic endocrine disruptors. Using a mouse gonad culture, we have studied the effects of estrogen on SOX9, a known target of estrogen and essential for testicular and Sertoli cell development, and somatic cell fate. E10.75 paired male mouse gonads were cultured in hanging drop cultures, exposed to either 0 nM (control) or 5 nM 17alpha-ethinylestradiol (EE2). After 48 hours, developing gonad and mesonephros were clearly defined and SOX9 staining was observed in the cytoplasm of somatic cells in the estrogen treated gonads, but not in controls. We showed by qPCR that this led to a suppression of male genes AMH and Dhh, and upregulation of granulosa cell genes Fst, Foxl2 and Msx1. We further examined the effect of estrogen on gonadal development by performing complete transcriptomics on estrogen treated and control cultured mouse gonads. We have identified several genes that are directly targeted by estrogen, downregulating male specific pathways. Conversely, genes specific to the female developmental pathway were significantly upregulated in male gonads following EE2 exposure. This study confirms that in mammals estrogen can suppress SOX9 through translocation to the cytoplasm and identifies key genes directly responsive to estrogen that push somatic cells towards a granulosa cell fate.

**S5.4 - New Insights into Ovarian Development and Function.** Dagmar Wilhelm, Julia Kaburaki, Pascal Bernard, Stefan Bagheri-Fam, Renata Phyland, Jeremy Le, JoAnne Richards, Andrew Pask

Normal development of the ovaries during embryogenesis is critical for their function and fertility of the adult female. The WNT4/RSPO1 signalling pathway is critical for normal ovarian development and function. Recently, it has been shown that the prorenin receptor (PRR), encoded by the Atp6ap2 gene, functions as a bridge between the WNT receptor LRP6 and the vacuolar H+-ATPase (V-ATPase). This interaction is crucial for canonical WNT signalling after binding of the ligand. These data, together with our observation that Atp6ap2 is highly expressed in the developing gonads, led to our hypothesis that PRR is important for ovarian development. In addition to the regulation of canonical WNT signalling PRR is best known for its role in the renin-angiotensin system. In addition, PRR is also important for other signalling pathways that play a role in ovarian development and/or function such as non-canonical WNT signalling and the ERK and p38-MAPK signalling pathways, demonstrating that PRR is a multi-functional protein. Hence, it is not surprising that complete loss of PRR in mouse, Drosophila, and zebrafish results in early embryonic lethality. To test our hypothesis that PRR is important for ovarian development we generated mice with conditional deletion of Atp6ap2 in the somatic cells of fetal gonads using the Nr5a1-Cre line. Here, we present the ovarian phenotype of these mice and show that PRR is important for granulosa cell differentiation and the cross-talk between granulosa cells and oocytes.
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Worldwide, 3 million pregnancies are lost to stillbirth every year. 50% of stillbirths are associated with fetal growth restriction (FGR) arising from placental insufficiency. There are no reliable screening tests for placental insufficiency, especially near-term gestation when the risk of stillbirth rises. The aim of our research is to identify novel biomarkers within the maternal circulation that could be used to predict which babies are FGR and have an elevated risk of stillbirth.

The Fetal Longitudinal Assessment of Growth (FLAG) study involved prospective collection of plasma from 1996 participants at 36 weeks’ gestation. We divided the cohort to discover, then validate circulating biomarkers (997 in cohort 1; 999 in cohort 2).

We initially screened 22 proteins in cohort 1 and identified a strong association between low circulating plasma serine peptidase inhibitor, Kunitz type-1 (SPINT1) concentrations and low birthweight (<10th centile), which we validated in cohort 2. Importantly, low SPINT1 concentrations were strongly associated with other clinical indicators of placental insufficiency, including increased uterine artery Doppler resistance, low placental weight and low lean body mass of the newborn. The association between low circulating SPINT1 concentrations and several clinical indicators of placental insufficiency validated in an independent cohort of high-risk pregnancies (n=291) from the United Kingdom. Mechanistically, hypoxia in vitro and in vivo reduced placental SPINT1 expression and silencing SPINT1 reduced placental cellular proliferation. In contrast, a SPINT1 mimetic enhanced placental cell proliferation.

Thus, using a large cohort of maternal bloods, we have screened 22 proteins and robustly demonstrated that low circulating SPINT1 is a marker of placental insufficiency. We have also developed a 4-tier screening system to demonstrate that low circulating levels of SPINT1 at 36 weeks’ gestation may identify pregnancies with fetuses at very high risk of being at a low weight centile, and at elevated risk of stillbirth.

**S6.2 - Understanding placental adaptation to maternal malnutrition.** Michael Satterfield

Maternal malnutrition is a common pregnancy condition with lifelong consequences for the offspring. Our objective was to evaluate lipid profiles throughout gestation of nutrient-restricted (NR) ewes with either small for gestational age (SGA) or normal (non-SGA) weight fetuses compared to well-fed controls. Singleton pregnant ewes (n=36) were generated by embryo
transfer and randomly assigned to receive either 100% (Control; n=8) or 50% (NR; n=28) of their National Research Council (NRC) nutritional requirements from Days 35 to 135 of gestation. Blood samples were collected on Days 35, 70, 105, and 135. Allantoic fluid was collected at surgery on day 70. Ewes were necropsied on Day 135 of gestation and additional samples obtained. NR ewes were segregated into upper (non-SGA; n=7) and lower (SGA; n=7) quartiles, for further investigation. Weights of Non-SGA fetuses did not differ from those of fetuses from control ewes (5.46±0.14 vs 5.61±0.13 kg), while weights for SGA fetuses were lower (P<0.0001) (3.84±0.14 kg). There was an interaction of treatment and day for concentrations of non-esterified fatty acids (NEFAs) (P<0.05) in maternal plasma. On Days 100 and 135 of gestation, NR non-SGA dams had higher concentrations of NEFAs in plasma. In the fetal circulation, concentrations of NEFAs were elevated (P<0.05) in NR non-SGA fetuses compared to controls. Allantoic fluid from NR SGA dams had greater concentrations of triglycerides on Day 70 (P<0.05) of gestation. Further, regression analyses identified a negative correlation between elevated triglycerides in allantoic fluid at Day 70 and fetal weight on Day 135. On Day 135, triglyceride levels in the fetal circulation were reduced (P<0.05) in NR SGA fetuses compared to NR non-SGA fetuses and control fetuses. Collectively, results identify dynamic changes in NEFA and triglyceride concentrations in fetal and placental fluids that may predict variable rates of fetal growth in nutrient restricted ewes.

**S6.3 - Morphological and Epigenetic Abnormalities in the Placenta Linked to Embryo Culture in a Mouse Model of In Vitro Fertilization.** Lisa Vrooman, Eric Rhon-Calderon, Olivia Chao, Duy Nguyen, Laren Riesche, Richard Schultz, Marisa Bartolomei

Assisted Reproductive Technologies (ART), which include in vitro fertilization (IVF), are associated with increased risk for adverse outcomes. We and others have demonstrated that ART may iatrogenically induce abnormal placentation, low birth weight, and DNA hypomethylation using the mouse as an experimental model. To elucidate the link between IVF procedures and abnormal placentation, naturally-conceived and IVF mouse concepti were examined at embryonic day (E) 12.5, 14.5, and 18.5. Groups controlling for superovulation, embryo culture, and embryo transfer were also assessed. Individuals (n=13-45) from a minimum of three litters were analysed for each experimental group. At E12.5 and E14.5, fetal weight was significantly reduced in all experimental groups but was most severe in embryo culture and IVF groups. By E18.5, fetal weight was still most dramatically reduced in embryo culture and IVF groups. Placenta weight at E12.5 was unchanged but vasculature abnormalities were observed in all ART groups. By E18.5, embryo culture and IVF placentas were 28-37% larger than embryo transfer and superovulation groups and 80-85% larger than natural concepti. Further, there was a disproportional increase in the junctional zone and differences in cell composition in embryo culture and IVF placentas. Global DNA methylation and methylation at select imprinting control regions was significantly altered at all time points in only the embryo culture and IVF placentas. Notably, the phenotypes associated with embryo culture were rescued if development occurred in vivo prior to the morula stage. Taken together, our results suggest that: 1) all ART procedures compromise fetal growth shortly after placental formation due to abnormal placental vasculature, 2) placental overgrowth observed in late gestation may be the result of compensatory mechanisms, 3) embryo culture from the 1-cell to morula stage induces the most severe fetal and placental phenotypes.
**S6.4 - Protective role of IL33 on negative pregnancy outcomes associated with lipopolysaccharide exposure.** Keisuke Kozai, Khursheed Iqbal, Regan Scott, Pramod Dhakal, Michael Soares

Preeclampsia is a pregnancy disorder characterized by maternal hypertension, fetal growth restriction (FGR), and preterm birth. Hallmarks of preeclampsia at the maternal-fetal interface include deficits in intrauterine trophoblast invasion and uterine spiral artery remodeling and heightened local inflammation. Although several studies demonstrated that disordered IL33 signaling is associated with preeclampsia, the role of IL33 signaling in the regulation of intrauterine trophoblast invasion and uterine spiral artery remodeling remains to be elucidated. To address this, we generated germline mutant rat models for components of the IL33/ST2 signaling pathway using CRISPR/Cas9 system. IL33 and ST2 deficiencies each interfered with lung inflammatory responses induced by intravenous Sephadex treatment, including the recruitment of eosinophils and Th2 cytokine expression. Male and female rats possessing either IL33 and ST2 deficits were fertile and did not differ from wild type rats according to an assortment of parameters of pregnancy and placentation. We next explored roles for IL33 signaling in pregnancy-dependent adaptations to lipopolysaccharide (LPS) exposure. Wild type and IL33 deficient pregnant rats were injected intraperitoneally with saline (1 ml/kg) or LPS (10 ug/kg on gestation day (gd) 13.5; 40 or 60 ug/kg on gd 14.5-16.5; n = 5-8 per group) and sacrificed on gd 17.5. Wild type pregnant rats treated with LPS satisfactorily adapted and did not differ significantly from control treated wild type rats. In contrast, LPS treatment decreased fetal survival rate, fetal and placental weights, and increased % FGR in IL33 deficient rats. IL33 deficiency also altered indices of intrauterine trophoblast invasion and uterine mesometrial interferon gamma (Ifng) expression in pregnant rats exposed to LPS. In summary, these results suggest that IL33 participates in the protection against LPS-induced fetal and placental growth restriction. Cellular and molecular mechanisms underlying IL33 action are unknown but may include impacts on intrauterine trophoblast invasion and the regulation of IFNG.

**S7.1 - Developmental Mechanisms for Protracted Female Fertility: Insights from the Naked Mole-Rat.** Ned Place

Naked mole-rats (NMRs) are renowned for being the longest-lived rodent, with an adult body mass of only 30-60 g and a maximum lifespan of >30 years. What is no less remarkable, is the fact that female NMRs show no age-associated decline in fertility or fecundity into their third decade of life. Our interrogations of NMR ovaries during early postnatal development have identified several fascinating features that likely contribute to their protracted female reproductive lifespan. Germ cells in NMR ovaries at postnatal day 1 (P1) number in the 100s of thousands, and expand to more than a million at P8. The P8 ovary is composed almost entirely of germ cell nests, with relatively few primordial follicles (PFs) having formed. By way of contrast, nest breakdown and PF formation is complete by P8 in mice, with an approximate ovarian reserve of 5,000-10,000 PFs. Whereas the combined number of germ cells and PFs markedly declines between P8 and P28 in NMRs, they still number in the 100s of thousands, and signs of continued germ cell division are evident in DDX4+ cells that co-label for mitotic markers. Many
other germ cells still express the pluripotency markers SOX2 and OCT4 at P28. In addition to germ cell numbers, we are also evaluating mechanisms that might attenuate age-associated ovarian fibrosis and inflammation in NMRs, including the roles of hyaluronan (HA) and the cytoprotective NRF2 pathway. NMRs produce an unusually high molecular weight hyaluronan (HMW-HA), and ovarian HA content is substantially greater in NMRs than in mice. Hepatic NRF2 signaling was shown by others to be more pronounced in NMRs than in mice, and we will determine if NRF2 signaling is enhanced in NMR ovaries as well. Collectively, our investigations are elucidating the means by which this longest-lived rodent can maintain female fertility for decades.

**S7.2 - The Conversion of a Homeostatic to Reactive Matrix: Hyaluronan and the Aging Ovarian Stroma.** Michele Pritchard, Jennifer Rowley, Cecilia Villanueva, Farners Amargant, Wendena Parkes, Lunan Zhou, Francesca Duncan

Female reproductive aging is characterized by a decline in gamete quantity and quality, but the role of the ovarian stroma in this process is unknown. We recently found that the aging mouse and human ovarian environment is fibrotic and inflamed. Hyaluronan (HA) is a large (>2MDa) glycosaminoglycan that contributes to tissue homeostasis and creates mechanically soft microenviroments. In extra-ovarian tissues, fragmentation-mediated HA loss drives inflammation, fibrosis, and aging. Therefore, we hypothesized that reproductive aging is associated with an overall loss of HA, in part due to increased fragmentation, which promotes increased stiffness, inflammation, and reduced oocyte quality. Here, we assessed the ovarian HA network in reproductively young (6–12 weeks) and old (14–17 months) CB6F1 mice, measured ovarian stiffness, and evaluated how HA fragments impact stromal inflammation and oocyte quality in vitro. Using QPCR, we interrogated the balance of HA synthesis and degradation through the expression of hyaluronan synthases (Has) and hyaluronidases (Hyal), respectively. Has3 (6-fold decrease) and Hyal1 (1.38-fold increase) expression changed in an age-dependent but estrous cyclicity-independent manner, suggesting a net loss of ovarian HA and increased fragmentation. Using a HA binding protein-based assay, we observed a significant age-associated reduction in total ovarian HA content specific to the stroma (p=0.008). To examine how advanced reproductive age affects ovarian micromechanical properties, we performed nanoindentation analysis. More force was required to indent ovaries from reproductively old (3.57±2.4kPa) compared to young (1.69±2.4kPa; p<0.001) mice, linking ovarian stiffness to advanced age and reduced ovarian HA content. Finally, in vitro exposure of ovarian stromal cells and follicles to HA fragments (200kDa) resulted in significant proinflammatory cytokine gene expression and poor-quality oocyte production, respectively. These results demonstrate that the ovarian HA network is dysregulated with advanced reproductive age, significantly impacting both the stroma and the follicle.

**S7.3 - Increased expression of Kifc1 and Kifc5b targeting endogenous-siRNA contribute to the age-related decline in oocyte quality.** Bettina Mihalas, Nicole Camlin, Miguel Xavier, Alexandra Peters, Janet Holt, Jessie Sutherland, Eileen Mclaughlin, Andrew Eamens, Brett Nixon
Oocytes are reliant on messenger RNA (mRNA) stores to support their survival and integrity during a protracted period of transcriptional dormancy as they await ovulation. Oocytes are, however, known to experience an age-associated alteration in mRNA transcript abundance, a phenomenon that contributes to reduced developmental potential. Here we have investigated whether the expression profile of small non-protein-coding RNAs (sRNAs) is similarly altered in aged mouse oocytes and whether these changes have the potential to influence subsequent gene expression. The application of high throughput sequencing revealed substantial changes to the global sRNA profile of germinal vesicle (GV) stage oocytes from young (4-6 weeks) and aged mice (14-16 months). Among these changes, 160 endogenous small-interfering RNAs (endo-siRNAs) and 10 microRNAs were determined to differentially accumulate within young and aged oocytes. We further demonstrated that these differentially accumulated endo-siRNAs targeted 39 unique mRNAs. More specifically, we discovered that two members of the kinesin protein family, Kifc1 and Kifc5b, were selectively targeted for expression regulation by three endo-siRNAs of elevated abundance in aged GV oocytes. Accordingly, we also revealed a reciprocal decrease in Kifc1 and Kifc5b mRNA expression as well as a reduction of their encoding protein, HSET, in aged GV and metaphase I stage oocytes. The implications of a reduction in functional HSET protein was explored using complementary siRNA-mediated knockdown of Kifc1 and Kifc5b (50µl/ml) and pharmacological inhibition of HSET (10 µM) from the GV stage, both of which led to increased rates of aneuploidy in otherwise healthy young metaphase II oocytes after in vitro maturation. Collectively, our data raise the prospect that altered sRNA abundance, specifically endo-siRNA abundance, may contribute to the age-dependent deterioration of oocyte quality.

S7.4 - The telomere length of spermatozoa as an epigenetic biomarker of sperm quality and aging. Mingju Cao, Peter Chan, Marie-Claude Léveillé, Bernard Robaire

Telomeres, repetitive DNA sequences that cap chromosome ends, maintain genomic stability and cell viability. In human somatic cells, telomere length (TL) shortens in every cell cycle. However, there are conflicting reports as to whether TL of male germ cells is affected during aging. Our objectives were to evaluate if and how TL of sperm is affected by age, and to assess the association of spermatozoal TL (STL) with ‘classical’ clinical semen parameters. A qPCR protocol to measure absolute telomere length (in kb) was optimized by using the human 1301 lymphoblastic cell line DNA with known TL of 70 kb for calibration. Semen samples from 276 men were analyzed, of these 154 samples were from a fertile control populations, and 122 samples from infertile men. The fertile control subjects were categorized into three age groups: young (18-38 yrs, n=55), intermediate (39-45 yrs, n=23) and old (≥46 yrs, n=76); the breakdown for infertile group was: young (18-38 yrs, n=66), intermediate (39-45 yrs, n=36) and old (≥46 yrs, n=20). Within the fertile and infertile groups, while no difference was found between the STL of young and intermediate-aged men, the STL in older men was significantly longer (fertile: 13.91±0.56 vs. 13.14±0.65, and 16.32±0.59, respectively, P<0.05; infertile: 15.24±0.47 vs. 14.56±0.49, and 19.96±1.06, respectively, P<0.001). Two-way ANOVA analysis revealed that STL in infertile older men was longer than that of older control men (P<0.05). Both paternal and maternal age at conception had no correlation with their subjects’ STL. A significant positive correlation was found between STL and sperm count (P=0.004), testosterone level (P=0.04) in infertile subjects, but not in fertile controls. Our results suggest that STL can serve as an
epigenetic biomarker of sperm quality and aging and that selection of spermatozoa from older men with shorter STL may be beneficial in fertility clinics. (Funded by CIHR-TE1-138298).

**S8.1 - Super-Mendelian inheritance mediated by CRISPR–Cas9 in the female mouse germline.** Hannah Grunwald, Valentino Gantz, Günnar Poplawski, Xiang-ru Xu, Ethan Bier, Kimberly Cooper

A gene drive biases the transmission of one of the two copies of a gene such that it is inherited more frequently than by random segregation. Highly efficient gene drive systems have recently been developed in insects, which leverage the sequence-targeted DNA cleavage activity of CRISPR–Cas9 and endogenous homology-directed repair mechanisms to convert heterozygous genotypes to homozygosity. If implemented in laboratory rodents, similar systems would enable the rapid assembly of currently impractical genotypes that involve multiple homozygous genes (for example, to model multigenic human diseases). To our knowledge, however, such a system has not yet been demonstrated in mammals. Here we use an active genetic element that encodes a guide RNA, which is embedded in the mouse tyrosinase (Tyr) gene, to evaluate whether targeted gene conversion can occur when CRISPR–Cas9 is active in the early embryo or in the developing germline. Although Cas9 efficiently induces double-stranded DNA breaks in the early embryo and male germline, these breaks are not corrected by homology-directed repair. By contrast, Cas9 expression limited to the female germline induces double-stranded breaks that are corrected by homology-directed repair, which copies the active genetic element from the donor to the receiver chromosome and increases its rate of inheritance in the next generation. These results demonstrate the feasibility of CRISPR–Cas9-mediated systems that bias inheritance of desired alleles in mice and that have the potential to transform the use of rodent models in basic and biomedical research.

**S8.2 - Editing the Non-Human Primate Genome.** Erika Sasaki

Nonhuman primates (NHPs) offer excellent models for preclinical research to develop novel therapeutic approaches because of their similarities of genetics, metabolism and physiological characteristics to humans. Not only preclinical study models, NHPs are also nice models for studying primate specific developmental biology. Most of mammalian early development including germ cell development has been understood by rodent models. However, recent studies including single-cell RNA-seq data of human embryos and non-human primate data have shown significant differences between primate and mice.

The common marmoset (marmoset) is a suitable NHP for studying development or creating disease models because of their unique reproductive characteristics such as multiple ovulation (2-3 oocytes/ovulate), short sexual maturity period (1.5-2 years) and relatively short gestation period (145–148 days). Furthermore, marmoset is an only creature that can collect naturally fertilized embryos.

Recent progresses in genetically modification (GM) technologies in NHPs including genome editing have enabled development of GM human disease NHPs models. Previously, only
exogenous gene over expression models by lenti or retro viral vectors were available for last decade. Not only NHPs but also the mammalian except mouse and rat, embryonic stem cells (ESCs) lack of the ability to contribute to the germline that is essential for producing targeted gene KO/KI animals. Therefore, traditional gene-targeting knock-out (KO)/knock-in (KI) technics could not be applied to NHPs.

However, the innovative genome-editing technologies such as zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALEN), and clustered regularly interspaced short palindromic repeat (CRISPR)/Cas9 has helped to resolve this issue, and it is now possible to generate target-gene KO animals without using ESCs. Consequently, it became possible to produce useful genetically modified non-human primate models to uncover unknown gene function, understand early development and develop new therapies for many diseases.

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S8.3 - In Utero Imaging of Clock Gene Expression Reveals the Development of the Circadian Clock. Keenan Bates, Ron McCarthy, Tatiana Simon, Jacob Amme, Sarah England, Erik Harzog

Circadian clocks drive daily fluctuations in physiological processes and behavior. Circadian rhythms are found in nearly every tissue of the human body and typically synchronize to environmental cues, such as the light-dark cycle. Rhythmicity is dependent upon the transcriptional-translational feedback loop involving the core clock genes, including Period2. In mammals, the circadian system is regulated by the suprachiasmatic nuclei (SCN) which is responsible for organizing the downstream peripheral oscillators found throughout the body. During pregnancy, there exists the possibility that three distinct circadian clocks interact to regulate gestation: the maternal clock, the fetal clock, and the placental clock. For example, daily rhythms in maternal melatonin may influence fetal development and circadian timing. By embryonic day 15.5 (E15.5), the fetal mouse SCN appears fully developed with intrinsic cellular daily rhythms in gene expression and may begin to influence maternal circadian rhythms. However, the role of each of these clocks and their effect on gestation are unknown. We aim to monitor and manipulate these daily rhythms in utero during gestation. We hypothesize that clock gene expression is detectable early on in pregnancy but is not rhythmic until SCN neurogenesis is complete. We utilized luciferase reporters to examine when clock gene expression begins, begins to cycle, and if it changes (e.g. the amplitude or time of daily peak) in utero. We detected PERIOD2 (PER2) expression beginning at E4.5 and diurnal rhythms beginning around E12.5 in the developing fetal tissues. Following cesarean section at E17.5, we observed higher expression of PER2 in the placenta than the fetus. We conclude that PER2 expression in utero begins by E4.5 and develops daily rhythms by E12.5. Ongoing studies aim to resolve if fetal rhythms depend on maternal rhythms. This work was supported by the March of Dimes Washington University Center for Prematurity.
**S8.4 - A genetic toolbox for functional in vivo and structural in situ analysis of the mouse testis.** David Fleck, Naofumi Uesaka, Justine Fischoeder, Lina Kenzler, Nadine Mundt, Jennifer Spehr, Marc Spehr

Spermatogenesis is an essential biological process that is critical for the survival of a species. However, knowledge about the physiological mechanisms that underlie testicular cell communication during spermatogenesis is still limited. Here, we introduce a novel toolbox to elucidate the structural and functional architecture of mouse testicular physiology. We combine the Cre-loxP system for conditional expression of genetically encoded fluorescent markers, reporters, and optogenetic tools, with whole organ clearing and *in vivo* multiphoton microscopy. Cell-type specific driver mice allow selective targeting of peritubular myoid, Sertoli, and germ cells, respectively. After establishing a testis-specific electrophoretic tissue clearing protocol based on the CLARITY method, we visualize the three-dimensional cytoarchitecture of each cell type in intact tissue preparations, allowing quantitative anatomical analysis. In addition, genetically encoded Ca$^{2+}$ indicators (GCaMP6) enable fast recordings of cell-type specific Ca$^{2+}$ signals *in vivo*. Our data indicate distinct spontaneous Ca$^{2+}$ signaling patterns within the seminiferous epithelium, which can now be analyzed as a function of developmental, (patho)physiological, or endocrine state.

**S9.1 - Molecular and Evolutionary Strategies of Meiotic Cheating by Selfish Elements.** Takashi Akera

Mendel’s Law of Segregation states that each allele has an equal chance to transmit to the gametes. However, this law can be violated by selfish genetic elements, which manipulate the production of gametes to increase their own rate of transmission. This genetic cheating in meiosis, meiotic drive, has significant impacts on genetics with associated fitness costs to the host (e.g., fertility issues). In female meiosis, selfish elements drive by preferentially segregating to the egg. Because centromeres direct chromosome segregation, they are the genetic elements with the best opportunity to cheat. However, the mechanisms of non-Mendelian segregation of selfish centromeres are unknown. Here we show that microtubule (MT) destabilizing activity makes centromeres behave selfishly in female meiosis. Using our hybrid mouse oocyte system, we find that selfish centromeres preferentially destabilize MT interactions on the cortical side of the spindle that would direct them to the polar body, thus promoting re-orientation from the cortical side to the egg side. Exploiting centromere divergence between species, we show that selfish centromeres in different species use the same molecular pathway but modulate it differently to enrich destabilizing factors. Our results indicate that increasing MT destabilizing activity is a general strategy for non-Mendelian segregation, but centromeres have evolved distinct mechanisms to increase that activity to exploit the asymmetry inherent in female meiosis.
**S9.2 - Phenotypic variation in female reproductive parameters in genetically diverse mice: a window to understand human infertility.** Ewelina Bolcun-Filas, Ruby Boateng

Detailed investigation of the genetic basis of gamete development will help us understand variation in female fertility and ultimately provide diagnostic and treatment options for patients with infertility. We use genetically diverse mice, the Collaborative Cross (CC), Diversity Outbred (DO) and their parental inbred strains, as a novel model to study genetic factors regulating female fertility in genetically heterogeneous population. The CC lines are multiparental recombinant lines from eight inbred founder strains. The DO mouse population was seeded via random breeding of incipient CC lines. Each CC line is a unique mosaic of eight parental genomes while all mice within a given line are genetically identical. In contrast, each DO mouse is genetically unique with a high level of allelic heterozygosity. The DO population is kept as heterogeneous stock, which, through a random breeding system, provides an unlimited source of novel allelic combinations, thus offering the best available mammalian model for genetic complexity of human population. The complementary use of CC and DO mice offers an unprecedented system to study the role of genetic factors in female reproduction which is challenging to address in human. We hypothesized that multiple genetic factors act in combination to regulate oocyte development and establish differences in oocyte quantity and quality among genetically diverse mice and humans. We analyzed fertility, early oocyte development and establishment of ovarian reserve in founder inbred strains (A/J, C57BL/6J, 129S1/SvJ, NOD/ShiLtJ, CAST/EiJ, PWK/PhJ, WSB/EiJ), CC lines (CC041, CC019, CC006) and DO females and observed significant differences which supports our hypothesis that development of healthy oocytes depends on heritable genetic factors. Interestingly, we found unique reproductive characteristics in lesser known inbred strains used for cancer, immunology or diabetes research that may have translational importance to human infertility. I will discuss our approach and results to date. Funded by NIH R01-HD093778 grant.

**S9.3 - Control of anaphase onset in mammalian female meiosis I.** Lenka Radonova, Michal Skultety, Kristina Kovacovicova, Jaroslava Sebestova, Thang Quang Dang-Nguyen, Michael Hopkins, Bela Novak, Martin Anger

Mammalian female gametes are prone to chromosome segregation errors, which are frequently resulting in aneuploidy. During the first meiotic division, the assembly of spindle apparatus and the attachment of kinetochores to the spindle microtubules is monitored by Spindle Assembly Checkpoint (SAC) and any irregularities in this process cause a delay in anaphase onset facilitated by blocking full activation of Anaphase Promoting Complex/Cyclosome (APC/C). In our study we focused on detailed quantitative analysis of SAC and APC/C activity during meiosis I in oocytes. Using micromanipulation and live cell imaging we assessed the activity of both pathways simultaneously in individual cells. Mad1 localization on kinetochores was used for quantification of the activity of SAC and the levels of securin and cyclins were used for measuring of the APC/C activity. Our results showed that during physiological conditions the onset of APC/C activity in meiosis I is preceded by removal of Mad1 from all kinetochores. However, our data also showed clearly that the ability of SAC to postpone anaphase in situations when chromosome attachments are incorrect is fairly limited and oocytes harboring such errors enter anaphase with only a short delay. Our results also revealed a new mechanism controlling
APC/C activity, which is not dependent on the attachment of the chromosomes to the spindle apparatus. These results are important for elucidation of control mechanisms of chromosome segregation in oocytes and for our understanding of etiology of conditions such as Down syndrome. Our project was supported by CSF projects 17-20405S and 19-24528S and by MEYS CR project CEITEC 2020 (LQ1601).

S9.4 - Interplay Between Caspase 9 and X-linked cellular Inhibitor of Apoptosis Protein (XIAP) In The Elimination of Mouse Oocytes During Fetal and Neonatal Ovarian Development. Xueqing Liu, Fatima Mansouri, Teruko Taketo

Female fertility is limited by the number and quality of oocytes in the ovarian reserve. Over 70% of the initial oocyte population gets eliminated during normal development, restricting the ovarian reserve. The cause or mechanism of this major oocyte loss remains an enigma. Our current study revealed two phases of oocyte surveillance during ovarian development by using various mutant mice with immunofluorescence (IF) staining of whole-mount ovaries or micro-spread ovarian cells and immunoblotting. We identified the first phase during fetal development, during which the oocytes underwent homologous chromosome synapsis, many oocytes were eliminated by the Caspase 9-mediated apoptotic pathway. The oocytes in the Casp9-/− ovary were accumulated at the pachytene stage with high levels of L1ORF1p expression and unrepaired double strain breaks (DSBs) in multiple foci along synaptonemal complex but with normal levels of asynapsis. Because most Casp9-/− mice die before birth, the fate of the oocytes retained in the Casp9-/− ovary was examined in the ovaries isolated at 16.5 dpc and cultured. The total number of oocytes increased in the Casp9-/− ovary after 3 days in culture (equivalent to 19.5 dpc), in consistent with the results in vivo, however, the number decreased to smaller than that in the Casp9+/− ovary after 7 days in culture (equivalent to 23.5 dpc). These results revealed the second phase of oocyte surveillance after birth, which was Caspase 9-independent. XIAP counterbalanced the Caspase 9 activity to regulate the oocyte dynamics; while XIAP overexpression mimicked Caspase 9 null, XIAP deficiency accelerated oocyte elimination. We conclude that the oocyte is equipped with multiple surveillance mechanisms to safe-guard the quality of oocytes in the ovarian reserve.

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S10.1 - Gonadotrope-specific Acvr2a and Acvr2b conditional knockout animals are hypogonadal and FSH-deficient. Gauthier Schang, Luisina Ongaro, Ulrich Boehm, Se-Jin Lee, Daniel Bernard

Activins are selective regulators of follicle-stimulating hormone (FSH) production by pituitary gonadotrope cells. In a gonadotrope-like cell line, LβT2 cells, activins stimulate FSH via the type II receptors ACVR2A and/or BMPR2. In vivo, global Acvr2a knockout mice have ~50% lower serum FSH levels than controls. In contrast, gonadotrope-specific Bmpr2 knockouts exhibit normal FSH. Another type II receptor, ACVR2B, can bind activins but appears dispensable for activin-stimulated FSH production in vitro. Acvr2b global knockout mice die soon after birth,
precluding their use to assess ACVR2B’s role in FSH production. In light of these previous results, we hypothesized that both ACVR2A and ACVR2B are required for normal FSH production in vivo. To investigate this idea, we crossed floxed *Acvr2a* or *Acvr2b* mice to GRIC mice, which express Cre recombinase specifically in gonadotropes. The resulting conditional knockout (cKO) animals were compared to littermate controls. Both *Acvr2a* and *Acvr2b* cKO females exhibited normal puberty onset (assessed by vaginal opening) and regular estrous cyclicity. However, when paired to wild-type males, *Acvr2a* and *Acvr2b* cKO females displayed ~70% and ~20% reductions in litter sizes, respectively. Similarly, *Acvr2a* and *Acvr2b* cKO males exhibited ~50% and ~20% reductions in testicular weights. Consistent with these phenotypes, serum FSH levels were lower in *Acvr2a* cKO males and females, as well as *Acvr2b* cKO males relative to controls. We failed to detect a decrease in FSH production in *Acvr2b* cKO females. Simultaneous deletion of both *Acvr2a* and *Acvr2b* in gonadotropes yielded females that were hypogonadal, acyclic, and sterile. Double knockout males were hypogonadal and had undetectable serum FSH levels. These data suggest that ACVR2A and, to a lesser extent, ACVR2B are the critical type II receptors through which activins (or related TGFβ ligands) signal to induce FSH production in mice in vivo.

**S10.2 - Conflicting desires: A POMC switch for breeding and feeding.** Jennifer Hill

The melanocortin pathway has been implicated in both metabolism and sexual function. When the melanocortin 4 receptor (MC4R) is knocked out globally, male mice display obesity, low sexual desire, and copulatory difficulties; however, it is unclear whether these phenotypes are interdependent. Female MC4R knockout mice were found to have reduced receptivity to copulation, as indicated by a low lordosis quotient. Our recent work shows that some, but not all, of these changes are independent of melanocortin-driven metabolic effects. In addition, we have identified key neuronal circuits responsible for these actions. These results raise interesting questions regarding how competing drives for food and sex are controlled in males and females.

**S10.3 - Activation of a classic feeding circuit slows neuroendocrine pulse generation controlling fertility: implications for PCOS?** Eulalia Coutinho, Melanie Prescott, Sabine Hessler, Christopher Marshall, Allan Herbison, Rebecca Campbell

The central regulation of fertility is carefully coordinated with energy homeostasis and infertility is frequently the outcome of energy imbalance. Likewise, life-style modifications to increase or decrease caloric intake can have a significant impact on overcoming infertility. This lecture will present recent findings investigating the impact of selectively activating NPY/AgRP neurons, critical regulators of metabolism, on the activity of gonadotropin-releasing hormone (GnRH) neurons, luteinizing hormone (LH) pulse generation, and reproductive function. Using both chemogenetic and optogenetic approaches coupled with serial sampling for LH in conscious mice, we found that selective activation of NPY/AgRP neurons decreased LH secretion and slowed LH pulse frequency in both males and females. An identical slowing was observed when optogenetic stimulation was restricted to NPY/AgRP fiber projections surrounding GnRH neuron cell bodies, and light stimulation of these fibers was also able to reduce GnRH neuron activity in an *ex vivo* brain slice preparation. Prenatally androgenized mice that model the LH
hypersecretion of polycystic ovary syndrome (PCOS) also respond to NPY/AgRP neuron activation with decreased LH pulse frequency. However, chronic activation of NPY/AgRP neurons did not ameliorate any of the reproductive deficits in these mice. Together, these data support a functional NPY/AgRP to rostral preoptic area circuit capable of reducing GnRH neuron excitability and slowing GnRH/LH pulse generation, potentially responsible for mediating the impact of caloric restriction on the reproductive axis.

\**S10.4 - Translational control in the gonadotrope: Musashi mediates crosstalk between metabolism and reproduction.** Ana Rita Silva Moreira, Tiffany Miles, Anessa Haney, Linda Hardy, Melody Allensworth, Michael Kharas, Christopher Lengner, Melanie MacNicol, Angus MacNicol, Gwen Childs, Angela Odle

Metabolic regulation of reproduction is mediated in part by the adipokine leptin, an appetite and energy-regulating hormone. We have reported that female mice lacking leptin receptors (Lepr-null) in gonadotropes are sub-fertile and show reduced GnRHR protein with normal levels of Gnrhr mRNA, suggesting a post-transcriptional role for leptin. An in silico analysis of the Gnrhr 3’UTR revealed binding elements for the translational regulator Musashi (MSI). In control female mice, leptin reduced MSI expression specifically in gonadotropes while increasing GnRHR expression. We have also shown that MSI binds Gnrhr in the mouse pituitary. Our hypothesis is that MSI repression, which is alleviated by leptin, regulates the normal expression patterns in GnRHR. To determine what role MSI plays in the normal peak in GnRHR, we developed and analyzed homozygous gonadotrope-Musashi-null mutant females (MUT) and littermate controls (CTL) on the morning of diestrus. The mutant females show a 1.6x increase in the pituitary levels of GnRHR protein (EIA), with no change in mRNA (qPCR). We measured pituitary and serum levels of luteinizing hormone (LH) and follicle stimulating hormone (FSH) (EIA) as well as Lh and Fsh mRNA (qPCR). Serum FSH levels are decreased by >50% in mutant females, but FSH stores and Fsh mRNAs are unchanged. Pituitary LH protein content is increased by 2.2x in mutant females with no change in serum LH or Lh mRNA levels. Mutant females have fewer pups per litter and longer delays between litters. Our studies show that, as a repressor of GnRHR translation, Musashi can regulate expression of gonadotropins. We propose that the increased expression of GnRHR in the gonadotrope Msi-null animals causes increased LH content (and perhaps disrupted FSH secretion) resulting in a higher serum LH:FSH ratio and subfertility. Collectively, these data suggest that MSI may be a critical regulator of female reproduction.

\**S11.1 - Embryo-maternal interaction prior to implantation in cattle.** Pat Lonergan, José Sánchez, Daniel Mathew, Claudia Passaro, Constantine Simintiras, Trudee Fair

Up to the blastocyst stage, the bovine embryo is relatively autonomous. Following hatching, the bovine blastocyst passes sequentially from a spherical- to ovoid-, then tubular- and finally filamentous-shaped structure. During this time, the conceptus secretes interferon tau (IFNT), the maternal pregnancy recognition signal, blocking the mechanisms that bring about luteolysis. In contrast to pre-hatching development, elongation is predominantly maternally-driven, dependent on substances in the uterine lumen fluid; blastocysts do not elongate in vitro and the absence of uterine glands in vivo results in failure of blastocysts to elongate following embryo
transfer. Limited evidence indicates an embryo maternal dialogue as early as the blastocyst stage. Bovine endometrial explants respond to the presence of 8-day old blastocysts by upregulating expression of classical interferon-stimulated genes (ISG). In contrast, exposure of endometrial explants to elongating conceptuses results in IFNT-dependent and independent changes in gene expression. IFNT, long or short age-matched (Day 15) conceptuses altered expression of 491, 498 and 230 transcripts, respectively, compared to control explants. We identified differentially expressed genes (DEG): (i) commonly responsive to IFNT and conceptuses, irrespective of size; (ii) commonly responsive to IFNT and long conceptuses only; and (iii) induced by a conceptus but independent of IFNT. Of the latter, the majority were exclusively induced by long conceptuses. Similarly, conceptuses produced from in vivo- and in vitro-derived blastocysts induced differential transcript changes in the endometrium which may reflect differences in pregnancy rate associated with such embryos. In conclusion, bovine endometrium responds differently to age-matched conceptuses of varying size as well as in vivo- and in vitro-derived conceptuses in both an IFNT-dependent and -independent manner, which may be reflective of the likelihood of successful pregnancy establishment.

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**S11.2 - Comparative Insights into How Uterine Glands Function in Pregnancy Establishment.** Thomas Spencer

All mammalian uteri contain glands in the endometrium that develop only or primarily after birth. Gland development or adenogenesis in the postnatal uterus is intrinsically regulated by proliferation, cell-cell interactions, growth factors and their inhibitors as well as transcription factors including forkhead box A2 (FOXA2) and estrogen receptor alpha (ESR1). Extrinsic factors regulating adenogenesis originate from other organs including the ovary, pituitary, and mammary gland. The infertility and recurrent pregnancy loss observed in uterine gland knockout sheep and mouse models support a primary role for secretions and products of the glands in pregnancy success. Recent studies in mice revealed that uterine glandular epithelia govern post-implantation pregnancy establishment through effects on stromal cell decidualization and placental development. In humans, uterine glands and, by inference, their secretions and products are hypothesized to be critical for blastocyst survival and implantation as well as embryo and placental development during the first trimester before the onset of fetal-maternal circulation. A variety of hormones and other factors from the ovary, placenta, and stromal cells impact secretory function of the uterine glands during pregnancy. This review will summarize new information related to the developmental biology of uterine glands and discuss novel perspectives on their functional roles in pregnancy establishment and success. This work was supported by Grants R01 HD096266 and R21 HD087589 from the National Institute of Child Health and Human Development of the National Institutes of Health.

**S11.3 - WNK1 in the Uterus: A Previously Undescribed Role In Implantation.** Ru-pin Alicia Chi, Nyssa Adams, San-pin Wu, Chou-long Huang, John Lydon, Francesco DeMayo
Through a global kinome profiling and gene knock down analysis, we previously identified With No Lysine Kinase-1 (WNK1) as essential for human endometrial stromal cell differentiation in vitro. WNK1 is a kinase associated with a renal function of controlling and maintaining ion homeostasis within the body, and a mutation in humans is associated with hypertension. In this study, we examined the in vivo role of WNK1 in uterine biology and pregnancy using a uterine Wnk1 deletion mouse model, where Wnk1-floxed mice (WNK1<sup>fr</sup>) were crossed to the PR<sup>Cre</sup> mice, generating mice with Wnk1 deletion in the PR positive cells (WNK1<sup>d/d</sup>). A 6-month breeding trial revealed WNK1<sup>d/d</sup> mice as severely subfertile with >50% reduction in the number of litters produced and the average litter size compared to the WNK1<sup>fr</sup> control mice. A closer examination during early and mid-pregnancy showed that subfertility was due to delayed implantation and increased embryo resorption. We demonstrate that the delayed implantation is associated with aberrant signaling in the luminal epithelium during the window of implantation (WOI) - including retained Progesterone Receptor (PR) expression and failure of FOXO1 nuclear localization, both of which are required for implantation. Subsequent RNA-sequencing analysis identified elevated PI3K/AKT signaling during the WOI, suggesting that the decreased nuclear FOXO1 may be attributed to its increased phosphorylation by AKT and hence the consequent cytoplasmic localization. Indeed, siRNA-mediated WNK1 inhibition in HEC1A cells increased the levels of phosphorylated (activated) AKT, and pharmacological inhibition of AKT was able to rescue WNK1 inhibition-induced FOXO1 cytoplasmic retention. Taken together, these results illustrated a critical role of WNK1 in mediating the PGR-FOXO1 signaling axis during the WOI, a disruption of which impaired the timing of implantation, leading to abnormal embryo development in utero and eventually resulting in increased resorption and subfertility.

**S11.4 - Intrauterine inhibition of chemokine receptor 4 signaling modulates local and systemic inflammation in ovine pregnancy.** Stacia McIntosh, Clara Maxam, Marlie Maestas, Kelsey Quinn, Ryan Ashley

During the first trimester, chemokines and growth factors coordinate inflammation within the fetal-maternal microenvironment to assist implantation and placentation. An inflammatory response is also evident in circulating blood during pregnancy, but mechanisms responsible are not explicitly understood. Our objective was to better characterize these phenomena and their functional implications by targeting intrauterine C-X-C motif chemokine ligand 12 (CXCL12) signaling through its receptor CXCR4. Because CXCL12-CXCR4 signaling encourages trophoblast viability and invasion, leukocyte migration, and inflammatory cytokine production, we hypothesized that intrauterine CXCL12-CXCR4 signaling governs local and systemic inflammatory potential during early gestation. Osmotic pumps were surgically installed for intrauterine infusion of a CXCR4 inhibitor, AMD3100, beginning on day 12 post-breeding in 15 ewes. Endometrial tissues collected on day 35 of gestation were evaluated for inflammatory potential, Akt activation, and autophagy induction. IL10 was localized to endometrial glandular epithelium, with lower abundance (P<0.05) when CXCR4 was antagonized. Endometrial Akt activation was less (P<0.05), while evidence of autophagy induction was greater (P<0.05). IL10 discourages autophagy through activation of Akt signaling and is implicated in homeostatic maintenance of endometrial cells by encouraging cellular proliferation, migration, and invasiveness. CXCL12-CXCR4 signaling may regulate endometrial cell viability important to placental development. To assess peripheral inflammatory activity, select cytokines were
measured in corpora lutea and spleen. Corpora lutea from ewes receiving intrauterine AMD3100 exhibited lower IFNG abundance (P<0.05). In spleen, IL10 abundance was greater (P<0.05) following infusion treatment, while IFNG was less (P<0.05). Our results provide compelling evidence that altering fetal-maternal CXCL12-CXCR4 signaling is a novel approach to modify local and systemic inflammation in the window when most pregnancy losses occur and impaired placental development transpires. Research supported by the New Mexico Agricultural Experiment Station (AES), an AES graduate research award, and an Institutional Development Award from the National Institute of General Medical Sciences, NIH P20GM103451.

**S12.1 - The orphan nuclear receptor NR5A2 regulates primordial follicle activation.** Marie-Charlotte Meinsohn, Anthony Estienne, H. Duygu Saatcioglu, David Pepin, Bruce Murphy

The orphan nuclear receptor, NR5A2, is required for female reproduction as mice with NR5A2 depletion in granulosa cells are infertile. Based on its role in granulosa cell proliferation, we hypothesized that NR5A2 could act as early as primordial follicle activation. We generated granulosa-specific knockout mice (Nr5a2<sup>fl/fl</sup>Amhr2<sup>Cre<sup>+</sup></sup>). Ovaries were collected at postnatal day 4 (PND4), end of formation of the primordial follicle pool. Histological analysis showed a significantly larger number of primordial follicles and a decreased population of primary follicles. Analysis by qPCR and in situ hybridization confirmed substantial depletion of Nr5a2 mRNA level in the cKO ovaries. Detection of NR5A2 proteins by quantitative immunofluorescence showed a significant decrease in expression overall in the ovary. Further, two subsets of primordial follicles were identified: those expressing NR5A2 and poised to activate and those negative for NR5A2 that are quiescent. These results were confirmed by the significant increase of granulosa and oocyte expressed quiescence markers (Cdkn1a, Cxcl1, Cxcl10, Egr1, Junb, Lin28a) and dysregulation of primordial follicle activation related genes (Foxl2, Foxo3, Pten) in the cKO ovaries. Moreover, as activation of granulosa cells in primordial follicles is characterized by a partial transformation from the epithelial to mesenchymal state, we investigated the role of NR5A2 in this process. We demonstrated a significant increase in the level of transcripts of epithelial state related genes (Cdhl1, Gja1) accompanied by a decrease of the mesenchymal characteristic markers (Snail1, Mmp9). To conclude, this study is the first implicating NR5A2 in the activation of primordial follicles. The mechanism includes regulation of quiescence and epithelial to mesenchymal transition markers and opens new perspectives into the maintenance and control of fertility via the ovarian reserve. CIHR to BDM.

**S12.2 - Post-Transcriptional Regulation of Germ Cell Development.** Guang Hu

The Ccr4-Not complex is the main deadenylase in eukaryotic cells, and it regulates mRNA poly(A)-tail length to influence mRNA stability and/or translation. Different subunits of the complex have been implicated in various diseases and developmental phenotypes, suggesting that the post-transcriptional regulation of the mRNA tail-length plays important roles. We have previously shown that Cnot1, Cnot2, and Cnot3 in Ccr4-Not are required for mouse Embryonic Stem Cell (ESC) maintenance, likely by controlling the deadenylation and degradation of differentiation gene mRNAs. To further dissect the function of the complex, we generated conditional deletion ESC lines for the core subunits, both individually and in combination. We
found that individual deletion of selected regulatory subunits, as well as combinatorial deletion of deadenylase subunits, resulted in ESC differentiation. In contrast, deletion of individual deadenylase subunits had no obvious effect. We are currently examining the mRNA steady-state abundance, decay rate, and polysome association to determine the impact of Ccr4-Not disruptions and the role of each subunit. Interestingly, in addition to ESCs, we found that deletion of Ccr4-Not subunits also impairs germ cell development, especially in the maintenance of male spermatogonial stem cells. Thus, mRNA-tail length regulation by Ccr4-Not may serve as a critical mechanism that controls stem cell fate.

S12.3 - TGFβ/SMAD signaling regulates transzonal projection (TZP) formation in growing follicles of the mouse ovary. Sofia Granados-Aparici, Qin Yang, Hugh Clarke

Shortly after the initiation of follicular development, the generation of an extracellular layer called the zona pellucida (ZP) physically separates the oocyte from the proliferating granulosa cells (GCs) that surround it. Since GC-oocyte contact-dependent communication is essential for oocyte development, GCs generate filopodia, termed transzonal projections (TZPs), which penetrate the ZP and establish contact with the oocyte plasma membrane. Understanding the molecular pathways regulating TZP formation may help identify novel markers of follicle/oocyte quality and provide insight into the causes of infertility. Growth-differentiation factor (GDF) 9, an oocyte-secreted TGFβ-family ligand, increases TZP number as well as the steady-state levels of Myo10 and Fscn1, which encode proteins implicated in filopodial assembly. We investigated whether the TGFβ signalling mediators SMAD3/4 regulate TZP formation by the granulosa cells during follicular development. Quantitative RT-PCR and immunofluorescent histological sections of mouse ovaries showed a significant increase in relative mRNA and protein levels of Fscn1 and Myo10 between day-4 and day-12 of age (enriched in non-growing and growing follicles, respectively). Moreover, ChIP-qPCR revealed that both SMAD3 and SMAD4, bound to the promoter region of Fscn1 and Myo10. The tamoxifen-inducible Cre-ER/floxed Smad4+/+ knockout (KO) system was used in neonatal ovaries and granulosa cell-oocyte complexes (GOCs) in culture to delete the Smad4 gene at early stages of follicle development. SMAD4 protein was depleted by ~50% in the tamoxifen-treated Cre+/floxed Smad4 neonatal ovaries and GOC cultures. This was accompanied by a striking decrease in the number of TZPs in both early-growth follicles of day-4 ovaries and GOCs of day-12 ovaries, although oocyte diameter was unchanged. In contrast, MYO10 and FSCN1 protein levels were not significantly altered. Our results identify a new role for SMAD signalling on the molecular mechanisms regulating TZP formation and germ line-somatic communication during early follicle development.

S12.4 - The critical roles of protein sumoylation in oocyte development. Stephanie Pangas

SUMOylation is a posttranslational modification that has widespread regulatory functions, particularly for transcription factors. During SUMOylation, a small ubiquitin-related modifier (SUMO) is conjugated to a substrate protein thereby altering its interaction with other proteins. The functional effect of SUMOylation depends upon the substrate; however, in general, SUMOylation regulates cellular processes such as protein localization, protein stability, or protein activity. SUMOylation in mammalian oocytes is known to effect meiotic maturation,
spindle formation, and chromosome segregation, but a role for SUMOylation and the identity of SUMOylation targets in oocytes during ovarian folliculogenesis is not known. We generated several novel mouse models to study the role of SUMOylation in developing oocytes, including stage-specific oocyte conditional knockouts of \textit{Ubc9}, a central component of the SUMOylation cascade. Loss of \textit{Ubc9} in oocytes beginning at the primordial follicle stage causes female sterility and depletion of the oocyte reserve in young adult mice. Loss of \textit{Ubc9} in oocytes beginning at the primary follicle stage also causes female sterility, but ovarian follicles remained present in the ovaries of older adult mice. Both models show defects in meiotic maturation. Transcriptome profiling indicates that loss of SUMOylation affects a number of key developmental processes and disrupts function of several oocyte-specific transcription factors, including newborn ovary homeobox (\textit{Nobox}) and spermatogenesis and oogenesis helix-loop-helix 1 (\textit{Sohlh1}). CRISPR-Cas9 gene editing of a NOBOX SUMOylation site in mice also results in fertility defects, indicating that some functions of NOBOX require post-translational modification. In total, our results provide insight into the regulation of key oocyte-specific transcription factors and allow for discovery of novel pathways in oocyte development and meiotic resumption. Importantly, these studies establish SUMOylation as an essential process in intraovarian oocyte development that are required for female fertility. These studies were funded by NIH R01 HD085994 (to SAP).

\textit{S13.1} - Genes and mechanism of androgenetic hydatidiform moles: an answer to a 40-year old question. Rima Slim

Androgenetic complete hydatidiform moles (AnCHM) are aberrant human pregnancies with no embryos and affect 1 in every 1400 pregnancies. They have mostly androgenetic monospermic genomes with all the chromosomes originating from a haploid sperm and no maternal chromosomes. AnCHM were first described in 1977, but how they occur have remained open questions. We identified bi-allelic deleterious mutations in \textit{MEI1}, \textit{TOP6BL/C11orf80}, and \textit{REC114}, with roles in meiotic double-strand breaks formation in women with recurrent AnCHM. We investigated the occurrence of androgenesis in \textit{Mei1}-deficient female mice and discovered that 8% of their oocytes lose all their chromosomes by extruding them with the spindles into the first polar body. We demonstrate that \textit{Mei1}^{-/-} oocytes are capable of fertilization and 5% of them produce androgenetic zygotes. Thus, we uncover a novel meiotic abnormality in mammals and a mechanism for the genesis of androgenetic zygotes that is the extrusion of all maternal chromosomes and their spindles into the 1st polar body. Our findings will contribute to the identification of the genetic causes of recurrent androgenetic moles and miscarriages as well as female and male infertility in more patients and offering them better management options tailored to their exact gene defect.

\textit{S13.2} - Genomic Insights into Reproductive Success. Pierre Ray

Infertility is a major health concern, affecting more than 50 million couples. In the Western world most infertile couples resort to assisted reproduction technology (ART) to try to conceive a child. Despite undeniable successes, near half the couples who seek medical assistance for infertility fail to achieve a pregnancy. ART, as it is currently proposed, is mainly palliative, with
little effort made to understand and specifically address the dysfunctions responsible for couple infertility. In these couples, a defective spermatogenesis is observed in half of the male partners. With over one thousand genes expressed almost exclusively in the testis, we believe that gene defects constitute the main cause for male infertility, especially for the most severe sperm defects (subjects with azoospermia or near 100% abnormal sperm).

Children conceived by ART have been shown to have an increased incidence of de novo chromosomal aberrations, genetic imprinting defects and birth defects. For these reasons, we believe that it would be imprudent to continue to support the entirely empirical ART approaches while neglecting parallel efforts to discover the causes of infertility and their consequences for the quality of gametes bearing the human genome to future generations.

Genetic testing for infertile men is currently largely inefficient. Here I will show that the use of whole exome sequencing (WES) is an effective strategy to obtain a genetic diagnosis for severe sperm defects such a non-obstructive azoospermia, multiple morphological anomalies of the flagella (MMAF) or globozoospermia. The next step is to understand the pathophysiology associated with a particular genetic defect and to improve our understanding of the basic mechanism underlying an efficient spermatogenesis. To that effect we used two different models: knock out mice created with the CRISPR/Cas9 mice technique and Trypanosoma brucei (T. brucei) which is one of the best models for sperm flagella studies as it has a 9+2 axonemal structure surrounded by para-axonemal fibers that are specific to the flagella and are absent from the other motile cilia. I will present some examples illustrating how these two models allowed us to understand the role of several proteins identified in infertile men and necessary for normal sperm production and function.

S13.3 - Dynamic evolution of male fertility genes in humans and other great apes. Marta Tomaszkiewicz, Arslan Zaidi, Danling Ye, Kristoffer Sahlin, Rahul Vegesna, Paul Medvedev, Kate Anthony, Corey Liebowitz, Michael DeGiorgio, Mark Shriver, Kateryna Makova

Specialized for spermatogenesis and male functions, the Y chromosome in humans harbors 27 classes of protein-coding genes, with one third of them being multi-copy male fertility genes, called ampliconic genes (AGs). In fact 60 out of 78 annotated genes on the human Y chromosome belong to nine AG families expressed exclusively in testis. Most of these genes are located on the Azoospermia Factor b and c (AZFb, c) regions of the human Y chromosome, which resulted from segmental duplications. This poses a major challenge in evaluating the copy number of these genes with high sequence identity (>99.9%) from sequencing data. None of the existing tools is capable of calculating the copy number of all AGs from whole genome sequencing data. We have recently demonstrated that the copy number of these genes measured using an experimental method, droplet digital PCR, varies from one man to another. We found 98 haplotypes based on the AG copy number among 100 men from 10 major Y-haplogroups world wide. Haplotypes were sometimes shared by males from different haplogroups, suggesting homoplasies. Two largest and most variable AG families, namely RBMY and TSPY, were the major contributing factors to the variability observed among haplotypes. Besides the high variability of AG copy numbers in human populations, we have also observed new AG
transcripts deciphered using targeted PacBio sequencing of AG RT-PCR products from testis of two men. Similarly, we observe substantial variability of AG copy numbers in non-human great apes: chimpanzees, bonobos, gorillas, and orangutans. Currently, we are deciphering the AG transcripts from great apes to study the trajectories of gains and losses in our closest relatives. During the talk I will point out the differences in their mating patterns and sperm competition levels and how this could affect the evolution of copy number and expression of AGs.

**S13.4 - Integrated epigenome, exome and transcriptome analyses reveal molecular subtypes and suggest homeotic transformation in uterine fibroids.** Jitu George, Huihui Fan, Benjamin Johnson, Anindita Chatterjee, Amanda Patterson, Julie Koewitz, Marie Adams, Zachary Madaj, Hui Shen, Jose Teixeira

Uterine fibroids are benign myometrial smooth muscle tumors of unknown etiology that when symptomatic are the most common indication for hysterectomy in the USA. We conducted an integrated analysis of fibroids and adjacent normal myometria by whole exome sequencing, Infinium MethylationEPIC array, and RNA-sequencing. Unsupervised clustering by DNA methylation segregated normal myometria from fibroids, and further separated the fibroids into subtypes marked by MED12 mutation, HMG2A activation (HMG2Ahi) and HMG1 activation (HMG1hi). Upregulation of HMG2A expression in HMG2Ahi fibroids did not always appear to be dependent on translocation, as has been historically described, and was associated with hypomethylation in the HMG2A gene body. Furthermore, we found that expression of HOXA13 was highly upregulated in fibroids and that overexpression of HOXA13 in a myometrial cell line induced expression of genes classically associated with uterine fibroids. Transcriptome analyses of the most differentially expressed genes between cervix and myometrium also showed that uterine fibroids and normal cervix clustered together and apart from normal myometria. Together, our integrated analysis shows a role for epigenetic modification in fibroid biology and strongly suggests that homeotic transformation of myometrium cells to a more cervical phenotype is important for the etiology of the disease.

**S14.1 - Use of endogenously produced BTB modifiers to enhance efficacy of non-hormonal male contraceptives – Lesson from adjudin.** C. Yan Cheng, Dolores Mruk

The efficacy of male contraceptives that exert their effects locally in the testis is dependent on the ability to penetrate the blood-testis barrier (BTB). Furthermore, multiple drug transporters at the BTB also pump drugs out of the testis. Thus, high doses of contraceptives are required and this would pose systemic toxicity after long-term use as illustrated in studies of adjudin. Interestingly, the testis is producing biomolecules endogenously that are capable of modifying the BTB permeability, improving the transport function of drugs. First, the F5-peptide derived from domain IV of collagen-γ3 chain at the Sertoli-spermatid interface during the release of sperm at spermiation was shown to induce BTB remodeling, making the BTB “leaky”. Second, the 80 kDa fragment derived from laminin-α2 chain at the basement membrane designated LG3/4/5 (laminin globular domains 3/4/5), was also shown to promote BTB integrity through the mTORC1 (mammalian target of rapamycin complex 1)/rpS6 (ribosomal protein S6)/Akt1/2 signaling pathway, making the BTB “tighter”. A knockdown of laminin-α2 was found to modify BTB by
making the barrier “leaky” through an activation of mTORC1/rpS6. Importantly, overexpression of a quadruple phosphomimetic (i.e., constitutively active) mutant, namely p-rpS6-MT, was capable of making the barrier “leaky”. The use of the F5-peptide (alone) vs. F5-peptide and p-rpS6 (combined) via overexpression in the testis, and with or without adjudin (at a low dose which by itself has no effects on spermatogenic function) in adult rats were assessed and compared on the BTB and transport function, and spermatogenic function. Combined use of these BTB modifiers and adjudin was effective to induce reversible male contraception wherein adjudin was at 1/10 of its effective dose when used alone. This thus provides a novel approach to deliver drugs to an organ behind a blood-tissue barrier for contraception or therapeutic purposes. [This work was supported by NIH, R01 HD056034]

S14.2 - testis interstitial antigens, Leydig cells, spermatogonia, regulatory T cell, spermiation, sertoli cell, blood-testis barrier, lactate dehydrogenase 3, zonadhesin, autoimmune orchitis, cancer/testis antigens, vaccines, DEREG mice. Kenneth Tung, Jessica Harakal, Hui Qiao, Claudia Rival, Liesbeth Paul, Daniel Hardy, Yan Cheng, Kathleen Schegg, Wei Yan, Erwin Goldberg

FOXP3+ regulatory T cells (Treg) are crucial in prevention of autoimmune disease development. Their function requires continuous Treg encounter with their cognate tissue antigens (Ag). Meiotic sperm Ag are hidden by the BTB and not protected by the Treg. Cancer-testis Ag expressed in human cancers and normal testis are sequestered, should be immunogenic as cancer vaccine Ag. However, these are unproven assumptions.

To investigate Treg as tolerance mechanism, we deplete them from DEREG mice that express FOXP3-Diphtheria toxin receptors. Treg-depleted mice spontaneously produce antibodies (Ab) to Leydig cells, including the steroidogenic enzymes 450scc and 17a-hydroxylase; and spermatogonial Ag. Because AIRE-deficient autoimmune polyendocrine syndrome patients respond to these testicular Ag, and spermatogonial Ag are used in human cancer vaccines, their tolerance status is clinically-relevant.

We also discover sperm Ag lactate dehydrogenase 3 (LDH3) but not zonadhesin continously egress normal seminiferous tubules: LDH3 forms immune complexes outside the BTB with serum Ab. These "exposed" sperm Ag are contents of residual bodies and cytoplasmic droplets, derived from spermatids discarded at spermiation. Sertoli cells degrade most residual bodies but the intact ones reach interstitial space. Importantly, Treg-depleted DEREG mice with autoimmune orchitis produce Ab to LDH3 but not zonadhesin. In contrast, vasectomized mice produce Ab to zonadhesin but not LDH3. To generalize the finding, Ab from vasectomized mice are found to react with the "hidden" sperm Ag in acrosome and not Ag in cytoplasmic droplets, whereas Treg-depleted DEREG mice produce Ab to the exposed Ag in cytoplasmic droplets but not sperm acrosome.

To conclude, complete testis Ag sequestration is invalid. Some testis and sperm Ag are normally exposed, and protected by the Treg. The exposed cancer/testis protected by the Treg may not be exceptionally immunogenic. Our results support a new paradigm for research on testis/sperm autoimmunity and cancer/testis vaccine.
**S14.3 - ATP-induced calcium signals and contractions in testicular peritubular cells.** Lina Kenzler, David Fleck, Nadine Mundt, Robert Moosmann, Jennifer Spehr, Marc Spehr

Generation of spermatozoa is the most fundamental process for male fertility. However, the underlying physiological principles are still largely unknown. The wall of the seminiferous tubules is composed of extracellular matrix proteins and flat smooth-muscle-like testicular peritubular cells (TPCs). It is generally assumed that TPCs mediate seminiferous tubule contractility. Accordingly, TPCs could play a fundamental role for sperm transport and, thus, male fertility. We recently identified ATP-sensitive P2 receptors in human TPCs. Here, we investigate ATP-dependent signaling mechanisms in mouse testicular peritubular cells (MTPCs). We use electrophysiological and live-cell imaging techniques in primary MTPC culture, acute seminiferous tubule sections, whole mount preparations, and in vivo experiments, allowing detailed physiological characterization.

Patch-clamp recordings from MTPCs (C57BL/6J) reveal ATP-induced currents that show moderate desensitization. Pharmacological studies suggest a heterogeneous expression of P2X2 and P2X4 receptors in distinct MTPC populations. Moreover, combining Ca\(^{2+}\) and brightfield imaging, we provide a quantitative analysis of purinergic Ca\(^{2+}\) signals in single MTPCs and identify their function in tubular contractions. Time-lapse live-cell imaging from acute tubular slices reveals ATP-induced cytosolic Ca\(^{2+}\) transients in MTPCs that generate tubular contractions. Signals are dose-dependent, require intracellular Ca\(^{2+}\) stores, and are modulated by extracellular Ca\(^{2+}\). Furthermore, we investigate seminiferous cycle stage dependency and directionality of both Ca\(^{2+}\) transients and contraction propagation in a whole mount preparation. Finally, ATP-induced Ca\(^{2+}\) transients and contractions are observed in vivo using multiphoton Ca\(^{2+}\) imaging.

Together, this research provides insight into the physiological signaling mechanisms in MTPCs. In the long-term, our data will help to elucidate mechanisms that regulate germ cell development and might identify reasons for male (in)fertility.

**S14.4 - The Role of ECM in Testicular Organoid Development.** Maxwell Edmonds, Hanna Pulaski, Kyle Orwig, Teresa Woodruff

Currently, male fertility preservation is limited to sperm banking, leaving many pediatric fertility patients who cannot provide a semen sample one option: testicular tissue cryopreservation. Unfortunately, there are no contemporary techniques to transplant or otherwise use this tissue for fertility restoration. One challenge towards this goal is the ex vivo reconstitution and maintenance of functional somatic testicular structures, a critical gap that limits both the value of cryopreserved tissues and our ability to investigate testicular biology.

We hypothesized that an in vivo-mimetic microenvironment is necessary for the de novo assembly and longevity of complex tissues in vitro, and that the basis of a synthetic mimetic should include extracellular matrices (ECM). To test this hypothesis, we investigated the use of the ECM Matrigel, for its utility to generate de novo testicular microtissues (organoids) compared with ECM-free controls. Testes from neonatal CD-1 mice were dissociated into single cells and cultured +/- Matrigel in 2D and 3D settings. Subsequent experiments explored organoid formation using cells from juvenile and adult aged mice.
Our studies demonstrate that ECM is dispensable for organoid formation, as long as 3D aggregation is enabled through other methods. Furthermore, our testicular organoid model has functionally proven to be mimetic of the native testis, in the development of seminiferous tubule-like structures and the secretion of testosterone and inhibin B. Currently our model requires juvenile or neonatal mice and has limited ability to maintain germ cells.

In conclusion, our study has demonstrated that ECM, while beneficial for organoid formation, is otherwise unnecessary, as long as aggregation is assured. Moreover, we have established an in vitro testicular organoid model which is useful for questions regarding de novo testicular morphogenesis and endocrine function. Work is ongoing to explore the functionality of our organoid model.

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*S15.1 - Epigenetic rewiring of ovarian cancer for cancer therapy*. Kenneth Nephew

High grade serous ovarian cancer, the most common ovarian cancer histological type, characterized by a p53 mutated signature, is initially a responsive tumor to platinum-based therapy. However, after meaningful near-complete responses and sustained remissions, most women experience relapse, tumors become chemo-resistant and ultimately fatal. It has been postulated that ovarian cancer progression to a platinum resistant state is intimately linked to accumulated epigenomic alterations, including increased DNA methylation and modifications of histone marks. Like genetic changes that affect to promote tumorigenesis through deletions or mutations, these epigenetic modifications act as inactivating “hits” during cancer initiation and/or progression, causing transcriptional silencing of tumor suppressor genes and of other genes associated with apoptotic responses to chemotherapy. We have conducted investigator initiated clinical trials using hypomethylating agents to re-sensitize ovarian tumors to platinum and established proof of concept for this innovative strategy of "rewiring" the ovarian cancer methylome. Our results point to hypomethylation of specific promoters leading to increased expression of tumor suppressor genes which were functionally linked to response to platinum. We identified these changes in tumors biopsied before and after treatment with hypomethylating agents. We associated genes and pathways with patient outcomes, with the goal of identifying patients that are most likely to benefit from epigenetic strategies. We are developing new strategies for patient selection and improving treatment strategies for a disease of critical need. Our overall goal is to improve our understanding of methylation events in chemotherapy resistance and the mechanism of action of hypomethylating agents in ovarian cancer and other solid tumors.

*S15.2 - Tackling endometrial cancer: A new frontier*. Julie Kim, Teerawat Wiwatpanit, Alina Murphy
Endometrial cancer remains the most commonly diagnosed gynecologic cancer and despite the overall decreasing trend of cancer incidence, the incidence of endometrial cancer continues to increase annually. This cancer is hormone dependent in that the growth promoting actions of estrogen accompanied by insufficient protection by progesterone drive the development and progression of endometrial adenocarcinoma. When endometrial tumors are established, their response to progestins is suboptimal, limiting the effectiveness of fertility sparing treatment options. We have demonstrated that hyperactive AKT signaling in endometrial tumors can blunt progesterone receptor action and that inhibiting AKT with chemical inhibitors could potentiate the efficacy of progestin treatment. Using this knowledge, we have begun to investigate how risk factors of endometrial cancer, including obesity and PCOS affect the progesterone response in the benign endometrium to favor neoplastic transformation. Such studies require unconventional model systems in order to investigate this ex vivo. We have established a human endometrial organoid system comprised of primary epithelial and stromal cells of the endometrium. These organoids retain characteristics and functionally behave like the native tissue. In addition, increased androgens found in PCOS, or the presence of adipocytes to mimic obesity, influenced endometrial organoid physiology, increasing proliferation and blunting differentiation. These data support the progesterone resistance hypothesis caused by abnormal paracrine signals found in PCOS and obesity. This would result in the loss of protection provided by progesterone, thereby promoting a pro-tumorigenic environment. Studies are ongoing to identify the mechanisms of progesterone resistance in the heterogeneous endometrial organoid using state-of-the-art microfluidic platforms and sequencing strategies.

S15.3 - Anti-apoptotic Based Fertoprotective Agents do not Protect the Ovarian Stroma from Radiation-induced Fibrosis in the Nonhuman Primate. Sharrón Manuel, Mary Zelinski, Brian Johnson, Megan Larmore, Michele Pritchard, Francesca Duncan

Cancer treatments, while life-preserving, can be gonadotoxic. Thus, efforts are underway to develop fertoprotective agents that prevent radiation-induced ovarian follicle destruction. In rhesus macaques, ovarian pre-treatment with anti-apoptotic agents, sphingosine-1-phosphate (S1P) and FTY720, preserved follicles against a single dose of 15 Gy X-ray radiation to the ovary, and live offspring were obtained from FTY720-treated animals. Whether these fertoprotective agents also protect the ovarian stroma is unknown. This is a critical concern, since fibrosis and vascular damage are late effects of radiation. We, therefore, evaluated the extracellular matrix and vasculature in ovarian histological sections from the rhesus macaque study in the following experimental cohorts: vehicle + sham irradiation (SHAM), vehicle + irradiation (OXI), S1P + irradiation (S1P), and FTY720 + irradiation (FTY720) (N=3 animals/cohort). One ovary from each animal was harvested prior to radiation exposure whereas the contralateral ovary was harvested 9-10 months post-treatment. Collagen I/III was assessed by Picrosirius Red (PSR) staining, hyaluronan by a hyaluronan binding protein assay, and vasculature by immunohistochemistry with a CD31 antibody. Although there were no differences in the hyaluronan matrix, quantitative digital pathology methods revealed significant (P<0.05) fibrosis in the OXI cohort relative to the SHAM, and neither S1P nor FTY720 treatment conferred a protective effect. CD31 staining revealed disorganized vessels in the medulla in both the OXI and S1P cohorts relative to the SHAM. However, the vasculature in the FTY720 cohort appeared similar to the SHAM cohort, which may partially explain its fertoprotective properties. Since S1P and FTY720 do not protect the stroma...
against fibrosis, the limited follicles remaining in the ovary following these treatments may only preserve reproductive function during a narrow window post radiation. Research efforts must focus on therapies that simultaneously protect both the follicle and the stroma to preserve fertility after radiation exposure.

**S15.4 - TGFbeta is critical for stromal and epithelial paracrine signaling in endometrial cancer.** Diana Monsivais, Maya Kriseman, Julio Agno, Ramya Masand, Chad Creighton, Martin Matzuk

The transforming growth factor beta (TGFβ) signaling pathway has critical roles in the transduction of signals that control development, reproduction, and cancer. Endometrial cancer is characterized by glandular epithelial dysfunction, yet paracrine signaling from the stroma may play a crucial role in epithelial cell quiescence. To study the tissue-specific contribution of TGFβ signaling in the endometrium, we developed mice with conditional deletion of ALK5 or SMAD2 and SMAD3 in the endometrial stroma and epithelium (using *Progesterone receptor-cre*, “*Pgr-cre*”), or in the epithelium (with *Lactoferrin-cre*, “*Ltf-cre*”). We identified that double conditional deletion of SMAD2 and SMAD3 in the endometrial stroma and epithelium (“*Smad2/3-Pgr-cre*”), resulted in endometrial hyperplasia by six weeks of age and loss of progesterone receptor (PR) expression by twelve weeks. These mice and ALK5-*Pgr-cre* mice developed aggressive endometrial tumors with lung metastases. The tumors were estrogen dependent, lost PR expression, and caused death by eight months of age in *Smad2/3-Pgr-cre* mice. The *Smad2/3-Ltf-cre* mouse model was used to assess paracrine communication in the endometrium. *Smad2-Ltf-cre* and *Smad3-Ltf-cre* mice displayed normal endometrial and myometrial morphology, with no glandular hyperproliferation or myometrial invasion. The *Smad2/3-Ltf-cre* double mutants developed endometrial hyperplasia by twelve weeks of age, but to a lesser extent than the *Smad2/3-Pgr-cre* model. *Smad2/3-Ltf-cre* mice also lost epithelial PR expression and had abnormal FOXA2 expression in the endometrial glands by 12 weeks of age. This suggests that while the tumors developed because of glandular epithelial proliferation in the *Smad2/3-Pgr-cre* model, signals from the stroma could help maintain epithelial cell function in the *Smad2/3-Ltf-cre* model. These studies suggest that TGFβ-mediated paracrine signals are critical to endometrial function and that perturbation of this balance results in endometrial cancer.

**S16.1 - Gene x Environment Interactions and Preterm Birth.** Jerome Strauss, III

**Context**

Studies on twins have revealed that both maternal and fetal genetics contribute to preterm birth, but environmental factors are proportionately the major drivers of gestational age at delivery. This suggests that gene x environment interactions lie at the foundation of preterm birth. Consistent with this notion, we previously reported an association between bacterial vaginosis, a maternal genotype linked with increased TNF alpha production and spontaneous preterm birth.

**Objectives:**
To identify genetic variants and environmental factors that interact with them that increase risk of preterm birth.

Methods:

Whole exome sequencing was performed on neonates born of women of African descent who delivered at term or prematurely as a result of preterm premature rupture of membranes (PPROM) to identify genetic risk variants. Metagenomic profiling of the vaginal microbiota was carried out on women of African descent who delivered at term or preterm to identify signatures associated with prematurity.

Main outcome measures:

Identification of nonsense mutations associated with the innate immune system and a microbiota signature associated with preterm birth.

Results:

Rare, African ancestry enriched nonsense mutations were identified in neonates born of pregnancies complicated by PPROM in the DEFBI and MBL2 genes, which encode antimicrobial peptides. Pregnant women of African descent who delivered preterm had a vaginal microbiota indicative of dysbiosis that differed significantly from the microbiota of women of European descent and women who delivered at term.

Conclusions:

Rare damaging genetic variants contribute to risk of preterm birth associated with PPROM. These variants are ancestry-specific, and may contribute to dysbiosis of the vaginal microbiota, enhancing inflammatory signals that lead to prematurity.

**S16.2 - Loss of REST in Uterine Leiomyoma leads to an Altered Progesterone Response.**
Ashley Cloud, Michelle McWilliams, Faezeh Koohestani, Sornakala Ganeshkumar, Sumedha Gunewardena, Vargheese Chennathukuzhi

Uterine Leiomyoma (UL) are benign tumors that arise in the myometrial smooth muscle layer of the uterus. UL are present in over 75% of women, often causing heavy bleeding, severe pain, and reproductive dysfunction. Despite their prevalence, there is no long-term pharmaceutical treatment for UL, due to the lack of understanding about the molecular pathogenesis of the disease. Extensive evidence has indicated an aberrant response to the steroid hormones, estrogen and progesterone, which play a critical role in the pathogenesis of UL. We have shown that REST (RE1 Silencing Transcription factor) is a tumor suppressor, and when lost in UL, it leads to de-repression of its target genes. We report here a critical novel link between loss of REST and an altered response to progesterone in UL. We found that a large number of REST target genes are also targets of progesterone receptor (PGR). Analysis of ChIP-sequencing data shows
conserved REST binding sites within 100 base pairs of PGR binding sites on approximately 200 target genes. Additionally, we generated a uterus specific conditional knockout mouse (cKO) model of REST. When REST is lost in our cKO mouse (Rest$f/fAmhr2^{+/Cre}$), we see a UL phenotype and an altered response to progesterone in the uterus. This phenotype consists of hyperproliferation in the uterus throughout the estrus cycle despite normal estrogen and progesterone levels. Furthermore, we identify a direct interaction between REST and PGR in the healthy myometrium. This interaction is disrupted in leiomyoma leading to aberrant regulation of progesterone receptor target genes. Collectively, our results identify a novel link between progesterone receptor and REST-regulated tumorigenic pathways in UL. Furthermore, we provide two important preclinical mouse models, which show loss of REST leads to a UL phenotype. This research was supported by NIH Grants 1R01HD076450and R01 HD094373.


Chorioamnionitis is a major risk factor for preterm birth, often associated with infection and characterized by neutrophil infiltration of the fetal membranes (FMs). We previously reported that FMs exposed to bacterial lipopolysaccharide (LPS) augment neutrophil migration, activation (degranulation and inflammation), and neutrophil extracellular trap (NET) formation. In this study, we investigated the mechanisms of FM-induced NET release. Peripheral blood neutrophils were exposed to conditioned media (CM) from normal FMs treated with or without LPS (1ng/mL). Media, LPS or phorbol myristate acetate (PMA, 100nM), a classic inducer of suicidal NETosis, served as controls. NETs were visualized and quantified using SytoxGreen and PicoGreen. Viability was measured by propidium iodide (PI) staining, and the CellTiter assay. Phagocytosis of *E. coli* bioparticles was quantified by flow cytometry. Untreated FM-CM induced NET release by 1.4±0.1-fold, and LPS-stimulated FM-CM further augmented this by 1.5±0.3-fold, compared to untreated neutrophils ($p<0.05$, $n=13$). Untreated and LPS-stimulated FM-CM induced NETs faster (5 mins) than PMA (30 mins, $n=3$). After 1hr, untreated and LPS-stimulated FM-CM had no effect on neutrophil PI-positivity compared to untreated cells, while PMA increased PI-positivity to 16.4±3.2% ($p<0.01$, $n=8$). After 24hrs, untreated and LPS-stimulated FM-CM increased neutrophil viability (2.4±0.3-fold) compared to untreated cells, while PMA reduced viability by 66.4±4.6% ($p<0.01$, $n=10$). Neutrophils exposed to untreated or LPS-stimulated FM-CM had ~1.5-fold more phagocytic activity compared to untreated cells, while PMA reduced phagocytic activity by 55.3±4.1% ($p<0.05$, $n=6$). This study demonstrates that in contrast to the more common suicidal NETosis, resting and LPS-stimulated FMs induce NETs via a vital pathway that maintains neutrophil viability and function. Thus, neutrophils through NET release may have a protective/surveillance role in the FMs during normal pregnancy, but in the presence of infection, prolonged viability combined with activation may lead to tissue injury, potentially contributing to preterm birth.

S16.4 - Pregnancy Length Parturition and the Telomere Gestational Clock. Mark Phillippe

The cellular and molecular mechanism(s) determining the length of gestation and the timing for the onset of labor (parturition) remain poorly understood. Although much is known regarding
the contributions of estrogens, progesterone, corticosteroids, prostaglandins and oxytocin related to the onset of parturition, none of these endocrine observations provide an explanation for the biologic clock that defines the length of pregnancy or the timing for the onset of labor. Progressive telomere shortening has been observed to be a key component in the multifaceted biologic clock that determines overall life-span in the adult; therefore, it is biologically plausible that telomere shortening could also serve a similar role in determining the life-span of gestational tissues (i.e. the placenta and fetal membranes) and their subsequent aging at term.

To address this possibility, my research is testing the telomere gestational clock hypothesis; a novel hypothesis that proposes a well-defined signaling pathway between the shortening of telomeres in gestational tissues leading to apoptosis and the release of increasing amounts of cell-free DNA (cfDNA), and the subsequent stimulation of a proinflammatory response mediated by cfDNA activation of Toll-like receptor 9 (TLR9). Thus, this telomere-activated gestational clock potentially provides the heretofore unknown intrinsic mechanism to stimulate the proinflammatory events that are well described as the final common pathway leading to parturition.

This telomere gestational clock hypothesis is supported by several mouse studies from my laboratory, by the research of other investigators, and by interesting associations in human pregnancies between maternal conditions (including stress, low education, increased pollution, poor neighborhood quality, and African-American race) and telomere shortening in the placenta and/or cord blood. In summary, these studies provide a foundation for future research regarding the potential role for a telomere-based biologic clock that determines the length of gestation not only in humans, but in other mammalian pregnancies.

S17.1 - Physiological mechanisms through which heat stress compromises reproduction in pigs. Jason Ross, Benjamin Hale, Jacob Seibert, Malavika Adur, Aileen Keating, Lance Baumgard

Seasonal variation in environmental temperatures imposes added stress on domestic species bred for economically important production traits. These heat-mediated stressors vary on a daily, seasonal, or spatial scale, and inevitably lead to reduced herd productivity in part due to depressed reproductive performance manifesting as delayed puberty onset, reduced farrowing rates, and extended weaning-to-estrus intervals. Understanding the effects of heat stress at the organistical, cellular, and molecular level is requisite to identifying mitigation strategies that could reduce the economic burden of compromised reproduction. Mechanistically, heat stress impairs the intestinal barrier, allowing translocation of the resident microflora and endotoxins, such as lipopolysaccharide (LPS), from the gastrointestinal lumen into systemic circulation. While much is known about the cellular function of heat shock proteins (HSP), the in vivo ovarian HSP response to stressful stimuli remains ill-defined. We have observed that infusion of LPS during the follicular phase mimics many of the ovarian molecular responses to heat stress suggesting that heat stress-induced breakdown of the gastrointestinal barrier explains part of the heat induced alterations occurring in reproductive tissues of pigs. Furthermore, we have observed that heat stress alters regulators of autophagy in both the ovary and the maturing oocyte implying specific biological
mechanisms are employed to mitigate the biological impact of heat stress in the ovary. Additionally, while heat stress elicits numerous molecular responses in reproductive tissues of pigs, hyperthermia experienced in utero also influences the postnatal phenotype of offspring in a tissue specific manner that culminates in altered body composition. Understanding tissue-specific molecular mechanisms through which heat stress confers suppressed reproductive ability is essential to the development of mitigation focused and hypothesis driven solutions. This work was supported by the National Pork Board, the Iowa Pork Producers Association, and the Agriculture and Food Research Initiative Competitive Grant number 2011-67003-30007.

S17.2 - Temperature-Dependent Sex Determination in Marine Turtles: Is the Future Female. Peter Dutton

Impacts from climate change are of particular concern for species with temperature-dependent sex determination (TSD), where the sex of an individual is determined by incubation temperature during embryonic development. In sea turtles, warmer temperatures produce females, and with continued temperature increases, many sea turtle populations are in danger of high egg mortality and female-only offspring production. I will discuss the implications of climate change to sea turtle, and show that in some cases populations have already become feminized, while others appear to still be stable.


Environmental heat stress (HS) is associated with mammalian ovarian dysfunctions and disruption of folliculogenesis. The impact of HS on the cellular and extracellular microRNAs (miRNAs) expression in granulosa cells (GCs) is still unclear. We aimed to assess the impact of HS on the expression of cellular and extracellular miRNAs in cultured GCs. For this, bovine GCs were aspirated from smaller follicles and cultured in F-12 media supplemented with 10% exosome-depleted FBS. Following initial incubation for 24 hours at 37OC, cells were cultured either at 37OC (control) or 42OC (heat-stressed) for another 24 hours and cells and spent media were collected. Extracellular vesicles (EVs) were isolated from the spent culture media using ExoQuick-TC kit and characterized using western blot, Nanoparticle Tracking Analysis, and electron microscope. MiRNA enriched total RNA was isolated from GCs and EVs using miRNeasy mini kit and Exosomal RNA isolation kit, respectively. MiRNA library preparation and NGS were performed by QIAGEN Genomic Services. Differential expression of miRNAs in GCs and EVs was determined using the EdgeR package. Results showed that HS induces the expression of heat shock protein genes (HSP70 and HSP90), oxidative stress (NRF2 and SOD) and endoplasmic reticulum stress-related genes (GRP78 and GRP94). There were no measurable differences in the size and morphology of the EVs due to HS. However, cells under HS released significantly more EVs into the culture environment. Sequencing results showed that 315 and 310 miRNAs were expressed in stressed and unstressed cells, respectively. Moreover, 3 miRNAs were down-regulated and 4 were up-regulated in the stressed GCs. Similarly, 2 miRNAs were
down-regulated and 4 were enriched in EVs of stressed GCs. Interestingly, miR-1246, which is abundantly expressed in stressed GCs, was also enriched in the corresponding EVs. This could suggest the role of EVs as potential indicators of the stress status of GCs.

S17.4 - Heat stress increases the incidence of uterine disease in dairy cattle without affecting the vaginal bacteria load. Paula Molinari, I. Martin Sheldon, John Bromfield

Bacterial infections cause metritis about 7 days after calving in approximately 30% of dairy cows, and uterine disease persists as endometritis in some animals beyond 21 days postpartum. Although heat stress reduces milk production and impairs immune function, little is known about its effect on uterine infection and disease. Our aim was to evaluate uterine disease severity and quantify the bacterial load in vaginal mucus collected from dairy cows on the same farm, during a cool (n=51, average maximum temperature: 22.5°C) season and a hot season when animals are subjected to heat stress (n=51, 31°C). We hypothesize that the incidence and severity of uterine disease is increased during the hot season, accompanied by an elevated bacterial load in the reproductive tract. All animals received heat abatement with fans and sprinklers after calving. Vaginal mucus was scored according to the abundance of purulent discharge and odor on days 7 and 21 postpartum, at the time of diagnosis of metritis and endometritis, respectively. Bacterial 16S rRNA was quantified by qPCR in vaginal mucus samples. During the hot season a higher proportion of cows had persistent uterine disease at both day 7 and day 21 (58.8% vs. 29.4%) and increased incidence of endometritis (64.7% vs. 43.1%). The concentration of bacterial 16S rRNA in vaginal mucus was higher in animals with endometritis compared with healthy cows at day 21 (2.35 vs. 0.04 ng/mg mucus) but did not differ between the hot and cool seasons (2.12 vs. 2.68 ng/mg mucus). In conclusion, our data indicate that despite a similar vaginal bacteria load, heat stress increased the incidence of endometritis. This work is supported by NICHD R01HD084316.

S18.1 - Sperm RNA code: How many more secrets? Qi Chen

Mammalian sperm RNA is increasingly recognized as an additional paternal hereditary information beyond DNA. Environmental inputs, including an unhealthy diet, mental stresses and toxin exposure, can reshape the sperm RNA signature and transmit certain paternally acquired traits to offspring with a converging effect on offspring metabolic performance. The expanding categories of sperm RNAs and associated RNA modifications has begun to reveal the functional diversity and information capacity of these molecules. However, the coding mechanism endowed by sperm RNA structures and by RNA interactions with DNA and other epigenetic factors remains unknown, and how sperm RNA-encoded information is decoded in early embryos to control offspring phenotypes remains unclear. Here, I discuss these key issues in light of emerging evidence.


**S18.2 - Role of RNA uridylation in germ line differentiation.** Marcos Morgan

Post-transcriptional RNA modifications are known to play a critical role in cellular differentiation. However, the physiological relevance of many RNA modifications still remains unknown. Here, I will present the importance of 3’ terminal uridylation during mouse germ line development, and other differentiation processes. To study the function of uridylation *in vivo*, I used conditional mutagenesis approaches where I remove TUT4 and TUT7 (TUT4/7), the two main terminal uridylyl-transferases, during early embryogenesis and the late stages of male and female gametogenesis. TUT4/7 can add non-templated 3’ Us to a number of RNA species such as miRNAs, piRNA and mRNA in many different tissues. However, TUT4/7 activity is only essential during specific stages of cellular differentiation. This work contributes to our better understanding of the role of RNA modifications, in particular, uridylation, in mammalian physiology.

**S18.3 - Single Cell RNA-Sequencing Reveals Critical Role of Interferon Signaling in Human Peri-Implantation Stage Embryos.** Rachel West, Hao Ming, Deirdre Logsdon, Rebecca Kile, Courtney Grimm, Sandeep Rajput, Jiangwen Sun, William Schoolcraft, Rebecca Krisher, Zongliang Jiang, Ye Yuan

Implantation is considered the ‘black box’ of human development as there is little insight into the signaling mechanisms that regulate this process. We used the recently developed extended culture system to grow human blastocysts *in vitro* to embryonic days 8, 10 and 12. Our objective was to investigate the molecular mechanisms that facilitate implantation in early trophoblast cells using single cell RNA-sequencing. A total of 139 cells were isolated from 12 embryos. Cells were categorized as “small” (cytotrophoblast), “large” (syncytiotrophoblast), or “migratory” (extravillous trophoblast; EVT) based on morphology. RNA-sequencing revealed some interferon related genes appear as early as D8 in cytotrophoblast cells. GO Pathway Analysis showed that both the Type I and Type II interferon pathways become upregulated from D8 to D12 in a time dependent manner. Additionally, EVT cells expressed the highest levels of all interferon related genes compared to other cell types. To validate RNA-sequencing data, 3D-confocal microscopy was used. Imaging divulged cells around the periphery of embryos that express high cytoplasmic levels of the Type I interferon receptor (IFNAR1), whereas IFNAR2 and the Type II interferon receptor subunit, IFNGR2, are localized throughout the embryo. Additionally, interferon stimulated gene (ISG) 20 is predominantly expressed in the nucleoli of most cells, and staining becomes more pronounced as development proceeds. In addition, ISG20 is more prevalent in EVT compared to cytotrophoblast. ISG15 is cytoplasmic and also becomes brighter at later stages of development. These data suggest that interferon signaling may play a critical role during implantation, providing previously unknown insight into the control of implantation in the human. Further analysis of our data will elucidate interactions between interferon signaling and other immunomodulation pathways in peri-implantation human embryos. This research was funded by CCRM and approved by WIRB (Study no: 1179872).
S18.4 - The roles of retrotransposon reactivation in mammalian preimplantation development. Andrew Modzelewski, Anne Biton, Gang Chen, Martin Kinisu, Yang Wan, Lin He

Retrotransposons are remnants of invading parasitic viral elements that are largely silenced in somatic tissues. However, a subset have maintained regulatory sequences and are strongly reactivated during the epigenetic reprogramming of mammalian pre-implantation development. Recent evidence suggests that retrotransposons have been employed by their host genomes for essential biological functions in development and perhaps disease. Through re-analysis of recently published single embryo RNA Sequencing datasets, we characterized the expression of retrotransposons during human pre-implantation development and identified distinct and precise waves of retrotransposon induction and characterize their potential effect on nearby genes.

Surprisingly, we found a number of stage specific retrotransposons exhibiting novel gene regulatory roles during pre-implantation development by influencing the generation of alternative transcript isoforms of adjacent protein-coding genes. These alterations include alternative promoters, 5’ leader sequences, exons, and 3’ UTRs. Interestingly, more than half retrotransposon-gene isoforms are predicted to alter open reading frames, through truncation or augmentation, yielding proteins with potentially novel functions. Of these, we more closely characterized instances in which novel transcript isoforms initiate within LINE, SINE or LTR elements as well as elements that serves as a new exons in developmental protein coding genes.

These isoforms differ from their canonical counterparts both structurally and functionally, demonstrating only a fraction of the possible mechanisms of retrotransposon based rewiring found in the embryo. This work provides some evidence for reactivated retrotransposon expression in pre-implantation development that may drive a novel and complex gene regulatory network. The rewiring of host gene expression and function through these embryo specific isoforms may serve critically important roles for proper human embryonic development.

S19.1 - The Role of Extracellular Vesicles in the Female Reproductive Tract. Patricia Martin-Deleon, Zeinab Fereshteh, Amal Al-Dossary, Pradeepthi Bathala

Extracellular vesicles (EVs), which are membranous nanoparticles, have been identified in the secretions of all 3 regions (oviduct, uterus, vagina) of the female reproductive tract. They have been documented in several species, including humans, and were first shown to carry specific fertility-modulating sperm proteins such as SPAM1 and PMCA4 whose expression is hormone-dependent. Expression levels are elevated during pro/estrus and generally elevated in the oviduct, compared to the uterus and the vagina which has the lowest expression. Oviductal EVs (OVS) have been shown to arise via the apocrine pathway and are expressed both in vivo and in vitro from cultured epithelial cells. Proteomic and transcriptomic analyses have shown that OVS carry a large variety of proteins, mRNA and miRNA that vary during the estrous cycle. These molecular components have potential roles in sperm functionality and embryo development, and EVs have been known to deliver specific proteins and miRNAs to sperm following co-incubation. Delivery occurs via a fusogenic mechanism involving integrin/ligand interactions, and impacts sperm function. Vaginal EVs deliver higher levels of proteins that inhibit premature
capacitation and the acrosome reaction, than those that promote them. Proteins delivered by OVS include those associated with sperm storage, hyperactivated motility, capacitation, and sperm-oocyte binding. Among the miRNAs that murine OVS can deliver to sperm after co-incubation is miR-34c-5p which is essential for the first cleavage and is solely sperm-derived in the zygote. In vitro produced bovine embryos were shown to internalize OVS, with beneficial effects on development. The discovery of the role of oviductal EVs as modulators of gamete/embryo-oviduct interactions has significantly advanced our understanding of the reproductive process, and holds promise for the use of exosome/microvesicle therapeutics for the treatment of infertility and to increase the success rate of assisted reproductive technologies. Supported by NIH #RO3HD073523 and 5P20RR015588.

**S19.2 - Reversing poor gamete quality and protecting embryogenesis in older fathers.**
Macarena Gonzalez, Haley Connaughton, Rebecca Robker

Increasing use of ART to treat age-related infertility in men necessitates new therapeutic strategies to improve success rates. Chaperone-inducing drugs improve oocyte quality and embryo development in obese mice, but whether these improve sperm quality has not yet been investigated. The aim of this study was to determine whether treatment of sperm with a chaperone-inducing drug can improve gamete quality in older fathers.

Sperm from C57BL6 male mice that were either “old” (>1-year-old), or “young” (<8 months old) was collected and treated in vitro during capacitation. Sperm quality assessments included motility, zona-binding capacity and mitochondrial activity. In parallel, sperm was used for IVF and embryo development was analyzed by time-lapse imaging.

Sperm from old males had reduced motility (N=9-12; P=0.03), lower mitochondrial membrane potential (N=6-11; P=0.04) and impaired zona-binding capacity (N=4-6; P=0.02) compared to young males. Each of these sperm quality parameters was improved by treatment. When sperm was used for IVF, embryos from old males had delayed time to first cleavage (N=21-27; P=0.01). Sperm from older males gave decreased 2-cell (N=7-12; P=0.04) and blastocyst rates (N=7-12; P=0.003). Drug treatment of ‘old’ sperm restored embryo development rates to those of sperm from young males.

To test the efficacy of this in vitro treatment in humans, sperm samples from 37 ART patients were treated in vitro for 30 minutes. Sperm from older men (>40 years old) had reduced motility (N=14; P=0.03), as well as increased levels of both mitochondrial ROS (N=10; P<0.0001) and DNA oxidative damage (N=15; P=0.004). Pharmaceutical treatment increased sperm motility by 10% in older men, while DNA damage levels were reduced in all patients independently of age (N=37; P=0.001).

These results demonstrate that male age negatively impacts sperm quality in both mice and humans but that drug/pharmaceutical treatment in vitro normalizes sperm quality and improves embryogenesis in mice.
S19.3 - Sperm-associated beta-defensin 22 influences the female immune response to seminal fluid and is a determinant of fertility and fecundity in mice. Sarah Robertson, Ricky Mathias, Honyueng Chan, Peck Chin, John Schjenken

Factors in seminal fluid interact with female tissues after mating to modulate the female immune response and promote receptivity to implantation. We have shown that sperm-associated molecules ligate TLR4 on uterine epithelial cells to initiate the female response. Here we investigated how DEFB22, a ligand of TLR4 present on the sperm glycocalyx, contributes to male fertility. Defb22/-/- mice were generated using CRISPR-CAS9 technology, and Defb22+/+ and Defb22/-/- mice were bred from Defb22+/- breeding pairs. Defb22/-/- or Defb22+/- littermate males were mated with Balb/c females. Females were killed on d3.5pc to measure T cell parameters by flow cytometry, and on d17.5pc to measure pregnancy outcomes (n=10-15 dams/group). Male reproductive organ and sperm parameters were measured at 20-22 weeks (n=16/group). Defb22/-/- males had reduced epididymal sperm, fewer progressive motile sperm, and fewer sperm with normal morphology, as well as reduced testicular weight and reduced seminal vesicle weight (all p<0.05) compared to wildtype males. Paternal DEFB22 deficiency resulted in 26% fewer viable pregnancies, 24% fewer viable pups per litter, and 5% reduced fetal weight and fetal:placental weight ratio (all p<0.05). This was associated with a 23% reduction in CD4+CD25+FOXP3+ regulatory T cells, and a 47% increase in CD4+FOXP3- effector T cells, in uterine lymph nodes at implantation (p<0.05). This study provides evidence that DEFB22 deficiency affects male gametogenesis, and affects fertility and fecundity after mating. Effects of DEFB22 on pregnancy outcome may be mediated through impaired fertilization and embryo development, and/or reduced capacity to elicit female immune tolerance.

S19.4 - Disruption of Semen Liquefaction in the Female Reproductive Tract: A Potential Novel Contraceptive. Brooke Barton, Jenna Rock, Devinae McNeill, Gerardo Herrera, My-Thanh Beedle, Wipawee Winuthayanon

Unintended pregnancy rates in North America are higher than in other developed countries, and associated medical costs in the United States alone are ~$5 billion/year. Although more effective contraceptives are available (i.e., pills, implants, or intrauterine devices), over-the-counter (OTC) contraceptives remain the most heavily used birth control method. Current OTCs, however, have two significant drawbacks: 1) high failure rates (19% for condoms and 28% for spermicides) and 2) vaginal toxicity from spermicidal use. Our laboratory has been investigating a novel non-steroidal on-demand contraceptive by targeting the semen liquefaction process in mouse and human models. Liquefaction is a post-ejaculation process that changes semen from a gel-like to watery consistency. Prostate-derived serine protease enzyme called kallikrein 3 is the primary enzyme cleaving the gel-forming protein in the semen. In our study, we developed a new method to suppress all serine protease activities in the semen and the female reproductive tract using a serine protease inhibitor (PI) to block the liquefaction process. We found that PI treatment significantly decreased the number of pups born in mice, inhibited sperm motility, and completely attenuated the fertilization rate after in vitro fertilization. Female mice gained fecundity status after stopping the treatment of PI. Additionally, PI treatment did not increase vaginal cell death compared to vehicle controls. Moreover, PI completely inhibit human sperm
motility in vitro. Thus, our findings could lead to a prototype development for a better OTC contraceptive choice for women (as vaginal gel or film).

**S20.1 - Investigating Embryonic Chromosomal Instability in Preimplantation Development.** Shawn Chavez, Kelsey Brooks, Brittany Daughtry

Multiple studies across higher-order mammals, including humans, established that in vitro-derived embryos suffer from frequent whole chromosomal losses and gains, or aneuploidy. While many aneuploid embryos will arrest at the cleavage-stage, some can still form blastocysts, depending on the chromosome(s) affected, type of error, and extent of the abnormality (mosaicism). Aneuploidy can arise either meiotically during gametogenesis or post-zygotically from cleavage divisions. Upon missegregation, chromosomes are sequestered into micronuclei and significant efforts have identified the key meiotic factors, but less is known about the specific contributors to mitotic aneuploidy. The unique process of cellular fragmentation is often, but not always, associated with human embryonic aneuploidy and its timing and degree is indicative of meiotic and mitotic progression. Using rhesus nonhuman primate embryos (N=50), which exhibit a similar incidence of aneuploidy, mosaicism, micronucleation, and cellular fragmentation, we recently demonstrated via single-cell/fragment DNA-seq that fragments may encapsulate whole and/or partial chromosomes lost from blastomeres. These chromosome-containing cellular fragments (CCFs) are almost equally maternal or paternal in origin and time-lapse imaging revealed that multipolar divisions at the zygote or 2-cell stage were frequently associated with CCF production. Because the DNA in CCFs was highly unstable in the absence of a nuclear envelope as evidenced by the accumulation of double-stranded breaks, we microinjected zygotes with fluorescently-labelled modified mRNAs (mCherry-H2B and mCitrine-LaminB1) to visualize micronuclei formation and potential sequestration by fragments in real-time. Live-cell multicolor confocal imaging demonstrated that micronuclei not only persist or rejoin the primary nucleus, but may also be eliminated from the embryo upon cytoplasmic pinching of fragments from blastomeres. In addition to chromosome elimination by cellular fragmentation at the cleavage-stage, we also show that CCFs and non-dividing aneuploid blastomeres exhibiting extensive DNA damage are prevented from incorporation into blastocysts, which may denote mechanisms to surpass aneuploidy and continue in development.

**S20.2 - Transcriptional and epigenetic reprogramming during bovine preimplantation development.** Pablo Ross

Preimplantation development ensues after two transcriptionally quiescent and highly differentiated gametes merge at fertilization. The morphologically simple cleavage divisions that follow are accompanied by extensive epigenetic remodeling and major activation of embryonic transcription at a species-specific developmental stage (e.g., 2-cell in mouse, 8-cell in human and cow). Much insight regarding biological mechanisms that govern early mammalian development is based on using a mouse model. Nevertheless, differences in timing of genome activation (e.g., a few hours for mouse vs a few days for cattle and humans) beckons use of a bovine model. Using next generation sequencing approaches to characterize transcript and chromatin conformation during bovine preimplantation development, as well as experimentally
manipulating transcription and specific regulators using in vitro-produced bovine embryos we are deciphering mechanisms that underlie genome reprogramming in a species in which the timing of genome activation is similar to human. Our results indicate that although major genome activation occurs during the 8-cell stage in bovine embryos, minor genome activation, which initiates during the 2-cell stage, is required for development. Furthermore, transcription from the embryonic genome is required to clear maternal transcripts and is associated with expression of small RNAs that could have a role in maternal transcript degradation. Finally, analysis of open chromatin, as well as embryonic gene expression, identified potential regulators of embryonic gene activation, some of which are conserved between mouse, human and cow, but many of which appear only to be important in human and bovine embryos. I will present these and other data that suggest the bovine model is highly appropriate to gain understanding of human preimplantation development.

**S20.3 - Unraveling the transcriptome by single cell RNA-Seq of porcine oocytes and parthenogenetic preimplantation embryos.** Piotr Pawlak, Natalia Derebecka, Arkadiusz Kajdasz, Zofia Madeja, Ewelina Warzych, Joanna Wesoly, Dorota Lechniak

The very first stages of mammalian embryonic development are governed by transcripts and proteins of maternal origin. At strictly species-specific time, embryonic genome activation occurs to take control over further development. At present livestock species like pigs and cows are proposed to be the best animal models for human reproductive medicine. As a result, we are aiming to demonstrate the molecular pathways governing early porcine embryo development. RNA from oocytes, IVM oocytes, 2-cell, 4-cell, 8-16 cell, morule and blastocysts (ICM and TE) stage embryos were isolated using Smarter Ultra Low Input RNA Sequencing kit (Clontech) followed by cDNA synthesis and amplification (3 to 8 biological replicates). Libraries were prepared using Nextera XT LP Kit (Illumina) followed by RNA-Seq on Illumina HiSeq2500 platform. We obtained on average 41 million 100-bp reads for oocyte/embryo samples, while on average 92% of all reads aligned to porcine genome (Sscrofa11.1). Mean number of detected transcripts through preimplantation development accounted for 14219 (+/-1149) representing 62% of all genes. In vitro maturation of oocytes resulted in 737 differentially expressed (DE) genes (374 up and 363 downregulated). Subsequent stages, 2-cell and 4-cell showed 38 and 47 differentially expressed genes (207 and 45 up and 151 and 2 downregulated respectively) indicating the use of maternal transcripts and cease in transcription. Eight cell embryos DE 5973 transcripts compared to 4 cell stage (1473 up and 4500 down) which is indicative for major embryonic genome activation. In morule 5889 (2320 up and 3579 down) transcripts were DE following 1345 in blastocyst stage (770 up and 575 down). In ICM and TE lineages we found 1364 differentially expressed genes (1058 up and 306 downregulated in TE). The ongoing GO analysis should help to understand the metabolism of preimplantation porcine embryos and allow more efficient derivation of porcine parthenogenetic embryonic stem cell lines.

**S20.4 - Investigating the role of zinc in murine preimplantation embryo development and the effect on cell fate determination in the blastocyst.** Julia Balough, Francesca Duncan, Thomas O'Halloran, Teresa Woodruff
Zinc is the most abundant transition metal in the mammalian oocyte, egg, and preimplantation embryo. Fluctuations in intracellular zinc content are necessary to drive early developmental milestones. Chelation of zinc during meiotic maturation or preimplantation embryo development results in cell cycle arrest. Conversely, increasing intracellular zinc using the zinc ionophore, zinc pyrithione (ZnPT) causes eggs to escape meiotic cell cycle arrest at metaphase of meiosis II. The effect of elevated zinc on the mitotic cell cycle during mammalian embryo development is not known; thus, we utilized ZnPT to perturb zinc content in murine preimplantation embryos and predicted that cell specification would be altered. First, we determined the dose range in which the ionophore was sublethal to embryonic development. ZnPT perturbs zinc in oocytes at concentrations of 10µM; however, we observed 10nM ZnPT in extended culture mimics control embryo development. To confirm zinc uptake as a consequence of ZnPT exposure, embryos were stained and imaged with labile zinc specific probe, ZincBY-1. 2-cell, cleavage, morula and blastocyst embryos were imaged to capture zinc fluorescence and analyzed for intensity. Unlike early embryos, the blastocyst consistently increased levels of labile zinc in ZnPT growing embryos. Next, we assessed RNA expression with RT-qPCR of cell fate markers. We observed 2-fold increases in gene expression of trophectoderm marker, Cdx2, and inner cell mass marker, Nanog, in treated embryos. Overall, we were able to perturb zinc content in the embryo and determined that increased zinc had an effect on the expression of both cell fate pathways in the blastocyst. The ultimate goal of this work is to determine if the increase in zinc seen in the blastocyst in response to zinc perturbation is a cause or consequence of cell fate determination.

This work is supported by the Woodruff Laboratory and NIGMS R01GM115848.

**S21.1 - Using a genetic model of epigenetic inheritance to determine the inter- and transgenerational impacts of paternal high-fat diet on the sperm epigenome and descendant phenotypes.** Anne-Sophie Pépin, Christine Lafleur, Vanessa Dumeaux, Deborah Sloboda, Sarah Kimmins

Obesity rates are at an all-time high, affecting over 650 million people worldwide. Known factors contributing to obesity risks include genetics, lifestyle and maternal factors. Epidemiological studies and animal model data suggest paternal diet may also affect offspring obesity risks. However, the molecular mechanisms underlying this inheritance of complex metabolic disease remain elusive. The Kimmins lab previously demonstrated using a genetic model of epigenetic inheritance, that sperm histone methylation is implicated in transgenerational epigenetic inheritance and offspring health. Using this genetic model our objective was to investigate whether there can be epigenome-environment interactions leading to enhanced offspring phenotypes.

Transgenic sires (F0) with a pre-existing abnormal sperm epigenome at the level of histone H3 lysine 4 methylation (H3K4me2/3) and wildtype sires, were exposed to either a low- or high-fat diet (10% or 60% kcal fat, respectively) for 10-12 weeks. Their sperm was examined for diet-induced changes to the epigenome by chromatin immunoprecipitation sequencing (ChIP-seq), targeting histone H3K4me3. Their regular chow-fed descendants (F1 and F2) were examined for inherited changes in metabolism and the liver for differential gene expression.
F0 males fed a high-fat diet became obese, glucose intolerant and insulin insensitive irrespective of their genotype. Intergenerational effects of paternal diet were observed in male offspring only, while metabolic functions in female descendants was not impacted by paternal diet. Males sired by obese transgenics had enhanced metabolic phenotypes compared to wildtype obese descendants. Interestingly, transgenerational effects of F0 diet were only observed in transgenic descendants. ChIP-seq data will be presented and links between diet and non-genetic transmission elucidated.

These findings suggest in an animal model that there can be epigenome-environment interactions leading to enhanced phenotypes. This may in part explain the higher incidence of complex disease observed in vulnerable populations where there are a multitude of potentially damaging exposures.

S21.2 - Metabolic Induction of Epigenetic Modifications in Early Embryos. Roger Sturmey

It is now widely accepted that events occurring prior to, and around the time of conception can alter developmental programming in ways that influence the lifelong health of the offspring. Compelling data, arising from molecular and cellular experiments through to epidemiological studies, suggest that these effects are mediated via the gametes and the preimplantation embryo. During the preimplantation period, the embryo undergoes a process of epigenetic reprogramming which may impact on the metabolic processes that sustain development. It is also apparent that the preimplantation phase is especially sensitive to environmental perturbation. While we have a mature picture of the profile of energy metabolism in early embryos, metabolic activity is dynamic and responds to changes in maternal nutrient provision and cellular demand for energy. It is now evident that metabolic activity alters epigenetic profiles which may provide a key sensing device through which the embryo responds to changes in its environment. Understanding the relationship between metabolism and epigenetics is vital to revealing the mechanisms by which conditions during preimplantation period can predispose to disease, and, how it may be possible to devise interventions that may reduce the likelihood of disease to ensure the best start in life.

S21.3 - Lasting Brain DNA Methylation Perturbations and Cognitive Impairments Following Preimplantation Alcohol Exposure. Lisa-Marie Legault, Mélanie Breton-Larrivée, Anthony Lemieux, Maxime Caron, Clara Amegandjin, Daniel Sinnett, Elsa Rossignol, Graziella DiCristo, Serge McGraw

Prenatal alcohol exposure (PAE) is known to alter cellular epigenetic profiles during brain development as well as being part of the molecular basis underpinning Fetal Alcohol Spectrum Disorder (FASD) etiology. However, the consequences of an early embryonic PAE (preimplantation) on the future embryo epigenetic landscape remain unknown. Our objective is to identify DNA methylation dysregulations, in the forebrain of late-gestation mouse embryos, initiated by early PAE, and uncover how early embryonic PAE leads to cognitive impairments in offspring. Using our preclinical model of early alcohol exposure, we exposed pregnant females
to binge-like concentration of ethanol at E2.5, and collected FASD and control E18.5 embryos. We established quantitative DNA methylation profiles in forebrains using Methyl-Seq and assessed differentially methylated regions (DMR) 100bp tiles with >±20% methylation difference between control and ethanol-exposed samples. To test cognitive capacity, we performed 3-chamber maze and novel object recognition tests with prenatally exposed pups, at P40. We observed 314 DMRs in forebrains, with a majority of DMRs being hypermethylated. Gene ontology analysis revealed enrichment for neuronal and synapse function or differentiation, suggesting that the dysregulation of DNA methylation associated with these processes persists through gestation. Behavioral assessment revealed that the exposure caused impairments in social interaction (3-chamber maze), spatial recognition and object recognition (novel object recognition). Our study reveals that an early acute alcohol exposure during the epigenetic reprogramming wave occurring in early embryos triggers long-lasting DNA methylation perturbations in the embryo, which leads to abnormal cognitive function in the developing offspring.

**S21.4 - Early life adversity: Impacts on the mother, the placenta and offspring. Deborah Sloboda**

In recent decades, epidemic levels of non-communicable chronic disease (NCD) states including obesity, and cardiovascular diseases, whose social and economic impacts have prompted a global investigation into their causes as well as their consequences. Though initially considered to be determined largely by genetic and lifestyle factors, this paradigm would ultimately be insufficient to explain the continued increased incidence of NCDs. We know that perturbations during critical developmental windows result in (mal)adaptations that confer disease risk. By extension, alterations in maternal physiology are implicated in this discovery, as it is the maternal environment that is the primary determinant of fetal growth during vulnerable critical windows. Paternal influences, although largely unexplored, have also begun to emerge as contributing factors in fetal and placental growth. In our work, we have investigated how early adversity impacts on reproductive and metabolic outcomes in offspring. An altered substrate and inflammatory profile is said to program offspring, resulting in increased disease risk. In this regard, we show that diet-induced obesity modifies maternal gut microbial communities, and that these shifts in gut community composition may be impacting maternal metabolism through altered production of bacterial metabolites, impacting intestinal permeability and immune function. Maternal metabolic compromise in turn results in an adverse fetal environment that impacts on placental function and ultimately will lead to (mal)adaptations in the fetus and postnatal offspring. In order to make real improvements in non-communicable disease risk, engagement with expectant mothers and their families as well as the services that support pregnant people are essential and will enable the translation of bench top biology into tangible policy-related improvements for women and children. In our community-based health study called *Mothers to Babies* we aim to develop a community-based formative knowledge transfer program of intervention, to improve diet, and body composition of women before and after conception.
**S22.1 - Photostimulated Ovarian Recrudescence: The Role of Gonadotropins.** Kelly Young

Ovarian cyclicity is not constant in the majority of vertebrates. Relative periods of inactivity induced by seasonal breeding cycles punctuate the female reproductive lifespan. In Siberian hamsters, exposure to short photoperiod reduces reproductive function centrally by decreasing gonadotropin secretion, whereas subsequent transfer of photoinhibited hamsters to stimulatory long photoperiod promotes FSH release leading to ovarian recrudescence. While the fluctuation in gonadotropin support systemically drives changes in ovarian function, the intraovarian factors needed to resume cyclicity in a quiescent ovary remain unknown. To address this question, we hypothesized that 1) the intraovarian transcriptome would vary across photoperiod exposure, 2) culturing photoregressed ovaries with gonadotropins would induce expression of key folliculogenic factors, and 3) ovarian processes independent of FSH may react differently to photoperiod changes than those dependent on gonadotropin support. Hamsters were assigned to photoperiod groups that maintained ovarian cyclicity (long day LD), induced ovarian regression (short days SD), or captured the photostimulated recrudescence period post-transfer from SD to LD (PT). Most of the differentially expressed genes in the short-read sequencing Illumina assays used to map and quantify the ovarian transcriptomes were noted between functional LD ovaries and regressed SD ovaries; however, multiple expression patterns were identified across photoperiod groups and were mirrored by immunostaining results. While ovarian mass increased in SD ovaries cultured with gonadotropins as compared to without gonadotropins, mRNA expression of intraovarian factors varied across gonadotropin stimulation and photoperiod groups. Among non-cultured ovaries, photoperiod-induced changes were noted in mRNA expression of late folliculogenic factors that interact with FSH signaling, but not in factors associated with early follicle growth that are largely FSH-independent. These results suggest that photoperiod broadly affects gene expression across the ovary, that early follicle development is not fully quiescent in photoinhibited ovaries, and that intraovarian factors in addition to gonadotropins are needed for photostimulated recrudescence.

**S22.2 - Progesterone Receptor Action in the Primate Ovulatory Follicle.** John Hennebold

The discovery of progesterone was based on its ability to maintain pregnancy through its action on the uterus. The subsequent discovery of a nuclear hormone receptor that selectively bound progesterone revealed that its site of action extended beyond the uterus. In primates and other species, expression of the nuclear progesterone receptor (PGR) increases in the granulosa cells of the ovarian follicle after the midcycle ovulatory surge. Studies conducted in rhesus macaques demonstrated that by blocking progesterone synthesis, rupture of the primate follicle did not occur in response to an ovulatory stimulus. Restoration of ovulation occurred when a synthetic PGR agonist was co-administered with the progesterone synthesis inhibitor. At this time, PGR was thought to be the primary, if not exclusive, receptor through which progesterone actions were elicited. However, studies over the past decade have revealed additional progesterone binding proteins exist within the ovarian follicle that are capable of regulating cellular function, including progesterone receptor membrane component-1 (PGRMC1). PGRMC1 is also highly expressed in the rhesus macaque follicle. Through direct delivery of viral vectors expressing small interfering (si)RNAs capable of inhibiting gene expression or protein translation into the rhesus macaque periovulatory follicle, the individual roles that PGR and PGRMC1 play in
Elevated cyclic AMP (cAMP) within the preovulatory follicle controls ovulation and oocyte maturation. Pharmacological elevation of cAMP in the oocyte and surrounding cumulus cells during oocyte in vitro maturation (IVM) improves oocyte developmental potential. Given that the substrate and degradation product of cAMP metabolism are ATP and AMP, respectively, we hypothesised that in vitro upregulation of cAMP in the mouse cumulus-oocyte complex (COC) impacts oocyte energy production. Accordingly, we investigated the oocyte’s ability to generate ATP from AMP via the adenosine salvage pathway. An established model of elevated COC cAMP was used, whereby a spike in COC cAMP production was induced in a short “pre-IVM” phase by 2h exposure to the cAMP modulators forskolin and 3-isobutyl-1-methylxanthine. Oocyte and COC adenine nucleotide (ATP, ADP, and AMP) levels and activity of the energy sensing enzyme AMPK were measured by LC-MS/MS and Western blotting, respectively. Cyclic AMP modulation during pre-IVM decreased ATP and the ATP:ADP ratio in the oocyte and activated AMPK (n=3 replicates). Conversely ATP in cumulus cells was higher (n=4). The capacity of the oocyte to utilise AMP from cAMP hydrolysis for ATP production via the adenosine salvage pathway was confirmed by tracing the metabolism of $^{13}$C$_5$-AMP. Oocytes cultured with $^{13}$C$_5$-AMP produced $^{13}$C$_5$-ATP and this was perturbed by preventing ADP to ATP metabolism by inhibition of creatine kinase (n=4), confirming participation of the adenosine salvage pathway in oocyte ATP metabolism. Intact COCs were 8-fold more efficient at generating ATP in the oocyte than oocytes denuded of cumulus cells and moreover blockage of COC gap-junctional communication led to 3.8-fold oocyte $^{13}$C$_5$-ATP (n=4). We conclude elevated cAMP alters oocyte metabolism, and that AMP contributes to ATP generation in the oocyte and that cumulus cells play an important role in that process.

**S22.4 - Cargo proteins in luteal extracellular vesicles have catalytic activity that may regulate activation of luteal resident immune cells.** Martyna Lupicka, Joy Pate

Extracellular vesicles (EV) are cup-shaped, membranous structures that contain specific cargo used for cell-to-cell communication. It was hypothesized that luteal steroidogenic cells communicate with luteal resident immune cells via EV. Extracellular vesicles were isolated from culture media of luteal steroidogenic cells isolated from day 10 (n=4) and regressing (n=4, 8 hr after PGF2A injection) bovine corpus luteum. Resident T lymphocytes and macrophages from day 10 (n=4) and regressing (n=4) luteal tissue were also isolated. Proteins from EV and resident immune cells were profiled using tandem mass spectrometry. Mann-Whitney Test was used for statistical analysis of the proteomic results and Benjamini-Hochberg method was applied for multiple test correction. Bioinformatic analyses were performed on the proteomic datasets using Ingenuity Pathway Analysis and Panther. In EV, a total of 1292 proteins were identified. The top molecular function identified with EV proteins was catalytic activity (36.2% of the total...
proteins). Among the identified proteins, enolase 1 was increased in abundance in EV during luteal regression (logFC=1.24, padj>0.05, p<0.01). Nine proteins identified in EV, among which two were more abundant in EV from regressing corpus luteum (p≤0.01), including enolase 1, were associated with glycolysis (p=0.0002). Enolase 1 was also increased in abundance in resident T cells during luteal regression (logFC=0.78, padj>0.05, p<0.01), indicating a potential shift of energy production from oxidative phosphorylation to glycolysis, allowing for more rapid activation of immune cells in hypoxic conditions. Overall, integration of the pathway and network analyses of the proteomic profiles showed that EV cargo can play a role in the differentiation and activation of immune cells within corpus luteum. This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2016-67015-24900 from the USDA National Institute of Food and Agriculture.

**S23.1 - Chromatin reprogramming in mammalian gametogenesis and early development.**  
Wei Xie

Drastic chromatin reorganization occurs during mammalian gametogenesis and early embryogenesis. Deciphering the molecular events underlying these processes is crucial for understanding both fundamental biology and infertility. Previously, we have developed a series of ultra-sensitive chromatin analysis methods. Using these technologies, we reported chromatin reprogramming during early mammalian development for chromatin accessibility, histone modifications, and 3D architecture. These studies unveiled highly dynamic and non-canonical chromatin regulation during maternal-to-zygotic transition and zygotic genome activation. In this talk, I will present our recent research progress in understanding chromatin regulation during mammalian gametogenesis and early development. We report that SETD2, an H3K36me3 methyltransferase, is a crucial regulator of the mouse oocyte epigenome. Maternal depletion of SETD2 results in oocyte maturation defects and subsequent embryonic lethality after fertilization. Detailed investigations using low-input chromatin analysis methods reveal how the oocyte epigenome is established through extensive crosstalks of chromatin modifications, and how its defects profoundly affect embryonic development.

**S23.2 - Understanding Human Pluripotent States and Their Applications.** Thorold Theunissen

Two distinct pluripotent stem cell (PSC) states can be isolated from mouse embryos: embryonic stem cells derived from the blastocyst represent an immature naïve state of pluripotency, whereas epiblast stem cells derived from the post-implantation epiblast represent a primed state of pluripotency. Accumulating evidence indicates that human PSC isolated under traditional conditions correspond to a primed pluripotent state, which is marked by repressive chromatin features and lineage bias. We and others have recently reported strategies to reprogram primed human PSC into a naïve state by exogenous expression of transcription factors or modulation of intracellular signaling pathways. The resulting naïve cells resemble the human pre-implantation embryo based on various molecular criteria, including the expression of transposable elements, globally reduced DNA methylation levels and X chromosome reactivation in female cells.
Nevertheless, important differences remain between naïve human PSC isolated \textit{in vitro} and pluripotent cells \textit{in vivo}, including the irreversible loss of parent-specific imprinting marks and genomic instability during long-term culture. I will discuss recent efforts to isolate naïve human PSC with enhanced stability, their advantages for modeling aspects of early human development that are intractable to dissect in traditional human PSC, and their differentiation potential. The isolation of naïve human PSC has the potential to transform our understanding of early human development and erase the variable lineage potential observed in primed PSC.

\textbf{S23.3 - Epigenetic Dysregulation of the Ido1 Gene Induced by BPA and TBBPA Exposure is Associated with Fetal Loss in Mice.} Jasmine Reed, Sarah Latchney, Philip Spinelli, Martha Susiarjo

Establishment of maternal-fetal immune tolerance, that is necessary to sustain a successful pregnancy, involves T-regulatory cell (Treg) expansion within the maternal-fetal interface. As disruption of this tolerance, associated with reduced Treg number, is linked to miscarriages, elucidation of mechanisms controlling this process contributes significantly to reproductive health. One proposed mechanism is tryptophan catabolism into kynurenine metabolites that are necessary for Treg differentiation. In mice, inhibition of indoleamine-2,3-deoxygenase 1 (IDO1), a tryptophan-depleting enzyme, results in early embryonic hemorrhaging that is linked to increased rates of fetal loss later in gestation, supporting the role of IDO1 in pregnancy maintenance. \textit{Ido1} is expressed in mouse placenta at embryonic day (E)9.5, and recently our laboratory found that \textit{Ido1} is a novel, maternally expressed imprinted gene with paternal allele-specific DNA methylation of the gene promoter. Interestingly, exposure to Bisphenol A (BPA) and Tetrabromobisphenol A (TBBPA), two endocrine-disrupting chemicals (EDCs), reduces \textit{Ido1} mRNA and protein; however, it is unknown if exposure is associated with changes in \textit{Ido1} DNA methylation and pregnancy loss. We hypothesize that EDC exposure-induced reduction of \textit{Ido1} is linked to increased DNA methylation in the placenta and increased rate of fetal loss. To measure DNA methylation, we performed bisulfite-pyrosequencing of the \textit{Ido1} promoter in controls and EDC-exposed placentas. We observed a significant difference in the variances of methylation in BPA-exposed placentas (N=10; P<0.05) and a trend towards a significant difference in TBBPA-exposed placentas (N=8; P=0.125). We also found that BPA and TBBPA-exposed embryos have higher rates of hemorrhaging at E7.5 (n=21-32; P<0.01). These findings demonstrate that perturbation of tryptophan catabolism by epigenetic dysregulation of \textit{Ido1} may reduce maternal-fetal immune tolerance. Future studies are aimed at determining if EDC exposure-induced reduction of \textit{Ido1} is associated with increased rate of fetal loss and reduced Treg number at the maternal-fetal interface.

\textbf{S23.4 - Role of Prmt6 and asymmetric dimethylation of H3R2 on mouse preimplantation embryos.} Shinnosuke Honda, Yuri Kunimoto, Naojiro Minami

MuERV-L (murine endogenous retrovirus with leucine tRNA primer) is a retrotransposon expressing in mouse early embryos. MuERV-L transcribed as chimeric transcripts with many genes during zygotic genome activation (ZGA) and knockdown of MuERV-L leads to the developmental defect at the 4-cell stage. Analysis of public RNA-seq data sets revealed that
Prmt6, which is a histone H3 arginine 2 (H3R2) methyltransferase, is transcribed as chimeric mRNA with MuERV-L in mouse 2-cell like ES cells. Because histone arginine methylation is thought to contribute to the heterogeneity of the 4-cell blastomeres and the cell fate determination in the later developmental stages, we hypothesized that MuERV-L controls cell fate determination via chimeric Prmt6 expression. In the present study, we obtained the profiles of Prmt6 transcripts by quantitative RT-PCR in embryos from the 1-cell to the blastocyst stage. Prmt6 mRNA dramatically increases from the early 2-cell to the late 2-cell stage, the time when the major ZGA occurs. We also investigated the localization of asymmetric dimethylation of H3R2 (H3R2me2a) by immunofluorescence in embryos from the 1-cell to the blastocyst stage. Levels of H3R2me2a are different among blastomeres from the 8-cell stage and, interestingly, cells strongly expressing H3R2me2a are preferentially localized in trophectoderm at the blastocyst stage. Western blotting analysis revealed that addition of PRMT6 inhibitor (EPZ020411) at a concentration of 10 µM in culture medium results in a dramatic decrease of H3R2me2a level in embryos at the 4-cell stage and the developmental arrest occurs before the blastocyst stage. These results suggest that the LTR promoter of MuERV-L drives Prmt6 expression and the resulting methylation of H3R2 plays important roles in preimplantation development and cell fate determination.

S24.1 - TRPV4 is the temperature-sensitive ion channel of human sperm. Nadine Mundt, Marc Spehr, Polina Lishko

A key competence of mammalian sperm to fertilize the egg is to gain hyperactivated motility. This is triggered by a highly interdependent orchestration of ion channels, which regulate membrane potential, intracellular pH, and cytosolic calcium. Previous studies unraveled three essential ion channels that regulate these parameters: (1) the Ca$^{2+}$ channel CatSper, (2) the K$^+$ channel KSper, and (3) the H$^+$ channel Hv1. However, the molecular identity of the sperm Na$^+$ conductance that mediates initial membrane depolarization and, thus, triggers downstream voltage-dependent signaling events is yet to be defined. Here, we functionally characterize DSper, the Depolarizing Channel of Sperm, as the temperature-activated channel TRPV4. It is functionally expressed at both mRNA and protein levels, while other temperature-sensitive TRPV channels are not functional in human sperm. DSper currents are activated by warm temperatures and mediate cation conductance, that shares a pharmacological profile reminiscent of TRPV4. Together, these results suggest that TRPV4 activation triggers initial membrane depolarization, facilitating both CatSper and Hv1 gating and, consequently, sperm hyperactivation.

S24.2 - Sperm-oocyte communication via PYK2 requires CD9 expression by the oocyte. William Kinsey, Huizhen Wang

Sperm-egg contact results in activation of the tyrosine kinase PYK2 within the egg cortex underlying the bound sperm. This signal transduction event occurs without gamete fusion and plays an important role in triggering actin-mediated sperm incorporation. However, the mechanism by which sperm binding could induce PYK2 activation remains unanswered. PYK2 is a member of the FAK family of protein kinases which suggests the possibility that PYK2
activated by the interaction of specific membrane proteins expressed on the sperm and oocyte surface. The objective of this study was to determine whether specific oocyte plasma membrane proteins are required for PYK2 activation at the sperm binding site. The experimental design was to compare the ability of oocytes that were null for known gamete-binding proteins to induce PYK2 activation at sperm binding sites. Zona-free oocytes collected from juno -/-, cd9 -/-, or wt female mice were incubated with a limiting concentration of capacitated wt sperm, then fixed at 20 mpi, just prior to the beginning of sperm incorporation in the wt oocytes. Activated PYK2 was detected with an antibody to the phosphorylated form of the activation site (Y402), then fluorescence was quantified by linescan analysis. wt oocytes exhibited little PYK2 response to most bound sperm, but a subset of sperm binding sites (25%) responded strongly with PYK2-PY402 fluorescence greater than 100% relative to adjacent cortex. Juno -/- oocytes were slightly less effective with 23% of sperm binding sites greater than 100% relative to adjacent cortex. However, in cd9 -/- oocytes, only 0.5% of sperm binding sites exhibited PYK2-PY402 fluorescence greater than 100% above adjacent cortex. This result is consistent with a model in which CD9 interaction with one or more sperm proteins is critical for activation of PYK2 in the oocyte cortex.

**S24.3 - Sperm signaling in ART and Science.** Pablo Visconti, Felipe Navarrete, Maria Gracia Gervasi

To become fertile, mammalian sperm must undergo a series of biochemical and physiological changes in the female tract known as capacitation. At the molecular level, capacitation involves the crosstalk between metabolic and signaling pathways. Previously, we have shown that a short incubation with Ca²⁺ ionophore A23187 is sufficient to rescue in vitro fertilizing capacity in sterile knock-out mice models. In this work, concentration of nutrients in the sperm capacitation media were modified. In these incubation conditions, sperm achieved significantly higher percentage of hyperactivated motility and displayed increased ability to fertilize in vitro. Surprisingly, the metabolically enhanced sperm promoted also higher rates of embryo development and, when blastocysts were transferred to pseudo-pregnant females produced more pups. This method also greatly increased fertilization and embryo development rates in sperm from Fe²⁺/DR/DR mice, which are severely sub-fertile in vitro, and when combined with a short Ca²⁺ ionophore treatment, induced high rates of in vitro fertilization and embryo development in the CatSper null sterile mouse model. Finally, when applied to bovine sperm, ICSI success (e.g., cleavage and blastocyst development) was significantly enhanced. Taken together these data suggest that this newly described “metabolic enhanced method” can be used to improve in vitro fertilization protocols in sub-fertile and infertile mouse strains and opens the possibility that similar treatments can be applied to other species including humans.

**S24.4 - Bouncer and SPACA4 - small proteins with big roles in fertilization.** Andrea Pauli, Sarah Herberg, Krista Gert, Yoshitaka Fujihara, Masahito Ikawa

Fertilization is fundamental for sexual reproduction, yet the molecular mechanism is poorly understood. We discovered Bouncer, a short, unannotated protein in zebrafish that is essential for fertilization. Bouncer is required for sperm-egg binding and sperm entry into the egg.
Remarkably, Bouncer functions as the gate-keeper of the egg by ensuring species-specificity of fertilization: it allows conspecific sperm to enter while keeping heterospecific sperm out (Herberg et al., 2018). While fish express Bouncer exclusively in the egg, the mammalian orthologue of Bouncer, Spaca4, is restricted to the male germline. Here, we will present our newest data on the roles of Bouncer and SPACA4 during fertilization.

S25.1 - The Versatile Fibroblast, Maestro of Endometrial Homeostasis and Pregnancy Success, is Derailed in Endometriosis. Linda Giudice

Endometrium is a dynamic tissue whose cellular components undergo cyclic proliferation and differentiation by highly coordinated spatiotemporal actions of ovarian-derived steroid hormones, preparing for pregnancy. In the absence of pregnancy it is shed and regenerates anew without scarring. It can compromise women’s health as it can compromise implantation, contribute to abnormal uterine bleeding and pelvic pain. Moreover, it has its own microbiome, is the target of infectious agents and endocrine disrupters, contains biomarkers for some gynecologic disorders, can cause infertility, affect pregnancy outcomes and is the site of the most common gynecologic malignancy, (endometrial cancer). Endometrium is comprised of luminal and glandular epithelium, an array of resident and transient immune populations, vascular cellular components, and stromal fibroblasts. The latter are unique among fibroblasts in that they express ER and PR and differentiate to an epithelial phenotype in response to progesterone (P4) after estrogen-priming. This results in a unique morphologic transition, altered transcriptome, epigenome, secretome, and extracellular matrix. These P4-induced changes are essential for normal embryo nidation and overall homeostasis of the tissue. Abnormal P4 response is a hallmark of endometriosis, adenomyosis, and polycystic ovarian syndrome and likely contributes to compromised fertility and pregnancy outcomes in affected women. The stromal fibroblast is a “master regulator” of implantation that interacts with invading cytotrophoblasts, secrets potent mediators of angiogenesis and also chemokines and cytokines that orchestrate the immune repertoire in pregnancy for immune-protection of the fetus. The decidua (endometrium of pregnancy) is largely comprised of P4-differentiated endometrial stromal fibroblasts, and recent evidence demonstrates compromised P4 action before and at delivery in some human pregnancy disorders. The focus herein is on the endometrial stromal fibroblast, the most abundant cell type in the tissue, that exhibits regulated or dysregulated responses ovarian-derived steroid hormones in endometriosis and changes its phenotype in an inflammatory environment and in pregnancy. Moreover, we have recently shown that the endometrial mesenchymal stem cell, progenitor of the endometrial stromal fibroblast, harbors abnormalities that affect the P4 responsiveness of its progeny.

S25.2 - Macrophages in Endometriosis Exhibit Phenotypic Heterogeneity and have Potential as Therapeutic Targets. Chloe Hogg, Beth Henderson, Prakash Ramachandran, Neil Henderson, Andrew Horne, Jeff Pollard, Erin Greaves

Endometriosis is a chronic inflammatory disorder associated with pelvic pain and infertility that affects ~176 million reproductive age women worldwide. It is defined by the presence of endometrial-like tissue outside the uterus (lesions). Current management is unsatisfactory and
there is an unmet need for new treatments. Macrophages play a key role in the growth, vascularization and innervation of endometriosis lesions, however our knowledge of macrophage origins, phenotype and heterogeneity in endometriosis is limited. We aimed to define the origin of lesion-resident macrophages and assess phenotypic heterogeneity using our unique mouse model. To assess infiltration of tissue-resident large peritoneal macrophages (LpM) into lesions, we adoptively transferred LpM (isolated from Csf1r-EGFP mice) into the peritoneal cavity of endometriosis mice. GFP+ cells were identified by immunohistochemistry in lesions after 2 weeks (n=4). To assess infiltration of Ly6C<sup>hi</sup> monocytes into lesions we performed Ly6C, F4/80 dual immunofluorescence. We identified both Ly6C<sup>+</sup> monocytes and F4/80<sup>+</sup> mature macrophages in lesions, which was also validated using flow cytometry (n=6). Next, we induced endometriosis in WT mice using donor endometrial tissue from Csf1r-EGFP mice; we identified that 16% (SEM± 4%) of lesion macrophages were GFP+ endometrial-derived macrophages (n=6). Using single-cell RNA-seq, we performed unbiased transcriptional profiling of CD45<sup>+</sup> cells in lesions (6006 cells), donor endometrial tissue (1306 cells) and peritoneal lavage cells from sham (5645 cells) and endometriosis mice (6720 cells). Clustering analysis identified 4 lesion-resident macrophage populations. Comparison of lesion-resident macrophage populations with macrophage clusters from endometrial tissue and the peritoneum confirmed that lesions contained macrophages from different origins. In summary, we demonstrate, for the first time, that macrophages in endometriosis lesions are heterogeneous in both ontogeny and transcriptional profile. We believe that this observed heterogeneity could be translated into clinical applications, such as targeted therapy for endometriosis-associated pain.

**S25.3 - The efficacy of niclosamide on the intra-abdominal inflammatory environment in endometriosis.** Kanako Hayashi, Mingxin Shi, Allison Whorton, Arpan Roy, Nikola Sekulovski, James MacLean

Endometriosis is a common gynecological disease, which causes chronic pelvic pain and infertility in women of reproductive age. Due to limited efficacy of current treatment options, a critical need exists to develop new and effective treatments for endometriosis. Niclosamide is an efficacious and minimally toxic, FDA-approved drug for the treatment of helminthosis in humans that has been used for decades. We have reported that niclosamide reduces growth and progression of endometriosis-like lesions via targeting STAT3 and NF-kB signaling in a mouse model of endometriosis. Niclosamide also disrupts macrophage induced proliferation and inflammatory activity in primary human endometriotic stromal cells. An aberrant inflammatory environment in endometriosis promotes disease symptoms such as pain. Various cytokines/chemokines in the peritoneal fluid (PF) can be peripheral pain sensitizers, linking the endometriosis microenvironment and induced peripheral pain. In order to better understand the inhibitory effects of niclosamide, we evaluated how niclosamide improves inflammatory environments in the PF, using a mouse model of endometriosis. Using MacGreen mice, which express EGFP in macrophages, as donor or recipient mice, GFP+ macrophages were detected in the PF from MacGreen recipients, but not from MacGreen donors, suggesting that macrophages are resident from recipients rather than lesions. Niclosamide treatment (200 mg/kg/day) for 3 weeks reduced not only macrophages but also total cell numbers in the PF. Protein array was then used to identify cytokine/chemokine profiles in the PF that were targeted by niclosamide. We found that ANGPT2, BDNF, CD40LG, FGF19, IFNG, IL1A, CXCL8, IL17A, KLK3, SPP1,
CXCL12 and CD71 were significantly reduced in the PF by niclosamide. Furthermore, niclosamide reduced sensory innervation in the lesions and the growth of dorsal root ganglion. These results suggest that niclosamide reduces inflammatory mediators in the PF that sensitize peripheral nociceptors indicating that niclosamide would have a high impact to improve endometriosis-associated pain. Supported by NIH/NICHD/R21HD092739.

**S25.4 - Understanding the role of endometrial hypoxia to improve management of heavy menstrual bleeding.** Jacqueline Maybin, Alison Murray, Philippa Saunders, Nik Hirani, Peter Carmeliet, Hilary Critchley

Heavy menstrual bleeding (HMB) is common and debilitating but remains a taboo subject. Its cause is undefined, resulting in non-specific hormone therapies with intolerable side effects.

Over 70 years ago it was proposed that progesterone withdrawal caused a transient endometrial hypoxia at menstruation. Subsequent research showed inflammation initiated menses and disputed the role of hypoxia.

By studying human endometrial tissue from women with normal and heavy menstrual blood loss and a mouse model of “simulated menstruation”, we revealed that hypoxia is present exclusively during menstruation. Hypoxia is not necessary for endometrial breakdown but is essential for timely repair of the denuded endometrial surface to limit bleeding. Furthermore, we demonstrated that pharmacological induction of the hypoxic response at menstruation significantly improved endometrial repair in our mouse model, revealing a promising, non-hormonal treatment for women suffering from HMB.

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**2019 Trainee Research Platform Competition Abstracts**

**PC1 - The program of maternal mRNA translation during oocyte meiosis: a genome-wide approach.** Xuan Luong, Enrico Maria Daldello, Gabriel Rajkovic, Cai-Rong Yang, Marco Conti

Complex changes take place in the oocyte throughout meiotic maturation. These alterations are highly dependent on the translation and degradation of accumulated maternal mRNAs as transcription is silenced during this time. This program of regulated translation is essential for assembling the molecular machinery required for proper meiotic progression, fertilization, and embryo development. Using RiboTag/RNA-Seq, we have constructed a detailed time course of ribosome loading (i.e. translation) onto maternal mRNAs from prophase (GV) through the late stages of metaphase I (MI) in mouse oocytes. By mining these data, we have uncovered novel properties of the translation program in GV-arrested oocytes and during progression through meiosis. Transcripts with high translational efficiency (TE) in GV-arrest have, on average, longer poly(A) tails and shorter 3’UTRs than transcripts with low TE. They are also depleted of cytoplasmic polyadenylation elements (CPEs) within 100 nucleotides of the polyadenylation
signal compared to transcripts with low TE. Upon meiotic resumption, mRNAs with high TE during GV-arrest become repressed (Class II), while messages with low TE become activated (Class III). For Class III transcripts, translation activation occurs at as early as two hours post-meiotic resumption, with progressive increases in ribosome loading thereafter. At the same time, Class II transcript undergo rapid translation repression. For a subset of Class II transcripts, the decrease in ribosome recruitment occurs early on at around pro-metaphase I, while total message levels remain stable for up to 12 hours post-meiotic resumption. This indicates that translation repression is uncoupled from message degradation. Preliminary experiments suggest that CDK1 activation is, at least in part, responsible for both translation repression and activation upon meiotic resumption. Thus, translational regulations in the mouse are triggered after reentry into the cell cycle at the time of CDK1 activation. This study is supported by NIH R01 GM116926 and P50 HD055764.

**PC2 - Location Matters: Compartmentalized Protein Translation In Sertoli Cells.** Ana Cristina Lima, Donald Conrad

Male reproductive health depends on the fine-tuned coordination of germ cell development. This process occurs in the seminiferous tubules of the testis, where individual Sertoli Cells (SCs) maintain tissue homeostasis by compartmentalization of many important functions within distinct cellular domains. How this is accomplished, however, remains elusive. We hypothesized that, similarly to neurons, translation of many RNAs is localized in SCs, so that specialized proteins are produced on-site as needed.

To detect and characterize patterns of translation, we adapted and optimized a technique that allows the visualization of nascent peptide chains – surface sensing of translation (SUnSET). This was applied to the TM4 SC line and to C57BL6/J mouse testes. The high co-localization of SUnSET signal with P-RPS6 (a partial proxy of protein translation) and Vinculin (SC cytoplasm maker) indicates that we are detecting translation sites restricted to SCs. Importantly, these sites also locate distally to the nucleus, in different subcellular compartments, strongly supporting our hypothesis.

Additionally, we generated RNA-seq data for proliferating and resting TM4 SCs. We mined these data and a published dataset of SC transcriptomes in 5 different stages of the mouse first spermatogenic wave. Briefly, we searched for SC-specific mRNAs differentially expressed during the first wave and generated a list of candidate drivers (RNA-binding and ribosomal proteins) and targets (cell polarity and adhesion proteins) of localized translation in the testis. Indeed, the protein of one such target, Claudin11, was detected in two distinct subcellular compartments. In stage VII-VIII tubules it can be found basally, at the blood-testis barrier, and adluminally, adjacent to elongated spermatids.

This novel approach will allow us to map the pattern of subcellular distribution of translation sites in the SCs of the testis and test hypotheses about how the regulation of localized translation is affected in different abnormal phenotypes.
**PC3** - **Intercellular Bridges Orchestrate Meiotic Initiation In Developing Mouse Ovaries.**
Bikem Soygur, Adam Fries, Rebecca Jaszczak, Necdet Demir, Ripla Arora, Diana Laird

Germ cell connectivity through stable intercellular bridges (ICBs) is highly conserved across many diverse organisms. ICBs interconnect developing spermatogonia and are essential for male fertility. The role of ICBs in female germ cells is well characterized in Drosophila, where a nursing mechanism supports oocyte development. Mammalian oocytes similarly develop in cyst structures and the movement of organelles through microtubule-mediated transport has been demonstrated, however the specific function of ICBs in fetal mouse oocytes remains unclear. To test the role of ICBs during the mitotic-meiotic transition of female germ cells, we used Tex14 mutant mice (Tex14-/-) which lack ICBs. Electron microscopy analysis confirmed the absence of ICBs in Tex14 mutants at E13.5. Using our newly developed methodology for 3D-imaging of the fetal mouse ovary, we quantified the spatial dynamics of meiotic initiation. We identified a novel radial wave of meiotic initiation as early as E13.0, in addition to the established anterior-posterior wave. Unexpected, the initiation of meiosis was premature in Tex14-/- mutant ovaries and the radial wave of meiotic initiation was absent while the anterior-posterior wave was intact, implicating ICBs in coordinating spatial meiotic initiation. We compared gene expression in Tex14+/- and Tex14-/- E13.5 ovaries by droplet-based, single-cell RNA sequencing to identify genes involved in the initiation and regulation of meiosis. Through the combined approaches of 3D image analysis and single cell transcriptomics, we aim to understand spatially-restricted developmental events and identify the regulatory factors shared through ICBs that prevent premature meiotic entry in the developing ovary.

**PC4** - **Epididymal Stem/Progenitor Basal Cells Express LGR5 and can Differentiate into Principal Cells.**
Laurie Pinel, Nick Barker, Daniel Cyr

The presence of epididymal stem cells has been controversial. Recent studies indicate that basal cells share common properties with adult stem cells. Stem/progenitor cells have the capacity to form organoids in culture and that these display similar characteristics to their tissue of origin. The objective of this study was to determine if epididymal cells can form organoids and if these display similar characteristics to the epididymis. 3D cultures of dispersed rat epididymal cells formed spheres consistent with organoids in other tissues. Purified basal cells from adult epididymis indicate that organoids could be derived from a single cell. Long-term culture of organoids indicates that these differentiate into cells that express AQP9, a principal cell marker. Short-term culture of organoids indicates that these express LGR5 whose expression levels decrease during differentiation. Co-localization of LGR5 with the basal cell marker TP63 in the adult epididymis indicates the existence of 3 basal cell types: LGR5+/TP63-, LGR5+/TP63+ and LGR5-/TP63+. The localization of LGR5 in basal cell was confirmed using a transgenic mouse model that expresses LGR5-LacZ. Lineage tracing studies indicate that LGR5+ cells can differentiate into principal cells in the short-term but that the staining is lost after 1 year, suggesting that these may represent a differentiating cell population in the adult. Together, the
data suggest that epididymal basal cells can form organoids and can differentiate into principal cells in vitro and in vivo.


Fertilization involves the fusion of the sperm membrane with that of the oocyte. To date, only one sperm protein (IZUMO) and two oocyte proteins (CD9 and JUNO) have been proved essential for this process. Herein, we report the identification of a second sperm protein required for gametes fusion: TMEM95. We have ablated this protein in mice by means of CRISPR and generated a colony of wild-type (WT), heterozygous (Hz) and knock-out (KO) individuals. Western blot confirmed the ablation of the protein in KO individuals, and immunohistochemistry localized TMEM95 protein in the acrosomal membrane of acrosome-intact WT sperm and on the sperm head following acrosome reaction. Fertility assessment by natural mating revealed that Hz individuals and KO females were fertile, but the eight KO males tested were unable to produce offspring after the observation of 24 copulatory plugs. Transmission electron microscopy did not detect obvious morphological differences between WT and KO sperm, and similar motility patterns were obtained following CASA analysis. However, sperm from Tmem95 KO mice were incapable of penetrating the oocyte following in vivo or in vitro fertilization, preventing zona hardening and causing an accumulation of TMEM95-deficient sperm in the perivitelline space. Artificial bypassing the membrane fusion step by intracytoplasmic sperm injection resulted in comparable developmental rates between WT or KO sperm (~40% blastocyst rate), isolating membrane fusion as the only process impaired by the ablation. Finally, a gamete fusion assay with Hoechst-loaded zona-denuded oocytes further confirmed that sperm-egg membrane fusion did not occur in the absence of TMEM95. These results highlight the indispensable role of this sperm protein during membrane fusion, a critical step during oocyte fertilization.

**PC6 - Chromatin remodeling during bovine preimplantation development indicates species-specific differences in regulators of genome activation in cattle, human, and mouse.** Michelle Halstead, Xin Ma, Richard Schultz, Pablo Ross

Extensive epigenetic remodeling is necessary during mammalian preimplantation development to erase oocyte-specific patterning and establish totipotency. However, mechanisms responsible for these changes remain unclear because the timing of embryonic genome activation (EGA) – initiation of embryonic transcription and degradation of maternal mRNAs – varies considerably between species. EGA in mice occurs during the 2-cell stage, whereas EGA in humans and cattle occurs during the 8-cell stage, suggesting that the regulatory network underlying EGA may not be conserved across mammals. ATAC-seq was used to profile the open chromatin landscape in bovine oocytes and in vitro-produced embryos at the 2-, 4-, 8-cell, and morula stages. Embryos were also cultured in the presence of alpha-amanitin to interrogate the relationship between transcription initiation and changes in chromatin accessibility. ATAC-seq data were then combined with gene expression data to infer putative regulators of bovine EGA. Accessible
regions in oocytes were largely closed in 2-cell embryos. Newly acquired open chromatin at the 8-cell and morula stages occurred primarily at intergenic regions, indicating that most remodeling happens at distal regulatory elements, e.g., enhancers. Over 90% of these newly accessible regions failed to open when transcription was inhibited, suggesting a causal relationship between transcription, or the products thereof, and chromatin remodeling at distal elements. Transcription factor binding motif analysis of newly accessible distal regions revealed enrichment for the binding motifs of Otx2, GSC, and KLF factors at the 8-cell stage, and enrichment for GATA-factor motifs in morulae. Potential key regulators of bovine EGA were identified based on expression of the corresponding transcription factors. KLF factors appear important to EGA in cattle, human, and mouse, indicating some regulatory mechanism conservation. Other potential regulators of EGA show higher concordance between cattle and human, suggesting that cattle constitute a more relevant model for human preimplantation development than mouse.

SSR 2019 Posters

**P1 - The Remain of the Male: Unexpected Contribution of the Male Tract Mesenchyme to the Female Reproductive Tract.** Humphrey Yao

Formation of the female reproductive tract is the first critical step in determining women’s reproductive health. At early fetal stage, female embryos contain both primitive male and female reproductive tracts, which are surrounded by their own different mesenchyme. During sexual differentiation, female embryos eliminate the primitive male tracts, and only maintain the primitive female tracts, which further differentiates into the oviduct, uterus, cervix and upper part of the vagina. Mesenchymal cells are critical for organ development and function. The degeneration of the primitive male tract in the female embryo leads to the logical assumption that the mesenchyme surrounding the male tract is eliminated as well, and does not contribute to female reproductive tract formation. To test this assumption, we developed a tamoxifen-inducible lineage tracing mouse model, where we labeled the male tract mesenchyme and traced the fate of labeled cells and their progeny during development. We found that, instead of being eliminated, the male tract mesenchyme remained and differentiated into smooth muscle and fibroblasts in adult female reproductive tract organs. Therefore, the mesenchyme surrounding primitive male tracts is another fetal origin of mesenchymal tissues in female reproductive tract organs. We then investigated whether the male tract mesenchyme was sufficient for maintaining the female tract formation in the absence of the female tract mesenchyme. We designed a Cre-mediated cell ablation model that removed cells from the female tract mesenchyme. When the female tract mesenchyme was ablated, the basic structure of the female reproductive tract organs was partially maintained, indicating that the male tract mesenchyme can compensate for the loss of the female tract mesenchyme. Taken together, our study unveils an unexpected contribution of mesenchymal progenitor cells surrounding primitive male tracts to the female reproductive tract formation. This discovery prompts a revision of our current model for female reproductive tract formation.
**P2 - Agarose-based-soft-culture-matrix (ABSCM) containing extracellular matrix proteins improves developmental competence of porcine oocytes.** Ji Eun Park, Minji Kim, Joohyeong Lee, Seung Tae Lee, Eunsong Lee

The three-dimensional organization of the ovary consists of a dynamic complex network comprising of a variety of extracellular matrix (ECM) components. Generally, mammalian ovarian follicles consist of ECM components including fibronectin, collagen, and laminin, and the quality of in-vivo-matured oocytes are found to be superior as compared to the in-vitro-matured oocytes. Accordingly, soft culture matrix containing ECM proteins constituting ovarian follicles are significantly required for artificially stimulating in-vivo-like ovarian follicles. To improve the developmental competence of pig oocytes, we first screened the efficacy of differing agarose stiffness in order to construct a soft culture matrix mimicking the stiffness of the ovarian follicle wall. Next, by mimicking the microenvironments of the ovarian follicle, we attempted to construct an ABSCM containing various concentrations of ECM proteins. The previously optimized 1% (w/v) ABSCM was reinforced by addition of each concentration of fibronectin (0, 5, 10, and 20 µg/ml), collagen (0, 5, 10, and 20 µg/ml), and laminin (0, 5, 10, 20, 40, and 60 µg/ml). Simultaneously, pig cumulus-oocyte-complexes (COCs) retrieved from medium antral follicles were matured in dishes coated with 1% (w/v) ABSCM, without or with each ECM protein. Cumulus expansion of the in-vitro-matured oocytes and their embryonic development after parthenogenetic activation were subsequently examined. Significant increases in blastocyst formation and cumulus expansion were detected in COCs matured on 1% (w/v) ABSCM containing 5 µg/ml fibronectin, 5 µg/ml collagen, and 20 µg/ml laminin, as compared with control. These results demonstrate that ABSCM containing optimized concentration of each ECM protein effectively improves the developmental competence of porcine oocytes.

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**P3 - Alternative Expression of PZP in Human and A2ML1 in Common Marmoset as a Pregnancy-Related Proteins.** Hirofumi Kashiwagi, Yoshie kametani, Sunichiro Izumi, Hitoshi Ishimoto

During pregnancy, pregnancy-related proteins protect fetus from the maternal immunity. Some of them are localized in the placenta, but others circulate in the whole body, although mothers’ immune system against systemic infection are not seriously dampened. Therefore, it is important to clarify the proteins contributing to the immune regulation of the whole-body during pregnancy. We examined the serum proteins and predicted their functions preserved evolutionarily through the comparison of kinetics between human and Common Marmoset (CM), a new world monkey species. The invasion of trophoblasts was confirmed by immunohistochemistry using GFP fetus and wild type mothers’ chimela placenta. GFP-positive
fetal cells infiltrated into GFP-negative decidua, suggesting that CM had invasive placenta similarly to human. Therefore, CM was used for further analyses as representative primate with invasive placenta. Human and CM plasma proteins were analysed by liquid chromatography-mass spectrometry. The most prominent proteins increased during pregnancy were Pregnancy zone proteins (PZP) in human and Alpha-2-Macroglobulin Like1 (A2ML1) in CM. These proteins belong to A2M family and were predicted to work as protease inhibitors capable of inhibiting multiple proteases. Sequence analysis of bait region which determines the accessibility of the proteases showed that bait region had very low homology compared to all amino acid sequences. Proteases which access to bait region and common to PZP in human and A2ML1 in CM were predicted to be Proline-endopeptidase (PE). Tertiary structure analysis showed that the sequence of PE specific region was exposed on the surface of those molecules. According to the computer analysis, both PZP and A2ML1 might inhibit PE activity in human, while CM PZP might not have such inhibitory function. These results suggest that PZP and A2ML1 were expressed alternatively and the protein function has been preserved evolutionarily between human and CM.

**P4 - Sex Determination in Xenopus.** Danielle Jordan, Benjamin Evans, Marko Horb

The mechanism of sex determination varies greatly among species. Amphibians use both ZZ, ZW and XX, XY systems. Amphibians belonging to the *Xenopus* genus do not have diverged sex chromosomes, but instead have sex determining loci on autosomes. *Xenopus* uses a ZZ, ZW system, where the female is the heterogametic sex. Both *X. laevis* and *X. borealis* are tetraploid and are closely related but have different sex chromosomes. The sex determining locus for *X. laevis* is on chromosome 8L, and on *X. borealis* it is on 2L. In contrast, another species – *X. tropicalis* is diploid, and its sex determining locus is on chromosome 7. Of the three species, only *X. laevis* contains the gene *DM-W*, which is located on the W chromosome; *DM-W* determines female sex by inhibiting *DMRT1*. Neither *X. borealis* nor *X. tropicalis* contain *DM-W*, and it is unknown if there is a single sex-determining gene responsible for female sex. In this poster I will present our efforts using gene-editing tools such as CRISPR-Cas9 and Meganuclease-mediated transgenesis to study the mechanistic aspects of how sex chromosomes and genetic sex determination evolve and work in *Xenopus* frogs. We aim to create knock-out and knock-in lines of *X. laevis*, *X. tropicalis*, and *X. borealis* to develop a comparative framework with which to better understand how the genetic basis of sex determination evolved, and how this system varies among closely related species.

**P5 - Identification and characterization of primordial germ cells in a vocal learning species, the zebra finch.** Young Min Kim, Anna Keyte, Matthew Biegler, Deivendran Rengaraj, Erich Jarvis, Jae Yong Han

The zebra finch (*Taeniopygia guttata*) has been used as a valuable vocal learning animal model for human spoken-language. It is a representative of vocal learning songbirds specifically which comprise half of all bird species, and of Neoaves broadly, which comprise 95% of all bird species. However, the low efficiency of germline transmission technologies makes them and other Neoaves species intractable. To improve germline transmission in the zebra finch, we identified and characterized its primordial germ cells (PGCs) that give rise to sperm and eggs,
and compared them to those of the more genetically tractable vocal non-learning, non-Neoaves chicken. We found striking differences between two species, including that zebra finch PGCs migrated slower, and were more widespread in early embryos before colonization into the gonads. We determined the optimal time and culture conditions for isolating zebra finch PGCs, were able to transfect them with gene vectors, and incorporate them into the gonads of host embryos. Our findings demonstrate important differences in the PGCs of a Neoaves species, and advance the first stage of creating PGCs-mediated germline transgenics of a vocal learning species.

**P6 - Lats1 and Lats2 are Required for the Maintenance of Pluripotency in the Mullerian Mesenchyme.** Guillaume St-Jean, Mayra Tsoy, Adrien Levasseur, Martin Morin, Charlène Rico, Marilène Paquet, Nicolas Gévry, Alexandre Boyer, Derek Boerboom

The development and function of the female reproductive tract requires the coordination of numerous signaling pathways, amongst which the Wnt and TGF-β pathways play essential roles. Although Hippo signaling has been known to interact with both aforementioned pathways, whether Hippo itself plays a role in female reproductive biology remains unknown. To test this hypothesis, we conditionally inactivated the key Hippo pathway kinases LATS1 and LATS2 in the müllerian mesenchyme by crossing mice bearing floxed alleles with the Amhr2(Cre) strain. The loss of Lats1 and Lats2 caused the müllerian mesenchymal cells to differentiate into a new cell type, resulting in profound reproductive tract development defects and sterility. Analysis of the urogenital tracts of e17.5 and newborn mutant mice showed that the targeted Lats1/2-null cells lose the expression of pluripotency markers such as Oct4 and Nanog. These cells expressed the markers α-SMA, vimentin and PTGS2, and this was accompanied by an increased deposition of collagen. Electron microscopy, RT-qPCR and microarray analyses were then used to confirm the Lats1/2-null cells to be myofibroblasts. Myofibroblast differentiation was attributed to increased YAP and TAZ expression, leading to direct transcriptional up-regulation of Ctgf and the activation of myofibroblast genetic program. The inactivation of Lats1/2 in fully differentiated uterine stromal cells in vitro did not compromise their ability to decidualize. In male mutant mice, the müllerian mesenchyme also underwent myofibroblast differentiation and failed to regress, which impeded the development of the male reproductive tract and testes. We conclude that Hippo is required to suppress the myofibroblast genetic program and maintain pluripotency in müllerian mesenchymal cells.

**P7 - LATS1 and LATS2 maintain the fate of somatic cells in the developing gonad.** Nour Abou Nader, Amélie Ménard, Guillaume St-Jean, Adrien Levasseur, Marie Le Gad-Le Roy, Derek Boerboom, Alexandre Boyer

In the Hippo signaling pathway, the Large tumor suppressor kinases 1 and 2 (LATS1/2) are functionally redundant serine/threonine-protein kinases that phosphorylate and inhibit YAP and TAZ, which are two transcriptional coactivators that play a major role in the regulation of cell proliferation and differentiation during embryonic development. In order to investigate the role of Hippo signaling in gonad development, we generated a mouse model
(Lats1flax/flax;Lats2flax/flax;Nr5a1cre/+), in which Lats1 and Lats2 were conditionally deleted in somatic cells of the developing gonad. Male and female gonads were characterized by a progressive accumulation of spindle-shaped cells. Immunohistochemistry and qPCR analyses demonstrated a progressive appearance of α-SMA+ as well as an increase in the expression levels of Acta2, Cald1, Cnn1, Cox2, and Spp1, suggesting that the spindle-shaped cells are myofibroblasts. An increase in the mRNA levels of Ctgf and Birc5, two downstream targets of YAP/TAZ, was also observed in the developing gonads of Lats1flax/flax;Lats2flax/flax;Nr5a1cre/+ mice suggesting that LATS1 and LATS2 normally act to restrict YAP/TAZ activity to maintain proper gonadal cell fate. These results define a crucial role for Hippo signaling in the development of the gonad.

P8 - Single-cell sequencing of neonatal uterus reveals an endometrial stromal progenitor indispensable for female fertility. H. Duygu Saatcioglu, Motohiro Kano, Heiko Horn, LiHua Zhang, Samore Wesley, Nicholas Nagykery, Minsuk Hyun, Rana Suliman, Joy Poulo, Jennifer Hsu, Caitlin Sacha, Dan Wang, Guangping Gao, Esther Oliva, Mary Sabatini, Patricia Donahoe, David Pepin

The embryonic Mullerian ducts give rise to the fallopian tube, uterus, cervix, and upper third of the vagina in the female. In the male, secretion of Mullerian inhibiting substance (MIS) from the fetal testes causes regression of the Mullerian ducts. This non cell-autonomous process is driven by mesenchymal cells acting on the adjacent ductal epithelium. The fate of this MIS type 2 receptor (Misr2) positive mesenchyme in the postnatal development of the uterus remains unknown.

Subluminal mesenchymal Misr2 expression persisted postnataley in the uterus in rodents, but receded by week 37 of gestation in humans as evidenced by RNAish. Using single-cell RNA sequencing, we described the cellular composition of the functional layers of the developing postnatal day 6 uterus, and validated novel endometrial stromal (Bmp7+, Wfikkn2+) and myometrial (Aldh1a2+, Mfap5+, Myh11+) cell subpopulations. By comparing gene expression in control and MIS-treated uteri, we characterized a persistent Misr2 subluminal cell type whose differentiation into endometrial stroma is blocked by treatment with MIS (inducing Smad6+, Msx2+, Bambi+). We confirmed that these subluminal mesenchymal progenitors (Misr2+), which normally form the endometrial stromal layers, undergo apoptosis when treated with MIS.

MIS treatment inhibited the Misr2+ mesenchymal progenitors only when administered before postnatal day 6 in rats, suggesting a developmental window for the specification of the endometrial stromal layers. Beyond postnatal day 6, Misr2 expression receded. The long-term consequences of exposure to MIS during this specification period were uterine hypoplasia, glandular defects, and complete infertility in the adult female rodents (n=3, p=0.0114). In conclusion, our findings suggest a mechanism of mesenchymal progenitor specification, with important implications on the etiologies of uterine hypoplasia, and uterine-factor infertility.

P9 - DNA Damage Responses in Mouse Primordial Germ Cells. Jordana Bloom, John Schimenti
The ability of organisms to pass their genetic information to subsequent generations is crucial for survival and propagation of a species. In mice, primordial germ cells (PGCs) rapidly migrate and proliferate to the location of the future gonads during embryonic development over the course of only a few days. Importantly, while DNA replication associated with rapid cell proliferation is often subject to spontaneous errors, the germline is highly refractory to mutation accumulation. To characterize how PGCs respond to altered genomic integrity, we examined PGC cell cycle checkpoint activation, generated a PGC-specific DNA double strand break-sensing transgenic mouse line and have assessed DNA damage responses in PGCs on a transcriptional level using RNA-seq.

At E11.5, prior to sexual determination, PGCs show no evidence of a G1 cell cycle checkpoint; rather the PGC population becomes dramatically enriched in G2/M phase cells. While most proliferating cells activate a G1 cell cycle checkpoint in response to DNA damage, mouse embryonic stem cells (mESCs) have also been shown to lack a G1 cell cycle checkpoint in response to DNA damage. Therefore, we hypothesize that PGCs at this stage of development employ similar cell cycle control and DNA damage response mechanisms to mESCs. Additionally, we examined the effect of irradiation-induced DNA damage in male and female PGCs post-sex determination at E13.5. At this developmental time point, female PGCs lack G1 cell cycle checkpoint activation in response to DNA damage. Transcriptionally, at E13.5 both male and female irradiated PGCs show a negative enrichment of pluripotency-related gene expression and a positive enrichment of gene expression related to retinoic acid signaling. Additional experiments underway aim to identify factors responsible for the distinct DNA damage responses in PGCs versus somatic cells, and if these differences persist throughout subsequent stages of germ cell development in both sexes.

**P10 - Multilayer programming via RB1 guides male germline stem cell establishment.**
Guihua du, Melissa Oatley, Yang Qi-En, Xin Wu, Jon Oatley

Retinoblastoma protein (RB1) is required for continuity of the spermatogenic lineage, yet the mechanisms underpinning its influence are undefined. Studies with mice demonstrated that expression in germ cells around the time of sex determination at embryonic day (E) 12.5 and an Rb1 null state leads to embryonic lethality at E14.5, but formation of the primordial germ cell (PGC) and prospermatogonial precursor populations are seemingly normal. Here, we further explored the role of RB1 using mouse models of conditional inactivation at either the PGC, prospermatogonial, or postnatal spermatogonial stages of development. Interestingly, knockout of Rb1 in the progenitor or differentiating spermatogonial stages did not alter spermatogenesis and males were of normal fertility. In contrast, as we reported previously, inactivation of Rb1 at the prospermatogonial stage leads to a normal first round of spermatogenesis but the germline is summarily lost upon aging. Examining this model further revealed that germline disruption begins at postnatal day (P) 2.5 with loss of spermatogonial stem cell (SSCs). Similarly, inactivation of Rb1 at the PGC stage leads to male sterility; but in contrast to inactivation at the prospermatogonial stage, a first round of spermatogenesis does not occur. In addition, we found that entry into quiescence that occurs normally around E16.5 is delayed to E18.5 in prospermatogonia derived from PGCs lacking RB1 and the population subsequently undergoes a massive wave of apoptosis leading to severe depletion by P3.5. Furthermore, lack of RB1 at
different stages of germline formation seems to disrupt the normal molecular program; as evidenced by impaired expression of NANOG in prospermatogonia derived from \textit{Rb1} knockout PGCs and altered cytoplasmic-to-nuclear translocation of FOXO1 in postnatal spermatogonia derived from \textit{Rb1} knockout prospermatogonia. Collectively, these finding suggest that RB1 functions to establish unique layers of programming in germ cells that are necessary for the normal PGC-Prospermatogonia-SSC developmental trajectory.

\textit{P11} - Testicular Endothelial Cells Promote Self-renewal of Spermatogonial Stem Cells in Rats. Yong-Hee Kim, Seok-Man Kim, Myeong-Geun Oh, Dong Ha Bhang, Bang-Jin Kim, Sang-Eun Jung, Gottfried Dohr, Sun-Uk Kim, Sandra Ryeom, Buom-Yong Ryu

Spermatogonial stem cells (SSCs) are the foundation of spermatogenesis in male because of their ability to reproduce in numbers by self-renewal and subsequent meiotic processes. However, since SSCs are present in a very small proportion in the testis, in vitro proliferation of SSCs in undifferentiated state is required for the research of germ cell biology.

In this study, we investigated minutely the effectiveness of various cell lines as a feeder layer for rat SSCs. Germ cells enriched for SSCs were cultured on feeder layers including SIM mouse embryo-derived thioguanine and ouabain-resistant cells (STO), C166 cells, and mouse and rat testicular endothelial cells (TeECs) and their stem cell potential for generating donor-derived colonies and offspring was assessed by transplantation into recipient testes. Additionally, Results showed that the rat germ cells cultured on TeECs increased mRNA and protein levels of undifferentiated germ cell markers. Rat SSCs derived from these germ cells underwent spermatogenesis and produced offspring when transplanted into recipients. Collectively, TeECs can play a role in an effective feeder layer that improves the proliferative and self-renewal capacity of cultured rat SSCs while preserving their stemness characteristics. This system may have broad applicability to human reproductive biology, for instance, in restoring male fertility.

\textit{P12} - Biochemical Characterization of Follicular Fluid in Buffalo (Bubalus bubalis). Ram Singh, Sukanta Mondal, V Varshney

The activity of the ovary is characterized by alternate phases of growth and regression between follicular and luteal structures. The follicular fluid, an exudates of serum, provides suitable micro-environment for the growth, development and maturation of oocytes. It contains specific constituents such as proteins, amino acids, enzymes, hormones, electrolytes and salts which play an important role in the physiological, biochemical and metabolic aspects of the nuclear and cytoplasmic maturation of the oocyte. The present study was designed to characterize buffalo follicular fluid and to correlate the changes in composition of follicular size in buffalo. Three hundred sixty two ovaries of non-descriptive and non-pregnancy buffaloes were randomly collected immediately after slaughter and stored in cold normal saline. Follicular fluid was aspirated from small (3-5 mm), medium (6-9) and large (10-12 mm) ovarian follicles. The supernatant fluids, devoid of cellular debries were collected by centrifuging the sample at 10000 rpm (4\textdegree C) for 15 minutes and stored at -80\textdegree C prior to assay. Follicular fluid samples were analysed for glucose, cholesterol, creatinine, proteins, triglycerides, bilirubin, BUN, acid phosphatase,
alkaline phosphatase and GGT. Results showed that follicular fluid concentrations of glucose, cholesterol and creatinine increased ($P<0.01$) from small to large follicles. The concentrations of proteins, triglycerides, bilirubin, BUN, acid phosphatase, alkaline phosphatase and GGT decreased as the follicle became larger. It can be concluded that the oocyte and granulosa cells of buffalo grow and mature in a biochemical environment that change from small to large follicles.

**P13 - Single-Cell RNA Sequencing Reveals Similarities Between Bovine and Human Primordial Germ Cell Development.** Delia Soto, Pablo Ross

Primordial germ cells (PGCs) are the progenitors of gametes and have a vital role in the reproduction of animals. To better understand the development of bovine PGCs we conducted single-cell RNA sequencing analysis (SC-RNAseq) of fetal gonads. Gonadal tissue from a 50 day old fetus was dissociated into single cells and processed for SC-RNAseq using the 10X Genomics platform. A total of 1,821 cells were sequenced at a depth of 112,728 reads per cell. Using dimensionality reduction by t-distributed stochastic neighbor embedding, we identified seven cell clusters that were classified based on cell type specific markers. A cluster representing 13% of the total cells corresponded to PGCs. Other clusters were identified as somatic gonadal cell precursors, erythrocytes, white blood cells, endothelial cells and smooth muscle cells. Gene expression analysis within the PGC cluster revealed that, at this time of development, bovine PGCs expressed pluripotency master genes (POUF51, NANOG), early germ line genes (BLIMP1, TFAP2C, NANOS3), and that a lower percentage of cells expressed late germ line markers (DDX4, DAZL). These results were confirmed by immunofluorescence staining of OCT4, BLIMP1, DAZL and DDX4. We also identified low expression of genes involved in mouse PGC development (SOX2, PRDM14) and higher expression of genes associated with human PGCs (SOX15, SOX17, EPCAM). Interestingly, low expression of meiosis (SYCP3) and oocyte markers (ZP3) was detected, indicating that bovine PGCs enter meiosis asynchronously, similar to what has been described in humans. Results of this study offer new insights into the mechanisms of PGC development in cattle, suggesting a higher similarity to human PGC development compared to the mouse system, and represent a useful resource for the in-vitro differentiation of bovine pluripotent stem cells into PGCs.

**P14 - The new biocompatible material for mouse ovarian follicle development in three-dimensional in vitro culture systems.** Hye Won Youm, Eun Jung Kim, Jung Ryeol Lee, Chang Suk Suh

The *in vitro* 3D culture system of ovarian follicle is an advanced method to maintain follicular morphology and physiology. Alginate is most widely-used biomaterial in 3D culture, however requires temperature control and lyase treatment for stable gelation due to its stiff property. Whereas the extracellular matrix-derived soft hydrogel (ES-hydrogel) is less stiff and degraded in room temperature with DW without toxic treatment. Therefore, this study aims to compare effects of new biomaterial, ES-hydrogel, and alginate on follicle growth and oocyte maturation. Mouse ovarian follicles were mechanically isolated and seeded on culture wells (2D) or encapsulated with alginate or ES-hydrogel (3D). Culture media was refreshed and collected every 4 days for E2/P4 evaluation. On the 10th day of culture, follicular survival and
pseudoantrum formation were examined followed by ovulation induction (hCG/EGF). After 17 hours, mature oocytes were collected and analyzed for diameter, normal spindle, reactive oxygen species (ROS) and mitochondrial membrane potential (MMP). To compare mechanical properties of two biomaterials, storage modulus was measured with the advanced rheometric expansion system. Our result showed that ES-hydrogel group was significantly superior to 2D and alginate groups in formation of pseudoantrum, COC, MII oocyte and normal spindle, and increase in E2 level. ES-hydrogel and alginate groups were not significantly differ in follicle survival rate, oocyte diameter, and P4, ROS and MMP levels. The storage modulus of ES-hydrogel was higher than that of alginate, suggesting that the better follicular function and oocyte maturation in ES-hydrogel group was based on the better hormone exchange through less stiff encapsulating material (ES-hydrogel). In this study, we verified that ES-hydrogel was efficient material comparable or superior to alginate for 3D follicle culture. Further studies for fertilization potential of 3D-cultured oocyte and biomaterial application with different property are required.

**P15 - Development of in vitro maturation rate in Korean crossbreed goat**

Dayeon Jeon, Sang Hun Lee, Jinwook Lee, Kwan-woo Kim, Yeoung-Gyu Ko, Dong-Kyo Kim, Sung-Woo Kim, Sung-Soo Lee

**Abstract**

In vitro maturation efficiency is one of the essential parts for successful in vitro embryo production. The aim of the present study was to improve the in vitro embryo productivity in the in vitro maturation step in Korean crossbreed goat. The total number of ovaries of pubertal and prepubertal goat used in this study were 93 and all are obtained from a slaughterhouse. For recovering the oocytes from ovary, syringe with 18G needle is used. Once recovered, oocytes were classified with grade A, B, C and D, and except grade D, all oocytes are mixed and incubated with IVM media. The percentage of mature oocytes was evaluated after 24h of maturation. The number of oocytes retrieved from each ovary of pubertal and prepubertal goat was 4.1±1.01 and 3.8±0.74, respectively. The results of oocyte retrieval efficiency for pubertal and prepubertal goat were 40.2% and 37.9%, respectively. And the proportions of oocyte by grade from pubertal ovaries were 24.0±0.03 in grade A, 26.7±0.04 in B, 35.9±0.06 in C and 13.3±0.03 in D. The proportions of oocyte by grade from prepubertal ovaries were 12.5±0.01, 27.9±0.03, 35.0±0.02, 24.6±0.01, respectively. The maturation rate according to concentration of goat serum(0%, 5%, 10%) and goat follicular fluid(0%, 5%, 10%) were investigated, also. In conclusion, oocytes from pubertal goat is more suitable for in vitro embryo production based on the ratio of oocytes in grade A and D. Also, addition of goat serum and goat follicular fluid to maturation media is helpful for the successful in vitro embryo production.

**P16 - Studies on the endocrinology of testicular development in the mule.** Alan Conley, Claudia Fernandes, Maria Alonso, Erin Legacki, Sarah Fingerhood, Amy McLean, Trish Berger, Kevin Keel
Few mammalian hybrids have been as successful as mules, supporting both agriculture worldwide and basic genetic studies into the limits of inter-species breeding and gametogenesis, focused primarily on meiotic failure. Surprisingly little is known about their reproductive endocrinology, especially the males, which are routinely castrated at a young age to control perceived masculine behaviors. We studied steroids in systemic blood of male mules using liquid chromatography-tandem mass spectrometry as reported (Legacki et al., 2016) with modifications to measure multiple pregnanes, androgens, estrogens and corticoids. Anti-Müllerian hormone (AMH) concentrations were also determined by validated immuno-assay. Blood samples included intact [(n=32;3-36 months old (mo)] and castrated (n=18;6-66 mo) males. Blood and testicular tissue samples (fixed and frozen) obtained from two mules castrated at ≈8 mo were used for tissue steroid determination and immuno-histochemistry (IHC) for 17α-hydroxylase/17,20-lyase cytochrome P450 (P450c17), a key enzyme necessary for androgen synthesis. Data were analyzed by ANOVA and linear regression. Concentrations (ng/ml) of pregnenolone (6.6±0.5 and 5.5±0.9), progesterone (0.4±0.1 and 0.1±0.1), cortisol (16.6±1.0 and 15.0±1.9) and corticosterone (2.6±0.3 and 2.4±0.3) were not different in serum from intact and castrate males, respectively. No androgens (testosterone, androstenedione, dehydroepiandrosterone), estrogens (estrone, 19nor-androstenedione) or other pregnanes were detected in serum. However, testicular tissue contained testosterone (109 and 106ng/g), androstenedione (8 and 7ng/g), dehydroepiandrosterone (10 and 7ng/g) and pregnenolone (37 and 49ng/g) among other trace pregnanes. Serum pregnenolone was not correlated with either cortisol or corticosterone. Prominent IHC staining for P450c17 was detected in Leydig cells sparsely scattered throughout the testes. AMH was higher in intact males than castrates (56.1±2.5 and 0.4±0.1ng/ml, respectively; P<0.01) but did not decrease with age in intact mules. These data suggest that mules do not initiate endocrine puberty by 3 years of age. Thus, puberty appears delayed in mules and the rationale for early castration remains unclear.

**P17 - Transcriptional Networks of Mammalian Female Reproductive Tract Development.**
Alejandra Elder Ontiveros, Rachel Mullen, Richard Behringer

The mammalian female reproductive tract includes the oviducts, uterus, and vagina. These organs are essential for embryo implantation and development of the fetus. Two pairs of genital ducts form within the embryonic kidney associated with the fetal gonads in male and female embryos: the Wolffian and Müllerian ducts. The female reproductive tract is derived from the Müllerian ducts (MD), a pair of epithelial tubes surrounded by mesenchyme. The MDs develop dependent on the Wolffian ducts. Little is known about the genetic and cellular processes that regulate MD formation.

*Emx2, Pax2* and *Pax8* are transcription factor genes expressed in MD and WD epithelium. *Emx2-*, and *Pax2*-null female mice lack reproductive tracts, including absent WDs and kidneys; *Pax8*-null mice have hypoplastic uteri. To determine the role of these genes specifically in the MD, we have used *Wnt7a-Cre* mice to knock them out. Using this approach, we showed that *Lhx1* acts cell autonomously in MD epithelium and is required for its formation. We are using this approach to determine the requirements of *Emx2, Pax2* and *Pax8* in the MD. Surprisingly, we find that MD epithelium-specific knockout of *Pax2* does not affect MD formation.
To determine the transcriptional networks that regulate MD formation we are performing RNA- and ATAC-seq on FACS-purified E14.5 male and female MD epithelial cells. Wnt7a-cre;Rosa26R-YFP mesonephroi were isolated, dissociated into single cells, and YFP-positive cells were purified. These studies will produce a list of genes expressed in the developing MD. They should also define the chromatin landscape to facilitate the identification transcriptional enhancers.

Our proposed studies should lead to an understanding of the transcriptional networks involved in the formation of the female reproductive organs. This may lead to new knowledge about the underlying pathophysiology of diseases such as infertility, recurrent miscarriage, and congenital uterine defects.

**P18 - Associations between plasma estradiol concentration, ovarian expression of KI67, P53 and PTGFR mRNAs, and fetal size in the pig.** Claire Stenhouse, Yennifer Cortes, Charis Hogg, Cheryl Ashworth

It is estimated that intra-uterine growth restricted (IUGR) piglets, with a weight of less than 1.1kg at birth, represent 25% of the total number of piglets born. Importantly, growth restricted female piglets have impaired reproductive performance as adults. It was hypothesized that alterations would be observed in plasma estradiol and expression of selected genes associated with apoptosis, proliferation and angiogenesis in the ovaries are associated with IUGR fetuses throughout gestation. Estradiol concentrations were quantified in plasma samples from the lightest, closest to mean litter weight (CTMLW) and heaviest female Large White X Landrace fetuses at gestational day (GD) 90 (n=5 litters). RNA was extracted from ovaries from the lightest and CTMLW female fetuses at GD45, 60 and 90 (n=5-6 litters/GD) and qPCR was performed. Gestational changes in gene expression were detected. Decreased ovarian CD31 mRNA expression was observed at GD90 compared to GD60 (P≤0.05). Inversely, expression of PTGFR (prostaglandin F2 alpha receptor) mRNA was greater at GD90 compared to GD45 and 60 (P≤0.001). Ovarian SPP1 (osteopontin) mRNA expression was greater at GD45 compared to GD60 and 90 (P≤0.01). Relative to fetal size, expression of KI67 (P=0.066) and P53 (P≤0.05) was less in ovaries of the lightest fetuses compared to CTMLW fetuses at GD60. Similarly, there was a decrease in expression of PTGFR in ovaries from the lightest compared to the CTMLW fetuses at GD45 and 60 (P≤0.05). Concentrations of estradiol were less in the lightest fetuses compared to the CTMLW fetuses at GD90. Overall, these findings suggest that differences in reproductive potential of IUGR females postnatally are programmed early in gestation. Further analyses of ovaries associated with fetuses of different size throughout gestation are expected to improve understanding of the relationship between prenatal reproductive development and postnatal reproductive performance. Funding: BBSRC and the University of Edinburgh.

**P19 - Loss of transzonal projections mediating germline-soma communication in the ovary is triggered by LHCGR-initiated signaling independently of oocyte meiotic maturation.** Karen Carvalho, Laleh Abbassi, Stephany El-Hayek, Qin Yang, Rafael Mondadori, Vileceu Bordignon, Hugh Clarke
During oocyte growth in mammals, the surrounding somatic follicular granulosa cells elaborate actin-rich filopodia, termed transzonal projections (TZPs), that traverse the zona pellucida separating the two cell types and contact the oocyte plasma membrane, enabling transmission of signals from the granulosa cells to the oocyte. During meiotic maturation, the TZPs are lost, freeing the oocyte from direct maternal control in preparation for fertilization. The molecular mechanisms regulating TZP loss, however, are unknown. We show here that, when cumulus-oocyte complexes are removed from antral follicles, the TZPs slowly retract over a period of 12 to 16 hours. This retraction can be completely prevented by conditions that maintain high levels of intracellular cyclic GMP (cGMP), mimicking the condition within intact antral follicles. Following injection of human chorionic gonadotropin into ‘primed’ mice or activation of epidermal growth factor receptor (EGFR) signaling in cumulus-oocyte complexes in vitro, TZPs retract between 4 and 8 hours later – much more rapidly than under unstimulated conditions. Unexpectedly, TZP retraction can be experimentally dissociated from oocyte maturation, indicating that it is controlled exclusively by signaling within the cumulus granulosa cells. EGFR-triggered TZP retraction requires activity of extracellular signal regulated kinase and of cGMP-specific phosphodiesterase. cGMP is known to inhibit Rho-associated kinase (ROCK), which regulates actomyosin contractility. Strikingly, pharmacological inhibition of ROCK fully prevents TZP retraction. We propose that high cGMP within granulosa cells inhibits actomyosin contraction, thereby maintaining TZPs; conversely, when the hormonal signals that initiate maturation cause cGMP to decrease, activation of actomyosin contraction triggers TZP retraction. Our results provide a molecular mechanism underpinning the developmentally programmed loss of contact and communication between the maturing oocyte and its follicular microenvironment.

**P20 - Maternal influenza A virus infection decreases the expression of T cell differentiation genes in the murine fetal thymus**

Thomas Hansen, Hana Van Campen, Jeanette Bishop, Gerrit Bouma, Quinton Winger, Leticia Sinedino, Christie Mayo, Richard Bowen

Maternal influenza infection may impair fetal immune competency and postnatal health as suggested by 1918 influenza pandemic studies. To test the hypothesis that maternal infection has detrimental effects on development of the fetal immune system, pregnant C57BL6 mice were inoculated intranasally with influenza A virus A/CA/07/2009 pandemic H1N1 (influenza) or PBS (control) at E3.5 (pre-implantation), E7.5 (peri-implantation) or E12.5 (complete placentation). Fetal thymuses were collected at E18.5 and pooled within each litter. Total RNA was submitted for RNA-Seq. Differentially expressed genes (Bioconductor software in R) were detected using the DESeq2 package and the Benjamini-Hochberg procedure to control the false discovery rate. IPA (Ingenuity) software package was used for pathway analysis. Out of 985 genes differentially expressed 2-fold (p<0.05), 957 transcripts were decreased and 28 genes were increased in fetal thymus RNA samples from E7.5 influenza-inoculated litters compared to controls. Top canonical pathways identified calcium signaling, tight junction, vasodilation and hepatic fibrosis. The immune-related canonical pathways were: B cell receptor signaling, Dendritic cell maturation, PI3K signaling in B lymphocytes, and PKC-theta signaling in T lymphocytes. The IPA disease and function analysis predicted: 1) decreased body size of
fetuses, 2) increased neonatal death and 3) increased risk of respiratory failure with influenza infection. The mRNA concentrations (RT-qPCR) of *mal*, *mal2* and *anxa1* were decreased ($p<0.03$) in fetal thymus from E3.5 influenza-inoculated litters compared to controls. *Mal* transcripts were also significantly decreased in fetal thymus RNA from E7.5 ($p<0.0001$). These differences were not found in E12.5 pregnancies. Maternal influenza A virus infection had a massive impact on the developing fetal thymus transcriptome and inhibition of selected T cell gene transcripts was greatest in RNA from litters inoculated at pre- and peri-implantation time points. NIH/NCATS Colorado CCTSI Grant UL1 TR001082 and CVMBS CRC Interdisciplinary Award.

**P21 - Computerized evaluation of jaguar (Panthera onca) frozen-thawed semen using Tris and ACP-117C extenders.** Herlon Silva, Thalles Nunes, Lívia Campos, Andréia Silva, Alexandre Silva, Lúcia Silva

Due to the risk of extinction, it is urgently necessary to develop reproductive strategies for the conservation of jaguars ( *_Panthera onca_*), the largest felid of Americas. Therefore, the objective of present study was to verify the effect of different extenders on the kinematic parameters of jaguar frozen-thawed sperm. Semen from five adult males was obtained through electroejaculation, totaling 8 semen samples. After collection in the Zoo, semen was only evaluated for subjective total motility and vigor and immediately processed for cryopreservation using extenders based on Tris (plus 20% egg yolk) or coconut water (ACP-117C; plus 10% egg yolk), both supplemented of 6% glycerol and further stored in liquid nitrogen. After two weeks, samples were thawed at 37 ºC and evaluated for kinetic motility patterns using an IVOS 7.4G (Hamilton-Thorne, EUA) according to the settings previously established for the domestic cat. Fresh samples presented $94.4 \pm 0.6$ total motile sperm with vigor $4.9 \pm 0.1$. After thawing, samples frozen in Tris presented higher values for total ($46 \pm 7.7\%$) and progressive ($4 \pm 1\%$) motility than those cryopreserved in ACP-117C ($20.9 \pm 5.4\%$ and $1.6 \pm 0.3\%$ respectively). Regarding subpopulations, Tris provided higher values for medium ($3.1 \pm 0.9\%$) and slow ($42 \pm 7\%$) sperm than the ACP-117C ($1.1 \pm 0.2\%$ and $16.5 \pm 5.2\%$, respectively). For other kinetic parameters, there were no significant differences between extenders, except for straightness that was better preserved in the use of ACP-117C ($84.8 \pm 1.3\%$) than Tris ($72.1 \pm 5.9\%$). In conclusion, we recommend the use of Tris extender for the jaguar semen cryopreservation since it provide a most adequate preservation of sperm kinetic motility patterns than ACP-117C. This research was supported by CAPES (Financial Code 01), Brazil.

**P22 - Potential roles of COUP-TFII positive cells in ovarian morphogenesis.** Ciro Amato, Kathryn McClelland, Humphrey Yao

Ovary morphogenesis is a highly coordinated process between oogonia, granulosa cells, and stromal progenitor cells. After onset of ovary differentiation in the embryo, these three cells types interact to form oogonial cords, which break down into individual follicles after birth. The stroma cells extend from the surface epithelium of the ovary to the mesonephros, a structure adjacent to the ovary, to help form the cords. Stroma cells eventually differentiate into theca
cells, which are required for steroidogenesis and ovulation. The main source of stromal cells is somatic cell progenitors present in both ovarian primordium and mesonephros at the onset of ovary differentiation. Concurrently, a population of stroma progenitors, which are positive for COUP-TFII (chicken ovalbumin upstream promoter transcription factor II or Nr2f2), cluster at the ovary-mesonephric boundary and intrude the ovary to form the stroma. We investigated sources of COUP-TFII+ cells within the fetal ovary and their involvement in ovarian morphogenesis. Using tissue-specific genetic mouse models that inactivate Coup-tfII in either the ovary or both the ovary and mesonephros, we determined which COUP-TFII+ population (mesonephros, ovary, or both) contributes to ovarian development. Mice with Coup-tfII inactivated only in the ovary still had few COUP-TFII+ cells. We did not observe COUP-TFII+ cells in the ovarian-mesonephric model. This suggests the mesonephros is a significant contributor of COUP-TFII+ cells in the ovary. Additionally, embryos lacking Coup-tfII in both ovary and mesonephros were stunted in growth, while the ovary only Coup-tfII knockout displayed normal morphology. Transcriptomic analyses of the ovary-mesonephros model revealed disrupted ovary differentiation. Stroma and granulosa cell genes were downregulated. Our results suggest the mesonephros as a potential source of COUP-TFII+ stroma cells in the fetal ovary, and these mesonephric-derived cells plays a critical role in establishment of ovarian morphology, differentiation, and ovigerous cord formation. Supported by NIEHS Intramural Research Funds.

P23 - The ancient NEMP protein family supports metazoan fertility, viability, and mechanical resistance of the nuclear envelope via interactions with LEM domain proteins. Helen McNeill, Andrea Jurisicova, Yonit Tsatskis, Robyn Rosenfeld, Julie Brill, Xu Sun, Xian Wang, Curtis Boswell, Rod Bremner

The double layered nuclear envelope (NE) encloses and protects the eukaryotic nucleus, and disruption of the NE or underlying lamina can lead to devastating diseases. However, the function of most NE proteins is unknown. Here, we use fly, fish, worm and mouse models to show that the poorly understood NE protein, NEMP is required for fertility across the animal kingdom. Loss of mammalian NEMP also causes anemia, and defects during erythrocyte enucleation. Super-resolution microscopy reveals NEMP proteins are localized at inner nuclear membranes and Atomic Force Microscopy (AFM) demonstrates NEMP proteins support mechanical stiffness of the nuclear envelope. Mass-spectrometry screens revealed interactive partnerships of human NEMP proteins with multiple LEM domain proteins, while genetic and AFM analyses confirmed these interactions support fertility and NE stiffness. Thus, NEMP plays conserved and essential roles in NE mechanical stability and organism fertility via interactions with LEM domain proteins.

P24 - Differential expression of LH receptor, LHR mRNA binding protein, bta-miR-222 and steroidogenic enzymes in the developing bovine ovary. Leonardo Mendes, Marina Chaves, Alan Giroto, Priscila Santos, Patricia Fontes, Anthony César Castilho

Steroids and gonadotrophins are essential for the regulation of antral follicular development and the late stages of preantral development. Although the luteinizing hormone receptor (LHR) has
been detected in the preantral follicles of rats, rabbits and pigs, the expression pattern of this receptor in bovine fetal ovary has not been demonstrated. The present study aimed to quantify the mRNA abundance of the genes LHR, LHR binding protein (LRBP), STAR, HSD3B1, CYP17A1, and CYP19A1 during the development of bovine fetal ovary, besides evaluated the immunolocalization of LHR in the fetal ovarian tissues. In addition, we aimed to identify and quantify the expression of bta-miR-222, a regulatory microRNA of the LHCGR gene. In summary, LHR was found in the preantral follicle in bovine fetal ovary, from oogonias to primordial, primary and secondary stages, and the mRNA abundance was lower on day 150 than day 60. Regarding LHR protein, the immunostaining was consistent throughout the ovarian development with strong staining in oocytes cells in all periods. On the other hand, the mRNA abundance of LRBP and bta-miR-222 followed the opposite pattern, with higher expression on day 150 than day 60 or 90 of gestation. With regard to the gene expression of steroidogenic enzymes, only the mRNA abundance of STAR was higher on day 150 than on day 60. In conclusion, these results suggested the involvement of LHCGR/LRBP regulation with mechanisms related to the development of preantral follicles, especially during the establishment of secondary follicles. Furthermore, the present data reinforced that the reduced expression of LHR mRNA in bovine fetal ovaries on day 150 was related to the higher expression of LRBP and bta-miR-222.


Bovine oogenesis and follicular maturation are initiated during in utero development. Maternal undernutrition and protein restriction during gestation reduce offspring ovarian follicle number and potentially ovarian function and reproductive longevity. Dietary supplementation of protein during gestation is common in agriculture to circumvent the seasonal reduction in forage protein content. However, the influence of maternal protein supplementation during gestation on the bovine ovarian follicular pool of offspring has not been investigated. We hypothesized that supplementation of protein to the dam during gestation would not alter the follicular pool of offspring. To characterize the effects of maternal protein supplementation, multiparous cows (Angus × Hereford; n = 8) were individually-fed isocaloric dietary treatments consisting of chopped forage top-dressed with a control or protein (80 g urea/animal/d) pelleted supplement. Diets met or exceed dietary requirements throughout gestation. Ovaries of offspring were collected at birth, paraformaldehyde-fixed, and frozen. Frozen tissue was sectioned, mounted on slides, and stained with hematoxylin and eosin. Follicles were classified and quantified under bright-field microscopy (n = 30 sections/animal). The effect of maternal dietary treatment on maternal morphometries and quantification of offspring follicles by classification were evaluated by ANOVA. Maternal dietary treatment did not influence (P ≥ 0.74) maternal body weight or ribfat thickness. Despite no differences in maternal morphometries, offspring from protein-supplemented dams had fewer (P < 0.0001) primordial and primary follicles than offspring from control dams. The number of secondary and tertiary follicles did not differ (P ≥ 0.07) between treatments. However, the number of atretic tertiary follicles was greater (P < 0.0001) in offspring from protein-supplemented dams. From these results we infer that maternal protein
supplementation induces precocious follicular activation and/or increases the number of follicles recruited during maturation. These findings highlight the influence of maternal diet during gestation on the follicular population of offspring.

P26 - Equine Preantral Follicle Population and Density. Benner Alves, Kele Alves, Gustavo Gastal, Melba Gastal, Jose Figueiredo, Eduardo Gastal

Recent studies in mares have reported preantral follicle features (population, density, and distribution) in ovarian fragments or the whole ovary (spatial distribution). However, the preantral follicle population and density considering the whole ovary still remains unclear. The only report available in the literature regarding follicle population in mares exclusively considered preantral follicles with large (>50 µm) diameters. Thus, the current study evaluated the preantral follicle population and density in the whole ovary according to: (i) age, (ii) follicular class, (iii) and ovarian portion (lateral and intermediary). Ovaries from young (4-9 years, n = 4) and old mares (>20 years, n = 4) were processed for histology. Preantral follicle population and density were determined for each histological section. The estimated overall mean of preantral follicle population per ovary was 82,206 ± 50,022 (range, 1,477 to 773,091), and differed (P<0.05) between young (152,663 ± 96,345) and old (11,749 ± 4,802) mares. In addition, the population of primordial, primary, and secondary follicles per ovary were 60,942, 21,323, and 165, respectively. Overall, the mean follicular density per cm<sup>2</sup> was 34-fold higher (P<0.05) in young (18.9 ± 2.8) than in old (0.5 ± 0.05) mares (overall mean, 9.5 ± 1.3 follicles per cm<sup>2</sup>; range, 0 to 525). In both young and old mares, the follicular density was greater (P<0.05) in the intermediary ovarian portion, with old mares having 2.7-fold higher (P<0.05) density in the intermediary than in the lateral portion. Collectively, these results suggest that preantral follicles located in the lateral ovarian portion can be more susceptible to follicular activation, supporting the less homogeneous follicular distribution previously reported in the ovarian parenchyma of old mares. Support: CNPq, CAPES, and SIU.

P27 - UNC5CL as a Possible Mediator of Sertoli Cell Proliferation. Lien Tu, Barbara Nitta-Oda, Trish Berger

Reduced estrogen signaling in the early juvenile interval increased porcine Sertoli cells approximately 25% at 6.5 weeks of age. The increase occurs between weeks 5 and 6.5 of age and appears due to a prolonged interval of proliferation; the miniscule rate of apoptosis present cannot explain altered population size. Preliminary RNAseq data on 2, 3, and 5-week-old pigs suggested a possible role for UNC5CL in the letrozole-induced proliferation of Sertoli cells. Immunohistochemical staining using an anti-UNC5CL primary antibody was performed on tissue samples of testes from boars at 5, 6, and 6.5 weeks of age. UNC5CL was localized within the seminiferous tubules and interstitial space. Labelling intensity scores were analyzed by ANOVA. An age effect on labeling intensity was observed (P < 0.01) with tissue from 6 week old boars having the most labelling; no difference in labelling of testes from letrozole-treated boars and vehicle-treated littermates was detectable. Gene expression was reduced as animals aged from 5 to 6.5 weeks as analyzed by qPCR (P < 0.05). At 5 weeks of age, gene expression
for UNC5CL appeared approximately 10 fold lower in letrozole-treated boars than in vehicle-treated littermates by qPCR analysis, supporting the preliminary RNAseq analysis (P < 0.05). These results are consistent with UNC5CL being part of the cellular machinery involved in prolonged proliferation following reduced estrogen signaling; these changes in gene expression might also be peripheral to the prolonged Sertoli cell proliferation. (Supported in part by NRICGP 2008-35203-19082, W3171 MSP, a W.K. Kellogg Endowment, and the infrastructure support of the Department of Animal Science, College of Agricultural and Environmental Sciences, and the California Agricultural Experiment Station of the University of California, Davis.)

P28 - Evaluation of cryopreserved murine testicular tissue following post-thaw in ovo CAM culture. Arpita Mohapatra, Patricia Byrne, Jonathan Molina, Thomas Jensen

Cryopreservation of gonads from deceased genetically valuable individuals is an effective approach to saving germplasm, although the process is damaging to tissues. Studies have shown the chicken CAM to be an excellent culture system with effective angiogenesis and support for mammalian tissues. We hypothesize that CAM culture following cryopreservation may counteract cryo-damage maintaining tissue survival, as well as seminiferous tubule structure. Cryopreserved mouse testes were thawed, transplanted onto chicken CAMs and incubated for seven days. Transplanted tissues were recovered, and either dissociated into single cell suspension, or fixed and paraffin embedded. The percent live cells and presumptive germline stem cells was determined by flow cytometry analysis of propidium iodide and SSEA-1, SSEA-3, and SSEA-4 antibody staining, respectively. Embedded samples were sectioned at 7mm followed by hematoxylin-eosin staining to qualitatively evaluate the effect of CAM culture on seminiferous tubule structure and vascularization. Fresh tissue survival was 72.46%±0.73 (average±sem) and CAM cultures tissue was 74.31%±1.31 (paired two-tailed t-test:p=0.41;t=1.04). The percent SSEA-1 presumptive germline stem cells was 0.01%±0.006 and 0.18%±0.08 (p=0.2;t=1.93), SSEA-3 was 0.037%±0.003 and 0.36%±0.16 (p=0.24;t=1.67) and SSEA-4 was 0.06%±0.02 and 0.04%±0.01 (p=0.29;t=1.43), for fresh and CAM cultured, respectively. Histological analysis showed a loss of normal seminiferous tubule structure and organization. Some seminiferous tubules contained live cells, but no active spermatogenesis, while others contained only dead spermatids with no other cell types visible. All CAM culture sections exhibited a high degree of vascularization derived from the CAM, as evident by nucleated RBCs. This study suggests that post-thaw mammalian testicular tissue maintain germline stem cell survival during CAM culture, although, the seminiferous organization appears to break down, likely due to the lack of adult concentrations of testosterone and FSH in the embryo. We hypothesize that seminiferous structure and function may be attained during CAM culture with the supplementation of testosterone and FSH.

P29 - Post-thaw survival of presumptive germline stem cells in cryopreserved quail testes following chorioallantoic membrane culture. Jonathan Molina, Patricia Byrne, Thomas Jensen

In endangered populations, genetically valuable individuals that die without reproducing pose a threat to the population’s genetic diversity. Gonadal cryopreservation of deceased animals
permits future reintroduction of genes to the gene pool. Gonadal tissues contain germline stem cells (GSCs), amongst others, that may be used in future advanced reproductive technologies, including GSC transfer, cloning, and iPS cells. However, freezing and thawing during cryopreservation damages tissues and cells, making a post-thaw recovery process imperative to maximize recovery of the genetic diversity represented by the sample. Chorioallantoic membranes (CAM) of developing chicken embryos are highly angiogenic, which, makes them ideal for studies of tumor development. Here, we utilized CAMs for post-thaw recovery of cryopreserved quail (Coturnix coturnix) testicular tissue. Quail testicular tissue samples were cryopreserved (10% DMSO), thawed using a step-wise cryo-protectant dilution protocol, and cultured in plating medium overnight (41°C;5% CO₂). Tissues were transplanted onto agitated CAMs and incubated for seven days. Transplanted tissues were recovered, dissociated into single-cell suspension and stained with propidium iodide and SSEA-1,-3, and -4 to determine percent live cells and presumptive GSCs, respectively. Fresh tissue survival was 75.34%±2.71 (average±sem) and CAM transplant was 88.74%±2.19 (paired two-tailed t-test: p=0.044). The percent presumptive GSCs, as detected by SSEA-1, -3, and -4 from fresh and CAM cultured were 31.34%±8.04 and 51.98%±16.12 (p=0.45); 0.15%±0.04 and 0.16%±0.10 (p=0.83); 0.08%±0.03 and 0.04%±0.02 (p=0.49), respectively. Histological analysis showed loss of normal seminiferous tubule architecture and pyknotic nuclei after CAM culture. This study suggests that post-thaw avian testicular tissue maintain cell and GSC survival during CAM culture, although, normal testicular function is lost. The lack of adult concentrations of testosterone and FSH in the embryo, likely is a major cause of this structural failure.

P30 - Highly profiling human sperm purification using sperm sorting chip with cervix mucous viscosity. Jung Kyu Choi, Jae Ho Lee

Swim-up technique refers to the method of harvesting motile sperm from supernatant liquid of semen pellet made by centrifugation. This technique is widely used in assisted reproduction techniques (ART) field because it has a relatively short processing time and does not require the treatment of special reagents. The female reproductive organ is playing the important role in selecting high quality sperm for the fertilization and healthy live birth. In the female reproductive organ, sperm is selected not only by sperm motility but also by structural action, chemotactic interaction of the oocyte and filtration due to the viscous resistance. That means, this sperm retrieval protocol is missing many natural sperm selection steps though female reproductive organ from cervix, uterus and oviduct tubule to oocyte. Therefore, we investigated that cervix mimic sperm chip for the harvest of highly efficient sperm with more cost-saving and time efficient system for the clinical application. We designed the cervix mimic microfluidic platform by 1% and 3% PVP as PVP filtering sperm chip to the treatment group. Then we set up the control group as regular sperm preparation media filling the passage of the sperm chip. Liquefied semen was dropped in the starting point of platform. Then we collected the sperm media in the sperm moving passage for sperm DNA fragment and kinetic analysis at the 5min, 10min, 20min, and 30min time point. Each sample we analysis motility profiling analysis by cell tracking plug-in ImageJ software. It showed high progressive motile sperm isolation perform from the debris and WBC contamination sperm. In conclusion, PVP filtering sperm chip has very simple and highly cost-effective for the motile sperm filtering. PVP sperm chips sperm directly sperm without sperm washing and wasting time for sperm purification.
The present study explored one of the most fundamental questions in the reproductive/developmental biology: do mammalian left and right fetal ovaries develop equally (symmetrically)? Although it is well known that during female chick embryogenesis asymmetrical gonad morphogenesis results in only one functional ovary on the left side, development of mammalian left and right ovaries has been widely assumed the same. However, recent studies in cows and women demonstrated that both the ovulation rate and the developmental competence of oocytes in the right ovary are superior to those of the left one. Causes of the asymmetrical development and functions of the adult left and right ovaries are unknown, but one possible mechanism could be the difference in the size of ovarian follicular reserve (i.e. the maximum number of primordial follicles). Primordial follicle formation and establishment of ovarian follicular reserve occur during fetal life in cows and women. Could the asymmetrical functions of the adult ovaries originate during fetal development? Using cattle as a model, we demonstrated, for the first time, that the difference in the ovarian weight (n = 42 pairs; P < 0.01) and the number of primordial follicles (n = 5 pairs; P < 0.05) between left and right bovine fetal ovaries was significantly asymmetric, with right fetal ovaries 1.2-fold heavier than the left ones and containing 20% less follicles. Furthermore, our studies suggested that these differences may have been induced by the asymmetric estradiol production (n = 12 pairs; P = 0.07) and FIGLA (factor in the germline alpha) mRNA expression (n = 11 pairs; P < 0.05) in the left and right bovine fetal ovaries during early embryogenesis. These findings provide important novel information on the left-right asymmetry in mammalian fetal ovarian growth and function, which is critical for a female’s reproductive lifespan and fertility.

**P32 - Amh and Activin B Synergistically Repress the Ovarian Program in the Mouse Fetal Testis.** Karina Rodriguez, Paula Brown, Barbara Nicol, Humphrey Yao

Differentiation of the gonadal primordium into a testis or an ovary defines the first morphogenetic event in mammalian sex determination. Y-chromosome-derived SRY gene and its downstream regulator SOX9 direct the bipotential somatic cells toward a Sertoli cell fate, rather than the ovarian granulosa cell lineage. Following differentiation, Sertoli cell fate must be maintained for proper testis development. A lack of the Sertoli cell-specific transcription factor DMRT1 leads to Sertoli-to-granulosa cell transdifferentiation in the adult mouse testis. In this study we demonstrate that anti-Müllerian hormone (AMH) and Activin B, two hormones produced by the Sertoli cells, are critical for maintenance of Sertoli cell identity. Fetal mouse testes lacking either one of these two hormones developed normally. However, Amh/Activin B double knockout (dKO) testes exhibited progressive sex reversal. Transcriptome analysis of dKO testis after sex determination uncovered the upregulation of the ovarian program, with increased expression of granulosa cell genes Rspo1, FoxL2, Fst and Runx1 and fetal female germ cell markers Stra8 and Synp3. Histological analysis confirmed that primary testis differentiation occurred normally in the dKO XY gonads with normal expression of SOX9 by E12. By E15
dKO testes exhibited ovarian domains in the poles of the gonads with disorganized cords, FOXL2-positive cells and meiosis entry of XY germ cells. This phenotype is maintained in the adult dKO testis with seminiferous tubules in the center and follicles in the poles. This genetic evidence reveals another level of somatic cell fate regulation in the fetal testis. While DMRT1 directly acts as a transcription factor to maintain Sertoli-cell identity, we show that Sertoli cell-derived AMH and Activin B act in an autocrine/paracrine manner to maintain Sertoli cell fate and prevent the emergence of the granulosa cell program in the fetal testis. This study was supported by NIH Division of Intramural Research program.

**P33 - Ovarian Influences on Postnatal Mouse Uterine Development.** Jessica Milano-Foster, Pramod Dhakal, Thomas Spencer

Postnatal uterine development involves differentiation and development of the endometrial glandular epithelium (GE) and luminal epithelium (LE) as well as development of the mesenchyme into the endometrial stroma and myometrium. This period of development is critical, because it influences the embryotrophic capacity of the adult uterus. In particular, uterine glands and, by inference, their products and secretions are essential for embryo implantation and influence stromal cell decidualization and placental development. In mice and sheep, the ovary influences growth and development of the uterus, but effects on uterine gland development are not clear in mice. Uterine gland genesis begins around postnatal day (PD) 9 and continues through weaning (PD21) to puberty (4-5 weeks). The overall hypothesis is that factors produced by the ovary, including estrogen, regulate development of glands in the prepubertal mouse uterus. Here, C57BL/6J and CD-1 mice were ovariectomized (Ovx) on PD15. Intact and Ovx mice from each strain were analyzed on PD 20, 30, 40 and 60 (n=5 per PD/treatment). Uterine weight was not different (P>0.10) on PD20, but was substantially lower (P<0.05) in Ovx mice after PD20. Although the uteri of ovx mice were hypoplastic, they were histologically similar to intact uteri at each time point regardless of strain. No differences in gland development were observed in Ovx mice as determined by immunofluorescence analysis of forkhead box A2 (Foa2), a transcription factor uniquely expressed in the GE of the uterus. Paradoxically, Foa2 mRNA levels were higher in uterus of Ovx mice regardless of strain. These data support the hypothesis that the ovary and, by inference, its products influence postnatal uterine growth but does not negatively impact differentiation and development of uterine glands. Supported by NIH R01 HD096266.

**P34 - The Window of Masculinization: Connecting Genetics, Testosterone Concentration, and Male Reproductive Tract Development.** Joan Jorgensen, Anbarasi Kothandapani, Kyle Krellwit, Abby Zacharski, Kyle Wegner, Chad Vezina, Elena Kaftanovskaya, Alexander Agoulnik, Emily Merton, Martin Cohn, Samantha Lewis, Jessica Muszynski

Cryptorchidism and hypospadias are the most frequent congenital birth defects in male children. It is established that Desert and Sonic Hedgehog (DHH, SHH) signaling via GLI1, GLI2, and GLI3 are essential for male reproductive tract development. During development, testicular testosterone must achieve critical concentrations to promote prostate bud formation, testicular descent, and differentiation of male external genitalia. We hypothesized that HH
signaling is required to achieve critical levels of testosterone to promote male reproductive tract development. Individual elimination of GLI1 or GLI2 had no effect on testis development; therefore, we focused on GLI3 and examined male Gli3<sup>xlJ</sup> mutant embryos. Results showed that Gli3<sup>xlJ</sup> mutant testes had fewer fetal Leydig cells and diminished testosterone production compared to wild-type samples. Gli3<sup>xlJ</sup> organ phenotypes included no difference in prostate bud numbers but delayed testicular descent and varying severity of hypospadias. Initial testicular descent requires INSL3 action on the gubernaculum and androgen-dependent degradation of the cranial suspensory ligament (CSL). Gli3<sup>xlJ</sup> testes synthesized less Insl3 and steroidogenic enzyme transcripts and disintegration of the CSL was impaired. External genitalia defects were similar to previous reports attributed to SHH with urethral exits positioned at points other than the distal tip. To test whether low testosterone production caused these defects, pregnant dams were treated with DHT or vehicle control. Hypospadias persisted, but CSL degradation was significantly increased in DHT treated mutants compared to controls. In summary, testosterone production was significantly lower in Gli3<sup>xlJ</sup> animals and caused a delay in testis descent and hypospadias but was sufficient for the onset of prostate bud formation. DHT replacement rescued CSL degradation but was insufficient or ill-timed to correct hypospadias. Thus, there are distinct concentration and timing requirements for androgen production to promote male reproductive tract development.

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**P35 - Gestation-dependent upregulation of thymic progesterone receptor expression by the maternal thymic epithelial cells in pregnancy.** Soo Hyun Ahn, Sean Nguyen, Geoffrey Grzesiak, Tae Hoon Kim, Jae Wook Jeong, Margaret Petroff

The thymus is the lymphoid organ responsible for generating self-tolerant T cells. During pregnancy, the maternal thymus undergoes dramatic regression in size and cellularity under the influence of progesterone. This process is thought to be mediated by thymic progesterone receptor (PGR) since its deficiency was previously shown to result in increased fetal resorption. While thymic Pgr expression is critical for pregnancy success, neither the cellular source nor its regulation of expression are known. To understand the relationship between Pgr and optimal pregnancy outcome, we sought to characterize Pgr<sup>+</sup> cells and quantify thymic Pgr expression across pregnancy. Determined by RT-qPCR, Pgr mRNA levels increased by ~9 fold (p=.004) at gestation day (GD) 16.5 (n=5) as compared to non-pregnant (NP) thymus (n=7). Western blot revealed this to be PR-A isoform at late gestation. Further, we generated double-fluorescent Cre reporter mice (mTmG) crossed with Foxn1<sup>cre/+</sup> (thymic epithelial cell (TEC) specific marker) or Pgr<sup>cre/+</sup> as a means to characterize Pgr<sup>+</sup> cells, and to quantify its expression across gestation. In these mice, Foxn1 or Pgr<sup>+</sup> cells become GFP<sup>+</sup> following Cre-mediated excision of membrane-targeted tandem dimer Tomato (mT), while Pgr- or Foxn1-negative cells remain mT. Using this approach, we observed increased percentage of Pgr<sup>+</sup> cells in the thymus from 0.015% of total nuclei (NP; n=2) to 0.045% (GD6.5; n=1), and 0.37% (GD14.5; n=2). Finally, immunofluorescence staining of the thymus from Foxn1<sup>cre/+ -mTmG</sup> females confirmed the identity of Pgr<sup>+</sup> cells to be Foxn1<sup>+</sup> TECs. In summary, we demonstrate that (1) Pgr mRNA and protein increase in pregnancy, (2) PGR is expressed by Foxn1<sup>+</sup> TECs, and (3) PR-A isoform is
expressed by the thymus in pregnancy. These results implicate the importance of TEC-specific PGR expression in ensuring pregnancy success by potentially driving maternal immune tolerance in pregnancy.

**P36 - Effect of OP and BPA on calcium signaling in cardiomyocyte differentiation of mESCs.** Jae-Hwan Lee, Seon Myeong Go, Yeong-Min Yoo, Eui-Bae Jeung

Endocrine-disrupting chemicals (EDCs) have similar structures with steroids hormones, which can interfere with hormone synthesis and normal physiological functions of male and female reproductive organs. Sex steroid hormones influence calcium signaling of the cardiac muscle in early embryo development. Progesterone (P4) has been reported to reduce blood pressure. To confirm the effect of P4, octyl-phenol (OP) and bisphenol A (BPA) on early differentiation of mouse embryonic stem cells (mESCs) into cardiomyocytes, P4, OP and BPA were treated at two days after attachment and media were replaced every two days. In addition, mifepristone (RU486) is a synthetic steroid that has an affinity for progesterone receptor (Pgr) and was treated for one day starting on day 11. To investigate the calcium signaling, the expression of calcium channel gene and contraction-related genes was analyzed. Beating ratio was decreased in P4, OP and BPA treatment. The Pgr mRNA level was significantly increased in P4, OP and BPA-treated group. However, the mRNA level of calcium channel gene, Trpv2, was significantly decreased in the P4, OP and BPA-treated group. In addition, expressions of contraction-related genes such as Ryr2, Cam2 and Mlck3 were significantly decreased in the P4, OP and BPA-treated group. Interestingly, treatment of RU486 rescues altered calcium channel gene and contraction-related genes. P4, OP and BPA treatments resulted in the reduction of intracellular calcium level. Taken together, these results suggest that OP and BPA may impact on the inhibition of cardiomyocytes differentiation of mESCs, results in disruption of cardiomyocytes differentiation of mESCs.

**P37 - NCKX3 depletion lead to abnormal motor function and social behavior in mice.** Dinh Nam Tran, Jae-Hwan Lee, Bo Hui Jeon, Eui-Man Jung, Eui-Bae Jeung

As a novel member of the family of K+-dependent Na+/Ca2+ exchangers, NCKX3 (sodium/potassium/calcium exchanger 3) is an important component of intracellular Ca2+ homeostasis. Ca2+ homeostasis has been extensively studied in various cell systems. Dysregulation of Ca2+ homeostasis can induce the excitotoxic and neurodegeneration in central nervous system. NCKX3 gene is highly expressed in thalamic nuclei, in hippocampal CA1 neurons, and in layer IV of the cerebral cortex in the mouse brain. Here, we examined the effects of NCKX3 deletion in mice. NCKX3 knockout (KO) mice at 6 week-age were used for behavior assays. NCKX3 KO mice show increased moving distances in the open field test. In the sociability test, NCKX3 KO mice have reduced time spent on general sniffing, anogenital sniffing, and following behavior but increased in fighting. In the rotarod test, there were abnormal in motor learnings in NCKX3 KO mice. There was no change in recognition memory in the novel object recognition test. During acquisition phase in the Morris water maze test, there was no different in escape latency time between wild-type and NCKX3 KO mice. This indicated
NCKX3 mutation did not impair to spatial learning in mice. These results suggest that NCKX3 mutation causes abnormal motor functions and social behaviors in mice.

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**P38 - Effects of dexamethasone on calcium channel and mucin gene expression in A549 cell line.** Bo Hui Jeon, Dinh Nam Tran, Yeong-Min Yoo, Eui-Bae Jeung

Calcium is important for physiological functioning in many tissues and is essential in mucus secretion. Previously reported, Mucin secretion is regulated predominantly by cytosolic calcium-dependent pathways. Cytosolic of calcium are regulated by calcium channels such as TRPV6, NCX1, and PMCA1. A549 cell line was treated with 10^{-8} M dexamethasone (DEX) and 10^{-6} M RU486. Subsequently, the expression of TRPV6, NCX1, and PMCA1 in A549 cell line were examined. There was no significant differences in PMCA1 expressions in DEX-treated groups, but TRPV6 was increased in DEX-treated groups and was recovered by DEX+RU486 treatment. NCX1 was decreased in DEX-treated groups and was recovered by RU486 treatment. In addition, mucin secretion, related genes MUC4 and MUC5AC, was also decreased by DEX treatment. Control of calcium channel gene expression may affect the control of mucus secretion in the lung. These results could be used for understanding the basis of treatment mucin secretion related disease such as cancer.

**P39 - Regulatory effect of systemic glucocorticoid on tracheal calcium processing proteins and mucosal secretion.** Bo Hui Jeon, Bonn Lee, Eui-Man Jung, Eui-Bae Jeung

Glucocorticoid inhibits mucin secretion considering the primary option for treating acute asthma exacerbation. However, the mechanism underlying glucocorticoid-induced decreased in mucosecretion is unclear. Recent studies have reported that dexamethasone exerts an inhibition on mucosecretion in the lung by modulating the expression of calcium-processing genes. However, the expression of the calcium-processing genes in trachea are not examined yet. Thus, the present study is the first to report glucocorticoid-induced regulation of tracheal calcium processing genes such as transient receptor potential vanilloid-4 (Trpv4), transient receptor potential vanilloid-6 (Trpv6), calbindin-D9k (CaBP-9k), and plasma membrane Ca^{2+}-ATPase (Pmca1) in the mice. In this study, mice were subcutaneously injected with systemic glucocorticoid dexamethasone for 5days, or injected with estradiol or progesterone for 3 days. The tracheal tissues were collected by dividing the trachea into cervical and thoracic sections based on its anatomical structure. Real-time PCR was performed to investigate mRNA expression of calcium-processing genes. Immunohistochemistry and immunofluorescence were performed to localize the calcium-processing proteins. Tracheal mucosubstances were detected by performing Alcian blue-periodic acid-Schiff staining. The localization of TRPV4, TRPV6, CaBP-9k, and PMCA1 proteins was detected in the tracheal epithelium, submucosal glands, cartilages and muscles. Dexamethasone treatment decreased the mRNA expression of the four calcium-processing genes and mucin 1, mucin 4, mucin 5ac, and mucin 5b genes. Dexamethasone inhibited in the secretion of mucosubstances in the trachea. The findings of the
present study suggest that glucocorticoids regulate the tracheal expression of calcium-processing genes and tracheal mucosecretion.

**P40 - Effect of steroid hormone on the calcium-processing proteins in the immature rat brain.** Seon Myeong Go, Seon Young Park, Jae-Hwan Lee, Eui-Bae Jeung

The cytosolic calcium concentration is regulated by calcium-processing proteins such as TRPV5, TRPV6, NCX1, and PMCA1. Those calcium-processing proteins are important for physiological functions in the brain. The effects of steroid hormones on calcium-processing protein expressions in the brains are undescribed. Thus, the effects of steroid hormones on the distribution, localization, and expressions of calcium-processing proteins in the brain were analyzed. Immature female rats were injected for 5 days with estrogen (E2), progesterone (P4), dexamethasone (DEX), and their antagonists (ICI 182,780 and RU486). The localization and expression of calcium-processing proteins in rat brain were observed by immunofluorescence and western blot analyses, respectively. We found that TRPV5 and TRPV6 proteins were highly expressed in the cerebral cortex (CT), hypothalamus (HY), and brain stem (BS) compared to that in the olfactory bulb (OB) and cerebellum (CB). Also, the NCX1 protein was highly expressed in CT and BS compared to that in OB, HY, and CB, and PMCA1 protein was highly expressed in CT compared to that in other brain regions. Furthermore, expression levels of calcium-processing proteins were regulated by E2, P4, and/or DEX in the CT and HY. In summary, calcium-processing proteins are widely expressed in the immature rat brain, and expressions of calcium-processing proteins in CT and HY are regulated by E2, P4, and/or DEX and can be recovered by antagonist treatment. These results indicate that steroid hormone regulation of calcium-processing proteins may serve as a critical regulator of cytosolic calcium absorption and release in the brain.

**P41 - Effects of steroid hormone on the calbindin-D9k (CaBP-9k) in the immature rat brain.** Seon Mi Park, Seon Young Park, Dinh Nam Tran, Eui-Bae Jeung

Calbindin-D<sub>9k</sub> (CaBP-9k), one of the major calcium-binding and calcium-buffering proteins, is important in the physiological functioning of organs. The neuroanatomical localization of CaBP-9k in the rodent brain has not been reported; thus, this study investigated the neuroanatomical distribution of CaBP-9k and the regulation of CaBP-9k expression on steroid hormones in the immature rat brain. To confirm the influence of steroid hormones on CaBP-9k expression, immature female rats were injected for 5 days with estrogen (E2), progesterone (P4), dexamethasone (DEX), and their antagonists (ICI 182, 780 and RU486). The localization and expression of the CaBP-9k protein in brain regions were identified by immunofluorescence and western blot assays, respectively. We observed that CaBP-9k expression was especially strong in hypothalamus, cerebellum, and brain stem. In addition, CaBP-9k was colocalized with mature-, GABAergic, dopaminergic, and oxytocinergic neurons. We also observed that the CaBP-9k protein level was significantly increased by P4 and reversed by antagonist RU486 treatment in immature rat brain. In summary, CaBP-9k positive cells have a wide distribution in the immature rat brain, and CaBP-9k expression is regulated by P4. We suggest that CaBP-9k expression
regulated by steroid hormone may serve as an important regulator of cytosolic calcium concentration in the brain.

**P42 - Progesterone signaling during pregnancy in the lab opossum, Monodelphis domestica.** Yolanda Cruz, Karin Yoshida, Joanna Line, Kobi Griffith, Alexandra Wooldredge

To investigate subtle pregnancy-associated changes in the lab opossum, *Monodelphis domestica*, an induced ovulator, we compared pregnant with non-pregnant and pseudopregnant animals with regard to serum P4 levels and progesterone receptor (PR) expression. Using video-verified, time-mated lab opossums as sources of biological material, we compared ovaries, uteri and sera obtained on odd-numbered days of the 14.5-day pregnancy in this animal. Females that mated successfully but did not produce embryos were classified as pseudopregnant. P4 levels differed significantly between pregnant (N=21) and either non-pregnant (N=3) or pseudopregnant (N=3) opossums, but not between the non-pregnant and pseudopregnant groups. A significant decline in serum P4 occurred between pregnancy days 3 and 5, coinciding with an elevated probability of pregnancy failure between days 5 and 9. PR was detected in the nuclei of uterine-gland epithelial cells on pregnancy days 5 and 7 as well as variably in the corpora lutea (CL) of animals on pregnancy days 3 to 11. PR expression in the CL suggests that P4 may be autostimulatory in lab opossums and that certain levels of this steroid are required during normal pregnancy. The significant day-3 drop in P4 may explain why pregnancy failure in this polyovular metatherian is likeliest to occur between days 5 and 9, an interval during which the extended period of blastocyst morphogenesis and expansion occurs. Taken together, these results suggest that P4 may have unrecognized signaling roles not only in pregnancy but perhaps embryonic development as well in the lab opossum.

**P43 - Conditional deletion of Fgfr1 in GnRH neurons directly impacts the hypothalamic-pituitary-gonadal axis during pubertal transition of male mice.** Cynthia Dela Cruz, Cassandra Horton, Pei-San Tsai

Neurons secreting gonadotropin-releasing hormone (GnRH) represent the most upstream neuroendocrine activators of the hypothalamic-pituitary-gonadal (HPG) axis and are indispensable for reproductive success. GnRH neurons arise at the tip of nose during embryogenesis, and a failure of the nasal region to develop results in the disruption of the GnRH system, leading to GnRH insufficiency and infertility. An important gene causally linked to GnRH insufficiency is *fibroblast growth factor receptor 1* (*Fgfr1*). It has been established that Fgfr1 is needed for the development of the GnRH system, but it is unclear if Fgfr1 signaling directly upon GnRH neurons contributes to their postnatal function. Our goal is to understand if decreased Fgfr1 signaling in GnRH neurons impacts the postnatal GnRH system and downstream reproductive functions. To address this, we generated a mouse with the conditional deletion of *Fgfr1* specifically in GnRH neurons (abbreviated *Fgfr1*-floxed mice) using the Cre-LoxP technology. Control and *Fgfr1*-floxed male mice were examined for the timing of balanopreputial separation, testicular and seminal vesicle (SV) mass, testicular histology (to assess the percent open seminiferous tubules and the mature sperm in seminiferous tubules), *GnRH* and *KiSS1* expression in the preoptic area, and pituitary and serum levels of
luteinizing hormone (LH) on postnatal day (PN) 25 (n=4/group), 30 (n=10/group) and 60 (n=8/group). Fgfr1-floxed males showed decreased SV somatic index (p=0.0003), gonadosomatic index (p=0.0484), and both pituitary and serum LH (p=0.0373 and p=0.0205, respectively) on PN25 but an increase in serum LH (p=0.0095) on PN30. No additional changes in the parameters examined and at other ages were observed. Together, these results suggest that the conditional deletion of Fgfr1 in GnRH neurons disrupts pubertal transition but does not alter the postnatal GnRH system and HPG axis in earlier adulthood. (Supported by NIH HD083260).

**P44 - Pleiotropic HIF1a regulates steroidogenesis and proliferation in bovine granulosa cells.** Vijay Baddela, Arpna Sharma, Jens Vanselow

Our recent transcriptome data indicated that HIF1a is one of the most abundantly expressed genes in FSH treated bovine granulosa cells (GCs), cultured under normoxic and hypoxic conditions. Such a high expression of HIF1a, under normoxia, is not explicable with previous studies. Therefore, we undertook the present investigation to elucidate the expression and function of HIF1a in bovine GCs. Immunohistochemistry of paraffin embedded bovine ovarian sections clearly showed that HIF1a protein levels are increased in the GC layer from primary to tertiary follicles. This suggested a prominent role of HIF1a in follicle maturation associated functions such as steroidogenesis and proliferation of GCs. To analyze these possible effects of HIF1a, primary GC cultures were treated with increasing concentrations of HIF1a inhibitor, echinomycin, and subjected to normoxic (21% O2) and hypoxic (1% O2) conditions. Echinomycin treatment significantly decreased the CYP19A1 expression and 17β-estradiol production without affecting HSD3B1 expression and progesterone production under normoxic conditions. However, it did not induce any significant changes in estradiol production under hypoxia though it down regulated the basal expression of CYP19A1. Further, Echinomycin caused down regulation of cell cycle regulator CCND gene expression both under normoxic and hypoxic conditions but the inhibition of S-phase DNA replication in flow cytometry analysis were observed only under normoxic condition. These findings were further validated by knock down of HIF1a gene expression using exogenous locked nucleic acid oligomers. A similar decrease in estradiol production, inhibition of cell cycle progression and the corresponding expression of genes by HIF1a specific antisense oligomers confirmed that HIF1a is essential for estradiol production and cell proliferation in GC cultured under normoxic conditions. These findings further reinforce that HIF1a may play an essential role in GC functionality in developing follicles.

**P45 - RFRP-3 Promotes Cell Death and Basal Lamina Degradation in Cultured Cat Ovarian Follicles.** Kathryn Wilsterman, George Bentley, Pierre Comizzoli

In addition to regulation of hypothalamic GnRH neurons and gonadotropes in the adenohypophysis, the neuropeptide RFRP-3 is produced in the ovary and can locally inhibit steroidogenesis and follicular development. Thus far, RFRP-3 production and function has only been described in ovaries from rodents, primates, and ungulates; no work has investigated RFRP-3 in the gonads of any carnivore species. Using the domestic cat as a model, our objective was to determine whether RFRP-3 suppresses ovarian follicle maturation in carnivores. We
predicted that RFRP-3 and its receptor would be expressed in the ovary, and that chronic exposure of ovarian follicles to RFRP-3 would dose-dependently decrease ovarian follicle integrity over time, as well as follicle survival *in vitro*. We first confirmed that RFRP-3 and its receptors were expressed in domestic cat ovaries by sequencing PCR products from RNA isolated from whole ovaries. We then isolated and cultured ovarian follicles in the presence of 10 mM (N = 81) or 1 mM (N = 93) RFRP-3 + FSH (1 mg/mL), and we compared these treatments with follicles treated with FSH alone (N = 82). We recorded percentage of morphologically viable follicles (based on basal lamina integrity) over 8 days and calculated percentage survival of follicles on day 8 (based on calcein-AM and ethidium homodimer-1, fluorescent markers for cell survival and death, respectively). 10 mM RFRP-3 had no effect on viability or survival. 1 mM RFRP-3 decreased the percentage of morphologically viable follicles (P < 0.04; FSH-only, 45%; 1 mM RFRP-3, 30%) and the percentage of surviving follicles on day 8 (P < 0.03; FSH-only, 46%; 1 mM RFRP-3, 30%). Our results are the first to investigate RFRP-3 in carnivores and action on the follicle as a functional unit. Our results support the conserved, inhibitory role of RFRP-3 in the mammalian ovary.

**P46 - Study On The Role Of Kisspeptin In The Regulation Of Motility Spectrum Of Ejaculated Spermatozoa Of The Buffalo Bull.** Muhammad Shahab, Lubna Khan, Shazia Shamas, Hira Zubair, Riffat Bano, Syed Murtaza Andrabi, Hussain Ahmad

Kisspeptin is a neurohormone and a potent regulator of the hypothalamic-pituitary-gonadal axis in vertebrates. Kisspeptin affects reproduction at the gonadal level also. Recently, stimulatory effects of human kisspeptin-10 have been observed on human spermatozoa. Such data are scarce in other species. Therefore, present study was designed to determine regulatory and functional roles of kisspeptin on motility parameters of buffalo bull spermatozoa by using Computer Assisted Sperm Analyser. Semen ejaculates were obtained from 6-7 years old buffalo bulls (n=3). Semen samples were diluted (1:100) under standard practices in PBS+BSA (0.2%). The aliquots of diluted semen (500 µl) were incubated for 2, 6, 12 and 24 minutes at 37°C with 0, 0.2, 2.0, 20 and 40 µM human kisspeptin-10. The parameters calculated in this study included rapid, hyperactivated, slow and poor motile cells for each aliquot, time point and dose. Additionally, ethanol fixed semen smears were processed for immunocytochemical detection of kisspeptin and its receptor, GPR54. It was evident that exogenous administration of human kisspeptin-10 had no effect on hyperactivated, slow and poor motile cells. However, the percentage of rapid motile cells was increased significantly with an increase in the dose of human kisspeptin-10. Spermatozoa collected from all bulls also showed the expression of kisspeptin and GPR54 in the tail region. Present study identified novel direct action of human kisspeptin-10 on buffalo sperm which can be potentially used to enhance quality of buffalo bull sperm being used through artificial insemination.

**P47 - Identification of the SLIT/ROBO signaling pathway as a new regulator of Leydig cell steroidogenesis.** Emmanuelle Martinot, Derek BOERBOOM

The SLIT ligands (SLIT1, 2, 3) are secreted glycoproteins which act *via* ROBO trans-membrane receptors (ROBO1, 2, 3, 4). First identified as important axon guidance molecules, the SLIT
ligands and ROBO receptors have since been found to be involved in the regulation of cell adhesion, proliferation and survival in many organs (brain, lungs, kidneys, heart, mammary glands, ovaries...), and appear to have important functions in the female reproductive system. However, very little is known about the potential role of this signaling pathway in the regulation of male reproductive functions, and of testicular physiology in particular. In this study, we determined for the first time that most members of the *Slit* and *Robo* families are expressed in several testicular cell types in mice, including Leydig cells. To study the function of SLIT/ROBO signaling in Leydig cells *in vitro*, the MA10 cell line and mouse primary Leydig cells were treated with exogenous SLIT ligands, which lead to a significant decrease in the expression of the genes encoding the steroidogenic enzymes *Star*, *Cyp11a1* and *Cyp17a1*. This was correlated with a decrease in progesterone (MA10) and testosterone (primary Leydig cells) concentrations in the culture media. This repression of steroidogenesis was found to be due to a decrease in the AKT-mediated phosphorylation (and thus the activity) of the transcription factor CREB, but was independent of PKA (n=3 triplicates for all experiments, p<0.05). Furthermore, our preliminary data show that the expression of *Slit1,2* and *3* and their receptors *Robo2* and *3* is enhanced by testosterone in Leydig cells. We therefore hypothesize that the SLIT/ROBO signaling pathway participates in the local negative feedback mediated by testosterone on its own synthesis. In conclusion, this work identifies for the first time the role of the SLIT/ROBO signaling pathway in testicular endocrine function in mammals.

**P48 - Steroid Receptor Expression and Cellular Proliferation in the Female Guinea Pig Reproductive Tract.** Amy Flowers, Alan Conley, Brian Reid

The guinea pig vaginal closure membrane is an unusual structure that completely seals the vaginal vault each luteal phase, opening during estrus in apparent response to ovarian steroids. This structure remains poorly understood, as is the mechanism of opening and closure. The direct tissue response to ovarian steroids was investigated by immunohistochemistry for proliferating cell nuclear antigen (PCNA), progesterone receptor (PR), and estrogen receptor alpha (ERα) during estrous and luteal phases. Reproductive tracts from eight female Hartley guinea pigs (n=4/stage) were formalin fixed, embedded, and sectioned at 5μm. After antigen unmasking, sections were incubated with primary antisera (24h, 4°C), conjugated secondary antisera (2h room temperature), and developed with Vector ImmPress reagent and Nuclear Fast Red counterstain. Negative controls were incubated without primary antisera. Numbers and intensities of nuclear staining were recorded, and least squares fit models analyzed with Tukey pairwise comparisons. In the vaginal epithelium, PR and PCNA both stained >70% of nuclei along the basement membrane during estrus (comparison, p=0.9998). During luteal phase, PCNA staining was reduced (vs estrus, p<0.0001; vs control, p=0.0191), and PR staining was absent. PR stained uterine epithelial and stromal cells moderately at estrus only, and didn’t stain ovary. PCNA strongly stained uterine epithelial and stromal cells at estrus, and stained ovarian follicles at both stages. ERα staining along the reproductive tract formed a gradient during estrus, with the greatest numbers and intensity of staining in the oviduct. Surprisingly, ERα staining was nearly absent in the vaginal epithelium in all sections examined at both cycle phases. Although sample sizes were restricted to a scant few days of the cycle, this absence is unexpected in a rodent,
particularly given the tissue level response to estradiol. Further exploration will be necessary to confirm the role of estradiol and ER in the guinea pig vagina.

**P49 - Elevated oleic acid concentration alter gene expression, steroid hormone production and ovulation in bovine preovulatory follicles.** Arpna Sharma, Vijay Baddela, Frank Becker, Dirk Dannenberger, Jens Vanselow

Negative energy balance (NEB) in dairy cows leads to increased concentrations of non-esterified fatty acids (NEFA) in serum and follicular fluid. Among them oleic acid (OA; C18:1) is known to increase towards significantly higher concentrations. In the present study, we investigated the effects of physiological concentrations of oleic acid on steroidogenesis in our serum free, estrogen producing granulosa cells (GC) culture model and simultaneously validated the effects of OA in a bovine in vivo animal model. In order to mimic in vivo conditions, OA was conjugated to bovine serum albumin (BSA). OA specifically increased the transcript levels of CD36, down-regulated the expression of CYP19A1 and STAR, followed by reduced 17β-Estradiol (E2) production in vitro. To validate the effects of OA in vivo, growing dominant follicles (10-19mm) were injected with BSA with/without conjugated OA. The follicular fluid was recovered 48 h post injection. As in our in vitro model, OA significantly reduced the intrafollicular E2 concentrations. Also, CD36 expression was significantly up- and that of CYP19A1 and STAR was significantly down-regulated in antral GC recovered from aspirated follicles. About 14 injected growing dominant follicles (one per animal) were kept for monitoring the ovulatory competence, 77% of those injected with BSA ovulated while only 20% of OA injected follicles reached ovulation within 96 h post injection. Although, not significant yet the ovulatory competence tended to be reduced following OA as compared to BSA injections. Gas chromatography analysis of follicular fluid samples obtained 48 h post intrafollicular injections revealed that the percentage of OA (46.5±5.8%) in OA injected follicles was significantly higher as compared to BSA (13.6±2.5%) injected follicles. Collectively, our results indicate that elevated OA concentrations specifically affect GC function both in vitro and in vivo which might specifically inhibit folliculo-luteal transition and ovulation in dairy cows during postpartum NEB.

**P50 - Androgen-induced Liver Dysfunction in PCOS.** Irving Salinas, Aierken Abudu, Sambit Roy, Kevin Childs, Jie Wang, Todd Lydic, Hanne Hoffman, Aitor Aguirre, Aritro Sen

In women, excess androgen causes polycystic ovary syndrome (PCOS), a reproductive and endocrine disorder, affecting millions of women worldwide. The health risks out of PCOS, however, go far beyond fertility problems, generally affecting the overall health of women. At present, there is much effort towards determining if fertility status can be used as a marker for overall health in women. Here, using a well-established (90d continuous-release DHT pellet-induced) PCOS mouse model, we elucidate the underlying mechanism of androgen effects on liver metabolism and its manifestation of liver dysfunction and development of non-alcoholic fatty liver disease (NAFLD) in PCOS. Results show that chronic high level of androgen significantly increases lipid accumulation and triglyceride levels in the liver. Furthermore, through RNA-sequencing we found 361 genes to be upregulated and 426 genes to be downregulated in DHT pellet vs placebo livers. Amongst these, 103 were upregulated and 133
were downregulatedmetabolic genes. We also performed metabolomics study that revealed a significant increase in total all lipids, total triglyceride and total glycerol in DHT-pellet animals compared to controls. However, there was a concomitant decrease in phospholipid abundance that was accompanied by a general higher incorporation of unsaturated and polyunsaturated fatty acids in the DHT group. Interestingly, sphingolipid and phosphoinositol synthesis were also lower in the DHT group which may affect numerous downstream signaling pathways. Given that excess androgen is the underlying cause of PCOS we have developed a hepatocyte-specific androgen receptor knockout (Hep-ARKO) mouse (female) model using AR-floxed and Alubumin-cre mice. Our aim is to determine if KO of AR would prevent liver dysfunction and development of NAFLD in DHT-pellet induced PCOS mouse model. This would establish AR as a potential therapeutic target for NAFLD associated with PCOS and a mechanistic link between fertility issues and NALFD associated with PCOS.

P51 - Luteinizing Hormone Actions on Primate Follicular Development and Function during Matrix-free Three-dimensional Culture. Shally Wolf, Maralee Lawson, Olena Tkachenko, Cecily Bishop, Jing Xu

A matrix-free three-dimensional culture system was established which supported primate follicle growth from the preantral to antral stage. Androgen production suggested the presence of theca cells in these in vitro-developed follicles at the antral stage. Since luteinizing hormone (LH) is postulated to promote theca cell-mediated steroidogenesis and follicle maturation, experiments were designed to investigate the effect of LH on growth and function of in vitro-developed follicles. Ovaries were obtained from adult cynomolgus macaques (Macaca fascicularis; 12 years old; n=3). Preantral follicles (diameter=140-225um) were mechanically isolated and cultured individually in the ultra-low-attachment plate containing alpha minimum essential medium supplemented with follicle-stimulating hormone and insulin at 20% O2 for 5 weeks. Follicles were randomly assigned to two groups (12 follicles/animal/group): (a) control media and (b) 0.4ng/mL recombinant human LH supplementation during culture week 3-5 (antral stage). Follicle survival and growth were assessed. Culture media was analyzed for estradiol, androstenedione, progesterone, anti-Müllerian hormone, and vascular endothelial growth factor concentrations. Data were analyzed using mixed models. Although follicle diameters were comparable between groups at the beginning of culture, more follicles were larger than 500µm in diameter, which could respond to hormone stimulation and yield mature oocytes, at week 5 with LH supplementation (50%) relative to the control group (~33%). While all antral follicles produced estradiol, more LH-treated follicles (75%) had androstenedione production detectable in the media, indicating sufficient precursors available for estradiol biosynthesis, compared with the controls (~53%). There were no significant differences in media concentrations of progesterone/peptide factors measured or diameters of oocytes harvested at week 5 between groups. LH supplementation appeared to facilitate primate follicle growth and androgen production in vitro. Larger sample sizes are needed to discern LH actions on oocyte maturation in cultured follicles. Supported by NIH/NIGMS UL1GM118964 (BUILD-EXITO), NIH/NICHD R01HD082208, NIH/OD P51OD011092.
Two experiments were conducted to determine if preovulatory estradiol affected luteal progesterone secretion. In experiment 1, ovulation was synchronized in nonlactating beef cows (n=53). Cows that exhibited estrus prior to GnRH-induced ovulation (d 0) had greater (P<0.01) peak concentrations of estradiol compared to cows that did not (11.5±0.8 vs. 6.2±0.6 pg/mL), but there was no difference in ovulatory follicle size (P=0.80) or interval to ovulation (P=0.23). Circulating concentrations of progesterone during luteal formation (d 3 to 7; P=0.70 and P=0.77) or mid-luteal phase (d 8 to 14; P=0.39 and P=0.12) were not affected by estrus expression or estrus by day of sample collection. During the regression phase (d 15 to 21), estrus (P=0.15) did not affect progesterone, but there tended (P=0.05) to be an estrus by day interaction with cows that did not exhibit estrus having decreased progesterone on d 17. Cows that did not exhibit estrus were grouped into high (>5.5 pg/mL) or low (<4.5 pg/mL) peak estradiol. Among these cows there was no effect of estradiol (P=0.51) or estradiol by day (P=0.43) on subsequent progesterone. In experiment 2, ovulation (d 0) was synchronized in nonlactating beef cows (n=13) and cows were allocated to three groups (control, vehicle injection, or ICI 182,780) and balanced for follicle size on d -2. Intrafollicular injection of vehicle (100 µl) or estradiol antagonist (25 µg ICI 182,780 in 100 µl) into the largest follicle occurred on d -2. Concentrations of estradiol increased (P<0.0001) from d -2 to 0, but did not differ among groups (P>0.50). Furthermore, plasma concentrations of progesterone on d 0 through 20 were not affected by treatment (P=0.86). These results indicate that preovulatory estradiol is not required to prepare granulosa cells for luteinization or subsequent luteal progesterone secretion in beef cattle. Supported by AFRI Grant no. 2013-67015-21076 from USDA.

In the bovine oviduct, estradiol (E2) is related to stimulatory effects on cell proliferation and secretion whereas progesterone has a suppressive effect. In this study, we evaluated the effects of two superstimulatory protocols (FSH alone or FSH combined to equine chorionic gonadotropin – FSH/eCG, n=5 animals/treatment) in the E2 levels of oviductal cells homogenate, and also in the mRNA abundance in oviductal cells at pre-ovulatory phase (before LH surge). E2 levels in oviductal cells were 1.86-folds higher in cows submitted to FSH/eCG treatment when compared to control group (single pre-ovulatory follicle synchronization, n=5 animals; P <0.05; tested by ANOVA, and means compared by Tukey–Kramer HSD test). We also observed higher mRNA abundance of steroid receptors (ESR1 and PGR) and genes related to the oviductal role on gamete interaction and polyspermy control (OVGP1, HSPA5, FUCA1, and FUCA2) in the infundibulum and ampulla segments of FSH/eCG group (P <0.05, tested by ANOVA, and means compared by Tukey–Kramer HSD test). There was no variation in progesterone levels or in the mRNA abundance in the isthmus segment (P >0.05). In conclusion, ovarian superstimulation using FSH combined with eCG increases oviductal E2 levels and stimulates genes associated to
gamete interaction. We may infer that such effects triggered by superstimulation with FSH combined with eCG could benefit fertilization.

**P54 - Gestational Endocrinology in Three Cetaceans; Killer Whales, Belugas and Bottlenose Dolphins.** Erin Legacki, Todd Robeck, Karen Steinman, Alan Conley

**Background:** Cetaceans are related to the Ruminatia a clade which, together with the Perissodactyla, comprises the Ungulata. There is surprising diversity in the endocrinology of pregnancy among ungulates, even closely-related ruminants, including progesterone metabolism. For instance, 5α-reduction of progesterone during pregnancy is prominent in horses and cattle, but not in sheep and goats. We investigated progesterone metabolites in pregnant cetaceans to expand our understanding of their gestational endocrinology.

**Materials and Methods:** Three cetacean species (killer whales, belugas and bottlenose dolphins); n=5/species) in captivity (SeaWorld Parks, USA) were bled during mid-luteal, early, mid- and late pregnancy. Serum was analyzed by liquid chromatography tandem-mass spectrometry (LC-MS/MS) using a previously validated method for progesterone, 20αOHP-progesterone (20αOHP), 5α-dihydroprogesterone (DHP) and related metabolites, 3β,20α-dihydroprogesterone (3β,20α-DHP), 20αOH-dihydroprogesterone (20α-DHP) and allopregnanolone (3α-DHP). Data were analyzed by ANOVA.

**Results:** The predominant mid-luteal pregnanes were progesterone in belugas, progesterone and 20αOHP in dolphins and progesterone and 3α-DHP in killer whales. For belugas (P<0.01), killer whales (P<0.001) and dolphins (<0.1) progesterone was two- to four-fold higher in early pregnancy than the mid-luteal phase, but decreased thereafter as 3β,20α-DHP increased (P<0.01) in mid- and late-gestation (from ≈1 to 40-90ng/ml). For killer whales, concentrations of 20αOHP and 3β,20α-DHP were similar at mid-gestation but 20αOHP declined in late-gestation. 20αOHP was lower than 3β,20α-DHP in belugas and dolphins throughout gestation. Other 5α-reduced metabolites were <10ng/ml throughout pregnancy. DHP and 3α-DHP decreased from early to mid-gestation in belugas, but changed little in killer whales and dolphins.

**Conclusions:** 5α-reduction of progesterone during pregnancy appears to be a conserved trait among these cetacean species, with 3β,20α-DHP being the major metabolite that increases significantly during pregnancy. As a metabolic route, 5α-reduction of progesterone during pregnancy is shared with horses and cattle, but not sheep and goats. Pregnancy diagnosis using LC-MS/MS analysis of blood samples may also be possible.

**P55 - A Novel Role for Hippo Signaling in Gonadotropin Synthesis.** Ariane Lalonde-Larue, Alexandre Boyer, Esdras Dos Santos, Guillaume St-Jean, Xiang Zhou, Daniel Bernard, Derek Boerboom, Gustavo Zamberlam
The gonadotropins FSH and LH are dimeric glycoproteins composed of a common α subunit (CGA) and unique β subunits (LHβ and FSHβ), which confer biological specificity. Activins are part of a family of ligands that were discovered based on their ability to stimulate FSH secretion by cultured pituitary cells. They signal via proteins called SMADs, which are essential for FSH synthesis in pituitary gonadotrope cells. The activity of the SMAD pathway can be altered by crosstalk with the Hippo pathway in several tissues and cell types, but whether the Hippo pathway is involved in regulating gonadotropin synthesis has not been determined. The core Hippo signaling pathway consists of a kinase cascade that regulates the activity of the functionally redundant transcriptional co-regulators YAP and TAZ. To elucidate the roles of YAP and TAZ in gonadotropin synthesis and secretion, we cultured pituitary cells from mice bearing Yap/Taz floxed alleles. Following treatment with an adenovirus to drive Cre expression (and thereby inactivate the floxed genes), cells were cultured in the presence of activin A. Basal Lhb expression and both basal and activin A-stimulated Fshb expression were increased in pituitary cells after Yap/Taz depletion. Interestingly, the depletion also increased basal mRNA levels for Cga and Gnrhr, which are important genes for the regulation of gonadotropins. Using a conditional gene targeting approach (cKO), we found that gonadotrope-specific inactivation of Yap and Taz resulted in increased circulating levels of FSH and LH in adult male mice, along with a slight sperm density increase. cKO female mice had augmented circulating LH (but not FSH) levels, which were associated with a hyperfertility phenotype characterized by higher ovulation rates and larger litter sizes. Together, these results indicate that YAP/TAZ, and by extension Hippo signaling, suppress gonadotropin synthesis in gonadotrope cells. Supported by CIHR, Canada.

**P56** - Differences in relative abundance of GnRH-I and GnRH-II in granulosa cells of bovine antral follicles at specific stages of follicular development. Jerica Rich, Emmalee Northrop, Kaitlin Epperson, Saulo Menegatti Zoca, Stephanie Perkins, Russell Daly, Robert Cushman, George Perry

A previous study reported that bovine follicles with greater follicular fluid concentrations of estradiol had decreased expression of GnRH-I and GnRH-II in granulosa cells (GC). The objective of this study was to characterize relative abundance of GnRH-I and -II mRNA within GC of follicles at specific stages of development. Beef cows were synchronized, and ovaries were collected at specific stages of follicular development [pre-selection (PRE), post-selection (POST), and post-selection 24 h after luteal regression (POST-PG)]. All surface follicles were classified as small (<5mm), medium (5-8mm), or large (>8mm) and aspirated to collect GC. Large follicles from each animal were kept separate and all other follicles were pooled by size within animal (n=27, 27, and 18 for small, medium, and large). Total cellular RNA was extracted, and RT-PCR was performed for GnRH-I, GnRH-II, and GAPDH. Data were analyzed using the MIXED procedure of SAS. Across all follicles, GnRH-I and GnRH-II were not influenced by stage \((P=0.27)\) but were influenced by size \((P<0.01)\). Smalls \((4.55\pm0.39 \text{ and } 3.91\pm0.44, \text{ respectively})\) had greater expression \((P<0.01)\) compared to mediums \((0.83\pm0.39 \text{ and } 1.41\pm0.44, \text{ respectively})\) and larges \((0.52\pm0.47 \text{ and } 2.12\pm0.54, \text{ respectively})\). There was also a stage by size interaction \((P<0.01)\). POST \((P<0.01)\) and POST-PG \((P<0.08)\) smalls had or tended to have increased expression compared to PRE smalls, but PRE mediums had increased expression \((P<0.03)\) compared to POST-PG mediums. When only the largest follicle for each animal was evaluated, stage of development influenced expression of GnRH-I \((P=0.03)\) but not
GnRH-II (P=0.91). For GnRH-I, PRE tended \((P=0.09; 2.29\pm0.55)\) to have increased expression compared to POST \((0.92\pm0.55)\) and did have greater expression compared to POST-PG \((P=0.01; 0.11\pm0.55)\). Thus, GnRH within antral follicles may be a key regulator of the follicle’s ability to produce estradiol. Supported by AFRI Grant No. 2018-67016-27578 from USDA. USDA is an equal opportunity provider and employer.


The objective of this study was to evaluate the necessity of a controlled internal drug releasing device (CIDR) in a fixed-time AI resynchronization protocol. Beef cows and heifers from eight herds were inseminated using the 7-day CO-Synch+CIDR protocol. On d 21 following the first insemination, the protocol was repeated, with animals either receiving a CIDR or no CIDR. Pregnancy status was determined on d 28 by transrectal ultrasonography and the IDEXX Rapid Visual Pregnancy Test. Non-pregnant animals by both methods \((n=183\) cows, \(n=351\) heifers) received an injection of PGF2α and were inseminated 54 (heifers) or 63 (cows) h later. Corpora lutea (CL) number and largest follicle diameter (LF) were recorded on a subset of non-pregnant animals \((n=101)\) from each herd and treatment at time of pregnancy diagnosis. Estrus expression was recorded at time of insemination. Pregnancy status was determined 31-78 d following the second AI. Statistical analyses were performed using the GLIMMIX procedure of SAS for CL, estrus, and pregnancy, and MIXED procedure for LF. There was an effect of treatment on estrus expression \((70\pm3\% \text{ CIDR vs. } 48\pm3.1\% \text{ no CIDR}; P<0.001)\), and on CL number \((0.96\pm0.07 \text{ CIDR vs. } 1.2\pm0.07 \text{ no CIDR}; P=0.01)\). But, there was no effect of treatment on follicle diameter \((11.8\pm0.35 \text{ mm CIDR vs. } 11.82\pm0.33 \text{ mm no CIDR}; P=0.90)\). There was no effect of treatment \((36\pm3.2\% \text{ CIDR vs. } 36\pm3.1\% \text{ no CIDR}; P=0.98)\) or a treatment by estrus interaction \((P=0.48)\) on second service pregnancy success. However, there was an effect of estrus expression on pregnancy success \((45\pm2.8\% \text{ estrus vs. } 26\pm3.4\% \text{ no estrus}; P<0.001)\). In conclusion, the use of a CIDR in this resynchronization protocol increased estrus expression and decreased CL number, but, it did not affect follicle diameter or overall pregnancy success. Project funded by Iowa Beef Industry Council.

**P58 - Bisphenol-A affects cell death and immune cell recruitment in the epididymis.** Yoo-Jin Park, Won-Ki Pang, Do-Yeal Ryu, Md Saidur Rahman, Won-Hee Song, Yoon-Jae Park, Bongki Kim, Myung-Geol Pang

The bisphenol-A (BPA) is a widespread environmental contaminant and it is associated with the male reproductive dysfunctions including deleterious effects on sperm motility, motion kinematics, and male fertility. However, the impact of BPA on epididymis that plays an important role during sperm maturation is remained unknown. To establish an optimal environment (acidic pH) for sperm maturation, clear cells secreted proton and principal cells reabsorbed bicarbonate and secreted proton. Also, the epididymal epithelial cells interacted with immune cells to maintain the epididymal immune homeostasis. Therefore, we described here the
role of BPA on the regulation of cell death pathway in epithelial cells lining the caput and cauda epididymal tube. We also tried to examine the immune cell recruitment in epididymis following BPA exposure. Five-weeks old male mice were exposed to BPA at a concentration of 50 mg/kg/mice/day for 6 weeks. And then the epididymides were collected and stained with caspase 3 and phospho-MLKL (pMLKL) as caspase and necroptosis marker, respectively. While pMLKL expression was increased in overall epididymal epithelial cells, caspase 3 expression was increased more specifically in clear cells of BPA exposed mice than control. In addition, the immune cell distribution was slight changed after BPA exposure. These data suggested that BPA may stimulate the alkalinization in epididymis through the induction of apoptosis in clear cells that secrete the proton, consequently induce the premature activation in epididymis and reduce the male fertility. Moreover, different in immune cell recruitment in epididymis between control and BPA-exposed mice indicate that BPA may break down the immune homeostasis and may disturb the clearance of pathogen in epididymis.

P59 - Membrane-localized estrogen receptor 1 (mESR1) regulates estrogen responsive genes and histone protein modifying transcripts. Ana Mesa, Jiude Mao, Theresa Medrano, Manjunatha Nanjappa, Madison Ortega, Paul Caldo, Jessica Kinkade, Ellis Levin, Cheryl Rosenfeld, Paul Cooke

Membrane and nuclear fractions of estrogen receptor 1 (ESR1) mediate 17β-estradiol (E2) actions. Mice expressing nuclear (n)ESR1 but lacking membrane (m)ESR1 (nuclear-only estrogen receptor 1 [NOER]) show reduced E2 responsivity culminating in female infertility. We examined whether expression of estrogen responsive genes and epigenetic regulators was affected by mESR1 ablation. Wild-type [WT] and NOER female mice were subcutaneously injected daily with diethylstilbestrol (DES, 1 mg/g body weight) or vehicle on postnatal day (PND) 1-5. Mice were ovariectomized at PND 60 and subsequently treated with E2 (10 ng/g BW) or vehicle. Uterine gene expression was determined for Esr1, Esr2, and several genes encoding epigenetic regulators. We used RNA-Seq to examine for global RNA changes, its biological relevance was interpreted by ranking magnitude of expression change in WT mice treated with E2 vs NOER with and without E2. Genes encoding epigenetic regulators were further analyzed based on functional enrichment analysis. Neonatal DES treatment of WT mice induced high levels of ovary-independent expression for estrogen-dependent genes, Ezh2, Ltf, Hat1 and Esr2, even after oil vehicle treatment, indicating they were neonatally imprinted by DES treatment. Expression of these genes was reduced in NOER mice; thus, mESR1 is critical for mediating epigenetic effects of neonatal estrogen. Correlation analyses revealed positive association between Ezh2 and Hat1 expression (r = 0.89, p < 0.0001), suggesting neonatal DES exposure simultaneously alters several histone marks. Hat1 and Ezh2 also positively correlated with Esr2 (r = 0.40, p = 0.01 and r = 0.50, p = 0.001, respectively). RNA-seq identified changes of epigenetic transcripts in multiple categories such as scaffold proteins and RNA modifiers. Pathways most affected pertain to PI3K, Notch, FGF and EGF signaling. Findings reveal a critical role of mESR1 in modulating uterine epigenetic responses and proteins involved in cell signaling and growth factor pathways.

P60 - Induction of ovulation in donkeys using GnRH or hCG. Ava Kent, Shelby Nester, Erik Peterson, Robert Gilbert, Hilari French
Ovulation induction agents are commonly used to manipulate the reproductive cycle in equine practice to optimize breeding management. Although this procedure is routinely used in horses, little information exists to support its use in donkeys. A pilot study was designed to develop a protocol for inducing ovulation in jennies to determine the time from administration of induction agents, hCG (Chorulon) and GnRH analogue (SucroMate) to observed ovulation. Eight reproductively sound, non-pregnant Caribbean jennies between the ages of 3 and 12 years old were examined daily via transrectal ultrasonography to monitor ovarian activity and follicular growth. The jennies were randomly assigned to each of three treatment groups (hCG, GnRH analogue, and control) and injected (hCG - IV, GnRH analogue - IM) at a follicular diameter of 27 mm or 30 mm. Twenty-four hours’ post-treatment, jennies were monitored every 6 hours via transrectal ultrasonography until ovulation. The number of jennies ovulating within 48 hours after treatment (or assignment to control group) induced at a follicular diameter of 27 mm was 1/8, 5/8 and 4/8 for control, GnRH and hCG. Results for induction at 30 mm follicular diameter were similar (2/8, 5/8 and 3/8). The mean time to ovulation was shorter after induction at 30 mm and the variation was less. These results indicate that ovulation can be induced in jennies. GnRH induced ovulation within 48 h more consistently than hCG, but at a follicular diameter of 27 or 30 mm, the effect was not as predictable as it is in horse mares induced at a follicular diameter of 35 mm. Waiting until the dominant follicle is larger may improve response to induction agents, but increases the risk of spontaneous ovulation.

P61 - Regulation of Steroidogenic Enzymes in the Fetal Testis: A Role for Activin A. Kate Loveland, Penny Whiley, Liza O'Donnell, Elizabeth Richards

Levels of several TGFbeta superfamily ligands are dynamic in mammalian fetal testes following sex determination, and they variously contribute to both germline and somatic cell growth and differentiation. We have new evidence of a crucial role for activin A in establishing the local hormonal milieu, including testosterone synthesis, at the onset of testis development. We used several gene expression profiling approaches (microarray, RNA-seq, Fluidigm and real time quantitative PCR) to compare fetal testes from activin A-deficient mice (Inhba-/-) and their wildtype (WT) littermates, on whole testes and FACs-purified somatic cells isolated from mice harboring an Oct4-GFP transgene (to remove germ cells); ages examined ranged from E12.5 to Day 0 (birth). Amongst the activin A gene targets were some we previously identified in cells of the postnatal testis, providing validation of our materials and methods. Two important targets were identified that encoding steroidogenic enzymes which are selectively expressed in fetal Sertoli cells and required for testosterone production. HSD17b1 and HSd17b3 were both significantly and dose-dependently lower in conditions of activin insufficiency. This reduction was also measured in short term gonad cultures exposed to SB431542, a pan-TGFbeta signaling inhibitor, indicating transcription of these genes is directly affected by activin levels. Evidence of disrupted steroidogenesis in the Inhba-/- testes was observed as the presence of abundant lipid droplets inside the developing cords. Most genes expressed in Sertoli and Leydig cells are unaffected. These outcomes indicate how compounds that perturb activin and/or TGFbeta superfamily signaling may alter fetal steroidogenesis around the crucial window of masculinization and thereby impair testicular and germline development. Comparison of these
observations of mouse testes with knowledge of the timing of TGFbeta superfamily ligand upregulation and gonad masculinization in the human provides a platform for understanding how the integrity of this signaling pathway in fetal life may influence adult fertility.

**P63 - Estrogen Receptor-eNOS Phosphorylation Partitioning: Temporal and Spatial Interactions within Uterine Endothelial Caveolae.** Mayra Pastore, Dongbao Chen, Maja Okuka, Ronald Magness

Pregnancy elevates estrogen biosynthesis, maintaining uterine perfusion and Nitric Oxide (NO) production by uterine artery endothelial cells (UAECs). The 4% of membrane estrogen receptors (ER-α, ER-β) induce nongenomic rapid vasodilatory responses via endothelial NO Synthase (eNOS). Caveolin-1 (Cav-1) interactions may influence estrogenic effects.

ER-α,ER-β maintain similar spatial partitioning between the plasmalemma and nucleus of UAECs and estradiol-17β (E₂β) induces temporal and spatial eNOS partitioning which reduces Cav-1/eNOS interactions, elevates E₂β-induced stimulatory phosphorylation and eNOS activation.

Electron microscopy revealed substantial UAEC caveolae structures. Immunogold labeling and subcellular fractionation identified ER-α,ER-β in plasmalemma and nucleus. Cav-1 immunosolization columns and sucrose density centrifugation demonstrated high affinity between ER-α, but not ER-β, with Cav-1 (n=4). Caveolae enrichment identified that total eNOS was located in caveolae and non-caveolae domains in control and E₂β (100 nmol/L; 10 & 20 min; n=4) groups. In control cells, stimulatory (Ser635/Ser1177 eNOS) and inhibitory (Thr495 eNOS) were detected in both domains. With E₂β treatments, Thr14 Cav-1 was rapidly elevated, stimulatory Ser635/Ser1177 eNOS levels were lowered in caveolae, but significantly higher in non-caveolae domains; inhibitory Thr495 eNOS was unchanged. Caveolin scaffolding domain peptide (Cav-SD) had no effect on Ser635 eNOS, but reduced basal NOx levels. Cav-SD pre-treatment completely abrogated E₂β-induced rises in Ser635 eNOS and NOx levels.

1) ERα,ER-β are localized to plasmalemma and nucleus; 2) ER-α, but not ER-β, displays protein-protein interactions with Cav-1; 3) ERs independently activate elevations in stimulatory eNOS phosphorylation sites, eNOS translocation from the caveolae to non-caveolae domains, and increase de novo NO biosynthesis; 4) Disruption of Cav-1-eNOS interaction by Cav-SD substantially reduced the E₂β-induced rise in Ser635 eNOS and the NOx biosynthesis, demonstrating post-ER caveolar convergence for regulating NO production that pertains to uterine perfusion during gestation.

**P64 - Endocrine function of the rat Leydig cells is most compromised by morning stress.** Tatjana Kostic, Marija Medar, Aleksandar Baburski, Silvana Andric

It is well known that immobilization stress (IMO) has ability to decrease Leydig cells steroidogenesis and serum testosterone level, but the effects of IMO on circadian rhythm of Leydig cells endocrine function are not completely clear. This study was designed to evaluate the
effect of acute (3 h daily) and repeated (3 h daily for 10 consecutive days) IMO, applied at different times during the 24h on circadian rhythm of testosterone secretion and expression of clock and steroidogenic genes in adult rat Leydig cells. The result showed that acute IMO changed transcription of genes involved in steroidogenesis (decreased mesor of Cyp11a1, Cyp17a1 and Star) and some core clock genes (increased rhythm robustness and mesor of Per1 and Reverba) which was associated with decreased testosterone secretion without circadian variation. Ten times repeated IMO also decreased and flattened oscillatory pattern of testosterone secretion and changed rhythm of steroidogenic genes transcription (decreased mesor of Cyp17a1, increased and initiated cyclic pattern of Hsd3β1/2). The transcription of clock genes were also deregulated (Bmal1, Per1, Cry1 and Cry2 decreased while Reverba increased). Altogether, presented data suggest severe effect of IMO on circadian rhythm of Leydig cell endocrine function. However, comparing IMO effects in different periods over 24 hours, it was found the IMO's strongest effect on rat Leydig cells was in the morning.

**P65 - The Calcium Binding Protein Secretagogin is Highly Expressed in GnRH Cells and Required for the Preovulatory Surge.** Chad Foradori, Laci Mackay, Arthur Zimmerman, Casey Read, B. White, Robert Kemppainen

A hierarchical hormonal cascade along the hypothalamic-pituitary-gonadal axis orchestrates reproduction with gonadotropin-releasing hormone(GnRH)-producing neurons positioned as the predominant regulator. The molecular mechanisms regulating phasic GnRH release remain poorly understood. Secretagogin(SCGN), a member of the hexa EF-hand superfamily of calcium-binding proteins, has been shown to be associated with secretory vesicles/docking proteins and has been thought to be preferentially expressed in neuroendocrine cells. However, brain expression mapping suggests a larger expression distribution and a previous investigation suggests only a small portion of neuroendocrine cells of the paraventricular nucleus express SCGN. To fully characterize the presence of SCGN in neuroendocrine cells of the preoptic area(POA) and mediobasal hypothalamus, adult ovariectomized Long-Evans rats were treated systemically with the retrograde tracer fluorogold followed four days later by central infusion of colchicine to provoke neuropeptide accumulation in somatic domains to aid in the identification of neuroendocrine cells. Analysis demonstrated that a small minority of neuroendocrine cells immunofluorescently positive for tyrosine hydroxylase(15%), somatostatin(6%), or growth hormone releasing hormone(9%) in the arcuate and periventricular nuclei were also immunopositive for SCGN. In contrast, nearly every GnRH positive cell examined(96%) was positive for the SCGN protein. A similar portion of GnRH mRNA expressing cells(94%) coexpressed mRNA for SCGN. SCGN’s comparatively low affinity(25μM) towards Ca\(^{2+}\) suggests it may be involved in excitatory release of GnRH. Indeed, acute silencing of SCGN expression(by siRNA) in the POA of ovariectomized hormone primed rats decreased luteinizing hormone levels associated with the pre-ovulatory surge. These findings suggests that SCGN deficiency might limit the translocation of GnRH to release sites and its calcium-dependent release into the portal circulation. A cursory examination of mouse, sheep and horse GnRH cells suggests the high portion of GnRH/SCGN colocalization is conserved across species. Cumulatively, these data define SCGN as a possible novel regulator of GnRH secretion.
**P66 - Propanil Acutely Changes Steroidogenic Enzymes in Heat-killed Streptococcus pneumoniae Immunized Female Mice.** Malia Berg, Ida Holásková, Jennifer Franko, Rosana Schafer, Robert Dailey

Propanil is a broadleaf herbicide used around the world. Propanil augments antibody responses to heat-killed *Streptococcus pneumoniae* (HKSP) immunization in ovary-intact, but not in ovariectomized female mice. Due to the immunostimulatory abilities of female sex hormones, the objective of this experiment was to determine if propanil modulates the expression of steroidogenic enzymes and immune regulating genes 24 and 72 hours after exposure in HKSP immunized mice. Based on preliminary microarray analysis of ovarian and oviduct tissues, we hypothesized that propanil would alter the expression of enzymes involved in the production of estradiol and progesterone, as well as other innate immune mediators. To test the hypothesis, 24 C57Bl/6 female mice were challenged with HKSP and assigned among four experimental groups (6 mice each) in a 2x2 factorial arrangement. The two factors included treatment (propanil and control) and collection times (24h and 72h). Based upon previous data, the expression of 22 different genes were examined in ovaries by quantitative real-time polymerase chain reaction (qRT-PCR), including those involved in: steroid synthesis, pro-inflammatory, anti-inflammatory, extracellular matrix remodeling, and immune regulatory. An interaction of treatment and time was found for Dhcr7 (P < 0.01), Cyp17a1 (P < 0.05), Lcn2 (P < 0.05), and Cxcl10 (P < 0.05) expression. Propanil had an overall effect on Dhcr7 (P < 0.01) and Cyp19a1 (P < 0.01) when compared to control over both time points. Specific comparisons detected propanil-mediated increases in Dhcr7 (P < 0.01) and Lcn2 (P < 0.05) and decreases in Cyp11b1 (P < 0.05), Cyp17a1 (P < 0.01), Cyp19a1 (P < 0.01), Hsd17b2 (P < 0.05), and Cxcl10 (P < 0.05) expression at 24 hours. In conclusion, propanil causes an acute effect on the steroid synthesis pathway in mice ovaries with minor inflammatory or innate immune factors activation.

**P67 - Sex differences in obesity-mediated impairment of reproductive function.** Djurdjica Coss, Nancy Lainez

Increase in the prevalence of obesity has coincided with an elevated risk of an array of health disorders including type II diabetes, cardiovascular disease and reproductive problems. Obese women experience menstrual irregularities and infertility due to anovulation, while obese men have low testosterone and decreased sperm count. To analyze molecular mechanisms of reproductive function impairment, we placed male and female mice on a high fat diet and control diet with equal sucrose levels. Male mice fed a high fat diet exhibit diminished LH levels, decreased intratesticular testosterone and reduced sperm count, similar to what is observed in obese human males. These mice display high levels of several inflammatory markers in the hypothalamus, as well as in circulation. Elevated inflammatory cytokines affect the expression of hypothalamic neuropeptides, either directly or alternatively, via changes in synaptic molecules that regulate activity-dependent gene expression. Female mice on the other hand are protected from inflammatory and neuroendocrine changes regardless of the presence of ovarian hormones. Ovariectomized females gained weight at the same rate as male mice, but failed to show a decrease in neuropeptide expression or an increase in inflammatory markers that we found in obese males. Therefore, contrary to our hypothesis, ovarian estrogens are not necessary for protection in females. We are analyzing other possible factors contributing to female protection.
Delineating the mechanisms by which inflammatory and metabolic signals influence reproductive function will provide insight into etiology of obesity-mediated disorders and highlight potential targets for pharmacotherapy.

**P68 - The farnesoid X receptor (FXR) is involved in ovulatory response to superstimulation.** Ikuo Tomioka, Yuka Tanahashi, Kentaro Takaie, Kanako Morohaku, Hiroshi Fujii

In metabolic organs, FXR is a well-known and key factor which regulates the bile acid synthesis, the fat metabolism, and the glucose homeostasis. Although we reported that FXR signaling in primary cultured granulosa cells was involved in steroidogenesis (J Reprod Dev. 65:47-55, 2019), the *in vivo* function of FXR in the ovary remains still unknown. Therefore, the purpose of the present study is to produce FXR-deficient mice using genome editing technique and to evaluate its role in ovarian function using FXR-deficient mice. To establish the FXR-deficient mice line, the plasmids expressing hCas9 and sgRNA targeted exon 3 of FXR genome were injected into paternal pronuclei, and the treated embryos were transferred into the oviducts of pseudopregnant females. A total of 129 embryos were subjected to plasmid injection, and 97 embryos were transferred to surrogates. Fifteen pups were obtained and two were revealed to be FXR-deficient mice after genomic PCR, genome sequence, and western blotting analyses. Then, FXR-deficient mice line was established by natural mating. Immature female mice in both wildtype and FXR-deficient were injected intraperitoneally with PMSG followed by hCG. At 18 h after hCG injection, oocytes were collected and counted under the microscopy. The number of ovulation in response to superstimulation in FXR-deficient mice was significantly increased compared to wildtype mice. Ovarian granulosa cells were also collected from superstimulated mice at 0, 24, and 48 hours after PMSG treatment and subjected to RNA extraction and quantitative RT-PCR. In the granulosa cells collected from FXR-deficient mice at 24 h after PMSG treatment, the gene expressions of Fshr, Hsd17b1, and Cyp19a1 significantly increased 1.6-, 1.8-, and 1.8-fold higher than wildtype mice, respectively. These results suggest that FXR in the ovarian granulosa cells inhibits the expression of estradiol synthesis-related genes, leading to the decrease in the number of ovulation.

**P69 - New Transgenic Mouse Models for Analysis of Classical and Non-classical Androgen Receptor Signaling.** Paul Cooke, William Walker

Androgens regulate differentiation, development and function of the male reproductive tract and other target organs via the androgen receptor (AR). Androgen binding to cytoplasmic AR triggers nuclear localization of the ligand-receptor complex and alters gene transcription through the classical response pathway. Androgens also act through AR to stimulate non-classical effects such as rapid activation of Src and MAP kinases and other signaling cascades. Defining relative AR-mediated classical and non-classical functions *in vivo* is technically challenging. To address this need, we produced transgenic mouse strains that lack endogenous wild-type AR expression but express either 1) an AR transgene lacking 11 amino acids essential for the non-classical pathway or 2) an AR transgene having a one amino acid substitution that blocks DNA binding
and the classical pathway. These two pathway-selective AR transgenes produced unique phenotypes in mice lacking endogenous AR expression specifically in testis Sertoli cells. Mice expressing transgenic AR retaining only classical activity had disrupted organization and localization of elongated spermatids but could complete spermatogenesis and were fertile. Transgenic AR having only non-classical activity had spermatogenesis halted during the round spermatid stage. We now report production of two new transgenic mouse lines that have endogenous AR globally deleted during early (epiblast) development but express a transgenic AR mediating either only classical or only non-classical signaling. These mice were produced by mating female mice having a floxed endogenous AR and one of the pathway-selective AR transgenes with male mice harboring the globally active Sox2Cre transgene. Phenotypes of male reproductive organs in mice expressing only classical or non-classical AR will be described. In summary, we have developed two novel transgenic mouse lines that provide unique tools to determine the relative functions of classical and non-classical AR signaling and facilitate our understanding of the mechanism of androgen action in reproductive and non-reproductive tissues.

**P70 - Exploring Necroptosis in Primate Luteolysis: A Role for Ceramide.** Konstantin Bagnjuk, Jan Stöckl, Thomas Fröhlich, Georg Arnold, Rüdiger Behr, Ulrike and Dieter Berg, Lars Kunz, Cecily Bishop, Jing Xu, Artur Mayerhofer

The mechanisms, which govern the fate of the Corpus luteum (CL) in humans are not fully understood. We reasoned that cellular studies, employing IVF-derived human granulosa cells (GCs), when complemented by studies of primate CL, may allow relevant insights. GCs die during culture by apoptosis and necroptosis, as shown before. Phosphorylated mixed lineage kinase domain-like pseudokinase (pMLKL) is a hallmark of necroptosis and is found in cultured GCs. By applying human chorionic gonadotropin, a molecule that prolongs CL lifetime in vivo, we were able to reduce MLKL phosphorylation in vitro. In CL samples of *Homo sapiens, Macaca mulatta* and *Callithrix jacchus* pMLKL was detected. As it was found only from the mid-luteal phase onwards in *M. mulatta* CL samples, we hypothesize that necroptosis may be involved in CL regression. In order to identify the underlying mechanisms, we conducted a proteomic analysis employing GCs. The expression levels of 50 proteins significantly changed after 5 days of culture. Cluster analysis indicated downregulation of cholesterol biosynthesis but upregulation of ceramide salvage pathway-associated proteins. Immunocytochemistry confirmed elevated levels of ceramide in GCs and qPCR studies validated transcriptome data of *M. mulatta* CL, and thus supported in vivo relevance of the ceramide salvage pathway. When we perturbed endogenous ceramide generation by fumonisin B1, cell survival was increased. In contrast, when we added soluble C2-ceramide, cell viability was decreased. Cell studies with the MLKL blocker necrosulfonamid and the pan-caspase blocker zVAD-fmk supported the assumption that the ceramide-induced cell death form is indeed necroptosis. Thus, our data pinpoint necroptosis in the physiological process of primate CL regression. This form of cell death is interlinked with the ceramide salvage pathway. Consequently, it may be possible to interfere with CL regression by targeting ceramide- or necroptosis pathways.
**P71** - Small extracellular vesicles from bovine follicular fluid exposed to low progesterone levels increase PTEN levels in cumulus cells. Ana Clara de Ávila, Alessandra Bridi, Flávio Meirelles, Felipe Perecin, Juliano Da Silveira

Small extracellular vesicles (EVs) are nanoparticle that mediate communication in bovine follicular environment. Recently, PI3K-Akt pathway was identified as involved in oocyte competence. The aim of this study was to evaluate the effects of follicular fluid EVs associated with progesterone (P4) levels and transcripts related to PI3K-Akt pathway in cumulus cells (CC) during IVM. Slaughterhouse pairs of ovaries were classified based on corpus luteum in early and late estrus cycle. Follicular fluid from small follicles (3-6mm) at early estrus cycle (n=4 pools) presented low P4 (63.62±13.59ng/mL), while late estrus cycle (n=5 pools) presented high P4 (158.8±39.07ng/mL; p=0.002). Follicular fluid was centrifuged at 119,700xg for 70 min twice to pellet EVs followed by dilution in maturation media. Cumulus oocyte complexes (COCs; n=25/group) were submitted to IVM supplemented with 10% of EVsfree-fetal calf serum (FCS) or 10% EVsfree-FCS supplemented with EVs from low or high P4. After 9 hours of IVM, COCs were denuded to obtain CC (n=6 replicates). Total RNA was extracted and reverse transcribed using High-Capacity (ThermoFisher) and miScriptII RT (QIAGEN), to study mRNAs and miRNAs, respectively. We performed RT-PCR to evaluate transcripts related to PI3K-Akt and mature miRNAs predicted to regulate PI3K-Akt genes. Results demonstrated that CCs exposed to EVs from low P4 have increased PTEN, a suppressor of PI3K-Akt pathway, compared to EVsfree-FCS (p=0.04). Additionally, from 18 miRNAs found statistically different between groups (p<0.05), four were potential regulators of PTEN. MiR-181a; miR-500 and miR-584 were downregulated in CCs exposed to EVs from low P4 compared to EVsfree-FCS. In conclusion, these results suggest that follicular EVs from low P4 have a suppressive effect of PI3K-Akt pathway in CC. This effect could be caused by delivery or changes in miRNAs upon EVs supplementation. Future experiments will elucidate the mechanisms involved in EVs modulation of PI3K-Akt pathway. Funding: FAPESP (2014/22887-0; 2017/02037-0).

**P72** - Transcriptomic analysis of major signaling pathways regulated by gonadotropins in the KGN line of human ovarian granulosa tumour cells. Patricia Tremblay, Marc-André Sirard

Female reproductive function largely depends on timing and coordination between follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Even though it is suggested that they act on granulosa cells via shared signalling pathways; mainly via protein kinases A, B and C (PKA, PKB, PKC) there is still very little evidence on how these signalling pathways are regulated by both hormones to provide such differences in gene expression through folliculogenesis. To provide a global picture of the principal upstream signalling factors involved in PKA, PKB and PKC signalling pathways, human granulosa-like tumour cells (KGN) were treated with FSH and specific activators (forskolin, SC79, PMA) for each signalling pathway to analyze gene expression with RNA seq technology. Normalization and cut-offs (FC 1.5, p≤0.05) revealed 3864 differentially expressed genes between treatments. Among the genes shown to be regulated by FSH, about 80% were common with at least one of the three principal signalling pathways (PKA, PKC, PKB) and 307 (~20%) were specific to FSH treatment only. Analysis of major upstream regulators showsthat PKA is a master kinase of cell differentiation being
responsible for early differentiation and the initiation of a complex pattern of intracellular signalling pathways and gene expression profile that accompany granulosa cell differentiation. Regarding PKC’s role in folliculogenesis, our data reveals that although this pathway operates in parallel with PKA, the activation of PKC in granulosa cell is a strong differentiation signal that could be controlling “advanced” differentiation in granulosa cells and inflammatory cascade that occurs in dominant follicle. For its part, PKB comes as a crucial support for PKA’s genes expression and is also involved in granulosa cells survival through follicular development. Taken together, our results provide new information on PKA, PKB, and PKC signalling pathways and their role in stimulating a follicle at the crossroad between maturation/ovulation and atresia.

P73 - Role of Vaspin in the ovarian follicles of Polish Large White pigs: signaling pathway and action on steroid synthesis via GRP78 receptor and kinases PKA and ERK1/2. Patrycja Kurowska, Ewa Mlyczynska, Joelle Dupont, Agnieszka Rak

Vaspin (Visceral adipose tissue-derived serpin) is an adipokine involved in the development of obesity, insulin resistance or pathogenesis of inflammatory reactions in mammals. Little is known about role in reproduction. Our previous data showed that gene and protein expression of vaspin in the ovary were dependent on fattening of animals and could be regulated by different factors like gonadotropins, steroids, insulin or IGF-1. The aim of the present study was to investigate vaspin in vitro effect on several kinases: ERK1/2, PI3K/Akt, Stat-3/JAK, AMPKα, PKA phosphorylation and NFKB factor protein expression, and also estradiol (E2) secretion. Ovarian follicles were obtained at days 10-12 of the estrous cycle of mature Polish Large White pigs, then ovarian cells were cultured. To study phosphorylation of the various kinases, vaspin (1 ng/ml) was added for 1 to 60 min and then kinases and NFKB protein expression were analyzed by immunoblotting. To determine the effect of vaspin on E2 secretion, different doses of vaspin (0.01 to 100 ng/ml) were added for 24 h and then steroid concentration and aromatase expression were analyzed by ELISA and immunoblotting, respectively. To check molecular mechanism of vaspin action in the ovarian cells, kinases PKA and ERK1/2 were blocked using KT570, PD98059 inhibitors, while GRP78 receptor was silenced. Finally, statistical analysis was performed by Graph Pad Prism 5 and a one-way ANOVA test. We observed that vaspin increased in a time dependent manner significantly activation of all kinases but decreased NFKB protein expression (n=3, p<0.05). Moreover, vaspin induced stimulatory effect on E2 and aromatase level by GRP78 receptor and kinases PKA and ERK1/2 activation (n=3, p<0.05). Taken together, vaspin is a new adipokine involved in the ovarian physiology by regulation of several kinases phosphorylation and steroid hormones synthesis.

P75 - In Vitro Effects of Follicular Stimulating Hormone (FSH) on Ovine Luteal Endothelial Cells (LEC); Implications for Regulation of Angiogenesis and Blood Vessel Functions. Anna Grazul-Bilska, Thanya Bunma, Chainarong Navanukraw, Dale Redmer, Sheri Dorsam

Follicle stimulating hormone is the major regulator of ovarian follicle functions, and its receptors (R) are present in the granulosa layer. FSHR are also expressed in other tissues of the
reproductive system including corpora lutea (CL), uterus and placenta. It has been suggested that FSH is involved in the regulation of angiogenesis. Our aim was to determine the in vitro FSH effects on angiogenesis in the CL measured by LEC proliferation, migration and tube formation. LEC isolated using enzymatic digestion and magnetic beads coated with lectin BS1 from mature CLs of two non-pregnant superovulated ewes (Beckman et al., Endocrine, 29:467; 2006), were used for mitogenesis, migration and tube formation assays. For each assay, cells were treated with 0, 0.3, 1, 3, 10, 30 and 100 ng/ml of ovine FSH. For mitogenesis, cells plated on 8-chamber slides were cultured with treatments for 72 h. For migration, Boyden chambers where bottom wells contained media with treatments and top wells LEC, were incubated for 4 h. Then, LEC were fixed and stained with DAPI, followed by image generation and analysis to determine cell number/area. For tube formation, LEC suspended in media containing treatments were incubated on Matrigel for 24 h followed by image generation. For each ewe, each assay was repeated three times; duplicates for mitogenesis, triplicates for migration, and once for tube formation for each repeat. FSH decreased (P<0.001) LEC proliferation (by 60-90%) in a dose-dependent manner. Migration was reduced (P<0.05) by ~30% in the presence of 100 ng/ml of FSH, and higher doses of FSH (30 and 100 ng/ml) prevented tube formation. These data demonstrate inhibitory effects of FSH on selected LEC functions, indicating that FSH is involved in the regulation of angiogenesis and blood vessel function in the ovine CL. USDA-AFRI grant 2011-67016-30174, and Hatch Projects ND01754 and ND01748.

P76 - Chronic (5 year) hyperandrogenemia and/or western style diet impact metabolic and reproductive outcomes in female rhesus macaques. Cecily Bishop, Diana Takahashi, Emily Mishler, Richard Stouffer, Ov Slayden, Cadence True, Jon Hennebold

Hyperandrogenemia is a hallmark of polycystic ovary syndrome (PCOS) and evidence suggests early (fetal) exposure to elevated androgen developmentally programs this disorder. To determine if postpubertal exposure also drives PCOS-like symptoms, female rhesus macaques were treated with testosterone (T), an obesogenic western-style diet (WSD) or the combination (T+WSD) beginning at menarche (n=8-10/group). To date this cohort has received 5 years of treatment and are 7-8 years of age. During the early follicular phase of a randomly selected menstrual cycle: 1) serum FSH levels were elevated in WSD and T+WSD groups compared to controls (p<0.05); 2) LH levels tended to be elevated by T and reduced by T+WSD (p=0.07); 3) the LH:FSH ratio was reduced in WSD and T+WSD compared to controls (p=0.003); and 4) AMH levels were reduced in the T+WSD cohort (p<0.015). The mid-cycle E2 surge was delayed in the T+WSD group (p=0.05). During the luteal phase: 1) progesterone levels were reduced by WSD (WSD, T+WSD; p<0.0003); and by the late luteal phase 2) serum LH and FSH were elevated in the T cohort (p's < 0.0003, 0.015); and 3) AMH levels were reduced by WSD (WSD, T+WSD, p=0.002). Metabolic outcomes such as increases in weight, BMI and body fat percentage were present in WSD and T+WSD cohorts. However, only T+WSD animals appeared hyperinsulinemic during a glucose tolerance test. The metabolic and reproductive outcomes after 5 years of T and/or WSD exposure are similar to those previously reported following 3 years of treatment; menstrual cyclicity continues but endocrine and possibly gametogenic defects likely contribute to impaired fertility observed following 4 years of treatment. Funded by P50-HD071835 (RLS/JDH), and P51-OD011092 (ONPRC).
**P77 - Ovarian impacts of Atm haploinsufficiency in response to phosphoramidemustard.**
Kendra Clark, Shanthi Ganesan, Aileen Keating

Phosphoramidemustard (PM) is used for autoimmune and malignant disease therapy, inducing DNA double strand breaks (DSB) to destroy rapidly dividing cells. PM exposure induces ovarian DNA damage and destroys primordial follicles. Ataxia telangiectasia mutated (ATM) protein activates cell cycle checkpoints and DNA repair proteins to recognize and repair DSBs, and mutations in *Atm* are implicated in increased risk for certain cancers, including breast and ovarian. We previously determined ATM inhibition prevents PM-induced follicular loss. The effects on oocyte quality remain unknown, thus, we hypothesized that DSB repair is blunted in *Atm*+/- mice and unhealthy follicles are retained within the ovary. Wild-type (WT) C57BL/6 or *Atm*+/- mice were dosed once intraperitoneally with sesame oil (95%) or PM (25 mg/kg) in the proestrus phase of the estrous cycle and ovaries harvested 3 days thereafter. *Atm*+/- mice spent ~25% more time in diestrus than WT (*P* < 0.05). LC-MS/MS on ovarian protein was performed and bioinformatically analyzed. Relative to WT, *Atm*+/- mice had 64 proteins and 243 proteins increased and decreased (*P* < 0.05) in abundance, respectively. In WT mice, PM increased 162 and decreased 20 proteins (*P* < 0.05). In *Atm*+/- mice, 173 and 37 proteins were increased and decreased (*P* < 0.05), respectively, by PM. Exportin-2 (XPO2) was localized to granulosa cells of all follicle stages and was greater 7.2-fold in *Atm*+/- than WT mice. Cytoplasmic FMR1-interacting protein 1 (CYFIP1) was decreased 6.8-fold in *Atm*+/- mice, with localization in surface epithelium and appeared deeper in the ovary post-PM exposure. PM induced pH2AX, but fewer pH2AX positive foci were identified in *Atm*+/- mice. Similarly, cleaved caspase-3 was lower in the *Atm*+/- PM-treated, relative to WT mice. These data support roles for ATM in ovarian DNA repair and atresia.

**P78 - MicroRNAs and their Diverse Roles in Ovarian Function- A Meta-analysis.**
Oluwatosin Adesina, Katie Woad, Victoria James, Nigel Mongan

MicroRNAs play regulatory roles in physiological and pathological processes. They are involved in many critical aspects of reproduction in humans and animals. MiRNAs mediate a wide-range of effects on ovarian function, hence the possibility of ovarian microRNAs being used as biomarkers for reproductive efficiency. We hypothesise that ovarian microRNAs mediating changes in angiogenic and steroidogenic pathways can be used as a measure of reproductive function. An analytical review of 66 published studies was performed to determine the expression and function of ovarian microRNAs in various aspects of reproduction (Folliculogenesis, follicular and luteal cell proliferation, luteinisation, angiogenesis, follicular apoptosis or atresia, luteolysis and steroidogenesis) in humans and animals. Five (miR-378, miR-21, let-7g, miR-150 and miR-96) of the 146 microRNAs reported were consistently expressed across the different aspects being investigated. Biological enrichment analysis (using FunRich; a functional enrichment analysis tool) demonstrated that the target messenger RNAs of these microRNAs were enriched in the following biological pathways: Glypican pathway, Platelet Derived Growth Factor Receptor signalling pathway, Signalling events mediated by Vascular Endothelial Growth Factor Receptor 1 and 2, Epidermal Growth Factor Receptor, TNF-Related
Apoptosis Inducing Ligand signalling pathway, Insulin-like Growth Factor 1 pathway, Interferon Gamma pathway, Signalling events mediated by Hepatocyte Growth Factor Receptor, Endothelins, Plasma membrane estrogen receptor signalling. These signalling pathways have established roles in ovarian function, therefore the modulation of microRNAs involved in these pathways is likely to be indicative of altered ovarian function. Further work will involve the use of functional studies to explore the exact roles microRNAs play in ovarian function in different aspects of reproduction.

P79 - Luteinizing hormone receptor deficiency in follicles harboring immature (germinal vesicle) oocytes after controlled ovarian stimulation. Maira Casalechi, Cynthia Dela Cruz, Fernando Reis

LH surge is the trigger for human oocyte meiotic resumption, leading to the ovulation of a fully competent oocyte. However, about 9% of oocytes retrieved for IVF do not respond to this stimulus and remain at germinal vesicle (GV) stage. Moreover, case reports have documented patients whose repeated IVF attempts yielded only GV oocytes despite the correct use of gonadotropins for controlled ovarian stimulation (COS) and ovulation triggering. Until now, there is no reasonable explanation to this failed meiotic resumption. This was a prospective cohort study including 39 women who underwent COS in a single IVF center. Luteinized granulosa cells (GC) and cumulus cells (CC) were collected from 96 preovulatory follicles and the maturation stage of the oocytes was immediately evaluated. LHR gene expression levels were measured by qPCR in GC and in CC surrounding each oocyte. The relative mRNA expression of LHR was compared between cells from follicles containing GV, metaphase I (MI) and metaphaseII (MII) oocytes using Kruskal-Wallis analysis of variance. LHR mRNA expression was detected in follicles containing MII or MI oocytes in both CC and CG, being four-fold more abundant in CC than in GC. Follicles containing GV oocytes had almost undetectable luteinizing hormone receptor (LHR) mRNA levels in both CC (fold change 0.52, p<0.01) and GC (fold change 0.13, p<0.01). These finding suggests that LHR deficiency is associated with GV phase arrest in oocytes retrieved for IVF. If these preliminary results are confirmed by protein levels of LHR and functional analyses, novel drugs acting beyond LHR might be developed to treat IVF patients with a high proportion of GV oocytes.

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P79 - Long-Term Hyperandrogenemia and/or Western-Style Diet Impairs Rhesus Macaque Oocyte Maturation, Fertilization, and Preimplantation Embryo Development. Sweta Ravisankar, Melinda Murphy, Nash Redmayne-Titley, Richard Stouffer, Shawn Chavez, Jon Hennebold

Elevated circulating androgens (hyperandrogenemia) are associated with reproductive pathologies that result in reduced female fertility, including polycystic ovary syndrome (PCOS). PCOS severity is associated with consumption of an obesogenic diet and development of
metabolic dysfunction. Thus, we established a nonhuman primate model whereby peripubertal rhesus macaque females were treated with testosterone (T), a western-style diet (WSD) or the combination (T+WSD) to determine how these factors drive PCOS-like symptoms (n=10/group). Treatments preceded the onset of puberty and continued over 5 years until 7-8 years of age. The impact of T and/or WSD consumption on ovarian function, oocyte maturation and fertilization, as well as preimplantation embryo development was assessed in each of the 4 groups (control diet n=10; WSD n=6; T n=7; T+WSD n=3) after animals underwent a controlled ovarian stimulation protocol to stimulate the development of multiple antral follicles. Oocytes were collected and assessed for re-initiation of meiosis, in vitro fertilization (IVF), and blastocyst formation. A reduction in fertilization and blastocyst formation rates were observed in WSD and T+WSD groups compared to the control and T only groups, despite the higher number of mature metaphase II oocytes in the T+WSD group. Immunostaining of WSD and T+WSD oocytes with the nuclear envelope marker, LMNB1, revealed a higher incidence of micronuclei formation suggestive of aneuploidy associated with reduced blastocyst formation that aligns with our findings. Our results suggest that the long-term diets are associated with reduced oocyte quality and in turn, responsible for impaired IVF outcomes, especially in the T+WSD group. Ongoing assessment will reveal chromosomal copy number variation in embryos that failed to form blastocysts, gene expression differences in blastocysts between treatment groups, the effect of the treatments on the intrafollicular environment, and initial mitotic timing post-IVF, multipolar divisions, and cellular fragmentation by time-lapse monitoring. Funded by P50-HD071835 (RLS/JDH) and P51-OD011092 (ONPRC).

**P80 - Effect of Mitochondrial Dynamin Like GTPase on Steroidogenesis in the Corpus Luteum.** Michele Plewes, Emilia Przygrodzka, Pan Zhang, Xiaoying Hou, John Davis

The corpus luteum is an endocrine gland that synthesizes/secretes progesterone (P4), which is essential for establishment/maintenance of pregnancy. Luteinizing hormone (LH) activates protein kinase A (PKA) signaling in luteal cells, increasing delivery of substrate to mitochondria for P4 production. Mitochondrial Dynamin-Like GTPase (OPA1) is a mitochondrial dynamin-related GTPase that promotes mitochondrial elongation. OPA1 has several functions based on posttranslational proteolytic cleaving. Constitutive proteolytic cleaving of OPA1 by OMA1 (Zinc Metallopeptidase) leads to two isoforms of OPA1: long (L-OPA1) and short (S-OPA1) isoforms. L-OPA1 mediates mitochondrial fusion, while S-OPA1 takes on a soluble form and modulates mitochondrial energetics. S-OPA1 also serves as an A-Kinase anchoring protein (AKAP) that associates with lipid droplets (LDs), which are abundant in steroidogenic luteal cells. Therefore, understanding the regulation of OPA1 in luteal cells is essential. We hypothesize that OPA1 serves as an AKAP and is required for optimal LH-induced steroidogenesis in luteal cells. The objectives were to determine 1) influence of knockdown of OPA1 on LH-induce P4 production, 2) effects of LH on localization of OPA1 with LDs and PKA, and 3) influence of knockdown of OMA1 on LH-induce P4 biosynthesis in luteal cells. Bovine ovaries were obtained from a local abattoir and corpora lutea were digested using collagenase. Dispersed luteal cells were enriched for small luteal cells via centrifugal elutriation. P4 secretion was measured by ELISA. Western blotting and confocal microscopy were used to determine localization of OPA1, PKA and LDs. Knockdown of OPA1 inhibited LH-induced P4 synthesis (P<0.05). Furthermore, OPA1 is present in LD fractions and LH increases colocalization of OPA1 with LDs (P<0.05). Moreover, OPA1 interacts with PKA
and LH further increases colocalization of PKA with OPA1 (P<0.05). Lastly, knockdown of OMA1 attenuated LH-induced P4 synthesis (P<0.05). Taken together, OPA1 may play a novel role in LH-induced P4 steroidogenesis in luteal cells.

**P81** - Effects of Hypoxia on Agonist-Induced Progesterone Synthesis in Fish Oil Supplemented Bovine Luteal Cells. Brian Krum, Patrick Burns

The corpus luteum is an ovarian endocrine gland that secretes progesterone, which is essential for the establishment and maintenance of pregnancy. We have demonstrated that dietary supplementation of fish meal decreases luteal sensitivity to intrauterine infusions of PGF2alpha, the endogenous luteolysin. A decrease in blood flow occurs during luteal regression and it is hypothesized that omega-3 fatty acids in fish meal are luteal protective during hypoxia. The objective of this study was to determine the effects of hypoxia on agonist-induced progesterone synthesis in fish oil-treated luteal cells. In experiment 1, mixed luteal cells were cultured for 72-h in control or 0.03% (vol:vol) fish oil supplemented medium to allow incorporation of omega-3 fatty acids into cellular membranes. Cells were treated with fresh medium containing forskolin (10 micromolar) and incubated under normoxic (20% O2) or hypoxic (5% O2) conditions. Medium was collected at 0, 12 and 24 h to determine progesterone synthesis. In experiment 2, cells were cultured in microscopy dishes containing control or fish oil supplemented medium for 72-h. Cells were then incubated for 0 or 24 h under a normoxic or hypoxic environment. Mitochondrial membrane potential was determined using tetramethylrhodamine, methyl ester, perchlorate staining and visualized using confocal microscopy. Forskolin stimulated progesterone synthesis under normoxic conditions, which did not differ between the two groups (P>0.10). Progesterone synthesis was decreased during hypoxia (P<0.05) and did not differ between the two groups (P>0.10). There was no difference in mitochondrial membrane potential when comparing fish oil-treated cells to control cells in either normoxic or hypoxic environments. Results show that fish oil supplementation is not luteal protective to progesterone biosynthesis under hypoxic conditions. This project was supported by National Research Initiative Competitive Grant no. 2013-67015-20966 from the USDA National Institute of Food and Agriculture to PDB.

**P82** - Effect of Fish Oil Supplementation on Progesterone Synthesis in the Bovine Following Intrauterine Infusion of PGF2alpha. Brian Krum, Jessica Cedillo, Jason Bruemmer, Terry Engle, Patrick Burns

We have reported that dietary supplementation of fish meal decreases luteal sensitivity to intrauterine infusions of prostaglandin (PG) F2alpha. Approximately 50% of the fish meal supplemented cows retained a functional corpus luteum (CL) following infusion. However, steady-state mRNA levels of key steroidogenic genes were decreased during the first 18 h following infusion of PGF2alpha despite the CL being able to synthesize adequate progesterone. The objective of the study was to determine if there is a rebound in steroidogenic gene expression following intrauterine infusion of PGF2alpha in fish oil supplemented cows. Cows were supplemented with either fish oil (n=5) or vegetable oil (n=4) for
approximately 60 days. Uteri were infused with PGF2alpha (0.5 mg) at h 0 and 12 on day 10-12 of the estrous cycle. Diameter of CL was measured using ultrasonography and luteal biopsies were collected at h 0, 18, 24, 36 and 48 h. Here, 60% of the fish oil supplemented cows retained a functional CL compared to 25% for cows receiving vegetable oil. Steady-state mRNA for STARD1, CYP11A1 and LDLR did not differ between cows with a functional or regressed CL at 0 or 18 h (P >0.10). Steady-state mRNA for STARD1, CYP11A1, and LDLR continued to decrease in tissue from cows receiving vegetable oil with a regressed CL, yet remained unchanged for cows supplemented with fish oil, regardless of CL function (P<0.05). Diameter of CL decreased for cows that had a regressed CL (P<0.05), but remained unchanged for fish oil supplemented cows retaining a functional CL. In conclusion, steroidogenic gene expression initially decreased and then stabilized in fish oil supplemented cows retaining a functional CL following PGF2alpha infusion. This project was supported by National Research Initiative Competitive Grant no. 2013-67015-20966 from the USDA National Institute of Food and Agriculture to PDB.

**P83 - Signaling Pathways Regulating Autophagy In Luteal Cells.** Emilia Przygrodzka, Michele Plewes, Xiaoying Hou, Pan Zhang, John Davis

Autophagy is a self-degradative process important for balancing sources of cellular energy at critical times in development and in response to nutrient stress, but can also lead to apoptosis. Several markers of autophagy were found to be elevated in the corpus luteum (CL) during luteolysis, however the signaling pathways regulating autophagy in luteal cells remain unknown. Mammalian target of rapamycin (MTOR) and 5' AMP-activated protein kinase (AMPK), key players in autophagy, are known to inhibit or activate autophagy, respectively. Previously, we reported opposite effects of luteinizing hormone (LH) on MTOR and AMPK activity in luteal cells. Thus, we hypothesize that LH inhibits autophagy by modulating the activity of these kinases. To test this hypothesis small luteal cells (SLC) isolated from mature bovine CL (n=3-5) were treated with LH (1-100 ng/ml), AICAR- (AMPK activator; 1 mM) or AICAR + LH (10 ng/ml). Additionally, SLC were incubated with forskolin (FSK; a Protein Kinase A (PKA) activator; 10 μM), H89 (PKA inhibitor; 30 μM) and rapamycin (MTOR inhibitor; 20 nM). Data were analyzed using one- or two-way ANOVA. Treatment with LH and FSK decreased (p<0.01) phosphorylation of Raptor (Ser792) and AMPK (Thr172), but increased (p<0.01) phosphorylation of p70S6K (Thr389) and ULK (Ser757), which are specific MTOR substrates. LH-mediated effects on AMPK, MTOR, and ULK were abolished after pre-treatment with H89 and rapamycin, respectively. In contrast, AMPK activator AICAR induced (p<0.01) phosphorylation of AMPK (Thr172) and RAPTOR (Ser792), and reduced (p<0.01) phosphorylation of ULK (Ser757) reflecting inhibition of MTOR. Additionally, AICAR significantly enhanced (p<0.05) phosphorylation of the autophagy mediator Beclin (Ser93). Pretreatment with AICAR prevented the ability of LH to phosphorylate the autophagy inhibitors MTOR and ULK. Our results suggest LH inhibits while AMPK activates key signaling pathways involved in autophagy. Supported by NIFA USDA grant no. 2017-67015-26450, VA and NIH R01 HD092263.
**P84 - Metabolic Pathways Triggered By Luteinizing Hormone In Luteal Cells.** Emilia Przygrodzka, Pan Zhang, Hou Xiaoying, Robert Powers, John Davis

Production of progesterone by the corpus luteum (CL) is fundamental for establishment and maintenance of pregnancy. Luteinizing hormone (LH) is crucial for the formation, function and maintenance of the CL, but the cellular metabolic changes induced by LH are unclear. We isolated highly steroidogenic small luteal cells (SLC) from bovine CL and treated them with LH (10 ng/ml) for 10, 30, 60 and 240 minutes. Cells and post-incubation media were harvested to determine metabolomics changes using GC/MS and LC/MS/MS platforms as well as NMR. Metabolomics changes were verified using Seahorse analysis. Additionally, glucose uptake was determined using bioluminescent method. Metabolomics data and results of Seahorse analysis as well as glucose uptake were analyzed using Welch’s two-sample t-test and t-student test, respectively. Analysis revealed 276 and 117 metabolites in cell extracts and media, respectively. Concentrations of progesterone, cAMP rapidly increased (p<0.05) in cells and media post-LH treatment. The content of lanosterol, a cholesterol precursor, was increased (p<0.05) in cells and media. Isocaproate, a product of CYP11A1, increased and cholesterol levels decreased (p<0.05) indicating cholesterol utilization for steroidogenesis. Glucose and fructose were significantly lower (p<0.05) in cells and media after LH treatment. Simultaneously, lactate was increased (p<0.05) in cells and media, while pyruvate was depleted (p<0.05). NMR analysis confirmed utilization of glucose by LH-treated SLC. Glutamine levels were depleted (p<0.05) from media and cells treated with LH. Additionally, glucose uptake was elevated (p<0.05) by cells incubated with LH. Seahorse XF Mito Stress Test showed LH stimulated (p<0.05) ATP production and basal oxygen consumption rate, an indicator of oxidative phosphorylation. Seahorse XF Glycolysis Stress Test indicated that LH enhanced glycolysis (p<0.05) and glycolytic capacity (p<0.05) in luteal cells. Metabolomics studies suggest that LH augments glycolysis supporting steroidogenic capacity of luteal cells. Supported by NIFA USDA 2017-67015-26450, VA and NIH R01HD092263.

**P85 - The role of FGF2/FGFR1 signaling on cumulus-oocyte complexes during in vitro maturation.** Chao Du, Hua Hua

Fibroblast growth factor 2 (FGF2) is a member of FGF family, and binds with high affinity to FGF receptor 1 (FGFR1) to initiate biological functions. The present study aimed to determine the expression patterns of FGF2 and FGFR1 and explore the role of FGF2/FGFR1 signaling on cumulus-oocyte complexes (COCs) during in vitro maturation (IVM). The results showed that the expression of FGF2 and FGFR1 mRNA increased during COCs maturation. Supplementation of FGF2 increased the rate of the first polar body extrusion, and inhibition of FGFR1 suppressed meiotic progress of oocytes. Moreover, inhibition of FGFR1 decreased the expression levels of genes c-mos [a regulatory subunit of mitogen-activated protein kinase (MAPK)] and cyclinb1 [a regulatory subunit of maturation/M-phase-promoting factor (MPF)]. Our results demonstrate that FGF2/FGFR1 signaling regulates oocytes meiosis by affecting MAPK and MPF activities.
Corpus luteum (CL) insufficiency is a multifactorial, endocrine disorder that is a substantial reason of early embryo mortality and implantation failure. To describe novel agents regulating equine CL function during early pregnancy we carried out global mRNA analysis using high throughput mRNA sequencing. Corpora lutea were obtained post-mortem at Day 8-12 after ovulation (n = 9) and from pregnant mares (n = 3/group) on Days 16-18 or 26-28 after insemination (DP). RNA isolated from CLs was used for library construction using TruSeq RNA Sample Prep v2 kit. The libraries were eventually sequenced in single 50-bp run (1×50bp) on the HiScanSQ system using TruSeq SBSv3 Sequencing kit. The differentially expressed genes (DEGs) were annotated in KOBAS 3.0 web server and analyzed in terms of their biological functions using Ingenuity pathway analysis (IPA). A total of 1116 and 102 DEGs were identified in CLs obtained from 16DP and 28DP as compared to mid-luteal CL, respectively. 824/1116 and 77/102 genes were successfully annotated. Functional analysis of DEGs revealed significant enrichment of molecular and cellular processes such as cell growth, lipid metabolism and molecular transport in pregnant CL and decrease in processes related to immune response. PTGER4, ACKR2, IL1RN (Z-score > 2.0) and IFNb, IL1b, PRL (Z-score < -2.0) were identified as upstream regulators of some of DEGs. Comparison of CL from 16DP versus 28DP revealed 540 DEGs of which 473 were annotated and linked to functions such as cellular movement, cellular growth and proliferation and inflammatory response. ADIPOQ, PRKAA1/2, CXCL12 (Z-score > 2.0) and TGF, VEGF (Z-score > -2.0) were identified as upstream regulators of many DEGs. These data reveal that changes in luteal transcriptome during early pregnancy is related to maintenance of luteal function and cell survival mechanisms.

Phthalates are used as solvents and plasticizers in a wide variety of consumer products. Most people are exposed to phthalates as parent compounds through ingestion, inhalation, and dermal contact. However, these parent compounds are quickly metabolized to more active compounds in several tissues. Although studies indicate that the phthalate metabolites reach the ovary, little is known about whether they are ovarian toxicants. Thus, this study tested the hypothesis that phthalate metabolites influence the expression of genes involved in cell division and cell death in mouse antral follicles in vitro. The selected metabolite mixture was based on concentrations in urine of pregnant women in the I-Kids study in Illinois; it included 36% monoethyl phthalate, 19% mono(2-ethylhexyl) phthalate, 15% monobutyl phthalate, 10% monoisopropyl phthalate, 10% monoisobutyl phthalate, and 8% monobenzyl phthalate. Antral follicles from adult CD-1 mice (32-42 days) were cultured in groups of 10-12 follicles for 96 hours with vehicle control (DMSO) or metabolite mixtures (0.1-500 mg/ml). Growth of follicles in culture was monitored every 24 hours. Total RNA was collected at 24 and 96 hours, extracted, and reverse transcribed. Real-time PCR was then performed for apoptosis-related genes Bax and Bcl2, and for selected cell cycle promoters, selected cell cycle inhibitors, and Ki67. The higher doses of the phthalate mixture inhibited follicle growth compared to controls. At the highest dose, the mixture
increased the Bax/Bcl2 ratio 4-fold above control, which is consistent with cell death. At the highest doses, the mixture decreased Ki67 expression compared to controls, which is consistent with a decrease in cell proliferation. Similarly, phthalate mixture metabolites at the highest doses decreased expression of a majority of cell cycle promoters and increased expression of a majority of cell cycle inhibitors. Supported by NIH T32 ES007326, NIH R56 ES025147, and NIH R01 ES028661.

**P88 - Investigating the impact of manganese supplementation on corpus luteum function.**
Jamie Studer, Zoe Kiefer, Aileen Keating, Lance Baumgard, Kristin Olsen, Zachary Rambo, Mark Wilson, Christof Rapp, Jason Ross

Functional corpora lutea (CL) are required for porcine pregnancy establishment and gestational maintenance, although CL function remains susceptible to environmental influences. Manganese (Mn) could be critical in regulating CL function since it is necessary for cellular function regulating Mn superoxide dismutase (Mn-SOD). We hypothesized that a more bioavailable dietary source would increase CL Mn content thereby influencing luteal function during the mid-luteal phase of the estrous cycle. Post-pubertal gilts (n = 32) were assigned to one of four gestation diets. The control diet (CON) met or exceeded NRC requirements and was formulated to contain 20 ppm added Mn in the form of Mn sulfate. Three additional diets included 20 (TRT1), 40 (TRT2) or 60 (TRT3) ppm of Mn from a Mn-amino acid complex (Availa-Mn; Zinpro Corporation) in place of Mn sulfate. Dietary treatment began at estrus synchronization onset and continued through D12 of the ensuing estrous cycle. Blood samples were collected at estrus onset (D0), D4, D8, and D12 and gilts were euthanized on D12. Luteal Mn content increased 19, 21 and 24% in CLs of gilts fed TRT1 (P = 0.06), TRT2 (P = 0.04), and TRT3 (P = 0.02), respectively, compared to CON. Relative to CON gilts, CL calcium (Ca) content decreased 36, 24 and 34% for TRT1 (P < 0.01), TRT2 (P = 0.07), and TRT3 (P = 0.01), respectively. Luteal content of copper, iron, magnesium, molybdenum, phosphorus, potassium, selenium, and zinc on D12 were unaffected by treatment (P ≥ 0.19) as were serum trace mineral levels (P ≥ 0.15). Serum progesterone increased (P < 0.001) from D0 through D12 but was unaffected by dietary treatment (P = 0.15) and there was no day x diet interaction (P = 0.85). Feeding a more bioavailable form of dietary Mn increases Mn and decreases Ca concentrations in luteal tissue.

**P89 - Proteomic analysis of the potential pathways mediating the temporal luteolytic responsiveness of porcine corpus luteum to prostaglandin F2alpha.** Karolina Lukasik, Pawel Likszo, Pawel Kordowitki, Dariusz Skarzynski, Beenu Moza Jalali
The estrus management and synchronization in pigs requires initiation of luteal regression induced by PGF2α. This can be however, achieved only on days 12 or more of estrous cycle. A single dose of PGF2α before day 12 is unable to induce luteolysis and regression of porcine corpus luteum (CL).

Aim of this study was to evaluate mechanisms by which porcine CL becomes responsive to external dose of PGF2α only on and after day 12 of estrous cycle. We used 2D-gel electrophoresis based proteomics and tandem mass spectrometry to address our aim. Protein lysates were prepared from luteal tissue slices collected on days 9 and 13 of estrous cycle (n = 4 each day) from estrus
synchronized pigs and incubated in medium with 1 mM PGF2a or without PGF2a (control) for 24 hours.
A total of 39 and 26 proteins revealed significantly altered abundance (P < 0.05 and fold-change of > 1.8 or < – 1.8) in PGF2a treated slices obtained from day 9 and 13 of estrous cycle and control, respectively. For biological functions analysis, identified proteins were subjected to Ingenuity Pathway analysis. PGF2a treatment resulted in decrease in abundance of proteins associated with cellular growth, oxidation stress and molecular transport on day 12. On day 9, proteins associated with cell function, protein synthesis and cell-cell signaling were induced by PGF2a. Decreased abundance of CD16 and MYC regulated proteins was observed only on day 12. A PGF2a induced expression of MAPK3, EIF3 and HSPb1 on day 9 and NME1 and ARHGDIA on day 12 might play a role in difference in responsiveness of CL to PGF2a.

In summary, dynamic differences in expression of proteins related to cell survival, acute phase response signaling and lipid metabolism in CL that are responsive versus refractory to the luteolytic actions of PGF2a were established.

**P90 - Synthetic agonist of PPARG inhibits dominant follicle development in cattle.** Juliana Ferst, Rogério Ferreira, Monique Rovani, Andressa Dau, Alfredo Antoniazzi, Bernardo Gasperin, Vilceu Bordignon, Dimas Oliveira, Paulo Bayard Gonçalves

The peroxisome proliferator-activated receptor gamma (PPARG, also called NR1C3) is a nuclear receptor of the peroxisome proliferator-activated receptor family (PPAR). PPARs are involved in the regulation of apoptosis, cell cycle, estradiol and progesterone synthesis, and metabolism. PPARG can be activated by synthetic ligands such as Thiazolidinediones (TZD, PPARG agonists). However, the regulation and function of this receptor during follicular development in monovular species is poorly understood. The aim of this study was to investigate the role of PPARG on development of follicle dominance using cattle as an in vivo model. First, cows (n=4 cows/day) had the estrous cycle synchronized and were ovariectomized on days 2 (before follicular deviation), 3 (follicular deviation) or 4 (after follicular deviation) relative to the follicular wave (Exp. 1). Follicular fluid and granulosa cells from two largest follicles were recovered to determine estradiol concentration and gene expression respectively. Second, cows had the emergence of a new follicular wave induced and when the largest follicle reached 7 to 8 mm an intrafollicular injection of 50 μM TZD (PPARG agonist) or saline was performed. The injected follicles were monitored daily by ultrasonography for three days (n=10; Exp. 2) or collected by ovariectomy at 24h after injection (n=11; Exp. 3). Differences in continuous data between treatments were assessed by paired Student’s t test (Exp. 1) or one-way ANOVA (Exp. 2 and 3). PPARG mRNA was not differentially regulated in dominant and subordinate follicles around deviation. When PPARG agonist (TZD) was intrafollicularly injected the follicles stopped growing and follicular size curve was statistically different from control group. The stimulation of PPARG inhibited follicular growth of all treated follicles and selectively downregulated CYP19A1 mRNA. In conclusion, PPARG activation downregulates CYP19A1 and inhibits dominant follicle development, suggesting that PPARG plays a role in the regulation of follicle growth and steroidogenesis.
**P91 - Dysregulated androgen-induced exosomal mir-379-5p release determines granulosa cell fate.** Reza Salehi, Brandon Wyse, Yunping Xue, Yoko Urata, Jose Vinas, Sahar Jahangiri, Kai Xue, Kevin Burns, Dylan Burger, Clifford Librach, Benjamin Tsang

We previously demonstrated androgenized rats exhibit lower granulosa cell (GC) rno-mir-379-5p expression, increased phosphoinositide-dependent kinase-1 (PDK1) content and proliferation. DHT reduced GC rno-mir-379-5p content by selectively packaging it into exosomes in GC from pre-antral follicle (PAF) but not in antral follicle (AF) *in vitro*. Increased exosomal rno-mir-379-5p in DHT-treated PAF GC was associated with increased AKT-mediated cell proliferation. However, the roles of exosome secretion in regulating cellular miRNAs content in exosome-secreting cells are unclear. We hypothesize that androgens regulate mir-379-5p exosomal content follicular stage-dependently, a process which determines granulosa cell fate. Our objectives are to determine whether: (a) inhibition or induction of exosomal release regulates cellular mir-379-5p and PDK1 content differentially in PAF and AF GC; (b) androgens regulate exosomal uptake and mir-379-5p cellular content; (c) the above observations are recapitulated in human samples by examining the extracellular vesicle content of mir-379-5p in follicular fluid from PCOS and non-PCOS patients. Inhibition of exosome release in PAF GC with GW4869 resulted in increased cellular rno-mir-379-5p content and reduced PDK1 and MCM2 (a cell proliferation marker) in the presence of DHT, suggesting that androgen-induced exosomal rno-mir-379-5p release may be a regulatory mechanism for granulosa cell PDK1 content and proliferation. Cortactin is involved in exosomal release and its overexpression in DHT-treated AF GC increased exosomal rno-mir-379-5p release and reduced its cellular content. DHT treatment failed to influence PAF GC exosomal uptake, but exosomes enriched in rno-mir-379-5p suppresses DHT-induced proliferation in PAF GC. In addition, hyperandrogen PCOS patients had lower hsa-mir-379-5p expression in microvesicles and exosomes from human follicular fluid compared to non-PCOS patients. These findings suggest that mir-379-5p inhibits PDK1-mediated granulosa cell proliferation, and androgen-induced mir-379-5p exosomal release removes its inhibitory action on PDK1, a survival mechanism specific for PAF GC (Supported by a CIHR grant and the Lalor Foundation Postdoctoral Fellowship).

**P92 - Genetic Variants Identified in Cows with an Excess Androgen Ovarian Microenvironment Provides Clues to Women with Polycystic Ovary Disease.** Alexandria Snider, Sarah Nafziger, Jeff Bergman, Scott Kurz, John Davis, Jennifer Wood, Jessica Petersen, Andrea Cupp

A naturally-occurring cow model of androgen excess (High Androstenedione; High A4) shares many metabolic and molecular phenotypes of Polycystic Ovary Syndrome (PCOS). High A4 cows have irregular estrous cycles, anovulation, and reduced calving rates but wean 24 kg heavier calves. Since producers retain females with heavier offspring to remain in the herd, it is possible High A4 cows have been preferentially selected. Thus, we hypothesize females with a PCOS-like phenotype have genomic variation linked to steroidogenic and metabolic phenotypes comprising the High A4 phenotype. To test this hypothesis, we genotyped 200 individuals with the GeneSeek Genomic Profiler Bovine 150K SNP array. While no loci achieved a genome-wide significance threshold determined by FDR of 0.01, 70 genes within 25 kb of genetic variants associated (raw p<0.01) with the High A4 phenotype were prioritized for investigation. Additionally,
approximately 17 million variants were identified in whole-genome sequence of 15 individuals (8 High A4, 7 Control). Annotation with SNPEff found 4,600 of these with predicted high impact. High impact variants were identified in seven of 70 genes identified in the initial GWAS. We have assayed expression of these, **CARN1**, **CCR6**, **GPR31**, **ALKBH6**, **CLIP3**, **SREBP1a** and **SREBP1c**, via ddPCR in ovarian cortex, theca, and granulosa cells, in High A4 compared to controls cows. In High A4 ovarian cortex, **SREBP1c** and **CLIP3** were significantly upregulated compared to controls (p≤0.05). There was a tendency (p≤0.10) for **CARN1** and **CCR6** to be downregulated in High A4 granulosa cells. These genes are involved in inflammation or oxidative stress, two processes upregulated in the High A4 phenotype. Understanding genetic variants that predict the High A4 phenotype can provide a potential selection tool for producers. Also, these genetic variants could allow us to better understand metabolic, growth and reproductive trait interactions in human disease like PCOS.

**P93 - Steroidogenic Factor 1 Is Essential For Reproductive Function In Mature Female Mice.** Olivia Eilers Smith, Marie-Charlotte Meinsohn, Fanny Morin, Bruce Murphy

As a growing population of women and couples choose to have children later in life, the lack of information on the causes of age-related infertility represents a major gap in our knowledge of reproductive biology. An integrated understanding of ovarian processes must be addressed using global analyses in order to develop innovative treatments for female infertility. The orphan nuclear receptor steroidogenic factor-1 (SF-1 or Nr5a1) has been identified as an indispensable regulator of ovarian follicle growth and ovulation. While it has been shown that granulosa cell-specific depletion of SF-1 in mice results in hypoplastic ovaries, impaired ovulation and infertility, its specific role in mature ovarian function remains to be determined. Using a progesterone receptor-related recombinase-Cre (Pgr), we have developed a new murine model characterized by conditional depletion of SF-1 (PgrCre-SF1f/f; cKO, SF1f/f; CON) in important reproductive tissues of the mature female; the pituitary gland and the ovary. The histological analysis of these tissues as well as steroidogenic gene and protein expression demonstrate that absence of SF-1 in peri-ovulatory events leads to smaller ovaries, absence of ovulation, impaired corpus luteum formation and infertility. Although sufficient to induce regular estrous cycles and normal sexual behaviour, reduced gonadotrope subunit synthesis by the pituitary gland is observed in cKO mature females. Exogenous delivery of hormones to induce ovulation does not successfully restore fertility in these cKO females, where dysregulated expression of steroidogenic enzymes and luteinization factors is observed in the ovary, resulting in impaired progesterone synthesis. This indicates the importance of SF-1 in ovulation, luteal function and pregnancy maintenance in mice. This novel model of female infertility demonstrates the critical role that SF-1 plays in reproductive function, identifying it as a potential target to help develop new targeted treatments for infertility in women.

**P94 - Neuregulin-1β (NRG1β), an anti-inflammatory peptide, signaling in granulosa Cell (GC) physiology.** Indrajit Chowdhury, Saswati Banerjee, Wei Xu, Sameer Mishra, Winston Thompson
Our previously published (PMID: 28938399) studies have demonstrated that NRG1β, a member of the epidermal growth factor family of proteins, is gonadotropin-dependent and progressively expressed in GCs during follicular maturation, and secreted in ovarian follicular fluid in the rat. Moreover, the gonadotropins-dependent expression of NRG1β is positively correlated with the synthesis and secretions of estradiol and progesterone. NRG1 is known to have neuroprotective properties with the suppression of pro-inflammatory gene expression in the brain. However, the detailed mechanism associated with the interplay of NRG1β and its receptors in GC function is not known. Therefore, we investigated the role and molecular pathways linked to the action of NRG1β and its receptors in the regulation of inflammatory cytokines and chemokine production, and support GCs survival. To understand the role of NRG1β in the regulation of pro-inflammatory gene expression in GCs, we employed siRNA interference to knock-down endogenous NRG1β in GCs collected from 25 days-old immature female rats treated with PMSG. Cell morphology was monitored by Phase contrast microscopy and revealed that changes in cell shape indicative of a differentiation phenotype occurred in cells transduced with scrambled shRNA and treated with LH. NRG1β knockdown GCs treated with LH showed increased levels of pro-inflammatory cytokines and chemokine production including tumor necrosis factor-α (TNFα), and increased activation of NFkβ-pathway with enhanced sensitization of these GCs to the induction of apoptosis. Further studies have shown that the treatment of GCs with exogenous TNFα promoted apoptosis in a dose and time-dependent manner. In contrast, the effects of TNFα was attenuated in the presence of exogenous-NRG1 in a dose and time-dependent manner through the increased levels of the anti-apoptotic proteins and activation of ErbB2-ErbB3-PI3K-Akt signaling pathway. Taken together, these studies provide new evidence supporting LH-dependent NRG1β upregulation in GCs is required to sustain GC functions.

(P95 - Molecular profiling demonstrates active luteal rescue in the cow and implicates calcium signaling, immune pathways, and retinoic acid biosynthesis. Camilla Hughes, E. Inskeep, Joy Pate

One view of ruminant luteal rescue implies passive maintenance of the corpus luteum (CL), but it is known that CL of pregnancy differ from CL of the estrous cycle. In addition to alteration of uterine signals, are changes within the CL required for luteal rescue during maternal recognition of pregnancy? To investigate this question, mRNA, proteins, and miRNA were profiled in CL from days 14, 17, 20, and 23 of pregnancy (n = 4/day). Among 15806 RNA identified by next generation sequencing, 1157 were differentially abundant (DA; Padj<0.15, likelihood ratio test in DEseq2). Pathway analyses (IPA; Qiagen) indicated inhibition of calcium, AMPK, thrombin, and MMP signaling over time, as embryonic signaling increased. Conversely, interferon signaling and DNA repair mechanisms seemed to be activated. Activation of immune-related pathways peaked at day 20 and subsequently declined. Among 992 proteins quantified by iTRAQ-based proteomics, retinal dehydrogenase (ALDH1A1) increased over time (Padj<0.15, Kruzkal-Wallis test in Scaffold). ALDH1A1 catalyzes a step in the conversion of beta-carotene, a highly abundant luteal carotenoid, to retinoic acid. Luteal steroidogenic cells (n=6) were treated with A37, an ALDH1A1 inhibitor (10 uM) and progesterone production was reduced compared to the control (P<0.01), indicating that increased ALDH1A1 may support progesterone production in pregnancy. Nanostring profiling detected 204 miRNA, among which 27 were DA (Padj<0.15,
ANOVA in R). Those that increased may target DA proteins associated with calcium homeostasis, while those that decreased were associated with regulation of LDL clearance. Together, these data indicate inhibition of calcium signaling and modulation of immune response in the CL during early pregnancy. Additionally, retinoic acid supports progesterone production and may be necessary for luteal rescue. This project was supported by USDA/Agriculture and Food Research Initiative Predoctoral Fellowship no. 2017-67011-26062 to CKH, and conducted as part of Multistate Project NE1727.

**P96 - Doxorubicin Obliterates Female Mouse Ovarian Reserve Through Promoting Primordial Follicle Atresia and Overactivation.** Yingzheng Wang, Mingjun Liu, Sarah Johnson, Gehui Yuan, Alana Arriba, Maria Zubizarreta, Shuo Xiao

Ovarian toxicity and infertility are major off-target effects of cancer therapy for young female cancer patients. We and others have demonstrated that doxorubicin (DOX), a widely used anti-cancer agent, has a dose-dependent toxicity on growing follicles. However, it remains unknown if the primordial follicles are the direct or indirect target of DOX. Twenty-one-day-old CD-1 female mice were intraperitoneally injected with PBS or clinically relevant of DOX at 10 mg/kg once and ovaries were harvested on days 1, 4, 7, and 14 for TUNEL assay, histology, and follicle counting. DOX significantly increased the number of atretic growing follicles and no corpus luteum was found in DOX-treated ovaries on day 14. Follicle counting results indicated that the numbers of primordial, secondary, and antral follicles in DOX-treated ovaries were significantly reduced. However, the number of primary follicles was reduced by 27.45% on day 1 but was greatly increased by 74.39% from day 1 to day 4, indicating that some primordial follicles might be activated. Additionally, the DOX-treated ovaries on days 1, 4, and 7 contained a significantly larger amount of early-growing primordial follicles, which were characterized by an enlarged oocyte surrounded by one layer of flattened pre-granulosa cells. We continued to treat 5-day-old CD-1 female mice with same exposure level of DOX as described above to further investigate the mechanism of DOX-induced primordial follicle loss, because their ovaries have a more uniform primordial follicle population. The result of follicle counting demonstrated that DOX treatment could also decrease the ovarian reserve in 5-day-old mice. Furthermore, immunostaining results revealed that DNA damage - TAP63a pathway was involved in DOX-induced primordial follicle apoptosis and Foxo3a was involved in DOX-induced primordial follicle overactivation. Taken together, these results demonstrated that the prepubertal DOX exposure obliterates mouse ovarian reserve through both primordial follicle atresia and overactivation.

**P97 - Effect of Heat Stress on the Corpus Luteum Proteome During Early Pregnancy Establishment in Pigs.** R Schultz, Katie Bidne, Matthew Romoser, Malavika Adur, Lance Baumgard, Aileen Keating, Jason Ross

Seasonal infertility is caused by chronic exposure to heat stress (HS), but the molecular mechanisms underpinning the phenotype are ill-defined. The porcine corpus luteum (CL) is required to establish and maintain pregnancy, and reduced CL viability and functionality is observed during HS. To test the hypothesis that HS during CL formation and early diestrus alters
the CL proteome on D12 following estrus, the study objectives were to identify differentially abundant proteins in CLs from gilts subjected to HS following ovulation and during the luteal phase. After behavioral estrus was observed, gilts were randomly assigned to diurnal HS (n = 12; 35 ± 1°C for 12 h/31.6 ± 1°C for 12 h) or thermoneutral (TN; n = 14; 21 ± 1°C) conditions 2d post-estrus (dpe) until 12dpe. A subset of gilts (TN: n = 7; HS: n = 5) were artificially inseminated before thermal treatment began to determine pregnancy’s effect on CL protein abundance. Protein was extracted from CLs (n = 3-4 per animal) and liquid chromatography-mass spectrometry (LC-MS/MS) was performed. A total of 1470 unique proteins were identified between the four groups. Thermal treatment and pregnancy resulted in 134 and 127 differentially abundant (P ≤ 0.05) proteins, respectively. Of the differentially abundant proteins related to thermal treatment, almost half (55/134) assembled into three protein classes: oxidoreductases, hydrolases and nucleic acid binding. Many of the differentially abundant proteins identified (e.g. SOD1, CAT, and HSPB1) are directly related to cellular stress response, the balance of reactive oxygen species, and protein folding. These processes play a crucial role in CL regulation and luteolysis, and mirror the negative effects of HS seen in other tissues and species. This project was supported by the Iowa Pork Producers Association and the AFRI Competitive Grant no. 2017-67015-26459 from the USDA-NIFA.

PP98 - Core Binding Factors are essential for ovulation, luteinization, and female fertility in mice. Somang Lee-Thacker, Yohan Choi, Hayce Jeon, Ichiro Taniuchi, Takeshi Takarada, Yukio Yoneda, CheMyong Ko, Misung Jo

Core Binding Factors (CBFs) are a small group of heterodimeric transcription factor complexes composed of DNA binding protein, RUNX (RUNX1, RUNX2, RUNX3) and a non-DNA binding protein, CBFβ. The expression of Runx1 and Runx2 is highly induced by the LH surge in ovulatory follicles, while Cbfβ is constitutively expressed. To investigate the physiological significance of CBFs, we generated a conditional mutant mouse model in which granulosa cell expression of Runx2 and Cbfβ was deleted by Esr2 Cre recombinase. Female Runx2gc-/-*Cbfβgc-/- mice were infertile and exhibited an irregular pattern of estrus cycles. In response to gonadotropin stimulation, follicles developed normally to the preovulatory follicle stage and underwent cumulus cell expansion, but failed to ovulate in mutant mice. Similarly, ovaries of 5-month-old mutant mice collected in the morning (1100h) of estrus displayed several large antral follicles with expanded COCs and were devoid of corpora lutea. RNA-seq analysis of mutant ovaries collected at 11h post-hCG unveiled 270 and 73 differentially down and up-regulated genes (2-fold, q<0.05), respectively. qPCR and in situ hybridization analyses verified differential expression of known and novel CBF-downstream genes that are associated with inflammation (Edn2, IL6, IL11, Ptgs1, Ptgs2, Ptgfr, Fabp4), matrix remodeling (Has1, Cldn18, Serpine1, Adams1, Adams4, Adams5, Timp1), Wnt signaling (Sfrp4, Wnt4, Wnt10b), and steroid metabolism (Ark1c18, Parm1, Hsd17b1, Cyp17a1, Cyp19a1). Analysis of ovaries collected consecutive days after ovulation indicated the failure of corpus luteum formation as evident by the lack of luteal marker gene expression (Lhcgr and Prlr), reduction of vascularization (PECAM-1), and excessive apoptotic staining (TUNEL) in the resultant poorly differentiated luteal structure, consistent with rapid reduction of serum progesterone. The present study provides in vivo evidence that CBFs play an essential role in ovulation and luteinization by
regulating the expression of specific key ovulatory and luteal genes in mice. Supported by P01HD71875 and R03HD095098.

**P99 - FOS/AP-1 is a Critical Transcriptional Regulator in the Ovulatory Process in the Human Ovary.** Yohan Choi, Mats Brännström, James Akin, Thomas Curry Jr, Misung Jo

FOS is a subunit of activator protein-1 (AP-1) transcription factor and functions by forming heterodimers with one of the JUN family proteins (JUN, JUNB, JUND). Fos null mice failed to ovulate even when given exogenous gonadotropins, suggesting that FOS/AP-1 plays a critical role in ovulation. Previously, we demonstrated that the expression of FOS and JUNs increases in human ovulatory follicles. The present study determined regulatory mechanisms controlling the expression of FOS and JUNs and function of the FOS/AP-1 using a revitalized human granulosa/lutein cell model. hCG increased FOS expression in a biphasic manner; the expression increased within 1h but decreased by 6h. The second rise was observed at 12h, but then the levels returned to control levels by 24h. Unlike FOS, hCG transiently increased JUNB mRNA levels only at 1h but had no effect on the levels of mRNA for JUN and JUND at any time points assessed. In contrast, Western blots revealed increases in all JUN proteins in hCG-treated cells although the temporal patterns are slightly different among them. The co-immunoprecipitation analysis showed the binding of FOS with all JUN proteins in hCG-treated cells, suggesting the presence of multiple forms of FOS/AP-1. Inhibitors of PKA (H89) and ERK (SCH772984) abolished hCG-induced increases in FOS, JUN, and JUNB proteins, revealing the involvement of the PKA and ERK pathways. Functionally, T-5224 (FOS inhibitor) reduced hCG-increased expression of genes involved in cell proliferation, differentiation, and metabolism (CCNA1, CDK16, FGF10, ID2, MRO, SOX9), cumulus expansion (PTX3), progesterone receptor signaling (PGR), and prostaglandin synthesis (PTGES) and transport (ABCC4, SLCO2A1). ChIP assay showed the direct binding of FOS to the specific site in promoter regions of selective key ovulatory genes. Based on these results, we concluded that FOS/AP-1 is involved in ovulatory changes in human granulosa cells. Supported by P01HD71875 and R03HD088866.

**P100 - Early Reduced Growth Rates Predict Delayed or Altered Puberty and May Adversely Affect Reproductive Longevity in Beef Heifers.** Jessica Keane, Sarah Nafziger, Mohamed Abedal-Majed, Sarah Tenley, Mariah Hart, Jeff Bergman, Scott Kurz, Jennifer Wood, Adam Summers, Andrea Cupp

A population of cows that have excess androstenedione (A4; High A4) in follicular fluid of dominant follicles and secreted from ovarian cortex media (30-fold>controls) has been identified. High A4 cows have similar theca molecular phenotypes to women with polycystic ovary syndrome (PCOS) including: irregular estrous cycles, increased ovarian inflammation, and reduced fertility. Because PCOS is identified at puberty, the manner heifers attain puberty was investigated. Heifers were classified using progesterone (P4) concentrations ≥ 1ng/ml to initiate puberty and continued cyclicity: 1) Typical- 378±2 day of age (DOA) (n=279); 2) Early- 317±4 DOA (n=143); 3) Start-Stop- P4 ≥1ng/ml at 265±4 but discontinued cyclicity (n=91); 4) Non-Cycling heifers- no occurrence of P4 ≥ 1ng/ml during sampling period (n=98). Start-Stop and Non-Cycling heifers also had excess A4 secretion from ovarian cortex cultures similar to High
A4 cows. Thus, our hypothesis was that early growth traits may be adversely altered in Non-Cycling and Start-Stop heifers leading to reduced reproductive maturation and performance compared to Early/Typical heifers. Weaning weight (p=0.017) was reduced in Start-Stop heifers and yearling weights (p=0.0074) was reduced in Start-Stop and Non-Cycling compared to Typical/Early heifers. Non-Cycling heifers had the greatest Antral Follicle Counts (p<0.0001) with reduced uterine horn diameter (p=0.0053) compared to Typical (Control, p<0.0001) heifers. There was a lower proportion of Start-Stop and Non-Cycling heifers with a reproductive tract score of 5 compared with Typical/Early heifers, and reduced response to prostaglandin synchronization resulting in fewer calves in the first 21 days of the breeding season in the Non-Cycling group. Interestingly, heifers in the Start-Stop group that do not regain cyclicity have similar growth and reproductive traits as the Non-Cycling heifers. Taken together, reduced growth and maturation observed in the Start-Stop and Non-Cycling heifers is initiated early in development and adversely affects timing of reproductive maturity and longevity in these heifers.

**P101 - Developmental programming: Prenatal testosterone-induced epigenetic modulation and its effect on gene expression in sheep ovary.** Niharika Sinha, Muraly Puttabyatappa, Vasantha Padmanabhan, Aritro Sen

Altered nutrition or intra-uterine exposure to adverse conditions during fetal development can lead to epigenetic changes in fetal tissues, predisposing those tissues to diseases that manifest when offspring become adults. Various animal models as well as epidemiological studies in humans show that prenatal androgen exposure may be an underlying cause of development of Polycystic ovary syndrome (PCOS) later in life. In women, PCOS is a common fertility disorder with comorbid metabolic dysfunction. In this study, using a sheep model, we elucidate the epigenetic changes induced by prenatal (30-90 day) testosterone (T) exposure and its effect on gene expression in fetal (90d) and adult (2yr) ovaries. We find that histone markers H3K4me3, H3K27ac, H3K9ac (gene inducing marks), H3K27me3 and H3K9me3 (gene silencing marks) to be downregulated in the prenatally androgenized fetal ovary compared to controls. In contrast, these markers were upregulated in the adult ovary of prenatally androgenized animals. We also looked at the corresponding epigenetic modulators that specifically regulates these epigenetic marks. Intriguingly, while there was no change in EZH2 (tri-methylates H3K27) levels, p300 (histone acetyltransferase) was downregulated both in fetal (90d) and in adult (2yr) ovaries. Histone demethylases, JMJD2b (demethylates H3K4) and JMJD3 (demethylates H3K27) were also downregulated in fetal ovaries but were not expressed in the adult. Furthermore, through RNA-seq studies we identified 298 upregulated and 566 downregulated genes in adult and 31 upregulated and 19 downregulated genes in fetal ovaries (T vs Control). Pre-natal androgen downregulates intraovarian steroidogenic genes (STAR, HSD3β, CYP17A1, CYP19A1). Currently, using these genes as endpoints, we are performing chromatin immunoprecipitation studies to determine prenatal testosterone-induced epigenetic modulation of the steroidogenic pathway in the ovary. This study provides mechanistic insights into prenatal androgen-induced developmental programming and its manifestation to ovarian dysfunction and development of PCOS in adult life.
**P102 - Quantification of KIT Ligand and Leukemia Inhibitory-Factor Expression in Multilayer and Small Antral Follicles in Cattle and Nonhuman Primate Ovaries.** Wilson Simmons, Shaina Jachter, Cecily Bishop

Disrupted follicular steroidogenesis is associated with persistent follicles in humans (polycystic ovary syndrome - PCOS) and in cattle (cystic ovary disease - COD). By investigating factors contributing to theca cell transformation and function, better therapies for these diseases may be developed. Both KIT ligand (KITLG) and leukemia inhibitory factor (LIF) may regulate theca cell differentiation and proliferation. To inform latter in vitro experiments, descriptive immunohistochemical analysis was performed on ovarian tissues of bovine (Bos taurus) and primate (Macaca mulatta) species. Paraffin-embedded tissues from rhesus macaques were obtained through ONPRC Tissue Distribution Program, bovine ovarian sections were purchased from a commercial source (AMSBIO). LIF expression in antral and multilayered follicles was quantified via corrected cell fluorescence (CTCF) using ImageJ. KITLG expression was quantified as present or absent for multilayer and small antral follicles (ImageJ cell counter). Each species had 100 follicles surveyed/protein. Positive staining for KITLG was detected in 69.2% of bovine and 31.6% of primate multilayered follicles. In bovine and macaque small antral follicles KITLG expression was present, but staining was minimal. In contrast, staining for the KITLG receptor c-Kit was extensive in granulosa and theca cells of small antral, but not detected in multilayer follicles. Secreted LIF was detected in multilayer follicles of both species at similar levels. However, LIF expression was absent in small antral follicles of both species. Further analysis of LIF receptor staining in follicles is ongoing. These factors appear to be suitable targets for future in vitro investigations into processes driving multilayer to antral follicle transition in 3D cultures in both cattle and macaque tissues. This work was supported by the USDA National Institute of Food and Agriculture, Hatch/Multi State project #1017763.

**P103 - Integrated analysis of genome-wide gene expression and histone modification in mouse granulosa cells undergoing luteinization during ovulation.** Yuichiro Shirafuta, Isao Tamura, Haruka Takagi, Ryo Maekawa, Hiroshi Tamura, Norihiro Sugino

We previously reported using RNA-sequence that gene expressions rapidly and dramatically change in granulosa cells (GCs) undergoing luteinization during ovulation after the LH surge, suggesting that GCs rapidly acquire a variety of functions during the luteinization. We also reported that histone modifications are involved in the regulation of steroidogenesis-related gene expressions, such as StAR, Cyp11a1 and Cyp19a1. However, genome-wide changes of histone modification in GCs undergoing luteinization are unclear. Here, we genome-wide investigated the change of H3K4me3 (active mark) by ChIP-sequence in mouse GCs undergoing luteinization. 3-week-old mice were injected with eCG followed by hCG. Luteinized GCs were obtained before (0h) and 4h, 12h after injection. Major changes were that H3K4me3 increased between 0h and 4h and decreased between 4h and 12h. Up-regulated genes with H3K4me3-increased regions at 4h showed significantly higher increase in mRNA expressions than those of up-regulated genes without H3K4me3-increased regions. Down-regulated genes with H3K4me3-decreased genes between 4h and 12h showed higher decrease than those of down-regulated genes without H3K4me3-decreased regions. These results suggest that the changes of H3K4me3 are associated with the changes of mRNA expressions during luteinization. Up-regulated genes
with H3K4me3-increased regions at 4h were associated with “MAPK pathway”, “Ovarian steroidogenesis” and "VEGF signaling pathway”, which are well-known activated pathways for the initial step of luteinization. Interestingly, some pathways involved in the regulation of cell morphology of GCs, such as “Hippo signaling pathway” were also found. Down-regulated genes with H3K4me3-decreased regions were associated with inhibition of apoptosis, which may contribute to cell survival for luteinization. In conclusion, our study showed that the changes in H3K4me3 were observed in a variety of regions in GCs during luteinization, which was closely associated with the change in mRNA expressions. Regulation of gene expressions by histone modifications contribute to the luteinization of GCs after the LH surge.

**P104 - RHOA is associated with ovarian follicular dynamics and regulated by activation of PKC-btea1.** Vaibhave Ubba, Swati Rajput, Rajesh Jha

Polycystic ovary syndrome (PCOS) is an endocrine disorder, affecting 5 to 10% of women during reproductive age. PCOS is characterized by anovulation, hyperandrogenized and multiple follicular cyst. The pathology of PCOS is not understood precisely. We previously reported the downregulation of RHOG and RAC1 in DHEA-induced PCOS ovary. Research findings have documented the presence of RHOA in luteal cells and involvement in progesterone synthesis, whereas the PKC has been reported in luteinization and oocyte maturation. Therefore, we speculated that PKC-RHOA signaling might be associated with ovarian follicular dynamics and PCOS pathogenesis. Thus, we investigated the involvement of RHOA and PKC-beta1 in ovarian folliculogenesis during estrous cycle in 90 days old and PCOS model in 21 days old *Sprague-Dawley* rats by employing western blotting and Immunohistochemistry. We found the upregulation of RHOA during the proestrus and estrus stages of estrous cycle (n=5) indicating its involvement during ovarian folliculogenesis and ovulation. Importantly, RHOA was primarily expressed in the oocyte, corpus luteum and thecal cells with mild appearance in GC cells. The PKC-beta1 was elevated during the diestrus, proestrus stage and optimum at estrus stage indicating PKC-beta1 importance in follicle development and ovulation. In the ovary of hyperandrogenized model of PCOS (n=5), PKC-beta1 (p<0.05), RHOA (p<0.005), phosphorylated RHOA (p<0.05), and PTEN (p<0.05) were downregulated, which suggest that the PKC-RHOA signalling is operational in the ovary. Further, we investigated the regulation of RHAO activation in PKC dependent manner in rat granulosa cell line (GC). The PKC-beta activity inhibition by its pharmacological inhibitor (Hispidin) in the rat GC line (n=5) resulted compromised expression level of RHOA (p<0.005), phosphorylated RHOA (p<0.05), PTEN (p<0.005), and RAC1 (p<0.05). These findings indicate the regulation of RHOA, PTEN and RAC1 signaling by PKC-beta1 in granulosa cells, and their dysregulation might be associated with PCOS.

**P105 - Amphiregulin Can Regulate the Expression of Cyp17a1 in Cultured Mouse Theca Cells.** Hanako Kakuta, Tomomi Sato

In ovarian theca cells, it is well known that CYP17A1, encoded by *Cyp17a1*, catalyzes progesterone to produce androgen. The expression of *Cyp17a1* is induced by lutenizing hormone
(LH), however, not many studies show that other factors can influence the expression of Cyp17a1.

Amphiregulin (AREG) is one of epidermal growth factor (EGF) like ligands induced by the LH surge in granulosa cells, and it induces ovulation related events such as the expansion of the cumulus cells-oocyte complex via phosphorylation of ERK1/2. Our previous study reveals a correlation between phosphorylation of ERK1/2 in granulosa cells and localization of CYP17A1 in theca cells after the hCG injection. In this study, the effects of AREG on regulation of Cyp17a1 was examined using cultured mouse theca cells.

Theca cells were isolated from immature female ICR mice, pre-cultured for 24 hours in 10% FBS in RPMI medium, and then cultured in serum free medium with or without LH (1-100 ng/ml) for 48 hours. The medium was changed and theca cells were cultured for additional 24 hours. Since an increase of Cyp17a1 expression by LH was determined at 48 hours and 72 hours in culture by real-time RT-PCR, this primary culture system of mouse theca cells was used for further experiments. Next, theca cells were cultured in serum free medium for 48 hours with or without LH (100 ng/ml), and AREG (0.1-100 ng/ml) was added to the medium for 24 hours.

AREG (10-100 ng/ml) suppressed an increase of Cyp17a1 expression by LH, whereas the effect of AREG was cancelled when AG1478 (10 nM) was added to the medium containing AREG and LH. Low doses of AREG (0.1-1 ng/ml) did not affect the expression of Cyp17a1. These results indicate that AREG can regulate the expression of Cyp17a1 in cultured mouse theca cells via EGF receptor.

P106 - Do activin A and FOXL2 co-regulate gene expression in ovarian somatic cells?
Andrew Childs, Pablo Hurtado Gonzalez, Rebecca Sumner

The formation and development of ovarian follicles is dependent upon extensive interactions between somatic (pre-)granulosa cells and developing oocytes, mediated by growth factor signals. Activin A has been shown to delay follicle assembly in mice, and regulates the production of key growth factors (such as BDNF and KL) by human fetal ovarian somatic cells, suggesting a role for activin signalling in early follicle development. In the pituitary, the activin-mediated induction of Fshb expression is dependent on the transcription factor FOXL2. As FOXL2 is also a key regulator of granulosa cell function and follicle development, we are investigating whether activin-SMAD2/3 and FOXL2 similarly converge to co-regulate gene expression in ovarian somatic cells.

SMAD2/3 was detected by fluorescence immunocytochemistry and western blotting in cultures of human fetal ovarian somatic cells. SMAD2/3 localisation was unchanged with human recombinant ActA treatment (50ng/ml), but nuclear localisation appeared reduced by treatment with ALK4/5/7 inhibitor SB431542 (0-10µM). To investigate ActA/FOXL2 co-regulation of gene expression, COV434 granulosa tumour cells were transfected with a FLAG-tagged FOXL2 expression vector, treated with ActA or vehicle, and expression assayed by RT-qPCR 24 hours later. An increase in putative ActA-regulated gene expression was detected following ActA treatment (TGFBI, FST and SERPINE2), and preliminary data indicate that ActA and FLAG-FOXL2 together increase SERPINE2 expression in COV434 cells to a greater extent than either factor alone. Consistent with this, endogenous SMAD3 could be co-immunoprecipitated with FLAG-tagged FOXL2 from transfected COV434 cells, suggesting that these proteins interact
directly in ovarian somatic cells. Together, these data suggest activin-SMAD2/3 and FOXL2 may co-regulate gene expression in ovarian somatic cells, and provide a basis for studies to explore how this interaction regulates granulosa cell function and follicle development. Supported by the UK Biotechnology and Biological Sciences Research Council.

**P107 - Balanced expression and activation of YAP1 in granulosa cells are vital for ovarian follicle development.** Xiangmin Lv, Cong Huang, Li Chen, Hongbo Wang, Cheng Wang

The Hippo signaling pathway controls organ size by regulating the downstream effectors such as YAP1 and TAZ. The role of YAP1 in development and tumorigenesis have been extensively studied, but its role in ovarian follicle development is largely unknown. The objective of this study was to examine the role of YAP1 in the ovarian granulosa cell functionality and follicle development. Several cellular models, including cultured primary human granulosa cells (hGCs), KGN and HGrC1 cell lines, were used to examine the role of YAP1 in granulosa cell proliferation and differentiation. Granulosa cell-specific Yap1 knock-in and knock-out mouse models were created to examine the role of Yap1 in follicle development. We found that the active form of YAP1 (nuclear YAP1) was predominantly expressed in proliferative granulosa cells, whereas the inactive form of YAP1 (cytoplasmic YAP1) was mainly detected in luteal cells (terminally-differentiated granulosa cells). Manipulating the expression and activation of YAP1 in cultured hGCs or cell lines significantly impacted the proliferation, differentiation and steroidogenic ability of these cells. Pharmacological inhibition of YAP1 activity disrupted mouse ovarian follicle development in vitro and in vivo. Foxl2 promoter-driven knockout of Yap1 in ovarian granulosa cells resulted in reduced ovarian size, decreased number of corpus luteum, increased apoptosis of granulosa cells, and severe subfertility. Interestingly, Cyp19 promoter-driven knockout of Yap1 in the luteinized granulosa cells had no effect on ovarian morphology and mouse fertility. Mechanistic studies demonstrated that YAP1 may interact with EGFR and TGFβ signaling pathways to regulate granulosa cell proliferation, differentiation, and survival. Our research results demonstrate that balanced expression and activation of YAP1 in granulosa cells is essential for follicle development and successful reproduction. YAP1 represents a promising target for the treatment of subfertility associated with abnormal granulosa cell function.

**P108 - Transcriptome of the corpus luteum of pregnant dairy cows during secretion of interferon-tau: implications for luteal maintenance.** Megan Mezera, Wenli Li, Rina Meidan, Caio Gamarra, Rodrigo Gennari, Andrea Edwards, Alexandre Prata, Milo Wiltbank

The corpus luteum (CL) is required for pregnancy maintenance in cattle, but in non-pregnant animals, it regresses near day 18 of the cycle. Luteolysis is prevented in pregnancy by interferon-tau (INFT), though the exact mechanism of CL maintenance remains undefined. To investigate the status of the CL in pregnancy, bihourly blood samples were collected for 74 hours from days 18-21 to monitor circulating progesterone and prostaglandin F2 alpha metabolite (PGFM). In conjunction with blood samples, daily CL biopsies were collected from inseminated and non-inseminated animals. Samples from animals confirmed pregnant on day 20 (P; n=5), and from non-inseminated animals with no PGF pulses in the 24 hours preceding the biopsy (NP; n=6, day
19.8±0.2) were submitted to whole transcriptome RNAseq analysis. Basal PGFM was low in P and NP (P: 11.6±0.7 pg/ml; NP: 9.9±0 pg/ml), and few pulses were noted prior to CL biopsy (P<0.05: P: 0.6±0.4 pulses/animal; NP: 0.7±0.3 pulses/animal). Those noted were much lower in concentration than those in non-inseminated animals that underwent luteolysis (P<0.05; P: 28.9±4.5 pg/ml; NP: 31.6±2.0 pg/ml; luteolysis: 79.3±14.7 pg/ml). In total, there were 87 differentially expressed genes (Q<0.05) between NP and P, with 60 upregulated in P, and 27 downregulated. Of the upregulated genes, a predominant portion of them (28%) were involved in interferon pathways, including the interferon stimulated genes ISG15 (4.3 fold greater, Q=0.01), MX1 (2.9 fold greater, Q=0.01), and MX2 (2.4 fold greater, Q=0.01), while 8% were related to cell proliferation, and another 10% related to cell motility. Of the downregulated genes, 22% were related to lipid balance within the cell. Thus, it appears the CL expresses a variety of INFT stimulated genes but is not exposed to high PGF in the first month of pregnancy, with low PGF likely contributing to CL maintenance in this period.

**P109 - Stimulatory effects of Transforming Growth Factor-alpha in bovine granulosa cells of small antral follicles.** Allie Lundberg, Nicole Jaskiewicz, David Townson

Intraovarian growth factors play a vital role in influencing the fate of ovarian follicles. They affect proliferation versus apoptosis of granulosa cells (GCs), and can influence whether small antral follicles continue their growth or undergo atresia. Transforming Growth Factor-alpha (TGFα), an oocyte-derived growth factor, is thought to regulate granulosa cell function, yet has been overlooked in favor of current interest in TGF-beta superfamily members such as bone morphogenetic proteins (BMPs) and anti-Mullerian hormone (AMH). Here, effects of TGFα on bovine granulosa cell (bGC) proliferation, intracellular signaling, and cytokine-induced apoptosis were evaluated. Briefly, all small antral follicles (3-5mm) from bovine ovaries were aspirated and the cells were plated in T25 flasks containing DMEM/F12 medium, 10% FBS, and antibiotic-antimycotic, and incubated at 37 degrees Celsius in 5% CO2 for 3-4 days. Once confluent, the cells were sub-cultured for experiments (in 96-, 12-, or 6-well plates) in serum-free conditions (insulin 100 ng/mL; transferrin 55 ng/mL; sodium selenite 6.7 pg/mL). 24 hour treatment of bGCs with TGFα (10 and 100 ng/mL) stimulated cell proliferation compared to control (p<0.05; n = 7 ovary pairs). This was accompanied by a concomitant increase in mitogen-activated protein kinase (MAPK) signaling within 2 hours of treatment, as measured by phosphorylated ERK1/2 expression (p<0.05, n = 3 ovary pairs). These effects were negated, however, by the MAPK inhibitor, U0126 (10uM, p<0.05). Additionally, pre-exposure of the bGCs to TGFα (100ng/mL) failed to prevent Fas Ligand (100ng/mL)-induced apoptosis, as determined by caspase 3/7 activity (p<0.05, n = 7 ovary pairs). Collectively, the results indicate TGFα stimulates proliferation of bGCs from small antral follicles via a MAPK-mediated mechanism, but may be incapable of promoting their persistence in follicles during the process of follicular selection/dominance. Supported by USDA-Hatch funding and the UVM Agricultural Experiment Station (ALL & DHT).

**P110 - Ovarian dynamics and gonadotrophins during emergence of the dominant follicle in postpartum lactating versus non-postpartum cycling mares.** Marilia Pastorello, Melba Gastal, Gabriella Piquini, Daniel Godoi, Eduardo Gastal

Intraovarian growth factors play a vital role in influencing the fate of ovarian follicles. They affect proliferation versus apoptosis of granulosa cells (GCs), and can influence whether small antral follicles continue their growth or undergo atresia. Transforming Growth Factor-alpha (TGFα), an oocyte-derived growth factor, is thought to regulate granulosa cell function, yet has been overlooked in favor of current interest in TGF-beta superfamily members such as bone morphogenetic proteins (BMPs) and anti-Mullerian hormone (AMH). Here, effects of TGFα on bovine granulosa cell (bGC) proliferation, intracellular signaling, and cytokine-induced apoptosis were evaluated. Briefly, all small antral follicles (3-5mm) from bovine ovaries were aspirated and the cells were plated in T25 flasks containing DMEM/F12 medium, 10% FBS, and antibiotic-antimycotic, and incubated at 37 degrees Celsius in 5% CO2 for 3-4 days. Once confluent, the cells were sub-cultured for experiments (in 96-, 12-, or 6-well plates) in serum-free conditions (insulin 100 ng/mL; transferrin 55 ng/mL; sodium selenite 6.7 pg/mL). 24 hour treatment of bGCs with TGFα (10 and 100 ng/mL) stimulated cell proliferation compared to control (p<0.05; n = 7 ovary pairs). This was accompanied by a concomitant increase in mitogen-activated protein kinase (MAPK) signaling within 2 hours of treatment, as measured by phosphorylated ERK1/2 expression (p<0.05, n = 3 ovary pairs). These effects were negated, however, by the MAPK inhibitor, U0126 (10uM, p<0.05). Additionally, pre-exposure of the bGCs to TGFα (100ng/mL) failed to prevent Fas Ligand (100ng/mL)-induced apoptosis, as determined by caspase 3/7 activity (p<0.05, n = 7 ovary pairs). Collectively, the results indicate TGFα stimulates proliferation of bGCs from small antral follicles via a MAPK-mediated mechanism, but may be incapable of promoting their persistence in follicles during the process of follicular selection/dominance. Supported by USDA-Hatch funding and the UVM Agricultural Experiment Station (ALL & DHT).

**P110 - Ovarian dynamics and gonadotrophins during emergence of the dominant follicle in postpartum lactating versus non-postpartum cycling mares.** Marilia Pastorello, Melba Gastal, Gabriella Piquini, Daniel Godoi, Eduardo Gastal
Compared to other livestock, the equine species has the shortest interval from partum to the first ovulation. The aim of this study was to evaluate the follicular dynamics and gonadotropin profiles around the emergence (≥6 mm) of the dominant follicle (DF) of the ovulatory wave in postpartum lactating (n=24) versus non-postpartum cycling (n=15) mares. Postpartum mares entered the study on the day of parturition and were paired with cycling mares. Data were collected for the postpartum group during the interval from partum to ovulation (IPOV) and interovulatory interval postpartum (IIOPP), and cycling group during two interovulatory intervals (IOI). Ovarian follicles (≥4 mm) were tracked daily by transrectal ultrasonography and blood samples collected simultaneously. The mean day and diameter of the DF at emergence (overall range: 3.9±1.8 to 19.1±9.4 days; 6.6±0.2 to 7.7±0.4 mm) did not differ (P>0.05) between groups. In postpartum mares the DF was larger (P<0.009) in the IPOV compared with the IIOPP. The mean intervals for the DF from emergence to deviation (overall range: 4.6±0.5 to 6.4±0.3 days) and emergence to ovulation (16.3±0.7 to 18.3±0.9 days), and the growth rates from emergence to deviation (2.4±0.1 to 3.1±0.2 mm/day) and emergence to ovulation (2.5±0.1 mm/day), were similar between groups. Systemic FSH and LH were lower (P<0.05-P<0.0001), during 11 days after emergence of the DF, in the postpartum compared with the cycling group of mares. This is the first study in which the ovarian dynamics during the puerperium and first IOI postpartum were profoundly studied and compared with cycling mares. Our results demonstrate a partum effect on the diameter of the DF in mares that ovulate during the foal heat. Although the gonadotropins levels were lower during the IPOV and IIOPP in postpartum lactating mares, this fact was not determinant to prevent ovarian cyclicity during the foal heat.

**P111 - Mutation of the conserved SUMOylation site in NOBOX leads to premature ovarian aging in mice.** Bethany Patton, Amanda Rodriguez, Stephanie Pangas

12% of U.S. women struggle with infertility caused by pathology or aging. One pathology, primary ovarian insufficiency (POI), affects 1% of the population and is characterized by premature loss of oocytes. Increasing maternal age is another factor contributing to infertility. Therefore, there is an increasing need to understand the mechanisms regulating intraovarian oocyte development and ovarian aging in order to develop clinically-relevant testing and treatment modalities. The oocyte-specific homeobox transcription factor, NOBOX, maintains the ovarian reserve; Nobox knockout mice lose all oocytes by postnatal day 14. In addition, NOBOX is one of the most mutated genes (6%) in women diagnosed with POI. SUMOylation, a ubiquitin-like post-translational modification, regulates protein function within the oocyte and in silico analysis indicated that NOBOX was a potential substrate. To determine if SUMOylation regulates NOBOX function, we mutated the predicted SUMOylation site of Nobox in mice using CRISPR/Cas9 gene editing. Forty founders were screened for the mutation via sequencing and one founder was selected to establish the line. Mutant females initially showed similar fecundity to controls. However, by four months of age, they began to have reduced litter sizes, resulting in a significantly decreased number of pups per litter. Compared to wild type ovaries, six-month old mutant ovaries were smaller, had fewer follicles, exhibited large areas of multinucleated macrophage giant cell infiltration, and showed increased fibrotic tissue, consistent with reproductive-age associated fibrosis. qPCR data showed that six-week-old mutant ovaries had
significant changes in a subset of known NOBOX target genes including *Rspo2*, while other NOBOX target genes, such as *Gdf9* and *BMP15*, were unchanged. In total, these data would indicate that without post-translational modification, NOBOX cannot regulate its full complement of genes, resulting in loss of germ cells and premature ovarian aging. These studies were supported by NIH/NICHD R01 HD085994 (to S.A.P.).

**P112 - Implications of Season on Proliferative, Angiogenic, and LH Receptors in the Follicle Wall in Mares.** Ghassan Ishak, Gabriel Dutra, Gustavo Gastal, Megan Elcombe, Melba Gastal, Seong Park, Jean Feugang, Eduardo Gastal

The effect of different seasons of the year on the expression pattern of growth factor and hormone receptors involved in follicle development was evaluated. A novel follicle wall biopsy technique was used to collect *in vivo* follicle wall layers and follicular fluid samples from growing dominant follicles, simultaneously, and repeatedly, using the same mares during the spring anovulatory (SAN), spring ovulatory (SOV), summer (SU), and fall ovulatory (FOV) seasons. The immunofluorescent expression patterns of epidermal growth factor receptor (EGFR), Ki-67, vascular endothelial growth factor receptor (VEGFR), and luteinizing hormone receptor (LHR) were evaluated in each follicle wall layer, in addition to intrafollicular estradiol. Proliferative proteins (EGFR and Ki-67) were highly (*P*<0.05–*P*<0.001) expressed during the SOV season compared to the SAN and FOV seasons. Lower (*P*<0.05–*P*<0.001) expression of both proteins was observed during SU compared to the SOV season. The expression of VEGFR was greater (*P*<0.05–*P*<0.01) in the theca interna of dominant follicles during the SOV season compared to the SAN and SU seasons. Similarly, in the overall quantification, the VEGFR expression was greater (*P*<0.001) during the SOV season. A higher (*P*<0.05) LHR expression was detected in the theca interna during the SOV season than in the SAN season. Furthermore, a higher (*P*<0.05–*P*<0.001) expression of LHR was observed in the granulosa, theca interna, and in the overall quantification during the SOV season compared to the SU and FOV seasons. Intrafollicular estradiol was higher (*P*<0.05) during SU than the SAN season, and higher (*P*<0.05) during the FOV season compared to SAN and SOV seasons. In conclusion, the synergistic effect of lower expression of proliferative protein, angiogenic, and gonadotropin receptors in at least some of the layers of the follicle wall seems to trigger dominant follicles toward the anovulation process during the spring and fall transitional seasons.

**P113 - Lipopolysaccharide Differentially Affects Pro-Inflammatory Responses in Theca Cells from Androgen Excess compared to Control Beef Cows.** Kerri Bochantin, Alexandria Snider, Shelby Springman, Scott Kurz, Jessica Keane, Sarah Nafzinger, Jeffery Bergman, Renee McFee, Andrea Cupp, Jennifer Wood

A population of cows with increased ovarian androstenedione (High A4; >40ng/mL) concentrations has been identified in the UNL physiology herd. Interestingly, circulating and follicular fluid pro-inflammatory cytokine composition is altered in High A4 cows suggesting chronic inflammation. Lipopolysaccharide (LPS) leak from the gut due to stress-dependent changes in microbial profiles induces expression of pro-inflammatory cytokines via activation of toll-like receptor 4 (TLR4) and the associated myeloid differentiation factor 88 (MyD88). Our
hypothesis is that theca cells from High A4 cows, which have similar gene expression profiles as women with PCOS, are chronically exposed to LPS resulting in differential secretion of pro-inflammatory cytokines compared to theca cells from Control cows. To test this hypothesis estrous cycles of High A4 and Control cows were synchronized and stimulated for 3 days with FSH (210 IU) prior to ovariectomy. Theca cells were micro-dissected from follicles >7mm and immediately cultured in untreated medium for 120 minutes or LPS-containing medium (50ng/ml) for 15 to 120 minutes. Western blot analysis showed progressive LPS-dependent increases in MyD88 in theca cells from Control cows. However, MyD88 expression was progressively decreased in theca cells from High A4 cows. Cytokine antibody arrays showed significant increases in pro-inflammatory cytokine concentrations (TNFα, IL-1α, and IL-21) in untreated medium from High A4 compared to Control theca cells. When Control theca cells were treated with LPS, TNFα, IL-1α, and IL-21 were significantly increased in a time-dependent manner. However, LPS treatment of High A4 theca cells resulted in significantly decreased levels of these same cytokines. These results suggest insensitivity of High A4 theca cells to LPS-mediated inflammatory response which may be due to chronic in vivo LPS exposure. Previous studies show that LPS increases ovarian steroidogenesis suggesting that chronic exposure of theca cells to LPS may be a mechanism for androgen excess in High A4 cows.

P114 - Expression and regulation of cysteine rich 61-connective tissue growth factor-nephroblastoma overexpressed (CCN1) in ovarian adenocarcinoma (OVCAR8) cells. Sarah Piet, Sarah Walker, Paul Tsang

Angiogenesis in the ovary is orchestrated by pro-angiogenic factors, e.g. CCN1, that contribute to tumorigenesis. Previously, using a granulosa tumor cell line (KGN), we reported that CCN1 is regulated by prostaglandin-F2alpha (PGF2α), mediated likely through the protein kinase C (PKC) pathway. In the present study, we sought to determine if similar expression and regulation of CCN1 exists in OVCAR8 cells. Using the protocol developed for KGN cells, the OVCAR8 cells were serum-starved for 2 hours, treated for 2 hours, and analyzed via quantitative PCR (qPCR). Treatment of OVCAR8 cells with fetal bovine serum (FBS) resulted in a 1.5 fold increase (p<0.05) in CCN1 expression over cells without FBS (negative control). Since PGF2α is a regulator of CCN1 in KGN cells, OVCAR8 cells were treated with PGF2α (0.1, 0.5, 1, 5uM). Interestingly, only the highest concentration of PGF2α (5uM) increased (p<0.05) CCN1 above the negative control (n=4). Since PGF2α is known to activate the PKC pathway, OVCAR8 cells were treated with phorbol 12-myristate 13-acetate (PMA; 0.1, 1, 10, 100nM), a pharmacological activator of PKC. Similar to PGF2α, only the highest concentration of PMA (100nM) increased (p<0.05) CCN1 expression (n=3). These modest responses led us to explore other regulators of CCN1 expression. The OVCAR8 cells possess high signal transducer and activator of transcription 3 (STAT3) activity, which is regulated by Janus kinase (JAK). In a pilot study, OVCAR8 cells were cultured in the presence or absence of a JAK inhibitor for 24 hours. Interestingly, the expression of CCN1 in FBS-treated cells was higher (p<0.05) in the presence of the JAK inhibitor. Moving forward, the effects of a shorter exposure time with the JAK inhibitor will be explored. In summary, OVCAR8 cells express CCN1 and its regulation appears to involve the PKC and JAK-STAT pathways.
**P115 - Complement components (C3 and C4) may be important in facilitating luteal rescue during early pregnancy in dairy cows.** Adelaide Hellmers, Camilla Hughes, Joy Pate

The complement cascade is an intricate pathway of the innate immune system discovered for its role in destroying pathogens. Complement C3 is the central protein in the pathway, while complement C4 is involved with the cleavage of C3 to initiate the cascade. Complement components have been found in the corpus luteum (CL), however the pattern of expression, regulation, and function of these proteins in this tissue remains unknown. Molecular profiling studies from this laboratory demonstrated that complement signaling may regulate luteal rescue. The objective was to evaluate abundance and hormonal regulation of C3 and C4 in the CL of early pregnancy. CL were collected from Holstein cows on day 17 and 18 of the estrous cycle and pregnancy. Protein (n=4) and mRNA (n=6) abundance of complement C3 and C4 were measured via western blots and qPCR, respectively. C3 protein abundance was numerically greater in the CL of the cycle compared to the CL of pregnancy on day 17 (P = 0.11), while C3 mRNA was greater in the CL of pregnancy compared to the CL of the cycle on day 18 (P < 0.05). C4 protein abundance was numerically greater in the CL of pregnancy compared to the CL of the cycle on day 18 (P = 0.12) with no changes on day 17. C4 mRNA did not differ. Cultured luteal cells (n=5) were treated with prostaglandin (PG) F2A (10ng/ml), PGE2 (10 ng/ml), and interferon-tau (IFNT; 1 ng/ml). C3 mRNA was decreased by PGF2A (P < 0.05), whereas C4 mRNA was increased by IFNT (P < 0.05), compared to control. These results demonstrate C4 abundance is positively regulated by IFNT and may modulate C3 abundance in the CL of pregnancy and facilitate luteal rescue. Project supported by: AFRI/USDA Grant no. 2016-67015-24900 and NIH Grant T32GM108563.

**P116 - Timing of luteolysis and conceptus expulsion after induced embryonic demise during the second month of gestation in beef cows.** Benjamin Duran, Sarah Battista, Martin Mussard, Alvaro Garcia-Guerra

Pregnancy loss during the second month of gestation can be initiated by embryo/fetal death or alternatively by luteolysis. The aim of this study was to test the hypothesis that embryonic demise at initiation of the second month of gestation will consistently result in luteolysis prior to the end of the second month. Pregnant non-lactating beef cows were randomly assigned to one of three treatments on gestation day 36: control (2 mL physiological saline IM; n=6), PGF (2 mL cloprostenol IM; n=6), and intrauterine hypertonic saline infusion (INF; 120 mL hypertonic saline; n=10). Corpus luteum (CL) volume and embryo/fetal viability were evaluated by B-mode ultrasonography daily until gestation day 41 and then thrice weekly until gestation day 67. Blood flow through the CL was evaluated by color doppler ultrasonography and luteolysis was defined as the day in which <25% of the CL contained color pixels. Corpus luteum and amniotic vesicle volume prior to treatment did not differ among groups (P>0.1). There was an effect of treatment (P<0.01), time (P<0.01) and treatment by time interaction (P<0.01) on CL volume. Volume of the CL was smaller in PGF-treated cows by 24h (P<0.05) following PGF treatment. Furthermore, CL volume was not different between control and INF cows between days 36 and 41 (P>0.10), however, INF-treated cows had a lesser CL volume starting at day 43 (P<0.05). Day of luteolysis differed (P<0.01) between INF (44.1±0.8) and PGF-treated (37.4±0.2) cows. Conceptus expulsion was detected earlier (P<0.01) in PGF (day 39.8±0.2) than...
INF-treated (day 44.6±0.8) cows. In conclusion, induced embryo demise on day 36 of gestation results in luteolysis, through ill-defined mechanisms, about 9 days later (~d45) and is associated with timing of conceptus expulsion. The induction of embryonic demise may be a valuable model to investigate the mechanisms regulating luteolysis during the second month of gestation.

**P117 - Regulation of the Angiogenic Inducer, Cysteine Rich 61-Connective Tissue Growth Factor-Nephroblastoma Overexpressed 1 (CCN1), in Bovine Luteal Cells.** Michael Goulet, Jake Donahue, Paul Tsang

Angiogenesis is a hallmark process that accompanies the growth and development of the corpus luteum (CL). We previously reported high *in vivo* expression of CCN1 in the young, 4-day-old bovine CL. The goal of the present study was to characterize the *in vitro* regulation of CCN1 in bovine luteal cells from day 4 corpora lutea. Luteal cell extracts were analyzed by quantitative polymerase chain reaction. To determine the temporal expression of CCN1, luteal cells (n=3) were incubated with 10% fetal bovine serum for 0, 2, 4, 8, or 24 hours. The expression of CCN1 was increased (p<0.05) by 2 hours. In subsequent experiments, cells were treated for 2 hours with a variety of substances in defined medium. Considering that luteinizing hormone (LH) is the primary luteotropin, luteal cells (n=5) were incubated with 10 and 100ng/mL recombinant human LH for 2 hours, but both were without effect (p>0.05) on CCN1 expression. Since the actions of LH are mediated through the protein kinase A (PKA) pathway, luteal cells (n=2) were treated with 10, 25, and 50μM forskolin (activator of adenylate cyclase) and with 0.1, 1, and 2μM dibutylryl-cyclic adenosine monophosphate (activates PKA), but neither had any effects on CCN1 expression. A pilot study was then performed to determine if the protein kinase C (PKC) pathway plays a role in regulating CCN1 expression in luteal cells. They were treated with 0.5, 1, and 5μM of prostaglandin F2alpha (PGF2a), and preliminary results showed that the highest PGF2a concentration (5μM) may stimulate CCN1 expression. Besides confirming these findings, future experiments will use phorbol ester, a pharmacological activator of PKC, to corroborate the effects of PGF2a on CCN1 expression. In summary, the regulation of CCN1 expression in dispersed bovine luteal cells does not appear to be mediated by the PKA pathway, but rather, the PKC pathway.

**P118 - Applying biomimetic rigidity via alginate hydrogel for in vitro domestic cat (Felis catus) ovarian follicle culture in a microfluidic chip.** Jennifer Nagashima, Helim Aranda-Espinoza, Rami El Assal, Utkan Demirci, Nucharin Songsasen

Ovarian follicle culture systems are needed for the development of efficient genome rescue protocols for endangered felids, and to capitalize on the domestic cat as a biomedical research model. While the importance of rigidity of a follicle’s microenvironment has been demonstrated in several mammalian species, to the best of our knowledge there is no published information on the rigidity of the cat ovary or its influence on *in vitro* folliculogenesis. Here, tensile testing via micropipette aspiration was performed using ovarian cortical and medullary tissue from 12 domestic cats (7 prepubertal, 5 peripubertal/adult). While high variability was observed among cortical tissues, a distinction between Young’s modulus values for cortical versus medullary tissue was observed (P=0.031, Wilcoxon test in JMP), where cortices averaged (±st.err.)
217.2±44.9 kPa, whereas medullas averaged 96.8±12.1 kPa. Micropipette aspiration was also performed using 0.25%, 0.5%, 0.75%, 1.0%, and 2% alginate hydrogels to identify biomimetically rigid concentrations. Cortical tissue was equivalent to a 0.29–2.14% alginate, and medullary tissue 0.18–0.42%. Per these results, we isolated cat follicles and embedded them in groups of 4-6 in 5 µl beads of either 0.25% (biomimetic medulla), 1% (cortex) or 2% (high-rigidity cortex) alginate. Encapsulated follicles were then placed in channels with collagen solution in a microfluidic chip to flow at either 0, 2 or 20 µl/min with culture medium (MEM-based, with 1 µg/ml FSH and 100 ng/ml EGF) for 3 days at 5% CO₂ and 38.5°C (n=13-23 preantral follicles/treatment). No difference in survival was observed among rigidities or flow rates (P>0.05), with moderate growth of follicles in 0.25 and 1% alginate and 0 and 20 µl/min flow. Use of biomimetic rigidity in a dynamic culture device represents a step toward developing an improved ovarian preantral follicle culture system for felines. This research was supported by NIH F32HD090854.

**P119 - VCAM1 is Induced by Androgens Within the Ovarian NR2F2+ Stromal Lineage.**
Nicholes Candelaria, Achuth Padmanabhan, Minerva Solis, Katharine Shelly, Jan McAllister, Sheng Wu, JoAnne Richards

Excess circulating androgens are thought to trigger Polycystic Ovarian Syndrome (PCOS), the leading cause of infertility in women. To model PCOS in mice, we implanted dihydrotestosterone (DHT) pellets (12.5 mg/pellet) into immature wild-type females for 1 week or as long as 2 months. Standard molecular techniques, such as genome-wide microarray studies, quantitative PCR, Western blotting, and immunohistochemistry (IHC) were utilized to characterize the molecular events driving the actions of androgens in our PCOS mouse model. Female mice treated for two months with DHT develop severe phenotypes in the ovarian stromal compartment, the cells of which became hyperplastic and lipid filled, and presented with abnormally nucleated stromal AR. Microarray studies revealed significant changes to the ovarian transcriptome, but most notable was the increased expression of a theca/Leydig cell marker (*Vcam1*). Induction of VCAM1 by androgens was confirmed by Western blot analysis, immunohistochemistry, and in situ hybridization, which demonstrated that VCAM1 induction was limited to the NR2F2 ovarian theca/stromal lineage, an effect that could be inhibited by hCG administration. To determine the function of VCAM1 within the NR2F2 stromal lineage, transgenic mouse lines bearing floxed VCAM1 and NR2F2 alleles were bred to the CYP17A1-icre mouse line to selectively target deletion of marked alleles to androgen producing cells within the gonads. Targeted VCAM1 deletion manifests in the development of an expanded interstitial compartment, the consequences of which are still unknown. Conversely, NR2F2 deletion triggers interstitial hypogenesis and is associated with abnormal VCAM1+ cells outside the theca layer in the interstitium in response to gonadotropins, further suggesting that NR2F2 and VCAM1 function within common signalling pathways. Together, the DHT androgen excess and transgenic mouse models reveal novel and surprising functions for VCAM1 and NR2F2 in ovarian function (NIH-HD-076980).
**P120 - To study the Association of ABO blood type with ovarian reserve in North Indian women with Subfertility.** Indu Lata, Prabhakar Mishra

**BACKGROUND:** Recent studies have examined the relationship and association between blood type and ovarian reserve, but have obtained contradictory results. We therefore collected and analyzed data from our center to investigate the association between blood type and ovarian reserve.

**OBJECTIVES:** The aim of this study was to assess the correlation of certain blood groups with ovarian reserve.

**METHODS:** Women attended the infertility clinic between Jan 2016 to Jan 2018 included and data, including age, BMI, blood type, types of subfertility, history of previous pregnancies was collected. A total of 241 women were included in the analysis. Day two or three serum FSH value were reviewed. DOR was defined as a day two or three serum FSH concentration > 10 IU/L. The association between blood groups and ovarian reserve using day two LH and FSH  and AMH levels.

**RESULTS:** Mean and median age of the patients were 29.42 and 29 respectively. Similarly mean and median BMI were observed 25.14 and 25.06 kg/m². In FSH, mean score was 7.84±7.58 and median score was 6.66 (range 0.1-83.2). Distribution of the Blood Groups were maximum were B+ (41.9%) followed by O+ (29.9%) in the Study Patients. There was maximum number of patients of Primary infertility (50.6%) followed by secondary infertility (49.4%). There was no statistically significant correlation was found between type of Infertility with the Blood groups (P =0.937) and FSH levels with Blood groups (P=0.771).

On comparison of primary and secondary infertility mean age (P<0.001)and FSH (p=0.043) was higher in secondary infertility group. As median score of the AMH and FSH among eight blood groups was compared, showed there was no correlation between the blood groups and AMH score (p=0.293) and blood groups and FSH score (p=0.142).

**CONCLUSION:** There was no significant association found between blood type and ovarian reserve.

**P121 - GDF-9 and BMP-15 Gene Expression In Canine Cumulus Cells Before And After In Vitro Maturation.** Georges Ramirez, Jaime Palomino, Monica De Los Reyes

The competence to undergo expansion is a unique characteristic of cumulus cells (CCs) which has been shown to be critical for normal oocyte maturation. This process is modulated in part by paracrine factors, including members of the transforming growth factor-beta superfamily, GDF-9 and BMP-15. Previously, we described these factors in oocytes and granulosa/theca cells in canines, but there are no reports in cumulus cells. Therefore, the aim of this study was to investigate the expression of GDF-9 and BMP-15 mRNA in canine cumulus cells in relationship with cumulus expansion. Cumulus cells were recovered from cumulus oocytes complexes (COCs n= 2,426) obtained from antral follicles at different phases of the estrous cycle, before and after in...
vitro maturation (IVM). Plasma progesterone analysis was used to confirm the reproductive status of each bitch. Quantitative real-time polymerase chain reaction (qPCR) was used to evaluate the relative abundance of GDF-9 and BMP-15 mRNA transcripts from CCs of non-matured COCs and CCs from expanded and non-expanded COCs after IVM. The results were analyzed statistically using ANOVA. There was a significant difference in GDF-9 and BMP-15 gene expression comparing CCs from immature and IVM COCs, with higher (P<0.05) transcript levels in CCs from IVM COCs compared to those from non-matured COCs at anestrus, proestrus and diestrus. In contrast, at estrus there were minor (P<0.05) mRNA levels of both paracrine factors after IVM. Also, the GDF-9 and BMP-15 gene expression were higher (P<0.05) in CCs from expanded versus non expanded COCs at anestrus, proestrus and diestrus. While all IVM COCs obtained in estrus were expanded showing low levels of GDF-9 and BMP-15 mRNA. This suggests an inverse relationship between the expression of these genes and CCs expansion previous ovulation in canines. Supported by FONDECYT 1171670.

**P122 - Ovine and bovine granulosa cells respond differently to Fibroblast Growth Factor 2.** Lauriane Relav, El Arbi Abulghasem, Christopher Price

Fibroblast growth factor-2 (FGF2) stimulates granulosa cell proliferation through MAPK, PKC and AKT pathways. A rapid and transient increase in MAPK3/1 phosphorylation is a typical mitogenic signal, and the duration of phosphorylation is regulated by dual specificity phosphatases (DUSPs). In rats, DUSPs have been implicated in the dephosphorylation of MAPK3/1 after FSH stimulation, but the role of FGFs in the regulation of DUSP expression in the ovary is unknown. The aim of the present study was to describe the regulation of DUSPs by FGF2 in ruminant granulosa cells. Ovine ovaries were collected from a local slaughterhouse and granulosa cells were harvested from antral follicles (1-4 mm diameter) for culture under serum-free conditions. On day 5 of culture, cells were stimulated with 10ng/ml FGF2 to measure MAPK3/1 phosphorylation by Western blot and abundance of mRNA encoding DUSPs and the FGF target gene, SPRY2, by RT-qPCR. Treatment with FGF2 significantly upregulated SPRY2 mRNA levels as expected, but curiously did not significantly increase in MAPK3/1 phosphorylation. We then employed a bovine granulosa cell culture system under the same conditions and observed a similar increase in SPRY2 mRNA levels and also a robust increase in MAPK3/1 phosphorylation. FGF2 caused an increase in DUSP5 mRNA abundance at all time points (1-8h) in bovine and in ovine granulosa cells. However, FGF2 increased DUSP1 mRNA levels after 1h of treatment in sheep cells which returned to control levels within 2h, whereas in bovine cells DUSP1 mRNA levels did not increase until 6-8h of treatment. In sheep cells, FGF2 increased DUSP6 mRNA levels only at 8h, but in bovine granulosa cells DUSP6 mRNA levels were increased at 2, 6 and 8h after FGF2 treatment. We conclude that FGF2 differentially regulates MAPK3/1 signaling and DUSP expression in these two ruminants, indicating species-specific fine control of follicular growth.

**P123 - The role of anti-Müllerian hormone (AMH) in ovarian steroidogenesis.** Alexandra Fontaine, Maxwell Edmonds, Sarah Wagner, Margrit Urbanek
Polycystic ovarian syndrome (PCOS) is a highly heritable reproductive disorder impacting 6-20% of reproductive aged women. Women with PCOS have amenorrhea, polycystic ovaries, and hyperandrogenism. PCOS can lead to anovulatory infertility and is associated with insulin resistance and diabetes. Hyperandrogenemia is predominantly due to excess testosterone production by ovarian theca cells. The increased levels of CYP17A1 expression seen in PCOS are predicted to increase androgen biosynthesis. In addition to elevated testosterone, Anti-Müllerian Hormone (AMH) levels are often elevated in PCOS. AMH is a member of the TGFβ superfamily and is secreted by the granulosa cells of preantral and small antral follicles. Interestingly, we recently identified 37 mutations in AMH and its cognate receptor, AMHR2, with impaired biological activity. The mechanism(s) behind these abnormalities remains unknown. We have created a research tool by optimizing a murine ovarian explant model that will allow us to test the impact of AMH levels on testosterone levels and CYP17 mRNA expression under both normal and PCOS-like conditions. We exposed ovarian explants to a range of AMH and insulin levels to determine optimal culture conditions. In ovarian explants cultured with high levels of AMH (400 and 800 ng/mL), a higher percentage of explants failed to secrete inhibin A (inhibin A < 100 ng/mL). Explants cultured with traditional yet super-physiological concentrations (1000 µIU/mL) of ITS [Insulin-Transferrin-Selenium]) produced less AMH and inhibitin A than explants cultured with lower concentrations (0, 10, 20 µIU/mL ITS). We have established ‘human-inspired’ culture conditions which mimic both PCOS (200 ng/mL AMH, 20 µIU/mL ITS) and ‘normal’ (0 ng/mL AMH, 10 µIU/mL ITS) conditions for use in murine ovarian explant culture. We are currently investigating the use of these models to study the effects of AMH on ovarian steroidogenesis.

**P124 - Granulosa cells of ovarian antral follicles exhibit distinct follicle size-related processes.** Natasja Costermans, Jaap Keijer, Evert van Schothorst, Bas Kemp, Nicoline Soede, Katja Teerds

Antral follicle size might be a valuable additive predictive marker for in vitro fertilization (IVF) outcome. To better understand consequences of antral follicle size for reproductive outcome, we aimed to obtain insight in follicle size-related granulosa cell processes, as granulosa cells play an essential role in follicular development via the production of growth factors, steroids and metabolic intermediates. Using the pig as a model, we compared gene expression in granulosa cells of smaller and larger follicles in the healthy antral follicle pool of sows which had a high variation in follicle size or a low variation in follicle size. Selected gene expression was confirmed at the protein level. Granulosa cells of smaller antral follicles showed increased cell proliferation, which was accompanied by a metabolic shift towards aerobic glycolysis (i.e. the Warburg effect), similar to other highly proliferating cells. High granulosa cell proliferation rates in smaller follicles might be regulated via increased granulosa cell expression of the androgen receptor and the epidermal growth factor receptor which are activated in response to locally produced mitogens. While granulosa cells of smaller follicles in the pool are more proliferative, granulosa cells of larger follicles express more maturation markers such as insulin-like growth factor 1 (IGF1) and angiopoietin 1 (ANGPT1) and are therefore more differentiated. As higher IGF1 and ANGPT1 both have been associated with better IVF outcomes, the results of our study...
imply that including smaller follicles for oocyte aspiration might have negative consequences for IVF outcome.

**P125 - Gonadotropin and Oxygen Regulation of Leukemia Inhibitory Factor Production by Rhesus Macaque Non-luteinized Granulosa Cells.** Heather Talbott, Adam Krieg, Jon Hennebold

Leukemia inhibitory factor (LIF) is required for rhesus macaque ovulation. However, it is unclear if granulosa cells within the ovarian follicle are capable of producing LIF and whether factors involved in coordinating ovulation also regulate LIF production. The object was to determine if rhesus macaque granulosa cells secrete LIF and whether its production is regulated by gonadotropins or oxygen (O2) levels. Non-luteinized granulosa cells (NLGCs) were collected from female rhesus macaques (n=4) that underwent a controlled ovarian stimulation protocol without human chorionic gonadotropin (hCG) administration. NLGCs were cultured with and without hCG (20 IU/mL) in the presence or absence of FSH (0, 0.5, or 2.5 ng/mL) at either 20% or 1% O2 for 24 h. Culture media was collected and assessed for LIF and progesterone concentrations. Rhesus macaque NLGCs responded to hCG treatment by increasing LIF secretion 3.4-fold relative to cells cultured in the absence of hCG (P=0.002). When NLGCs were cultured in 1% O2, LIF secretion was increased 1.3-fold compared to cells cultured at 20% O2 (P=0.0026). Furthermore, the combination of hCG and 1% O2 interact to synergistically increase LIF secretion (P=0.0011). As expected, NLGC stimulated with hCG increased progesterone secretion (113-fold; P<0.005) in the absence of FSH. In the presence of both FSH and hCG, NLGCs did not increase LIF or progesterone levels above what was observed when the treated with hCG alone. In the absence of hCG, NLGC produced progesterone in a dose-dependent response to FSH (P=0.016). Thus, in vivo it is likely the granulosa cells are a significant source of the LIF that is found in follicular fluid after an ovulatory stimulus. This project was supported by NIH R01 HD020869 and NIH P51 OD011092.

**P126 - A modified form of human GDF9 with cumulin-like Smad-2/3 activity.** William Stocker, Kelly Walton, Karen Chan, Georgia Goodchild, Craig Harrison

Human growth differentiation factor-9 (GDF9) is expressed almost exclusively by oocytes, but is secreted in a latent form. Recent studies have indicated that human GDF9 is activated by forming a heterodimer (called cumulin) with the closely related molecule bone morphogenetic protein-15 (BMP15). Cumulin added to in vitro maturation (IVM) media of immature porcine or human oocytes markedly increases subsequent embryo yield, identifying this molecule as an attractive therapy for the treatment of female infertility. However, the heterodimeric nature of cumulin makes mass production difficult and prone to batch-to-batch variability in potency. To address this, we sought to generate an engineered form of GDF9 with ‘cumulin-like’ ability to activate the Smad-2/3 transcription pathway. Site-directed mutagenesis was used to introduce targeted mutations into the GDF9 homodimer with the goal of conferring cumulin-like activity. Modified GDF9 protein was produced in human HEK293 cells and analysed via Western blotting. Collectively, the introduced mutations were found to be non-disruptive to GDF9 in vitro production. GDF9 analogues were then purified by IMAC. Excitingly, the modified GDF9 forms were capable of inducing Smad-2/3 activity in human granulosa COV434 cells, with some
having even greater potency than cumulin. Replacement experiments indicated that multiple mutations are required for maximal activation of the Smad-2/3 pathway. Ongoing studies will determine if, like cumulin, our modified GDF9 can improve oocyte quality and embryo yield.

P127 - Expression Of Terra Differs In Early Bovine Embryonic Development In A Stage-Dependent Manner- A Possible Link To Maternal Ageing. Pawel Kordowitzki, Isabel López de Silanes, Maria Blasco, Dariusz Skarzynski

OBJECTIVE: Maternal aging-associated reduction of oocyte viability and age-related subfertility is a common feature in women, and still poorly described. Late exit from the developmental line during oogenesis presumably contributes to telomere shortening. When telomeres are transcribed they generate non-coding RNAs known as Telomeric Repeat-Containing RNA (TERRA), a very recent discovery. To our knowledge, there are no reports about the expression of TERRA during early bovine embryo development, which is very similar to the human species. Therefore, we studied TERRA expression dynamics in oocytes, and throughout early embryonic cleavage stages up to blastocyst formation in a bovine model.

MATERIALS AND METHODS: Bovine GV oocytes were aspirated from collected ovaries at a local abattoir, matured, fertilized, and cultured in vitro until day 7 in a standard laboratory protocol. Samples were collected starting from the GV, MII, zygote until blastocyst stage and fixed on glass slides. TERRA foci were stained by a RNA-FISH protocol, and RNase treated samples were considered as negative controls, and pictures were captured with the help of confocal microscopy. Relative TERRA spots were determined by the summatory fluorescence intensity per nucleus.

RESULTS: TERRA is localized in the GV stage inside of the germinal vesicle, in the MII stage in the ooplasm, and from the zygote to blastocyst stage the foci are nuclear. Mean number of TERRA foci from GV to the 4-cell embryos were counted as 2.43. After this stage, namely in the 8-cell and later cleavage stages, there was a significant 3-fold increase(P<0.01) in mean TERRA foci with 7.452 counted spots per nucleus.

CONCLUSIONS: Our data show for the first time that TERRA expression is activated at the 8-cell cleavage stage in bovine embryos, a time of significant telomere reprogramming and embryonic genome activation, what could be a possible pathway for a better understanding of reproductive ageing.

P128 - New links and genetic connections between menarche and antral follicle count in African American and European American women. Sonya Schuh, Julia Kadie, Mitchell Rosen, Barbara Sternfeld, Renee Reijo Pera, Marcelle Cedars

Women have a finite reproductive window that spans from sexual maturation and the first menstruation, or menarche, to menopause. These complex reproductive traits are highly variable, both within and between ethnic groups, and are highly heritable. Here, we aimed to identify new genetic variants, and replicate previous variants, associated with menarche and
antral follicle count (AFC), and also examine relationships between these traits and anthropometric factors including body mass index (BMI), in a multi-ethnic, community-based cohort of normative women. Blood samples, questionnaires, and anthropometric data were collected from 518 African American and European American women, aged 25–45. AFCs were measured by transvaginal ultrasound during early follicular phase. Ethnicity was self-reported and genetically validated. Genome-wide scans were performed on age of menarche, in both ethnic groups independently, as well as replication of previous variants. We found that earlier age of menarche was associated with both higher BMIs and higher AFCs in adulthood, with control for female age. The follicle difference between early (<12 years) vs. late (>15 years) initiation of menarche in both white and black women was equivalent to a significant ~20% increase in AFC. We identified several novel nominally significant SNPs linked with menarche in the RORA gene in African American women. Several variants of the LIN28B gene, previously associated with height and menarche, were validated in this cohort and were directionally and proportionally consistent. Specific variants linked with AFC were also associated with age of menarche. This study provides the first evidence that timing of menarche may influence follicle number, and hence fertility, and that there are shared genetic links between menarche and AFC. This work underscores the importance of data on underrepresented populations and better understanding the genotypic variation of the reproductive lifespan, which may enhance our ability to screen and treat various reproductive-related diseases.

P129 - Metformin Abrogates Age-Associated Ovarian Fibrosis in Women. Barbara Vanderhyden, Curtis McCloskey, David Cook, Brendan Kelly, Jeremy Upham, Dominique Trudel, Mary Senterman

Age and the number of life-time ovulations are the primary non-hereditary risk factors for ovarian cancer, but the reasons for this remain largely unknown. Ovulatory risk is especially curious since ovarian cancer incidence increases in post-menopausal women, after ovulations have ceased. To determine how age and the accumulation of ovulatory events underlie ovarian cancer risk, we first validated the development of age-associated murine ovarian fibrosis and generated further support for the development of chronic inflammation with age in murine ovaries. To expand our findings into human aging, and with research ethics board consent, we assembled a cohort of normal human ovaries (N = 18) over a wide age range (21-71 years old). Using Masson’s trichrome staining and second harmonic generation imaging, we discovered that ovarian fibrosis also develops in human post-menopausal ovaries. RNA was isolated from the cortex of each of the human ovaries and subsequent Nanostring gene expression analyses and immunohistochemical validation showed that fibrotic ovaries have enhanced pro-inflammatory gene expression with an increased CD206+:CD68+ ratio, and increased abundance of CD8+ T cells and pro-fibrotic DPP4+αSMA+ fibroblasts. Fibrosis, fibroblast activation, CD8+ T cells, and macrophage polarization were reversed or inhibited in ovaries from post-menopausal women taking metformin, providing a novel mechanism to explain its proposed ability to reduce ovarian cancer risk. Ovaries from both pre- and post-menopausal BRCA mutation carriers have collagen architecture similar to post-menopausal fibrotic ovaries, suggesting that ovarian fibrosis may develop at an earlier age in BRCA mutation carriers. These results provide support for a novel hypothesis that unifies the ovarian cancer risk factors of age, ovulation, and BRCA mutation
through the development of ovarian fibrosis, which in turn generates a premetastatic niche that can be inhibited by use of metformin.

**P130 - Profiling the age-associated transcriptomic changes in mouse ovarian tissue.** Zijing Zhang, Lynae Brayboy, Maria Schlamp, Xiaotian Wu, Haley Clark, Gary Wessel

The fertility of women declines sharply after age 35 and is essentially lost upon menopause at age 50. The ovary plays an important part in age-associated changes in women’s physiology since it is an essential component of the reproductive and the endocrine system of a female. The ovary undergoes major transformations over the course of aging. The ovarian follicle reserve, which is a source of germ cells and hormonal production, is gradually depleted with each ovulation cycle. Also, the molecular and biochemical composition of the ovary is altered drastically as the female ages. Previous studies show that the ovary from reproductively aged mice exhibits distinct signs of fibrosis compared to their reproductively young counterparts, and aged follicles show elevated expression of genes associated with chronic inflammation. These changes in the ovarian environment may underlay the marked decline of oocyte quality in aged individuals. To identify gene expression changes that take place in the ovary during aging, we performed detailed transcriptomic analysis of whole ovaries from mice of six different age groups (3 months, 6 months, 9 months, 12 months, 15 months and 18 months). In addition to the identification of more than 5000 genes with significant up or downregulation over the course of aging, we learned that a major shift in the ovarian transcriptomic profile takes place between 9 and 12 months of age. Consistent with previous reports, we found upregulation of inflammation-associated genes (i.e. Ifngr1, Cnr2, Cxcl16, etc.) and downregulation of genes associated with metabolic processes (i.e. Pgm3, Tet3, Plk1, etc.) in ovaries from mice with advance reproductive age. Further examination of these differentially expressed genes provides clues about the nature and development of chronic inflammation, and the progression of metabolic

**P131 - Effect of Aging on Meiosis Progression, Developmental Competence and DNA Double-strand Breaks in Mouse Oocytes.** Gao Lei, Jia Gongxue, Huang Zhengyuan, Yue Mingxing, Zhang Chao, Zhu Shien, Fu Xiangwei

This study investigated the effect of aging on meiosis progression, embryo developmental competence and DNA double-strand breaks (DSBs) in mouse oocytes and resultant early embryos. Germinal vesicle (GV) oocytes were first cultured to monitor the progression of germinal vesicle breakdown (GVBD) and polar body extrusion (PBE) during *in vitro* maturation (IVM), then the harvested metaphase II (MII) oocytes were parthenogenetically activated to evaluate pronuclear (PN) formation of parthenogenetic embryo and embryo development. The cytoplasmic maturation was examined by measuring the intracellular reactive oxygen species (ROS) and glutathione (GSH). DNA DSBs were examined by immunostaining of pi-H2AX, the marker of DNA DSBs. The results showed that the GVBD rates were similar in oocytes of young and aged mice. Polar body extrusion was significantly delayed in aged mice (P < 0.05), however the rate of polar body extrusion was similar to that of young mice at 16 h of IVM. Moreover, PN formation of parthenogenetic embryo was significantly delayed in aged mice (P < 0.05). Afterward the two groups obtained similar results with respect to the percentages of activated oocytes, 2-cell embryos and blastocysts. The cytoplasmic maturation of MII oocytes and
blastocysts in aged mice were significantly compromised to those of young mice (P < 0.05). Furthermore, GV oocytes, 2-cell embryos and blastocysts showed significantly higher relative intensities of pi-H2AX in aged mice (P <0.05). Taken together, our result indicate that aging disturbed oocyte maturation and parthenogenetic embryo development, which could be related to insufficient cytoplasmic maturation and worsening DNA DSBs in oocytes and early embryos.

**P132 - Age-specific Fully- and Hypo-glycosylated Follicle Stimulating Hormone Glycoforms are Bioactive in Isolated Murine Secondary Follicles.** Leah Simon, T. Rajendra Kumar, Francesca Duncan

Female reproductive aging is characterized by a rise in FSH levels during the peri-menopausal period. The relative abundance of N-glycosylated FSH glycoforms shifts during reproductive aging from hypo-glycosylated FSH21 in younger women to fully-glycosylated FSH24 in older women. Previous in vitro studies in granulosa cell lines showed that hypo-glycosylated FSH21 was more bioactive and in vivo studies in Fshb null mice showed that both glycoforms elicited bioactivity. However, the physiological function of FSH glycoforms in directly regulating the ovarian follicle has not yet been determined. We investigated whether FSH21 and FSH24 have differential effects on activation of downstream FSH-responsive signaling pathways in the murine follicle. Secondary follicles from pre-pubertal mice were isolated and treated with 0-, 5-, 10- and 25mIU/mL of either recombinant human FSH (rhFSH) (80:20 mixture of fully- and hypo-glycosylated glycoforms), or 20- and 100ng/mL of individual purified FSH glycoforms. Treatment times were for one-hour or overnight representing acute and chronic exposures respectively. Immunoblot and immunofluorescence techniques were used to analyze the activation status of downstream PKA and MAPK signaling pathways. Induction of phospho-PKA substrates and phospho-p-44/42 MAPK in follicles were dose- and time-responsive to rhFSH relative to the untreated control. At both time points, treatment using 20- and 100ng/mL purified FSH glycoforms resulted in similar trends of increased expression of phosphorylated PKA substrates relative to control. Our results indicate that purified FSH glycoforms induce signaling events in isolated follicles. Studies are ongoing to determine the effects of these glycoforms in follicle gene regulation, steroidogenesis and growth dynamics. This research will advance our understanding of reproductive aging in relation to FSH glycosylation trends, and possibly optimizing ART protocols, which rely heavily on FSH usage for follicle growth. Supported by M.S-RSM program at Northwestern University and Makowski Endowment, University of Colorado Anschutz Medical Campus.

**P133 - Characterization of Akt isoforms in the mouse ovary and their impact on the Ovarian Reserve.** Dadou Lokengo, Pascal Adam, Laurence Tardif, Sophie Parent, Eric Asselin

Ovarian reserve (OR) is one of the fertility determinants. Its alteration results in ovarian aging and infertility. In recent decades, women tend to postpone their first childbirth beyond 35 years, thus reducing fertility due to ovarian aging. Exhaustion of OR involves atresia and follicular growth, processes involving the PI3K/Akt kinase signaling pathway. The emphasis on therapeutic perspectives concerning the management of ovarian aging in the context of infertility involves elucidating the mechanisms of onset of this alteration. Studies establish the role of Akt
in folliculogenesis but not those of its specific three isoforms. However, they possesses specific roles. In this respect, this study seems useful because it may pave the way for specific therapies for women with early ovarian aging who attempt to conceive. Our goal is to characterize these Akt isoforms in the ovary and determine their impact on the OR. With wild-type and utero-ovarian PR-Cre specific Knock Out mice for all Akt isoforms, and their combination at 45 and 100 days old, we studied the expression of Akt isoforms by comparing the number of follicles and the expression of specific Akt downstream targets by immunohistochemistry. The results tend to show that these isoforms show variable expression (persistent expression of Akt2 in some phases) and location (nuclear for Akt1; cytoplasmic for Akt2 and 3) through the estrous cycle and significant OR alteration of their cancellation at 100 days. It appears that it is the double cancellation of specific isoforms (1 and 3) which alters the OR. Some Akt targets (Bad, Fox03a) are phosphorylated by specific isoforms (Akt3 and Akt1) than by others (Akt2) for the protection of OR. These three isoforms keep redundancy on phosphorylation of others Akt targets (GSK3β specifically in metestrus). This specific implication of Akt isoforms on OR could lead to the development of therapeutic targets.

**P134 - Dehydroepiandrosterone on follicular fluid IGF-I level and IVF outcomes in diminished ovarian reserve women.** Xiaokui Yang, Yonglian Lan, Feiyan Zhao, Qin Wang, Shuyu Wang (Corresponding authors)

**Background:** Diminished ovarian reserve (DOR) is associated with poor response to ovarian stimulation and adverse pregnancy outcomes. The present study was designed to investigate the effect of dehydroepiandrosterone (DHEA) pretreatment regimens on follicular fluid Insulin-like growth factor-I (IGF-I) levels, and evaluate the relationship between IGF-I level and ovarian reserve, and pregnancy outcomes of DOR women undergoing in vitro fertilization (IVF) treatment.

**Methods:** A total of 197 cycles of DOR women undergoing IVF/ intracytoplasmic sperm injection - embryo transfer (ICSI-ET) due to fallopian tube factors and/or male factors were enrolled in the study from August 2015 to August 2017. All patients were distributed into DHEA group (102 cycles) and control group (95 cycles). The ovarian reserve, ovarian response to stimulation and IGF-I levels in follicle fluid were compared. Further analysis was done to illustrate the correlation of IGF-I levels and ovarian reserve.

**Results:** DHEA significantly increased the antral follicle counts (AFC) and IGF-I levels in follicular fluid, compared with the control group ($P<0.05$). There was no significant difference between two groups in the following indices, such as total dosage of gonadotropin (Gn), the estradiol ($E_2$) levels on hCG day, the number of oocytes retrieved, the fertilization rate and clinical pregnancy rate ($P>0.05$). The IGF-I levels in follicular fluid were negative correlated with age ($P<0.05$), and were positively correlated with the number of fertilization and clinical pregnancy outcomes ($P<0.05$). Furtherly, all DOR patients were distributed into the pregnant subgroup and the non-pregnant subgroup. The levels of IGF-I in the pregnant subgroup were significantly higher than that in the non-pregnant subgroup ($P<0.05$).

**Conclusion:** DHEA can improve AFC and the follicular fluid IGF-I levels in the DOR women. And higher levels of IGF-I are beneficial for pregnancy outcomes.
**P135 - Poor ovarian reserve is associated with the increased levels of FBXO31 in human granulosa cells**

Yonglian Lan, Feiyan Zhao (co-first author), Zhimin Xin, Shuyu Wang, Xiaokui Yang (Corresponding author)

Premature ovarian insufficiency (POI) is an ovarian disorder of multifactorial origin that affect female reproductive health due to infertility and premature menopause. However, the etiology of POI remains undefined. Abnormality of granulosa cell apoptosis, reduced number of follicles formed during ovarian development and increased rate of follicle loss may contribute to POI. Our study showed that poor ovarian reserve was associated with the decreased levels of miR-106a in serum and granulosa, as well as increased expression of FBXO31 in Granulosa cells. To better understand the pathogenesis of POI, in present study, we analyzed miR-106a target gene by bioinformatic analysis, and FBXO31 was verified as the target gene of miR-106a by Dual Luciferase Reporter Assay.

**P136 - The disorder of folliculogenesis in ob/ob mice.** Hee-Seon Yang, Mohammad Mollah, Kil Soo Kim, Hyo-il Jung, Yong-Pil Cheon

Obesity is considered as disease concerned with metabolic disorders associated with reproductive dysfunctions such as sterility and subnormal number of ovarian follicles. The leptin-deficient (Ob/Ob) female mice is infertile but administration of leptin can restore the fertility. Mega-data studies show that the obesity has negative correlation with fertility but it is controversy. In this study, the histological analysis of ovary was performed in ob/ob mice by aging (3, 6, 9, 12, and 24 wk) and the quantitative mRNA expression was analyzed with real-time PCR. The relative ratio of atretic follicles was increased in ob/ob mice from 3 wk. The relative ratio of primary and secondary follicles was decreased from 9 wks. Mis mRNA levels were decreased in ob/ob ovary as compared to lean ovary same as other groups from 3 wks. Stra8 and Dazl mRNAs were decreased in ob/ob ovary as compared to lean ovary but Dazl mRNA level of 6 wk ob/ob was higher than that of 6 wk lean mice. Sohlh1 and Nobox mRNA levels were decreased in ob/ob ovary as compared to lean ovary. Sohlh1 and Msy2 mRNAs were almost not expressed in ob/ob ovary. Zp3 mRNA was significantly decreased in ob/ob ovary as compared to lean ovary. Though needed more studies, those results suggest that the obesity is not reason of the aging of ovary. It may be disorder of ovarian follicle activation in ob/ob mice.

**P137 - Kinetics of FSH Glycoform interactions with the FSHR and activation of intracellular signaling events.** Xiaoying Hou, Vladimir Butnev, Pan Zhang, Jeffrey May, George Bousfield, John Davis

FSH plays a central role in female reproduction. Recent studies indicate that FSH β-subunit glycoforms (based on the presence or absence of either FSHβ Asn7 or Asn24 oligosaccharides) are differentially expressed. Partially glycosylated FSH variants (FSH18 and FSH21, respectively) are more prevalent in young women, while fully glycosylated FSH24 is more prevalent in women approaching menopause. Importantly, FSHR binding studies showed that
FSH21 exhibits higher association rates and greater occupancy than FSH24. However, no studies have been performed evaluating FSH glycoform responses in a homologous model under identical conditions. Our hypothesis is that differences in FSHR association rate and receptor occupancy are reflected in rates of cellular response and signal strength, respectively. Using a CHO cell line expressing human FSHR, binding assays were performed to determine dose dependence and kinetics of $^{125}$I-hFSH21 or $^{125}$I-hFSH24 association with FSHR. Western blots were performed and temporal and concentration-dependent cell signaling responses to FSH21 and FSH24 were quantified. FSH21 consistently occupied 2.3-fold more FSHR binding sites than FSH24 after 15 min of incubation with 15 ng/ml hormone ($P<0.05$). Under these conditions, FSH21 induced significantly greater phosphorylation of CREB, YAP, and P70S6K than FSH24. Our studies also revealed that binding was linear with increasing concentrations at each time point. At 5 min, 5 ng/ml FSH21 binding was 0.93% of total cpm added, whereas 15 ng/ml FSH24 was required to reach to a similar level. FSH21 provoked rapid and concentration-dependent increases in the phosphorylation of CREB, YAP, ERK, and P70S6K ($P<0.05$). Maximal signaling responses were observed with 15 ng/ml of FSH21. The cellular signaling responses to FSH24 (CREB, YAP ERK, and P70S6K phosphorylation) were slow and modest compared to FSH21. These findings using a homologous system indicate that FSH21 exerts greater bioactivity than FSH24 and may be useful for improving fertility. Support P01AG029531.

P138 - Telomere Dynamics Throughout Spermatogenesis. Heather Fice, Bernard Robaire

As delayed parenthood is an increasingly pertinent issue within our society more information is required regarding the influence of advanced parental age on offspring health. The field thus far has shown that offspring from aged fathers have increased incidence of many multigene disorders, potentially due to a disruption in chromatin integrity. This disruption may come from a variety of sources including: oxidative stress, accumulating DNA damage, and conflicting evidence suggests that the length of telomeres may also be altered in sperm of aged fathers. The present study aims to assess telomere dynamics across spermatogenesis during the aging process in both outbred and inbred rat models. Sprague-Dawley (SD) and Brown Norway (BN) rats were aged to 4 and 18 months, with each population representing young and aged groups respectively. The absolute telomere length (aTL) has been measured for pachytene spermatocytes (PS), round spermatids (RS), as well as spermatozoa from the caput and cauda epididymidis using qPCR. No significant differences were found when comparing PS, RS, caput and cauda sperm of young and aged SD rats; this is likely due to high variance observed among individuals. A significant age dependent decrease in aTL was observed from 115.6 kb ($\pm$6.5) to 93.3kb ($\pm$6.3) in caput sperm ($p=0.04$) and from 142.4 kb ($\pm$14.6) to 105.3 ($\pm$2.5) in cauda sperm ($p=0.01$) from BN rats. Additionally, a trend toward increased telomere length during epididymal maturation was observed in all groups, strikingly from 115.6 kb ($\pm$6.5) to 142 kb ($\pm$14.6) in young BN rats; this is the first instance of telomere length changing during transit through the epididymis. These results suggest telomere length decreases in an age dependent manner, consistent with other
rodent models for advanced paternal age. This research has been funded by the CIHR Institute for Gender and Health Team Grant TE1-138298

**P139 - A-to-I RNA editing landscape during human folliculogenesis reveals age associated defects in RNA editing.** Nehemiah Alvarez, Pavla Brachova, Lane Christenson

Fully grown human oocytes and eggs are transcriptionally quiescent, and therefore have a unique RNA environment in which cellular processes depend on post-transcriptional gene regulation. RNA editing of adenosines into inosines (A-to-I) by adenosine deaminase acting on RNA 1 (ADAR1) is a common post-transcriptional regulatory mechanism, yet it has not been systematically studied in human oocytes. Our goal was to characterize the A-to-I RNA editing profile in human oocytes and metaphase II (MII) eggs during folliculogenesis. We identified transcriptome-wide A-to-I RNA edit sites in transcriptionally active, growing oocytes from primordial (613±218, n=4), primary (1,238±153, n=10), secondary (1,205±297, n=4), and antral follicles (2,622±502, n=8), and transcriptionally quiescent MII eggs from young women (≤30yrs; 11,669±795, n=3) and women of advanced maternal age (≥40yrs; 6,492±150, n=2; p<0.05, one-way ANOVA). Advanced age was associated with a decrease in A-to-I RNA edit sites and was correlated with decreased Adar transcript abundance (young 32.6±12.4 vs advanced age 2.4±2.4 transcripts per million). Within the coding sequence (CDS), A-to-I RNA edit sites increased in oocytes isolated from primordial (221±59), to primary (340±42), secondary (188±35), and antral (411±81) follicles, with a maximum level observed in MII eggs from young women (869±31). In contrast, MII eggs from women of advanced maternal age had reduced (553±37) A-to-I RNA editing levels in the CDS. We observed a majority of A-to-I coding region edits in MII eggs from women of both young and advanced maternal age occurring at the codon wobble position. Thus, our analysis demonstrates an age-associated decline in A-to-I RNA editing. Selective A-to-I editing of the codon wobble position has the potential to fine tune post-transcriptional regulation through altering codon usage and mRNA stability to ultimately impact translational efficiency.

**P140 - Involvement of NFκB Signaling in the Rate of Mammalian Primordial Follicle Growth Activation and Ovarian Aging.** Evelyn Llerena Cari, Jeryl Sandoval, Sarah McKenna, Elise Bales, Leanna Nguyen, Alex Polotsky, Clyde Wrigth, Joshua Johnson

NFκB signaling has been associated with the timing of menopause in a large human genome-wide association study. However, our knowledge of factors that activate and execute NFκB signaling within ovarian follicles is incomplete. Tumor necrosis factor alpha (Tnfα) activates NFκB via its receptor Tnfr2. This results in degradation of the inhibitor of kappa-B (IkB) proteins, NFκB subunit nuclear translocation, and downstream gene transactivation. Tnfrα:Tnfr2 activity stimulates primordial follicle growth activation (PFGA), where loss of function resulted in slowed PFGA and ovarian aging. NFκB signaling has not been interrogated downstream of Tnfrα:Tnfr2 signaling, nor has the potential involvement of other NFκB-activating factors, including those that bind Tnfr2. Ovaries from wild-type (WT) ICR mice and the mouse granulosa cell line OV3121 were used to screen candidate NFκB-activating factor expression by RT-PCR and immunostaining. “A knockout/B knock-in” (AKBI) mutant mice, whose IkBα allele has been replaced with IkBβ were used to test the effect of “blunted” NFκB signaling on PFGA, follicle development, and survival.
Histomorphometric follicle analyses were performed on AKBI and WT control ovaries at postnatal day 8 (pn8), 9.5 weeks, and 6 months. Along with Tnfα, the Tnfr2-binding ligands淋巴毒素 alpha and beta (Lta, Ltb) and also the ligand Light/Tnfsf14 are expressed in granulosa cells and the cell line. Lta was upregulated in the granulosa cells of atretic follicles. AKBI mice exhibit significantly slowed PFGA, with no difference in primordial follicles at pn8, but nearly double the number of primordial follicles at 9.5 weeks and 6 months vs. WT controls (p<0.05, Student’s T test). The AKBI phenotype shows that the regulation of NFκB activation by the specific IκB proteins present greatly impacts ovarian aging. These data suggest that multiple NFκB-activating factors may signal at the level of primordial follicles, contributing to the lifetime rate of PFGA.

P141 - Ovarian somatic cells and their influence on post-menopausal health. Tracy Habermehl, McKenna Walters, Kyleigh Tyler, Steven Gawrys, Jeffrey Mason

Reproductively cycling females have a significant health advantage over similarly aged males. However, at menopause, their ovaries become senescent and the risk of health-related diseases increase. Germ cell-depleted ovarian transplants displayed a further extension of life and health span than germ cell-containing ovaries. Replacement of young, ovarian somatic cells allows the ovaries to support improved health in aged females. In the current experiments, controls contained their original ovaries and included mice at: 1) 850 days old (n=5), 2) 600 days old (n=6), 3) 250 days old (n=6) and 4) 150 days old (n=19). Treated mice (600 days old) all received transplantations and included: 1) mice with young, intact ovaries (n=9), 2) mice with young germ cell-depleted ovaries (n=10), and 3) mice with young ovarian somatic cell injections (n=5). Each mouse is participating in several health span assays associated with aging kan post-menopausal conditions. We expect to see changes in cognition, metabolic and immune function, musculoskeletal capabilities, and cardiovascular function within and between treatment and control groups. The suggested expectations arise from the well-established enhancement of health in germ cell-depleted primitive species. The upregulation of FOXO signaling and the preservation of the somatic cells are critical for the improvement of health in those organisms. The FOXO gene plays a role in several cellular pathways, and in the absence of the germ line, FOXO upregulates the pathways needed for survival, thus improving health. Therefore, in the absence of the ovarian germ cells or with the addition of young ovarian somatic cells, FOXO signaling should help to improve those aspects of health influenced by aging. Understanding the communication between the somatic and germ cells with the FOXO signaling is hoped to be a key factor in restoring and improving the health aspects of post-menopausal women in the future.

P142 - Mitochondria DNA methylation programming in oocytes and embryos. Marc-André Sirard

There is an increasing interest to study embryos epigenetic status to understands intergenerational influence of the male or the female environment. Few studies have analyzed DNA methylation on the genomic DNA as the amount is very limited but no study have explored the analysis of mitochondrial DNA (mtDNA) with the same purpose. In this study, methylation
status of mtDNA was investigated bovine oocytes and blastocysts recovered from cows subjected to ovarian stimulation (OS) or from non-stimulated abattoir ovaries (AO) followed by IVF and culture. Pools of 10 GVO or 10 blastocysts were digested with proteinase K and restriction enzyme was used (200 U of SalI for 4 h at 37°C) to linearize the bovine mtDNA. Samples were treated with 130 µL of Pico Methyl-Seq Library Prep kit (Zymo Research). Whole Genome Bisulfite libraries were made with 10 pg to 1 ng of DNA with 10 cycles for two PCR amplification rounds and sequenced. Oocytes and early embryos, contains high number of mitochondria (>200,000) resulting in very high coverage (140-4000x) and very low p values. Overall Methylation level was lower in oocytes compared to blastocysts and was not restricted to CG sites but was found also at CHG and CHH sites. The OA oocytes showed 72, 106 and 1045 hypermethylated sites (P<0.05) for the CpG CHG and CHH cytosines respectively compared to the OS oocytes. The correlation between cytosines in OS oocytes and OS blastocysts was 0.81 (p<0.001). When compared to level of gene expression of 12 mitochondrial genes (ATP6-8, COI-III, CYB, ND1-2-3-4-4L-5-6) obtained by RNAseq an inverse correlation of -0.71 was obtained for oocytes and -0.74 for blastocysts. Collectively, our findings show a conserved pattern of mtDNA methylation which could play a programming role during gametogenesis and would be subject to epigenetic regulation according to the maternal environment.


The embryoid body test (EBT) is a developmental toxicity test method that assesses the half inhibitory concentrations of substances in the area of embryoid bodies (EBs), and in the viability of mouse embryonic stem cells (ESCs) and fibroblasts (3T3 cells). In the previous pre-validation study evaluated the predictive accuracy of the EBT using 26 coded test substances and highly accurate (above 80%) when substances were classified using the predictive model. EBT used two same endpoints as EST, the half inhibition concentrations for cell viability of mouse ESCs (IC50 E14) and 3T3 fibroblasts (IC50 3T3), but replaced the half inhibition concentration for cardiac differentiation (ID50 CM) with the half inhibition concentration for EB area (ID50 EB). We used the hanging drop method to form an embryoid bodies. In order to verify the proposed EBT method in this study, inter-laboratory reproducibility (5 substances in common) and predictive capacity (10 substances in each laboratory) tests were performed. To ensure reliability of the study results, the tests were conducted using identity-coded test substances. The results of statistical analysis of the inter-laboratory reproducibility test indicated that reproducibility accuracy 87%, sensitivity 78%, and specificity 100%. The results of statistical analysis of the predictive capacity test indicated that the lead laboratory had reproducibility accuracy 80%, sensitivity 86%, and specificity 67%. Participatory laboratory 1 had reproducibility accuracy 80%, sensitivity 71%, and specificity 100% and participatory laboratory 2 had reproducibility accuracy 80%, sensitivity 86%, and specificity 67%. The results of the intra- and inter-laboratory 2tests were highly accuracy 83%, sensitivity 80%, and specificity 89% when substances were
classified using the predictive model. EBT can accurately classify various embryotoxicants in a short time with less effort and greater validation.

**P144 - Calbindin-D9k prevent endoplasmic reticulum stress induced pancreatic beta cell death.** Dinh Nam Tran, Changhwan Ahn, Jae-Hwan Lee, Yeong-Min Yoo, Eui-Bae Jeung

Intracellular calcium ion is tightly regulated to maintain cellular function and cell survival. Signals have been proposed to activate signal for hormone secretion. Calbindin-D9k (CaBP-9k) is responsible for regulation of the distribution of cytosolic free calcium ion. The previous study demonstrated that calcium binding protein CaBP-9k contribute to control signal-dependent NAD(P)H formation, respiration, and ATP changes in intact cells. Those regulation determine cell survival and secretory function. Furthermore, in the latest article demonstrated that CaBP-9k expression in insulin secreting and CaBP-9k depletion cause hypoinsulinemia. CaBP-9k KO mice accumulate only a few amounts of abdominal fat compare to wild-type mice result from hypoinsulinemia. Decreased insulin levels impede fat storage into the adipose tissues and other metabolic organs like liver and skeletal muscles. On the other hand, insulin resistance leads to an increase in the amount of fatty acids in the blood circulation due to the loss of insulin's ability to suppress lipolysis. Therefore, the phenotypes in aged CaBP-9k KO mice are related with decrease insulin secretion or production. 6 months old CaBP-9k KO mice showed decreased islet volume, increased cell death marker such as capase-3 and TUNEL staining resulting from endoplasmic reticulum stress which can lead pancreatic β cell death. Collectively, our findings indicate that CaBP-9k play a critical role for protection of pancreatic β cell survival from ER stress which contribute to glucose homeostasis accompanying lipid metabolism.

**P145 - Adult Exposure to Di(2-ethylhexyl) Phthalate and Diisononyl Phthalate Negatively Affects Fertility in Female Mice Twelve Months after Initial Exposure.** Catheryne Chiang, Saniya Rattan, Emily Brehm, Liying Gao, Daryl Meling, Jodi Flaws

Di(2-ethylhexyl) phthalate (DEHP) is a plasticizer that has become a ubiquitous environmental contaminant. Some manufacturers have replaced DEHP with other plasticizers such as diisononyl phthalate (DiNP). However, little is known about the reproductive effects of DEHP and DiNP exposure. Thus, this study tested the hypothesis that adult exposure to DEHP or DiNP negatively affects female reproductive health. To test this hypothesis, adult female CD-1 mice (age 39-40 days) were orally dosed with either vehicle control (corn oil), DEHP (20 or 200 µg/kg/day or 20 or 200 mg/kg/day), or DiNP (20 or 100 µg/kg/day or 20 or 200 mg/kg/day) for 10 days. Twelve months following dosing with no additional exposure, females were vaginally lavaged daily for two weeks to assess their cyclicity. Following cyclicity monitoring, females were paired with untreated male mice for breeding trials. DEHP increased time spent in estrus (20 and 200 µg/kg/day) and decreased time spent in metestrus and diestrus (20 µg/kg/day) compared to control. DiNP increased time spent in estrus (20 µg/kg/day). DEHP increased pregnancy loss (20 µg/kg/day and 200 mg/kg/day) and average live weight of pups (200 µg/kg/day and 200 mg/kg/day) and decreased litter size (200 µg/kg/day) and percent female pups (20 µg/kg/day and 20 mg/kg/day) compared to control. DiNP decreased time to mating and percent of female pups (20 µg/kg/day) and increased pregnancy loss (20 mg/kg/day) and
gestation length (200 µg/kg/day and 200 mg/kg/day) compared to control. Additionally, DEHP and DiNP (20 µg/kg/day) both reduced the percent of females who were able to produce offspring compared to control. These data show that short-term exposure to DEHP and a common DEHP replacement, DiNP, can have negative effects on female fertility long after exposure has stopped. Supported by R56 ES 025147 (JAF), R01 ES 028661 (JAF), Billie A. Field Fellowship (CC), and T32 ES 007326 (CC).

**P146 - Propylparaben exposure affects mouse cultured antral follicle growth and steroidogenesis.** Ayelet Ziv-Gal, Kristene Gedye, Zelieann Craig, Arnon Gal

Parabens are chemicals prevalently used as antimicrobial agents in various products including cosmetics, pharmaceuticals, and foods. Recent studies suggest that parabens may act as endocrine disruptors and may affect reproductive function. However, their direct effects on the mammalian ovary are unknown. We hypothesized that propylparaben exposure of mouse cultured antral follicles inhibits follicle growth and reduces steroidogenic capacity. To test this hypothesis, we mechanically isolated antral follicles (Swiss, aged 32-42 days) and cultured them in dimethylsulfoxide as vehicle control or propylparaben (0.01, 0.1, 1, 10, 100µg/ml) up to 72 hours. Every 24 hours, follicle diameter was determined as a measurement of follicular growth. At 24 and 72 hours, follicles and culture media were collected for gene (Star, Cyp11a1, Hsd17b1, Cyp19a1) and hormone (estradiol, testosterone, progesterone, dehydroepiandrosterone) analyses. Statistical evaluation (ANOVA) indicates that follicle growth was significantly inhibited compared to controls only at 100µg/ml at all times (n=5, p<0.05). Gene levels at 24 hours were similar across treatment groups (n=3, p>0.1). At 72 hours, levels of Star (100µg/ml) were significantly increased, compared to controls (n=3-5, p<0.05); whereas levels of Cyp11a1, Hsd17b1, and Cyp19a1 were similar to controls. Changes in hormone levels at 24 hours include increased dehydroepiandrosterone (10µg/ml) and decreased estradiol (100µg/ml), compared to controls (n=4, p<0.05). At 72 hours, testosterone (10µg/ml) and estradiol (10µg/ml) levels were decreased (n=4, p<0.03); whereas propylparaben (100µg/ml) increased estradiol levels compared to controls (n=4, p=0.07). These data suggest that propylparaben exposure directly affects mouse cultured antral follicles by altering steroidogenesis and inhibiting follicle growth.

**P147 - Metabolic regulation of sex differentiation under starvation in medaka.** Yu-ta Sakae, Yuki Sugiura, Akira Oikawa, Masatoshi Mita, Toshiya Nishimura, Minoru Tanaka

Medaka employs an XX/XY sex determination system and does not display sex reversal in natural conditions. However, we found that 20% of XX medaka (n=173) developed into male by 5 day-starvation after hatching. The amount of pantothenate increased in starved XX medaka, and the expression level of Pank, encoding the rate-limiting enzyme of pantothenate metabolism pathway, was downregulated, suggesting the involvement of suppression of pantothenate metabolism in the sex reversal. Supporting this, 15% of XX medaka (n=75) treated with Pank inhibitor caused female to male sex reversal. One of the important metabolites from pantothenate metabolism is coenzyme A (CoA), and it is generally known that CoA affects lipid metabolism. HPTLC analysis indicated that triglyceride dramatically decreased among the lipid components under starved conditions.
This suggests that synthesis of fatty acid and/or triglyceride is important for sex differentiation. As expected from the results, inhibition of fatty acid synthesis by C75 showed female to male sex reversal (13%, n=57). *Dmrt1*, an essential gene for male development, was upregulated by starvation, Pank inhibitor and C75 treatment while starvation increases the expression level of both of *foxl2* and *aromatase*. Interestingly, the increase of *dmrt1* expression was suppressed by administration of a certain type of fatty acids. This study suggests a novel mechanism on sex differentiation, and the sex of medaka with genetic sex determination system can be affected through the change of metabolism. This research was partly supported by a grant from the Project of the NARO Bio-oriented Technology Research Advancement Institution (Research program on development of innovative technology), a Grant-in-Aid for Scientific Research on Innovative Areas (17H06430) and a Grant-in-Aid for Scientific Research (A) (16H02514).

P148 - Prenatal Exposure to an Environmentally Relevant Phthalate Mixture Alters the Ovarian Steroidogenic Pathway in the F1, F2, and F3 Generations of Adult Cycling Female Mice. Emily Brehm, Kathleen Leon, Changqing Zhou, Liying Gao, Jodi Flaws

Phthalates are used in personal care products and plastic products. Previous studies have mainly focused on single phthalate exposure, however, humans are exposed daily to mixtures of chemicals, including phthalates. Previously, our laboratory developed a phthalate mixture made of 35% diethyl phthalate, 21% di(2-ethylhexyl) phthalate, 15% dibutyl phthalate, 15% diisononyl phthalate, 8% diisobutyl phthalate, and 5% benzylbutyl phthalate that mimics human exposure. We tested the effects of prenatal exposure to this mixture in the F1, F2, and F3 generations of female mice and found that it impaired reproductive outcomes in multiple generations. Impaired reproductive outcomes could be due to abnormalities in the ovarian steroidogenic pathway. However, it is not known whether this phthalate mixture affects this pathway in females. Thus, we tested the hypothesis that prenatal exposure to a phthalate mixture alters the ovarian steroidogenic pathway in a multigenerational manner in female mice. Pregnant CD-1 dams were orally dosed with vehicle control (tocopherol-stripped corn oil) or a phthalate mixture (20 and 200 µg/kg/day, 200 and 500 mg/kg/day) daily from gestational day 10 to birth. Adult F1 females born to these dams were used to generate the F2 generation by mating them with unexposed males and adult F2 females were used to generate the F3 generation by mating them with unexposed males. Tissues and sera were collected at postnatal day 60. Sera were used to measure hormone concentrations. Ovaries were subjected to RNA isolation, cDNA synthesis, and qPCR analysis. The phthalate mixture altered hormones in the F1, F2, and F3 generations. Further, the mixture altered the expression of ovarian steroidogenic enzymes in a multigenerational and transgenerational manner. Overall, prenatal exposure to a phthalate mixture is detrimental to ovarian steroidogenesis in a multigenerational and transgenerational manner in female mice. Supported by NIH P01 ES022848, EPA RD-83459301, and NIH R25 ES025059.

P149 - Low-doses Bisphenols exposure affecting follicle development in next generation in mice. Luhan Jiang, Hongjie Fan, Ernest Ng, William Yeung, Kai-Fai Lee
Bisphenols (BPA, BPS and BPF) are group of endocrine disruptive chemicals (EDC) because of their weak estrogenic effect. However, whether these bisphenols exert similar estrogenic effect to mammalian reproductive functions remains largely unknown. Recently, an in vivo study in female mice (F0) showed that exposure of chronic low-dose BPA before and during gestation impaired fertility of their offspring (F1). Their ovaries became vulnerable to challenges of environmental pollutants. We hypothesized that exposure to low-dose bisphenols during embryonic development altered ovarian development and functions, leading to impaired fertility in adulthood. In this study, female mice were treated with bisphenol A, S, F and A+F at 500 mg/Kg/day for 30 days before pregnancy and throughout gestation. The ovaries of their female offspring were collected at 6-8 weeks of age and serial sectioning were performed. The number of developing follicles at different developmental stages were counted. Apoptosis and cell proliferation of the developing follicles were quantitated by TUNEL and Ki67 staining, respectively. We found that the number of activated follicles (i.e. secondary, pre-antral and antral) were significantly increased in BPA (p<0.01) and BPF (p<0.05) group when compared to controls while activated follicle number in BPA+BPF group is comparable to control. Apoptosis in granulosa cells were significantly higher in BPA (p<0.05), BPF (p<0.05) and BPA+F (p<0.01) groups than that in the control. No significant difference was found in granulosa cells proliferation among all groups. Although more primordial follicles were activated in adult F1 mice, the apoptosis level of the activated follicle was also increased, indicating more developing follicles underwent atresia before ovulation. Therefore, low-dose bisphenols may lead to early primordial follicle pool exhaustion in adulthood resulting in impaired fertility in the F1 generation. [This project is supported in part by GRF 17120415 to KFL]

P150 - The role of beta-carotene metabolism in maternal cardiac remodeling: findings in mice lacking beta-carotene 9',10'-oxygenase (BCO2). Chelsee Holloway, Youn-Kyung Kim, Loredana Quadro

High intake of fruits and vegetables, main vitamin A sources, is associated with improved adult cardiac function and may affect heart remodeling and function during pregnancy. Beta-carotene, the most abundant dietary precursor of vitamin A, is cleaved by beta-carotene 15,15'-oxygenase (BCO1) and beta-carotene 9',10'-oxygenase (BCO2). BCO2 is the only beta-carotene cleavage enzyme expressed in adult hearts. During pregnancy, cardiac Bco2 expression is elevated at mid-gestation in wild-type (WT) mice when the heart is hypertrophic. However, in Bco2/-/- mice, the maternal heart fails to enlarge. We aimed to elucidate the role of BCO2 in maternal cardiac hypertrophy and determine if metabolic pathways in the heart are disrupted by loss of BCO2. We hypothesized that BCO2 contributes by affecting cardiac homeostasis of retinoic acid (RA). WT and Bco2/-/- mice raised on a chow diet were sacrificed at pregnancy day 14.5. WT mice have cardiac RA deficiency during pregnancy. While, Bco2/-/- mice already showed cardiac RA deficiency pre-pregnancy. Bco2/-/- females had reduced PDK4 mRNA expression and enhanced PDH activity (phosphorylation) in the heart, that was reversed earlier, at mid-gestation. Bco2/-/- mice exhibited increased cardiac Glut1 mRNA expression and reduced triglyceride levels. Lipid regulatory genes, including Pgc1a and Scd1, were increased at mid-pregnancy in the hearts of WT dams but not in Bco2/-/- mothers. Our data indicate that RA may be involved in modulating cardiac hypertrophy during pregnancy. We determined dysregulation of RA homeostasis in the heart of Bco2/-/- females resulted in metabolic adaptations that makes the heart of non-pregnant
females preferentially dependent on glucose as an energy source. This suggests that during pregnancy retinoic acid deficiency in the Bco2-/- heart may induce an earlier attenuation of PDH activity, facilitating utilization of fat over glucose as energy substrates. Thus, BCO2 may play an essential role in regulating cardiac hypertrophy and function during pregnancy.


Di(2-ethylhexyl) phthalate is a commonly used plasticizer and known endocrine disruptor. Although studies show that it causes transgenerational reproductive toxicity in female rodents, the mechanisms of action are unknown. Therefore, this study examined the effects of prenatal and ancestral DEHP exposure on various ovarian pathways critical for cell growth, proliferation, and function and DNA methylation pathways in mouse ovaries from the F1, F2, and F3 generations. Pregnant CD-1 dams (F0 generation) were orally exposed to corn oil (vehicle control) or DEHP (20 µg/kg/day–750 mg/kg/day) daily from gestation day 10.5 until birth. F1 females were mated with non-treated males to produce the F2 generation and F2 females were used to produce the F3 generation. At postnatal day 21 from each generation, mice were euthanized and ovaries were removed for gene expression analysis of steroid hormone receptors, DNA methyltransferases (Dmnt), and ten-eleven translocation enzymes (Tet) as well as 5-methyl cytosine (5-mC) levels. In the F1 generation, prenatal DEHP exposure increased the expression of estrogen receptor 2 (Esr2), decreased the expression of androgen receptor (Ar), Dnmt1, and peroxisome-proliferator activating receptor gamma (Pparg), and increased the percentage of 5-mC in the ovary. In the F2 generation, DEHP exposure decreased the expression of Ar, Tet1, Tet2, and Tet3 in the ovary. In the F3 generation, ancestral DEHP exposure decreased the expression of Esr2, Ar, Dnmt1, Dnmt3a, Dnmt3b, Tet2, and Tet3 and decreased the percentage of 5-mC in the ovary. Collectively, these data indicate that prenatal and ancestral DEHP exposure decrease the expression of hormone receptors and alter 5-mC levels in the ovary. These results provide insight into various mechanisms of DEHP-mediated toxicity in the ovary across generations. Supported by the Billie A. Field Fellowship in Reproductive Biology (SR), NIH P01 ES 022848 (JAF), EPA RD83 543401 (JAF), F31 ES030467 (SR), and T32 ES007326 (SR).

**P152 - Iodoacetic Acid Inhibits Follicle Growth and Alters Expression of Genes that Regulate Apoptosis, the Cell Cycle, and Ovarian Steroidogenesis in Mouse Ovarian Follicles.** Andressa Gonsioroski, Daryl Meling, Liying Gao, Michael Plewa, Jodi Flaws

The disinfection of drinking water was arguably the most important public health achievement of the 20th century. However, the reaction between disinfectants and organic matter in water generates water disinfection by-products (DBPs). Iodoacetic acid (IAA) is an emerging, unregulated DBP and it is cytotoxic and genotoxic in mammalian cells. IAA was reported as a reproductive toxicant, but its effects on the ovary are not well known. This study determined whether IAA exposure affects ovarian follicle growth and expression of genes that regulate apoptosis, the cell cycle, and steroidogenesis. Antral follicles from CD-1 mice were cultured in
dimethyl sulfoxide (DMSO; vehicle control) or IAA (2-15 µM), and follicle growth was measured every 24 h. After 96 h, RNA was extracted, reverse transcribed, and subjected to quantitative polymerase chain reaction (qPCR) to analyze expression of apoptosis regulators (Bax and Bcl2), cell cycle regulators (Ccna2, Ccne1, Ccnb1, Ccnb2, Cdk4, Cdkn1a), steroidogenesis regulators (Star, Cyp1b1, Cyp11a1, Cyp19a1, Hsd17b1, and Hsd3b2), and estrogen receptors (Esr1 and Esr2). IAA exposure significantly decreased follicle growth compared to controls, beginning at 72 h and continuing through 96 h of culture. Further, IAA exposure decreased expression of the cell cycle regulators Ccnd2 and Ccna2 and it decreased expression of the anti-apoptotic factor Bcl2. In addition, IAA exposure increased expression of the pro-apoptotic factor Bax and the cell cycle regulator Cdk4 and it increased expression of the cell cycle inhibitor Cdkn1a. IAA exposure also decreased expression of Hsd17b1, Cyp1b1, Cyp19a1, Esr1, and Esr2. In contrast, IAA exposure increased expression of Star, Cyp11a1, and Hsd3b2. Collectively, these data show that IAA exposure inhibits follicle growth and alters expression of anti- and pro-apoptotic factors, cell cycle inhibitors, cell cycle regulators, and factors involved in the steroid synthesis pathway. Supported by NIH R21 ES028963 and NIH T32 ES007326.

P153 - Mitotic and Meiotic Germ Cells in the Developing Ovary Are Equally Sensitive to Benzo(a)pyrene-Induced Germ Cell Death. Kelli Malott, Melody Lee, Laura Ortiz, Ulrike Luderer

Polycyclic aromatic hydrocarbons (PAHs), such as benzo(a)pyrene (BaP), are products of incomplete combustion of organic materials. Primordial germ cells (PGCs), the embryonic precursors of oocytes, arise in the mouse yolk sac at 7.25 days postcoitum (dpc). PGCs proliferate before and after arriving at the gonadal ridge 10.5 dpc, entering meiosis 13.5 dpc. Now referred to as oocytes, they arrest in the first meiotic prophase beginning at 17.5 dpc. This finite oocyte pool is the primary determinant of female fertility and reproductive lifespan. We have shown that in utero exposure of female mice to 2 or 10 mg/kg/day BaP during a dosing window that spans the proliferative and meiotic stages of PGC development results in depleted follicle numbers postnatally. We hypothesized that PGCs are more sensitive to BaP exposure during rapid proliferation, starting at 6.5 dpc prior to entry into meiosis at 13.5 dpc. We exposed timed-pregnant female mice to 2 mg/kg/day of BaP or oil vehicle, by oral pipetting, from 6.5-11.5 dpc and 12.5-17.5 dpc corresponding to PGC mitosis and meiosis, respectively. One female from each litter was euthanized for ovarian follicle counts on first vaginal estrus, assessed by vaginal cytology.

Our preliminary data for small follicles show that the females exposed to BaP, regardless of timing, were sensitive to PGC depletion (N=3-8). We observed statistically significant decreases in primordial and primary follicle counts with BaP dose (P< 0.005, 2-way ANOVA), but no statistically significant effects of dosing window or of the interaction between BaP dose and window (P>0.48). We see little change in number of large follicles for both dosing windows. These preliminary results suggest that PGCs are equally sensitive to BaP-induced cell death during proliferation and meiosis. These results demonstrate that prenatal exposure to BaP depletes the finite ovarian reserve. This research was supported by NIH R01ES020454.
P154 - Perturbed tryptophan-serotonin pathway induced by vitamin B6 deficiency is linked to gestational glucose intolerance in mice. Ashley Fields, Philip Spinelli, Martha Susiarjo

Gestational diabetes, affecting 10% of pregnancies, can adversely influence maternal health. Although the etiology remains unclear, reduced maternal serotonin has been linked to gestational diabetes in mice. In pancreatic islets, catabolism of tryptophan into serotonin and the subsequent activation of serotonin receptor 2b (Htr2b) play a key role in beta cell proliferation during pregnancy and the regulation is crucial for maternal glucose homeostasis. Factors that reduces serotonin synthesis (e.g. dietary tryptophan restriction) and perturbs Htr2b signaling (e.g. Htr2b deletion) are associated with reduced beta cell number and gestational glucose intolerance. In this study, we investigate the effects of vitamin B6 deficiency (vitB6-def) on glucose tolerance in mice as the tryptophan-serotonin pathway is dependent on vitB6 bioavailability. Despite ~40% of women of reproductive age being vitB6-def, its impact on maternal glucose homeostasis is poorly understood. We hypothesize that gestational vitB6-def will decrease maternal serotonin through inadequate depletion of tryptophan; these changes will reduce beta cell proliferation by decreased Htr2b activation and results in gestational glucose intolerance. To test this, we treated mice with various doses of vitB6-def diets. Mild (0.5 mg/kg vitB6; n=6) and severe (0.1 mg/kg vitB6; n=10) vitB6-def dams had reduced serum serotonin at gestational day (GD)16.5 (p=0.0045). Additionally, the reduced serotonin in mild (p=0.020) and severe (p=0.024) vitB6-def dams was associated with glucose intolerance. To determine if the vitB6-def induced glucose intolerance was associated with decreased Htr2b activation, we treated mild vitB6-def dams with an Htr2b agonist (GD9.5) and measured glucose tolerance (GD16.5). Preliminarily, the Htr2b agonist rescues gestational glucose intolerance in mild vitB6-def dams (n=5, p=0.576) compared to control. These findings demonstrate that vitB6-def induces glucose intolerance in a serotonin-dependent mechanism. Future studies are aimed at determining if vitB6-def reduces beta cell number through insufficient islet serotonin and decreased Htr2b activation by LC-MS and western blot, respectively.

P156 - Alterations in expression of transcription factors in bovine granulosa cells exposed to oxidative stress. Mohamed Taqi, Samuel Gebremedhn, Mohammed Saeed-Zidane, Dessie Salilew-Wondim, Michael Hoelker, Karl Schellander, Dawit Tesfaye

Cells respond to intra or extracellular challenges to establish cellular homeostasis by activating or repressing several transcription factors (TFs). These physiological and molecular events are believed to occur in many organs including ovaries. Therefore, this study was aimed to unravel the TFs regulation in bovine granulosa cells (bGCs) subjected to oxidative stress (OS). Accordingly, sub-confluent bGCs were treated with 5µM H2O2 for 40 min to induce OS. 24 hrs later stress-related phenotypes, lipid accumulation and expression of candidate TFs (Nrf2, c-FOS, E2F1, SREBF1, SREBF2, KLF4, KLF9 and NOTCH1) as well as KLF4 downstream (CCNB1, CCND1, BAX and CDKN1A), endoplasmic reticulum (ER) stress (Grp78 and Grp94), apoptosis (CASP3, BCL2L1) and differentiation (CYP11A1, CYP19A1 and STAR) marker genes were assessed. Additionally, the Nrf2 was selectively knocked down using siRNA to investigate its potential cross-talk with candidate TFs. Biological triplicates were used and results have been statistically analyzed using student’s two-tailed t-test at $P \leq 0.05$. Treatment of bGCs with H2O2 increased the intracellular reactive oxygen species and lipid accumulation as
well as reduced the mitochondrial activity accompanied by upregulation of ER stress marker genes. Moreover, H$_2$O$_2$ challenge induced the expression of candidate TFs, except KLF4 and its downstream gene CCNB1. Consequently, the pro- and anti-apoptotic marker genes were up- and downregulated, respectively, being associated with the reduction in cell proliferation. Meanwhile, the differentiation marker genes were upregulated in stressed group. Knockdown of Nrf2 led to concomitant reduction of KLF9, SREBF2 and c-FOS. However, the mRNA expression, protein level of KLF4 and its downstream genes CCND1, CDKN1A and BAX were upregulated upon Nrf2 knockdown. Thus, these data indicate that bGCs response to OS by differential activity of various TFs associated with differentiation, apoptosis and antioxidant activity, which are important during folliculogenesis.

**P157 - Gestational Diabetes-associated Epigenetic Modifications involved in Developmental Origins of Ovarian Dysfunction.** Olivia Nave, Christina Seger, Aritro Sen

Maternal perturbations or sub-optimal conditions during development are now recognized as contributing to the onset of many diseases manifesting in adulthood. Gestational diabetes (GD), that is characterized by hyperglycemic intra-uterine environment and fetal hyperinsulinemia, is one of the most common conditions for the ‘fetal origins of adult disease’. Here we elucidate the underlying mechanism(s) of epigenetic modifications induced by adverse intrauterine environments associated with GD and its manifestation as ovarian dysfunction later in life. Using two well-established GD mouse models (STZ- and high fat high sugar, HFHS-induced), we have found that female offspring from GD dams are predisposed towards fertility problems later in life. Our studies show that this predisposition to fertility problems is due to fetal epigenetic modifications involving glucose- and insulin-induced micro-RNA, miR-101 and PI3K/Akt pathway. These signaling events regulate three epigenetic modifiers: Ezh2 (promotes histone H3 lysine 27 trimethylation-H3K27me3, associated with gene silencing); MLL (promotes methylation of histone 3 on lysine 4 -H3K4me3- a gene activation mark); and CBP/ p300 (promotes acetylation of histone 3 on lysine 3 on lysine 27-H3K27ac, associated with transcription activation) in the fetal ovary. We find that these epigenetic regulators reprogram the expression of a peptide called Cocaine-and Amphetamine-Regulated Transcript (CART), that is known to negatively affect folliculogenesis. The CART promoter (in the fetal ovary) gets reprogramed into a “super-promoter” through changes in specific gene silencing (H3K27me3) and transcription activating (H3K4me3 and H3K27ac) epigenetic marks. Previously we reported that obesity-induced hyperleptinemia directly induce the expression of CART. Here we further show that the GD-induced reprograming of the CART promoter in the fetal ovary makes the offspring more sensitive to leptin levels, such that in adult life when GD offspring are fed with low fat diet, even if they do not become obese, they develop ovarian dysfunction and fertility problems, compared to control (non-GD offspring) animals.

**P158 - Transglutaminase activity in mammalian gametes after heat stress.** Muhammad Mustafizur Rahman Chowdhury, Yeoung-Gyu Ko, Sung Soo Lee, Sung Woo Kim

Transglutaminase (TGs) belongs to enzymes liable for catalyzing Ca$^{2+}$-dependent acyl-transfer reactions between the substrate proteins. In this study, we have traced the transglutaminase 2 activity in mammalian gametes after heat stress.
(TGM2) and anti-N epsilon gamma-glutamyl lysine (Nε-γ-glutamyl lysine) activity in heat stressed mouse and bovine oocytes and sperm. The heat treated oocytes were stained pattern TGM2 and Nε-γ-glutamyl lysine activity after 6 h. Both TGM2 and Nε-γ-glutamyl lysine intensity were significantly (P < 0.05) higher but the Nε-γ-glutamyl lysine was 2.5 fold more in heat stressed mouse oocytes compared to control and also 1.5 times higher in case of Nε-γ-glutamyl lysine and 2 fold higher for TGM2 expression in bovine when oocytes were subjected to HS at 6 h of IVM at 39 ºC. These results recommend that, the cytosolic accumulation of the TGM2 might play a vital role throughout the oocyte maturation process in cytosol during folliculogenesis and early embryonic development. Nevertheless, even after three replications, there was no remarkable intensity of TGM2 enzyme observed in case of bovine sperm (both control and treated group), only in the equatorial region of the sperm head, neck and tail slightly show the Nε-γ-glutamyl lysine activity. Our hypothesis is that TGM2 and Nε-γ-glutamyl lysine may be correlated with the cellular physiology of mouse and bovine oocyte and also aids in the detection of the principal proteins as well as the pathogenic mechanism with altered physiology. Whereas TGM2 may not be involved in the sperm physiological activities, another isotype of TGs would be convoluted and still need to clarify. We need to approve this through Western blotting with associated marker antibodies. Further studies are going on to elucidate this with detailed molecular mechanisms.

**P159 - Impacts of Thermal Neutral Housing on Murine Reproduction and Metabolism.**
Katie Bidne, Rong Fan, Alana Rister, Eric Dodds, Soonkyu Chung, Jennifer Wood

When male and female mice fed a western diet are housed at thermal neutral temperatures (30-32º C), the metabolic phenotypes that develop better mimic human obesity than when mice are housed at traditional temperatures (control, 20-23º C). However, there is no information regarding female reproduction in this model. When we housed female C57/BL6J mice fed normal rodent chow at thermal neutral temperature, they had decreased post-plug pregnancy rate (30%) compared to control mice fed normal rodent chow (70%). We hypothesized that increased temperature reduces oocyte quality due to metabolic stress. To test this hypothesis, female C57/BL6J mice were placed in either thermal neutral (n = 8) or traditional housing (n = 9) at weaning and fed normal chow until 8 weeks of age. Ovulation was stimulated and rectal temperatures were taken. After euthanasia ovulated oocytes, body weight, serum, and tissue samples were collected. Thermal neutral housing significantly increased white and brown fat mass compared to control-housed mice. There were no differences in rectal temperature or blood glucose between the thermal neutral and control groups. Liquid chromatography-mass spectrometry of serum showed a tendency for increased corticosterone concentrations (p = 0.07) in the thermal neutral group compared to control mice. Oocytes from both groups were fertilized in vitro and cultured to blastocysts. There were no differences in the number of embryos that developed from the 2-cell to blastocyst stages between experimental groups. Previous studies in the literature demonstrated that exogenous corticosterone administration in mice impaired fertility via reduced implantation without altering oocyte competence or pre-implantation embryo development. In addition, elevated corticosterone downregulates thermogenesis while increasing lipid droplet accumulation in brown adipose tissue. Collectively, these results suggest
that thermal neutral housing induces metabolic and thermal stress which decreases female reproduction, likely due to decreased uterine receptivity.


Hypospadias, a developmental defect of the penis, is one of the most common congenital malformations in humans. Its incidence has rapidly increased over recent decades, and this has been largely attributed to our increased exposure to endocrine disrupting chemicals. Penis development is primarily an androgen driven process, however estrogen and xenoestrogens are known to affect penis development in both humans and mice. Here, we investigated the role of estrogen in the developing penis. Using a novel penis culture system we showed that exogenous estrogen directly targets the developing penis to cause hypospadias. In addition, we also uncovered an unexpected endogenous role for estrogen in normal penis development and showed that a loss of estrogen signaling results in a mild hypospadias phenotype, the most common manifestation of this disease in humans. Our findings demonstrate that a delicate balance of androgen and estrogen signaling is intrinsically required for normal urethral closure. These findings confirm that penis development is not an entirely androgen driven process, but one in which endogenous estrogen signaling also plays a critical role and has implications for the impact of endocrine disrupting chemicals on hypospadias in humans.

**P162** - *Proteomic signature of BPA-induced transgenerational effects on male reproduction in a mouse model.* Md Saidur Rahman, Do-Yeal Ryu, Shehreen Amjad, Won-Ki Pang, Sarder Arifuzzaman, Won-Hee Song, Yoo-Jin Park, Myung-Geol Pang

Bisphenol A (BPA) is a man-made ubiquitous endocrine disrupting compound. BPA in the food, beverages, air, dust, and water are the common sources of human exposure. Developmental exposure to BPA alters neurological and reproductive phenotypes. Here, we demonstrate that male mice exposed to BPA during puberty affect reproductive functions in a transgenerational fashion. BPA deteriorated sperm count, motility parameters, and intracellular ATP levels via decreased in the number of type VIII seminiferous epithelium in testis and reduction of protein kinase A and tyrosine phosphorylation activities in spermatozoa subsequently affects male fertility. These harmful effects persisted up to second filial generation (F2) or beyond those progenies having never been exposed to BPA. Proteomic study and subsequent data analyses revealed 14 differentially regulated proteins in the spermatozoa of F0 male between BPA-exposed and control mice. In addition, we used Western blotting to predict changes in these proteins in different generations. The data showed that BPA-mediated effects on male reproduction are related to parallel proteomics alterations in spermatozoa, which may impair ATP generation, oxidative stress response, and fertility. These results may shed light on how pubertal exposure to BPA causes transgenerational effects on male fertility. Given these results, we suggest that the differentially expressed proteins may be a biomarker indicative of pubertal BPA exposure thus could be considered for the theranostic purposes for the environmental perturbation.
**P163 - BPA and BPS affect microRNA expression during bovine oocyte maturation and early embryo development.** Reem Sabry, Leanne Stalker, Jonathan LaMarre, Laura Favetta

Endocrine disrupting chemicals (EDCs), such as Bisphenol A (BPA) and Bisphenol S (BPS), are commonly used plasticizers. BPA decreases oocyte maturation, resulting in poor embryo development. Our lab has shown that oocytes treatment with physiologically relevant doses of BPA results in poor oocyte maturation, low cleavage and blastocyst rates. Due to its adverse effects, BPA has been replaced with its analog, BPS. Unlike BPA, not much is known about the effects of BPS. MicroRNAs are key regulators of gene expression during oocyte maturation and embryo development. BPA targets specific microRNAs, however, the exact mechanism is unknown.

This research aims to identify BPA/BPS target microRNAs and to investigate how BPA and BPS affect their expression. Bovine oocytes were matured *in vitro* under four treatment groups: control, vehicle (0.1% Ethanol), BPA (0.05 mg/ml in 0.1% Ethanol), BPS (0.05 mg/ml in 0.1% Ethanol). Pools of 40 cumulus-oocyte complexes (COCs), denuded oocytes, and their corresponding cumulus cells were analyzed after maturation. Additionally, COCs were fertilized and embryos were collected at key developmental stages (2-4 cell stage, 8-16 cell stage, blastocyst stage). qPCR results showed BPA significantly upregulates miR-21 expression in COCs and cumulus cells, but not in denuded oocytes (p=0.02). miR-155 is significantly upregulated only in BPA treated denuded oocytes (p=0.03). miR-34C was significantly downregulated in BPA treated cumulus cells, but not in COCs or denuded oocytes (p=0.02). Preliminary results in the embryos show a significant increase in miR-34C expression at the 8-16 cell stage after BPA treatment (p=0.001). BPS shows no effects on the microRNAs analyzed, suggesting that BPS might act through a different mechanism and might potentially be a good substitute to BPA.

Further experiments are being conducted on downstream targets of the affected microRNAs to understand how the effect of BPA and BPS on microRNAs may regulate changes during early development.

**P164 - Combined therapeutic approach of interferon-tau (IFNT) and arginine decreased body white-fat gain and adiposity in obese Zucker Diabetic Fatty (ZDF) rats.** Erin Posey, Guoyao Wu, Fuller Bazer

Interferon-tau (IFNT), a Type 1 interferon, was discovered as the maternal of pregnancy signal in ruminants. Since its discovery, IFNT has also been shown to decrease adiposity in obese rat models by decreasing formation of white adipose tissue (WAT) and increasing lipolysis. ARG, a conditionally essential amino acid, also decreases adiposity in rat models by increasing oxidation of fatty acids in fat depots in the body. ARG and IFNT also stimulate brown adipose tissue (BAT) formation. This study tested the hypothesis that the combination of ARG and IFNT would decrease body WAT greater than either therapeutic alone in the Zucker Diabetic Fatty (ZDF) rat. 41-day-old male ZDF rats were assigned randomly to one of four treatments groups:
(1) alanine (isonitrogenous) control, (2) IFNT + alanine, (3) ARG, and (4) IFNT + ARG. Animals had *ad libitum* access to Purina 5008 diet throughout. Treatments were administered via drinking water over the course of 9 weeks. Individual rat body weights were determined each morning. Food intake did not differ (*P* > 0.05) among the treatment groups. All three treatments, including IFNT + ARG (*P* < 0.05), decreased body weight gain without affecting skeletal muscle mass, compared to the control. ARG + IFNT decreased (*P* < 0.05) body weight gain to a greater extent than ARG alone or IFNT alone. There was a similar trend for treatment effect to decrease overall WAT in the ZDF rats. The combination of ARG + IFNT increased BAT 1.5 fold, compared to the alanine control. Overall, results of this study indicate that a combined therapeutic approach of IFNT plus ARG decreases overall body weight and WAT, and increases BAT to a greater degree than either IFNT or ARG individually. This research was supported by the T3 Grant Program from the Texas A&M University President’s Excellence Fund.

**P165 - Successful Detection of Marijuana-derived Phytocannabinoids in Human Follicular Fluid by Mass Spectrometry.** Brandon Wyse, Noga Fuchs Weizman, Miranda Defer, Mugundhine Sangaralingam, Sahar Jahangiri, Isabel Wiesenfeld, Clifford Librach

Cannabis/Marijuana is the third most commonly used substance by women of childbearing age. Therefore, for those users requiring IVF to conceive, knowledge of its potential effect on IVF treatment and outcome is of utmost importance. The endocannabinoid system is required for successful reproduction and can potentially be disrupted by phytocannabinoids. Previous studies in humans exploring the effects of THC on female fertility have relied on self-reporting, which introduces significant bias. The objective of this study was to develop a methodology that enables the measurement of delta9-THC and its metabolites in follicular fluid (FF). Twenty-seven consented patients undergoing IVF at CReATe Fertility Center (Toronto, CA) were enrolled in the study. Patients were allocated to either the test group (previous cannabis use) or control group (no previous use), based on self-reporting. FF from both leading and subordinate follicles (75ul) were assayed for delta9-THC, and two of its metabolites 11-OH- and 11-COOH-delta9-THC by LC-MS/MS at Analytical Facility for Bioactive Molecules (Hospital for Sick Children; Toronto, ON). Proteins were precipitated using 1:1-methanol:water, pelleted, and the supernatant measured by LC-MS/MS using a QTRAP 5500 and Agilent 1290 HPLC. Calibration curves (0.001-200ng) were generated to permit absolute quantification. Data acquisition and quantification was performed with Analyst 1.6.2 software. All control samples were negative for all phytocannabinoids. Among test samples, 23.1% were positive for both delta9-THC (12.8-75.1nM) and 11-OH-delta9-THC (1.7-16.4nM); 38.5% were positive for 11-COOH-delta9-THC (3.1-111.5nM). Interestingly, subordinate FF had consistently higher concentrations of all phytocannabinoids, with THC being significant (*p*=0.001), and a trend in 11-COOH-delta9-THC (*p*=0.10). This is the first study, to our knowledge, to assess phytocannabinoids in FF. We were able to determine levels of THC and two of its metabolites in FF with high sensitivity. This study was underpowered to determine the impact phytocannabinoids have on IVF cycle outcomes, which we are currently investigating.
P167 - Oxidative Stress Alters the Expression Profile of Dppa3 in Oocytes and Decreases Di-Methylation of Histone H3K9 in the Pre-Implantation Embryo. Alison Ermisch, Kelsey Timme, Jennifer Wood

Increases in obesity rates are correlated to poor fertility in women. To mimic human obesity, female mice are fed a high fat and/or high sucrose diet which causes ovarian inflammation and oxidative stress. These obesity phenotypes lead to reduced oocyte quality. Data from our lab showed altered expression of the maternal effect gene *Dppa3* in oocytes from obese mice. We hypothesized that oxidative stress dependent changes in the expression and/or localization of *Dppa3* transcripts alters DPPA3 protein expression and methylation profiles in embryos which could have long-term effects on embryonic and fetal development. To test this hypothesis, murine cumulus oocyte complexes (COCs) were *in vitro* matured (MII) in the absence or presence of 100µM hydrogen peroxide (H₂O₂). Overall *Dppa3* mRNA abundance was significantly decreased in H₂O₂ exposed compared to control MII oocytes. However, localization of *Dppa3* mRNAs, measured by fluorescence in situ hybridization (FISH), was significantly increased in the sub-cortical maternal complex region of the H₂O₂ exposed oocytes. Both control and H₂O₂-matured oocytes were subsequently *in vitro* fertilized and cultured under normal conditions. There was no difference in *Dppa3* abundance in 2-cell or 4-cell embryos between the experimental groups. However, DPPA3 protein was significantly increased in 2-cell embryos from the H₂O₂ matured oocytes (i.e. H₂O₂ embryos). Importantly, 4- and 8-cell H₂O₂ embryos tended to have decreased levels of demethylated H3K9. These data indicate that exposure of oocytes to oxidative stress during maturation alters the expression of *Dppa3* mRNA and DPPA3 protein which in turn decreases methylation of H3K9. This epigenetic change may shift the timing of embryonic genome activation and have long term effects on the embryo which could contribute to poor fertility rates in obese women.

P168 - Exposure to Di (2-ethylhexyl) phthalate (DEHP) and high fat diet during early pregnancy disrupts placental development and affects fetal growth in mice. Athilakshmi Kannan, Liying Gao, Juanmahel Davila, Jodi Flaws, Milan Bagchi, Indrani Bagchi

Phthalates are endocrine-disrupting chemicals found in numerous consumer products, and DEHP is found ubiquitously in the environment. Despite constant exposure to phthalates, their impact on early pregnancy and embryo development is not well understood. We conducted studies where pregnant mice were exposed to an environmentally relevant level of DEHP during early gestation to evaluate its effect on placental development. Our results indicate that DEHP exposure causes disorganization of the trophoblast layers and abnormal vascular patterning, indicating defects in placental development. Previous studies indicated that DEHP interacts with members of the peroxisome proliferator-activated receptor family. PPAR<sub>gamma</sub> is expressed in all trophoblast subtypes and is required for proper placental function. In PPAR<sub>gamma</sub>-null mice, labyrinthine trophoblast precursors fail to undergo terminal differentiation, causing defects in blood vessels permeation and compromised maternal-fetal exchanges leading to embryonic lethality. Interestingly, mice exposed to DEHP and fed high fat diet during pregnancy showed exacerbated placental defects, including impaired terminal differentiation of labyrinthine trophoblast cells and vascularization of the placenta. Our studies further revealed dysregulated expression of PPAR<sub>gamma</sub> targets, including
PAPP-A and MUC-1, in the placenta in response to DEHP exposure. A decline in PPAP-A expression impairs trophoblast invasion into the maternal decidua while a reduction in MUC-1 expression adversely affects the expansion of fetal blood vessels. GLUT 1 is expressed in spongiotrophoblast and syncytiotrophoblast layers of the labyrinth. DEHP exposure attenuates the expression of GLUT1 in the placenta thereby affecting proper nutrient transport to the fetus. Consequently, DEHP and high fat diet exposed fetuses are either growth retarded or die <i>in utero</i>. Feeding a high fat diet alone did not adversely affect placental development and fetal growth. Collectively, these results indicate that phthalates and high fat diet disrupt PPAR<sub>gamma</sub>-dependent signaling pathways in the placenta to severely affect its development during pregnancy, causing fetal morbidity and mortality.

**P169 - Ovulatory Prostaglandin Synthesis and Metabolism is Altered in Human Granulosa Cells by an Environmentally Relevant Phthalate Metabolite Mixture.** Patrick Hannon, James Akin, Thomas Curry, Jr.

Humans are exposed to phthalates daily because these environmental toxicants are found in common consumer products. This is a public health concern because phthalates have been shown to disrupt ovarian function. However, these previous studies utilized single phthalate exposures to rodents and little is known about the effects of phthalates on human ovulation. This is alarming because ovulatory defects are the leading cause of female infertility. The present hypothesis is that an environmentally relevant phthalate metabolite mixture alters the levels of periovulatory enzymes involved in prostaglandin production and metabolism in human granulosa cells. In granulosa cells, luteinizing hormone (LH) or human chorionic gonadotropin (hCG) induces prostaglandin synthases and inhibits prostaglandin metabolic enzymes to increase prostaglandin levels, which is requisite for oocyte release. For these experiments, human granulosa-lutein cells from follicular aspirates of IVF patients were acclimated in culture to regain LH/hCG responsiveness. Prior to hCG treatment, cells were exposed to vehicle control (dimethylsulfoxide) or a mixture of six phthalate metabolites (MPTmix; 1-500μg/ml) for 48hr. The MPTmix was derived from urinary phthalate measurements in pregnant women. Cells were treated with or without hCG and collected at 0-12hr post-treatment (n=4/group; p≤0.05) to measure mRNA levels of synthases (<i>PTGS2</i>, <i>PTGES</i>, and <i>AKR1C1</i>) and a metabolic enzyme that renders prostaglandins inactive (<i>HPGD</i>). Treatment with hCG+MPTmix decreased the levels of <i>PTGS2</i> at the 6hr (500μg/ml) and 12hr (1-500μg/ml) time-points and decreased <i>PTGES</i> levels at 6hrs (10-500μg/ml) when compared to hCG alone. Further, hCG+MPTmix increased both <i>AKR1C1</i> and <i>HPGD</i> levels at 6hrs (100-500μg/ml) and 12hrs (10-500μg/ml) when compared to hCG. These data suggest that exposure to phthalates may inhibit ovulatory prostaglandin production by decreasing the levels of synthases and increasing metabolism. Because prostaglandins are indispensable for ovulation, exposure to phthalates may impair human fertility. Supported by K99ES028748 and P01HD071875.

**P170 - Impact of high fat diet-induced obesity on ovarian DNA damage repair proteins in rats.** Bailey McGuire, María González Alvarez, Karl Kerns, Peter Sutovsky, Aileen Keating
Obesity is associated with heightened ovarian cancer risk and reproductive dysfunction. Using a model of progressive obesity, we have discovered reduced primordial follicle number, basal DNA damage and a blunted response to genotoxicants due to obesity. DNA damage precipitates induction of DNA repair proteins, including Ataxia telangiectasia mutated (ATM) and phosphorylation of histone 2AX (γH2AX). Since we have determined that obesity alters the ovarian response to DNA damaging chemicals, we hypothesized that high fat diet-induced obesity would also alter ovarian DNA damage protein abundance. Female Wistar rats were fed a high fat (60% Kcal) diet from 4 to 22 weeks of age. Ovarian protein was isolated and western blotting performed to quantify abundance of phosphorylated histone 2AX (γH2AX), breast cancer type 1 susceptibility protein homolog (BRCA1), RAC-alpha serine/threonine-protein kinase (AKT1), DNA (cytosine-5)-methyltransferase 1 (DNMT1), histone deacetylase 1 (HDAC1), histone H3 [trimethyl Lys 9] (H3K9me3) and superoxide dismutase [Cu-Zn] (SOD1). There was no effect of HFD-induced obesity on protein abundance of γH2AX, AKT1, BRCA1, HDAC1, H3K9me3, DNMT1 and SOD1 (P > 0.05). These results suggest that contrary to our findings in other models, that HFD-induced obesity in rats at this level does not affect the overall abundance of protein markers of DNA damage. These data raise the possibility that a threshold of obesity may be required for observance of basal DNA damage. Supported by funding from the Iowa State University Bailey Career Development Award to AFK, the Fulbright Foreign Student Program to EGA and the University of Missouri F21C program funding to PS.

P171 - Impact of high fat diet-induced obesity on ovarian chemical metabolism proteins in rats. María González Alvarez, Bailey McGuire, Karl Kerns, Peter Sutovsky, Aileen Keating

Obesity affects ~20% of girls and 40% of women in the United States with higher rates in some minority populations. Obesity causes negative female reproductive effects including infertility, poor oocyte quality, miscarriage and offspring birth defects. Chemical-induced ovarian dysfunction is largely dependent on whether chemicals are metabolized to a greater or lesser toxic form. We have discovered that hyperphagia-induced obesity alters abundance of ovarian proteins involved in chemical metabolism. We hypothesized that high fat diet-induced obesity would also alter ovarian chemical metabolism protein abundance. Female Wistar rats were fed a control diet (CT) or a 60% Kcal high fat diet (HFD) from 4 to 22 weeks of age at which time the body weight was increased in the HFD-fed rats. Ovarian protein was probed via western blotting to quantify the abundance of the insulin receptor (INSR), glutathione S-transferase Pi (GSTP1), cytochrome P450 2E1 (CYP2E1), and microsomal epoxide hydrolase 1 (EPHX1). Densitometric analysis was completed using Image J software. There were no HFD-induced effects on ovarian abundance of INSR, GSTP1 or CYP2E1 (% > 0.05). Relative to CT rats, however, EPHX1 was increased (P < 0.05) in the ovaries of the HFD rats. We have previously demonstrated that ovarian EPHX1 can detoxify or bioactivate chemicals in chemical-specific manner. The effects of obesity on EPHX1 protein agree with our previous findings in other models and support that obesity may alter ovarian chemical metabolism contributing to negative reproductive phenotypes associated with elevated body weight. Supported by funding from the Iowa State University Bailey Career Development Award to AFK, the Fulbright Foreign Student Program to EGA and the University of Missouri F21C program funding to PS.
Epigenetic reprogramming and transgenerational inheritance of epimutations in medaka. Ramji Bhandari, Xuegeng Wang

Postfertilization epigenome reprogramming erases epigenetic marks transmitted through gametes and establishes new marks during mid-blastula stages. A mouse embryo undergoes dynamic DNA methylation reprogramming after fertilization, while in zebrafish the paternal DNA methylation pattern is maintained throughout early embryogenesis and the maternal genome is reprogrammed in a pattern similar to that of sperm during the mid-blastula transition. Here we show DNA methylation dynamics in medaka embryos during epigenetic reprogramming of embryonic genome. The sperm genome is hypermethylated and the oocyte genome hypomethylated prior to fertilization. After fertilization, the methylation marks of sperm genome are erased within the first cell cycle and embryonic genome remains hypomethylated from zygote until 16-cell stage. The DNA methylation (5-mC) level gradually increased from 16-cell stage through the gastrulation stage. The 5-hydroxymethylcytosine (5-hmC) levels showed an opposite pattern to DNA methylation. The pattern of genome methylation in medaka was thus very similar to mammalian genome methylation but not to zebrafish. We also screened for environmental bisphenol A-induced epimutations that survived epigenetic reprogramming in primordial germ cells (PGCs) and represented in sperm. The present study suggests that a medaka embryo resets its DNA methylation pattern by active demethylation and gradual remethylation similar to mice. BPA-induced epimutations can survive reprogramming, and additional unique epimutations are established de novo during gametogenesis. Our results provide new information regarding endocrine disruption and environmental epigenetics research.

DNA methylation dynamics during BPA induced transgenerational inheritance of reproductive phenotypes in medaka. Ramji Bhandari, Xuegeng Wang, Frederick vom Saal, Donald Tillitt

Exposure to environmental chemicals can have far reaching health effects especially when it occurs during the sensitive windows of embryonic development. In medaka, embryonic BPA exposure at F0 generation resulted in impaired fertility in males at F2 and F3 generations. To further understand the mechanisms associated with transgenerational inheritance of impaired male fertility, we examined epigenetic reprogramming of primordial germ cells (PGCs) and surveyed bisphenol A-induced epimutations in PGCs and sperm across three generations. Here, we show that the pattern of genome methylation in medaka is similar to mammalian genome methylation. We also show that medaka PGC reprogramming undergoes a global erasure of methylation marks from the 8 day post-fertilization (dpf) stage until 15 dpf stage, and de novo methylation starts at 25 dpf stage and ends at the time of gametogenesis (50 dpf), during which several additional new methylation marks are also established. Moreover, the present findings suggest that BPA-specific epimutations that escape epigenetic reprogramming in PGCs and additional epimutations are established in PGCs due to embryonic BPA exposure. Some of the BPA-specific epimutations that escape reprogramming or that are established during gametogenesis are subsequently inherited by sperm. The BPA exposure that occurred three generations ago resulted in reduced fertility by 25%. Specifically, we found that the epimutations on androgen receptor alpha can transfer from germline to soma at F2 generation leading to
suppression of ARalpha mRNAs in the testis; this suggests that transgenerational epimutations can transfer to testicular somatic cells affecting male reproductive physiology and impacting subsequent generations. The present results show that the medaka embryo resets its DNA methylation pattern by active demethylation and gradual remethylation similar to mice. In addition, the BPA-induced epimutations can survive reprogramming, and germ cells establish de novo unique BPA-specific epimutations during gametogenesis.

**P174 - Hexavalent Chromium (CrVI) induced cell cycle arrest and apoptosis of immortalized rat trophoblast cell line Rcho-1.** Sakhila Banu, Jone Stanley, John Wu, Joe Arosh, Pramod Dhakal, Michael Soares

Environmental contamination with hexavalent chromium (CrVI) has been increasing in the drinking water of the USA and developing countries. Remy et al., (2017) reported for the first time that environmental exposure to CrVI causes various health problems including infertility in women and developmental problems in children for two generations. Previous reports from our laboratory indicated that increased Cr burden in the human placenta is directly associated with increased oxidative stress, decreased levels of antioxidants resulting in the activation of apoptotic pathways. To explore the underlying mechanism, we used a well-characterized immortalized trophoblast cell line from rat, the Rcho-1 cell line. The Rcho-1 trophoblast stem cell line was derived from rat choriocarcinoma and is an effective tool for elucidating regulatory mechanisms controlling trophoblast cell cycle and apoptosis in response to endocrine disrupting chemicals (EDCs) such as CrVI. In the current study, we treated Rcho-1 with various doses (0.01, 0.1, 0.1, 0.5, 5, 10, 15 and 30 μM) of potassium dichromate (CrVI) for different time-points (0, 15, and 30 min, 1, 3, 6, 12 and 24 h). We chose 15 μM as the LD50 value and 24h time-point as the optimal treatment duration for further analyses. We analyzed expressions of mRNA and protein that regulate cell cycle and apoptotic pathways. CrVI decreased cell proliferation as a result of cell cycle arrest and down-regulated cyclin-dependent kinases (CDK), cyclins, and PCNA while up-regulating CDK-inhibitors and several apoptotic proteins. Overall, CrVI altered AIF, Bax, cytochrome-c, cleaved caspase-3, and p53 (apoptotic pathway); Bcl-2, Bcl-XL, phospho-AKT, phospho-ERK, PCNA, XIAP (cell survival pathway); and cyclin B1, CDK-2, -4 and -6 (cell cycle regulatory pathway). Overall, the current study indicate that CrVI could adversely affect trophoblast (TB) cell survival and increase TB cell apoptosis which could negatively influence the growth and development of the fetuses.

**P175 - Impact of heat-induced hyperthermia on certain proteins and cytokines in periovulatory follicle of lactating dairy cows.** Louisa Rispoli, Rebecca Payton, Chelsea Abbott-Finn, Ky Pohler, Lannett Edwards

Heat-induced hyperthermia occurring after a pharmacologically-induced LH surge altered follicular fluid proteome of the periovulatory follicle in lactating dairy cows. Objective of this study was to further examine kininogen and transferrin levels in the follicular fluid and serum of thermoneutral cows kept at a THI of 65.9 ± 0.2 or heat-stressed (HS) cows becoming hyperthermic after exposure to changes in THI to simulate what may occur during an acute heat stress event (71
to 86 THI). Follicular fluid (aspirated ~16 h after GnRH) and serum levels at GnRH administration and follicle aspiration were evaluated. Bradykinin, active cleavage product of kininogen, was higher in follicular fluid (P = 0.05) but not in serum (P = 0.93) of HS cows. Intra-follicular transferrin (P = 0.02) was higher in HS cows; serum levels approached significance (P = 0.07). Nine of 15 different cytokines evaluated were detected in follicular fluid using a custom bovine cytokine panel (MilliporeSigma; St. Louis, MO); all 15 cytokines were detectable in serum. Heat-induced hyperthermia only increased intra-follicular levels of IL-6 (P = 0.02). No differences in the 15 circulating cytokines evaluated were detected (P > 0.2). Follicular fluid changes in certain proteins and cytokines may explain some of the negative consequences of hyperthermia on the developmental competence of the maturing oocyte housed within the periovulatory follicle. This project was supported by Agriculture and Food Research Initiative Competitive Grant no.2016-67015-24899 from the USDA National Institute of Food and Agriculture.

**P176 - Blood serum extracellular vesicle coupled microRNAs expression analysis in metabolically divergent lactating cows.** Tsige Hailay, Mikhael Poirier, Michael Hoelker, Samuel Gebremedhn, Franca Rings, Dessie Salilew-Wondim, Karl Schellander, Dawit Tesfaye

Extracellular vesicles (EVs) are evolutionarily conserved communicasomes and fitted to carry various molecules including miRNAs. The current study aims to investigate the effect of postpartum metabolic stress on EV-coupled miRNAs content in blood serum as noninvasive indicator of metabolic stress in Holstein-Friesian cows. Blood samples were collected from 30 cows during the week 5 - 10 of postpartum and 8 control heifers. Energy balance status of postpartum cows was assessed based on their blood metabolites (NEFA and β-hydroxybutyrate), body weight loss and overall energy output. Based on these criteria, cows were categorized as transient (TCs), always negative cows (ANCs), and always positive cows (APCs). Blood serum samples at week 5 & 6 were pooled and used for EVs isolation by ultracentrifugation. EV-coupled miRNAs were analyzed using Next-generation sequencing. Result has revealed the presence of 179 known and 118 novel miRNAs. The comparison between the ANCs vs APCs revealed differential expression of 5 miRNA with downregulation of bta-miR-145 and miR-375 and upregulation of bta-miR-133a, miR-9-5p, and miR-184 in ANCs. Moreover, the comparison between ANCs and heifer’s revealed downregulation of 18 out of 29 miRNAs. Interestingly, when compared to heifers, several miRNAs including (bta-miR-192, miR-215, miR-107, miR-130a, and miR-199a-3p) were found to be downregulated in metabolically stressed cows (ANCs, TCs) while the same miRNAs were upregulated in APCs. The comparison between ANCs vs TCs showed upregulation of all 9 differentially expressed miRNAs including bta-miR-133a, miR-206, and miR-122 in ANCs. These miRNAs could be potential indicators of cows either remained in metabolic stress or recovered in the late weeks. Target prediction analysis of these miRNAs revealed their involvement in TGF-beta signaling, central carbon metabolism, p53 signaling, steroid biosynthesis, ECM-receptor interaction, lysine degradation, and AMPK signaling pathways. This study identified EV-coupled miRNAs in serum as potential indicators of metabolic stress in dairy cows.
**P177 - Paternal di(2-ethylhexyl) phthalate (DEHP) exposure leads to altered developmental gene expression in embryos.** Chelsea Marcho, Alex Shershbehev, Haotian Wu, Alexander Suvorov, Jesse Mager, J. Richard Pilsner

Phthalates, a class of man-made, non-persistent endocrine disrupting compounds, are ubiquitous environmental contaminants. Exposures are widespread and are reported to cause both developmental and reproductive health issues. In particular, phthalate exposure in males is associated with poor sperm quality and longer time to pregnancy. Therefore, gaining a mechanistic understanding of the effect of phthalate exposure in the adult male on reproductive health is of significant importance.

Our objective was to determine the effects of adult exposure to di(2-ethylhexyl) phthalate (DEPH) on sperm DNA methylation, as well as changes to epigenetic reprogramming and development of the embryo. Eight-week old C57BL/6J male mice were exposed to either a vehicle control, a low dose of DEPH (2.5mg/kg/weight), or a high dose of DEPH (25mg/kg/weight) for 70 days. Following the exposure, males were mated with unexposed, 8-12-week old, DBA/2J females. To assess the effects of DEHP, sperm males was collected for methylation analysis. We also isolated gastrulation stage embryos from unexposed, pregnant females to assess methylation and expression in embryonic and extra-embryonic lineages.

We found differential expression of genes related to important developmental processes, including tissue morphogenesis, blood vessel development, placenta development, and cell fate commitments, in the embryonic and extra-embryonic lineages following paternal DEPH exposure. This includes critical developmental genes, such as *Hox* cluster genes, *Cdx* genes, *Shh*, and *Bmp* receptors. Surprisingly, while we found altered methylation in the embryonic and extra-embryonic tissues following exposure, this was not directly associated with specific changes in genes expression. This indicates that in addition to changes in DNA methylation status, there must be additional epigenetic and regulatory changes driving changes in embryonic expression.

**P178 - The JAK-STAT Pathway is affected by Heat Stress in the Corpus Lutea of Post-Pubertal Crossbred Gilts.** Crystal Roach, Katie Bidne, Matthew Romoser, Jason Ross, Lance Baumgard, Aileen Keating

Heat-stress (HS) occurs when environmental and metabolic heat production exceeds heat dissipation, increasing body temperature. Porcine reproductive dysfunction results from HS and we have previously identified HS-induced reduced corpora lutea (CL) weight. In the ovary, prolactin (PRL) influences luteolysis via binding with its receptor (PRLR) and inducing the JAK-STAT pathway, resulting in activation and phosphorylation of janus kinase (JAK2) and signal transducer and activator of transcription (STAT) proteins. We have previously demonstrated that HS elevates circulating PRL in swine, thus, we hypothesized that the CL is hyper-responsive to PRL; a molecular scenario that could contribute to luteolysis. Fourteen post-pubertal crossbred gilts were synchronized and subjected to thermal neutral (TN; 20±1°C; 35-50% humidity; n = 7) or cyclic HS (35 ±1°C for 12h/31.6°C for 12h; 20-35% humidity; n = 7) conditions from 2 days post estrus (dpe) until peak CL function at 12dpe. The protein abundance of PRLR, pJAK2, JAK2, pSTAT3, STAT3, pSTAT5α/β, and STAT5α were quantified by
western blotting. No treatment effect on levels of PRLR, pJAK2, JAK2, pSTAT3, pSTAT5α, and STAT5α/β were observed ($P > 0.14$). Ovarian STAT3 protein abundance was greater (11%; $P < 0.01$) in the CL of gilts exposed to HS, relative to TN treated gilts. There was no impact of HS on levels of pJAK2 and pSTAT5α/β ($P > 0.05$), however, pSTAT3:STAT3 ratio was decreased ($P < 0.06$) in the CL of HS relative to TN gilts. These data suggest that HS alters ovarian signaling pathways during the luteal phase that could contribute to fertility dysfunction. This project was supported by the Iowa Pork Producers Association.

**P179 - Neonatal Genistein Exposure Results in Uterine Implantation Deficits Due to Impaired Gland Formation and Function.** Elizabeth Padilla-Banks, Wendy Jefferson, Lindsey Royer, Ripla Arora, Carmen Williams

The phytoestrogen genistein (GEN) is found in soy products including soy-based infant formulas. Exposure of neonatal mice to GEN causes infertility due in part to uterine implantation defects. Because embryo implantation relies on precise hormonal regulation of reproductive tract gene expression and tissue architecture, we hypothesized that neonatal GEN exposure disrupts these characteristics. Indeed, uterine weight gain in response to 17β-estradiol treatment or a decidualization regimen was dampened in GEN-exposed mice compared to controls. However, expression of candidate estrogen-regulated and decidualization-responsive genes was largely unaffected, suggesting hormone signaling was relatively intact. Histological analysis of uteri from pregnant GEN-exposed mice (gd4) revealed a marked increase in extracellular matrix deposition in muscle and stromal compartments, a reduction in glandular epithelium and abnormal appearing luminal epithelial cells. Confocal imaging and 3D reconstruction of uteri from 28-day-old prepubertal mice revealed that half of the GEN-exposed mice had very few glands, whereas the rest had gland numbers similar to controls. There were 1,339 differentially expressed genes (DEGs) between control and GEN-exposed uteri by microarray analysis. One highly down-regulated gene was *Foxa2*, which is critical for normal uterine gland development and function. To determine if the reduction in *Foxa2* in GEN-exposed uteri impacted gland function, we compared gd4 GEN DEGs to DEGs in two genetic mouse models of conditional *Foxa2* uterine deletion [Foxa2 flox/PgR-cre+ (FP; glandless) or Foxa2 flox/Ltf-icre+ (FL; gland dysfunction)]. Of the 1,339 GEN-specific DEGs, 40% overlapped with FP DEGs and 20% overlapped with FL DEGs. Leukemia inhibiting factor, which rescues the implantation deficit in FP and FL mice, also partially rescued the implantation defect in GEN-exposed mice. These findings suggest that reduced *Foxa2* signaling in GEN-exposed mice alters gland formation and function and contributes to implantation defects.

**P180 - Sexual Dimorphism in Susceptibility to Age-dependent Metabolic Disorders and Lifespan in Mice Born through ICSI.** Yue Wang, Tong Zhou, Huili Zheng, Wei Yan, Zhuqing wang

Intracytoplasmic sperm injection (ICSI), as an assisted reproductive technology (ART), has been increasingly used as a treatment for human male infertility in fertility clinics. Consequently, numerous so-called “test-tube babies” are born through ICSI nowadays. Many studies have shown that epigenetic alterations could occur in early ICSI embryos, most likely due to extensive
in vitro manipulation and/or the superovulation protocol for egg collection. Although these ICSI babies are born seemingly “healthy”, the potential long-term adverse effects of ICSI-based ART on the health perspectives of these individuals have not been reported due to the relative short history of ART application in humans (~40 years). We sought to address this question by examining metabolic status (GTT: glucose tolerance test and ITT: Insulin tolerance test) and lifespan of mice derived from ICSI in comparison to those from natural mating. Our data revealed sex- and age-dependent predisposition of metabolic disorders. Male ICSI mice displayed insulin insensitivity at 3 months of age and both impaired glucose tolerance and insulin insensitivity at 10 months of age. Female ICSI mice showed both impaired glucose tolerance and insulin insensitivity at both 3 and 10 months of age. Interestingly, both sexes showed normal GTT and ITT results at the age of 6 months. Moreover, male ICSI mice displayed normal growth with their body weight gain comparable to controls, whereas the body weights of female ICSI mice were much greater than controls at 3 months and thereafter. Female ICSI mice were more sensitive than male ICSI mice to high fat diet (60% fat contents), as demonstrated by greater weight gain and aberrant ITT results. Our data suggest that ICSI offspring display sexual dimorphism in susceptibility to metabolic disorders in mice.

P181 - The Detrimental Impact of Phthalates and Plasticizers on Fertilization and Early Embryo Development. Liliya Gabelev Khasin, John Dela-Rosa, Polina Lishko

Phthalates and plasticizers are ubiquitous environmental toxins (ET) that have been linked to reduced fertility. We have tested the effect of four commonly used ET: DEHP, DEP, DMP, and BPA on pre-implantation embryo development and sperm fertilizing ability. Here we show that all phthalates at the concentration of 10 µM are deleterious to embryo development. Using mouse in vitro fertilization assay, we found DEHP to have the strongest impact on sperm fertility. While spermatozoa retained their fertilization potential at 1µM, at 2µM a significant decrease in blastocyst formation was observed (59.15%±0.85 vs 85.16%±2.1). Moreover, at 10µM all blastocyst formation was completely abolished. Further evaluation of DEHP’s effect on sperm revealed that 10 µM DEHP inhibits acrosome reaction and triggers a change in protein tyrosine phosphorylation dynamics during sperm capacitation. We conclude from these experiments that pre-exposure of spermatozoa to DEHP poses a threat to male fertility.

P182 - Differential effect of Bisphenols (BPA, BPF and BPS) in regulating gene expression and receptivity in endometrial epithelial Ishikawa cells. Kai-Fai Lee, Hongjie Fan, Chris Wong, William Yeung, Ernest Ng

Bisphenol A (BPA) is commonly found in epoxy resins used in the manufacture of plastic coatings in food packaging and beverage cans. There is growing concern about BPA as a weak estrogenic compound that can affect human endocrine function. Chemicals structurally similar to BPA including BPF and BPS have been developed as substitutes in the manufacturing industry. Whether these bisphenol substitutes have adverse effects on human endocrine systems and reproductive health remains largely unknown. This study aimed to investigate if BPA, BPF, and BPS affect endometrial stromal cell proliferation, receptor expression, gene transactivation and spheroid attachment in vitro. All three bisphenols inhibited Ishikawa cell proliferation at 100 µM with
potency BPA>BPF>BPS. Bisphenol from 1-100μM could suppressed the expression of estrogen receptors (ERα and ERβ) and membrane receptor GPR30. BPA and BPF have stronger transactivation activities than BPS on ERE-Luciferase reporter assay in the transfected Ishikawa cells. Addition of ICI 182,780 (ERα antagonist) or MPP (ERα-specific antagonist), but not G15 (GPR30 antagonist) could nullify the transactivation activity of the reporter construct. Furthermore, microarray analysis of BPA, BPS and BPF treated Ishikawa cells induced similar transcriptomic changes, although the expression of several nuclear receptors and focal adhesion molecules were found to be differentially regulated. Importantly, the expression of progesterone receptor was up-regulated by bisphenols at micromolar concentrations. Taken together, BPA, BPS and BPF share similar structural and biological properties. Changes in steroid receptor expressions modulate various down-stream signaling pathway resulting in changes in receptivity of the endometrial epithelial cells in vitro. [This project is supported in part by grants from CRCG/HKU and GRF/RGC 17120415 to KFL]

P183 - Human relevant level of Bisphenol A and di-(2-ethylhexyl) phthalate inhibit steroidogenesis in MLTC1 cells line. P Chaturvedi, Pramod Kumar, Surabhi Gupta, R Sharma

Endocrine-disrupting chemicals (EDCs) affect human health including male reproductive health. EDCs affect gene-environment interactions and produce effects in exposed individuals and even in their descendants. di-(2-ethylhexyl) phthalate (DEHP) and Bisphenol A (BPA) are most commonly used as plasticizers in construction and food industry which can cause male infertility. Occupational human exposure to these compounds through intake or inhalation increases body burden levels. It has been observed that higher dose exposure EDCs inhibit testosterone production in animal model or in leydig cells. However, the effect of EDCs exposure on steroidogenesis is debatable due controversial observations by various groups. Here, we aimed to explore effects of BPA and DEHP exposure in biologically (human) relevant levels on steroidogenesis in leydig cells to simulate the closure effects of these compounds on male reproductive health.

We have used MLTC-1 cell lines for the study of leydig cells function following HCG (10 mIU/mL) induction and BPA and DEHP treatment (24 hrs). DEHP concentration was selected in biologically relevant range that has been observed by our group in semen of Indian population. BPA concentration was selected based on the available literature. Testosterone and estradiol production were measured using chemiluminescence. The optimal production of testosterone was observed after 24 hrs following HCG induction. BPA (10ng/mL) and DEHP (0.4 µg/mL) significantly inhibited biosynthesis of testosterone in MLTC1 cells by 44% and 40% respectively. Production of estradiol was varying following exposure of BPA and DEHP. However, the testosterone/estradiol ratio was decreased significantly in EDCs treated groups following HCG induction. The invitro study based on human relevant concentrations of EDCs is in support of the previous study which revealed association of EDCs (semen) on steroidogenesis inhibition in males

P184 - Effects of dibutyl phthalate (DBP) exposure on the expression of transcription factors in the mouse ovary. Franchesca Nunez, Jazmin Beltran-Gastelum, Zelieann Craig
Dibutyl phthalate (DBP) is an endocrine disruptor previously linked to adverse reproductive outcomes in females. We have previously shown that environmentally relevant exposures to DBP significantly increase the expression of pro-apoptotic genes Bax, Bad, and Bid in the mouse ovary. To begin elucidating the effects of DBP on gene regulation, the expression of transcription factors implicated in the regulation of these genes was explored using the transcriptional regulatory database TRRUSTv2. Peroxisome proliferator-activated receptors (Ppara and Pparg) and estrogen receptors (Esr1 and Esr2) were also selected for analysis due to their functional importance in follicular development and known interactions with phthalates. Female CD-1 mice (60 days old; n=14-16 per treatment) were orally administered daily doses of tocopherol-stripped corn oil (vehicle) or DBP at environmentally-relevant doses (10 and 100 µg/kg/day) or a classical high dose (1000 mg/kg/day) for 10 days. After dosing, ovaries were collected and processed for qPCR. Seven activating (Nfat5, Runx2, Sall2, Tnfaip3, Trp53, Trp73, E2f1) and four repressing (Foxo1, Gfi1, Pou4f1, Ezh2) transcription factors identified through TRRUSTv2 were tested. There were no statistically significant differences in transcription factor expression between treatment groups. Interestingly, trends for decreased expression of Ezh2 (p=0.058) and Runx2 (p=0.097) were observed in the DBP 1000 mg/kg/day group. Although expression of Pparg, Esr1, and Esr2 did not differ between treatments, a trend for increased Ppara (p=0.113) was observed in the DBP 10 µg/kg/day group. Though the trend observed for less Ezh2 expression fits the profile of increased Bad expression in a previous study, our findings suggest that DBP-induced changes in gene expression do not involve disrupting transcript levels for these key transcription factors. Future studies will test whether changes in transcription factor activity better explain the effects of DBP on ovarian gene expression. Supported by NIH R01ES026998.

**P185 - Environmentally Relevant Exposure to Dibutyl Phthalate and Ovarian Gene Expression: Effects of Terminal Estrous Cycle Stage.** Jazmin Beltran-Gastelum, Estela Jauregui, Zelieann Craig

Dibutyl phthalate (DBP) is a plasticizer commonly used in cosmetics and oral medications which has been detected in human urine and ovarian follicular fluid. We have previously shown that daily exposure to environmentally relevant levels of DBP results in alterations in ovarian gene expression related to folliculogenesis and steroidogenesis. Many studies have exposed mice for a specific number of days and collect samples on the same stage of the estrous cycle (e.g. estrus), but no studies have tested whether results vary according to the stage of estrous at collection (i.e. estrus vs. diestrus). In a previous study we measured ovarian mRNA expression for aromatase (Cyp19a1) and insulin-like growth factor 1 (Igf1) in mice treated with DBP at environmentally-relevant levels (10 and 100 µg/kg) or a classical high dose (1000 mg/kg) for 20-25 days and collected in estrus. In that study, gene expression results were variable and included a trend for decreased Cyp19a1 expression in response to treatment with DBP at 10 µg/kg/day but no significant changes in Igf1 expression. The present study was designed to determine whether similar results would be obtained if the ovaries were collected in diestrus. Adult female CD-1 mice (n=12/treatment) were given daily oral doses of tocopherol-stripped corn oil (vehicle) or DBP as in the previous study but ovaries were collected in diestrus and processed for qPCR. In contrast to the previous study, variability in the expression of each gene was reduced and the expression of Cyp19a1 significantly decreased at all levels of DBP treatment. There was also a
trend for decreased *Igf1* mRNA expression ($p=0.1$) in the DBP 100 µg/kg/day group. These results reveal that the sensitivity of these genes to DBP exposure varies according to stage and that analyses throughout the estrous cycle be considered in toxicological studies. Supported by NIH R01 ES026998.

**P186 - SNF5, a SWI/SNF chromatin remodeling complex core subunit, is required for porcine embryo development.** Yu-Chun Tseng, Jennifer Crodian, Birgit Cabot, Ryan Cabot

The SWI/SNF family of chromatin remodeling complexes has been shown to impact transcription during early mammalian embryogenesis. SWI/SNF chromatin remodeling complexes are composed of multiple protein subunits. All SWI/SNF complexes have an ATPase as their catalytic subunit, either BRM or BRG1 and utilize the energy from ATP hydrolysis to reposition nucleosomes. Additional protein subunits that associate with the ATPase provide each SWI/SNF complex with unique functions. SNF5 is one of these core subunits and can be found in all traditional SWI/SNF complexes. The objective of the present study was to determine the developmental requirements of SNF5 during porcine embryo development. We hypothesized that SNF5 depletion would lead to developmental arrest during cleavage development in the porcine embryos. An RNA interference assay was used to knockdown endogenous SNF5 level to test this hypothesis. *In vitro* produced porcine zygotes were assigned to one of the three following treatments: 1) microinjection of duplex interfering RNAs to target SNF5 (SNF5 RNAi), 2) microinjection of a duplex RNA containing a scrambled sequence (control RNAi), 3) non-injection (non-injected). The number of nuclei was determined in each treatment group following seven days of embryo culture. Embryos in the SNF5 RNAi treatment possessed an average of 1.7 nuclei per embryo, while the control RNAi and non-injected treatments possessed an average of 7.8 and 7.6 nuclei per embryo, respectively ($p<0.05$). Moreover, the reduction of SNF5 led to an increased abundance of transcripts encoding *NANOG* and *OCT4* as compared to control groups. Our results support our hypothesis and suggest that an alteration in SNF5 can lead to changes in transcript abundance during early cleavage development. This work was supported in part by the Eunice Kennedy Shriver National Institute of Child Health and Human Development of the National Institutes of Health (award number R01HD084309).

**P187 - Karyopherin alpha 7 imports BRD7 to the nucleus in early porcine embryo development.** Jennifer Crodian, Yu-Chun Tseng, Jillian Bouck, Birgit Cabot, Ryan Cabot

Mammalian embryos must regulate gene expression to ensure proper development. SWI/SNF (Switch/Sucrose non-fermentable) chromatin remodeling complexes are active during early embryogenesis. SWI/SNF complexes are multi-subunit complexes consisting of a core catalytic ATPase, either SMARCA4 or SMARCA2, and additional subunits that direct the complexes to specific loci; BRD7 is one of these subunits. The objective of this work was to determine if karyopherin alpha 7 (KPNA7) serves a role in the nuclear import of BRD7 in porcine embryos. We hypothesized that KPNA7, a nuclear transport receptor expressed exclusively in oocytes and cleavage stage embryos, serves a role in trafficking BRD7. An RNA interference assay and a dominant negative over-expression assay were used to test this hypothesis. Using
immunocytochemistry, we found a significant difference in the localization patterns of BRD7 46 hours after treatment with KPNA7 interfering RNAs (KPNA7 RNAi), as compared to our two control groups (injection with a control RNAs consisting of the scrambled sequence used to target KPNA7 (control RNAi) and non-injected embryos (non-injected). Where the majority of embryos in the KPNA7 RNAi treatment group (n=24/28) showed a reduced amount of BRD7 in the nucleus, the majority of embryos in the control RNAi (n= 16/22) and non-injected (n= 11/15) treatment groups showed an even distribution of BRD7 between the cytoplasm and the nucleus (p <0.05). BRD7 was found enriched in the cytoplasm of embryos injected with mRNA encoding a dominant negative KPNA7 mutant, as compared to non-injected embryos and embryos injected with mRNA encoding GFP (p<0.05). Our findings support our hypothesis and indicate that KPNA7 is involved with the nuclear import of BRD7 during cleavage development. This work was supported in part by the Eunice Kennedy Shriver National Institute of Child Health and Human Development of the National Institutes of Health (award number R01HD084309).

**P188 - The Accuracy Of Gene Expression In Blood To Assess Early Pregnancy And Embryo Loss In Dairy Cattle.** Irene Malo Estepa, Dayle Johnston, Michael Diskin, Mark Crowe

An early pregnancy biomarker would improve reproductive efficiency. Alterations in the expression of 8 potential pregnancy biomarker genes in blood during early pregnancy and after embryo loss were investigated. Twenty seven dairy cows were assigned to control (n=10), pregnant (n=9) or bred-aborted (n=8) groups. The pregnant and aborted groups were inseminated (AI; day 0) and the aborted group received an injection of prostaglandin on day 21. Blood samples were collected on days 16, 21-24, 26 and 28 post AI. Pregnancy was confirmed on day 35 by ultrasound. Relative gene expression was analysed by qPCR. Data were analysed by ANOVA, least square means and Tukey test. The optimal cut-off values, sensitivity and specificity were calculated with ROC curves and the ability of the candidate genes to identify animals undergoing embryo loss was evaluated. On day 21, all the genes showed higher expression in pregnant and bred-aborted compared with control cows, 4 of which showed higher expression in bred-aborted compared to the pregnant cows. On days 22 and 23, all the genes were up-regulated in the pregnant and bred-aborted groups. On day 23, 2 genes had higher expression in the bred-aborted group compared to the pregnant, and 1 gene had an intermediate expression of the bred-aborted group between the pregnant and the control. On days 24 and 26 all the genes in the pregnant group remained up-regulated, while all genes except for gene 7 returned to control levels within the bred-aborted group. For pregnancy detection, 4 genes on day 22 and 7 genes on day 23 showed both a sensitivity and specificity close or equal to 100%. An additional gene (Gene 1) achieved sensitivity and specificity of 89% and 100%, respectively, on day 26. This panel of genes could be used to determine early pregnancy status and differentiate pregnant animals.

**P189 - Dramatic epigenetic changes in the genome impact gene expression and the differentiation of ovarian granulosa cells.** Tomoko Kawai, S.A Hoque, JoAnne Richards, Masayuki Shimada
During follicular development, granulosa cells proliferate, differentiate and acquire the ability to respond to the LH surge and ovulate. Recently, we reported that induction of the LH receptor is dependent on demethylation of the LH receptor (Lhcgr) promoter and that this is mediated by retinoic acid suppression of DNA methyltransferase1 (Dnmt1) expression. We hypothesized that not only the Lhcgr promoter but also promoters of other genes expressed in granulosa cells of preovulatory and periovulatory follicles would be coordinately demethylated. Comprehensive changes in the DNA methylation status in the whole genome of granulosa cells during follicular development were analyzed by MeDIP (Methylated DNA immunoprecipitation) sequencing. Methylation of promoter regions was significantly decreased in more than 40% of the genes in all chromosomes except the X chromosome; genes expressed in granulosa cells of preovulatory follicles, Cyp19a1, Ar, Nr5a1 and periovulatory follicles, Hsd3b1, Snap25, Ptx3 were included in this list. Expression of the top 10 genes was analyzed further by RT-PCR, methylation by bisulfite sequencing, histone modification (H3K27 acetylation) by chip assays and genome structure by FAIRE (Formaldehyde-Assisted Isolation of Regulatory Elements) assays. Results showed epigenetic regulation of the promoters of these genes, including DNA demethylation, histone acetylation and open DNA structure, indicating that each of these mechanisms contributes to induced gene expression. Treatment of granulosa cells with a cell cycle inhibitor suppressed these molecular changes, suggesting that increased DNA demethylation by the reduction of DNMT1 activity might be linked to the epigenetic changes in granulosa cells. Our results provide compelling novel information that the down-regulation of DNMT1 and the induction of granulosa cell proliferation by FSH+retinoic acid leads to changes in DNA structure in whole genome to induce the expression of specific genes in granulosa cells including Lhcgr and also LH-targeting genes allowing preovulatory follicles to respond to the LH surge.

P190 - Highly efficient genome editing using CRISPR/Mb3Cpf1 in mice. Zhuqing Wang, Yue Wang, Hayden McSwiggin, Shawn Wang, Kimberly Castaneda-Garcia, Huili Zheng, Wei Yan

The Cpf1 endonuclease has the following advantages over Cas9: 1) Cpf1 targets T-rich regions, and is guided by a single short CRISPR RNA (crRNA). 2) It can efficiently process its own crRNAs. 3) it has much lower on-target and off-target effects compared to SpCas9. However, practical applications of Cpf1 have been severely hindered due, at least in part, to its strict requirement for the TTTV PAM sequence. Given that Moraxella bovoculi AAX11_00205 (Mb3Cpf1) edits HEK293 cells at high efficiency through TTTV PAM, we explored whether Mb3Cpf1 could be utilized for efficient genome editing in mouse zygotes. Microinjection of Mb3Cpf1 mRNA and one crRNA with 20nt spacer targeting Prps1l1 with TTTV PAM sequence showed that Mb3Cpf1 worked efficiently in mouse zygotes. We further demonstrated that Mb3Cpf1 could process their own crRNAs in mouse zygotes by microinjecting one crRNA harboring two 20nt spacers recognizing two TTTV PAM sequences at Saraf locus. Successful targeting of the same Saraf gene by microinjection of one crRNA harboring two 20nt spacers recognizing two TTV PAM sequences suggest that Mb3Cpf1 can target genomic DNA with TTV PAM sequences as well. Comparison between spacers recognizing both TTTV and TTV PAM sequences in the Mrvi1 locus suggested that Mb3Cpf1 prefers the spacer targeting the TTTV PAM sequence. The editing efficiency was further improved by optimizing the crRNA length from 20 to 23nt. We used a crRNA containing three 23nt spacers separated by 20nt direct
repeats to target miR-10b. All of five founders were edited by the two spacers targeting the TTTV PAM sequence (100%), and one of them was edited by the one recognizing the TTV PAM sequence (20%). In summary, our data demonstrate that Mb3Cpf1 can edit the murine genome independent of TTTV PAM sequence with minimal on-target mutations and very high targeting efficiency.

**P191 - FSH-regulated histone modification and gene expression in mouse granulosa cells.**  
Ejimedo Madogwe, Milena Taibi, Yasmin Schuermann, Audrey St-Yves, Raj Duggavath

An improper dose of follicle stimulating hormone (FSH) as part of assisted reproductive technologies is associated with low follicular response, inferior embryos and abnormal endometrial receptivity. Therefore, it is important to understand the regulatory mechanisms of FSH in ovarian follicles to develop precision doses for individual patients. Because FSH regulates follicular growth through a specific gene expression program, we set out to profile FSH-regulated transcriptome and H3K4me3 modification in granulosa cells during follicular development. We used super-stimulated immature mouse model to collect granulosa cells prior to and 48h after stimulation with equine chorionic gonadotropin (eCG). We profiled the transcriptome using RNA-sequencing (N=3/time-point) and genome wide trimethylation of lysine 4 of histone H3 (H3K4me3; an active transcription marker) using chromatin immunoprecipitation and sequencing (ChIP-Seq; N=2/time-point). Across the mouse genome, 14,583 genes had an associated H3K4me3 peak and 63-66% of these peaks were observed within <1kb promoter region. There were 72 genes with differential H3K4me3 modification at 48h-eCG (abs[logFC]>1; FDR<0.05) relative to 0h-eCG. Transcriptome data analysis showed 1463 differentially expressed genes at 48h-eCG (abs[logFC]>1; FDR<0.05). Among the 20 genes with differential expression and altered H3K4me3 modification, Lhcgr had higher H3K4me3 abundance and expression, while Nrip2 had lower H3K4me3 abundance and expression. Intriguingly, 52 genes had differential H3k4me3 modification without altered expression, and 1444 genes were differentially expressed without altered H3K4me3 modification. Transcript analysis using Kallisto-Sleuth tool revealed 875 differentially expressed transcripts at 48h-eCG (b>1; FDR<0.05). Isoform-Switch analysis showed that 266 genes, including Bmp1 and Oxtr, had differential transcript usage. Pathway analysis demonstrated that TGFβ signaling and steroidogenic pathways were regulated at eCG48h. Thus, FSH regulates gene expression in granulosa cells through multiple mechanisms namely altered H3K4me3 modification, inducing specific transcripts and differential transcript usage. These data form the basis for further studies investigating how these specific mechanisms regulate granulosa cell functions.

**P193 - Role of sex steroids in intergenerational effects of general anesthetic sevoflurane in young adult rats.**  
Anatoly Martynyuk, Ling-Sha Ju, Jiao-Jiao Yang, Ning Xu, Timothy Morey, Nikolaus Gravenstein, Christoph Seubert, Barry Setlow

The adverse effects of anesthetic exposure in adults during their prime reproductive period remain largely unexplored. This study uses a rodent model to investigate whether a commonly used anesthetic in clinical practice, sevoflurane, affects the exposed subjects and their future offspring. Sprague-Dawley postnatal day 56 (P56) rats (generation 1, G1) were anesthetized with
2.1% sevoflurane on 3 alternate days and mated 25 days later to produce offspring (G2) of all combinations of exposed parents. The G1 rats were sequentially evaluated in the elevated plus maze (EPM) starting on ~P125, prepulse inhibition (PPI) of the acoustic startle response on ~P135 and Morris water maze starting ~on P145. The G1 rats were restrained for 30 min on ≥P160 to measure corticosterone stress responses, followed by collection of tissue samples. The G2 rats were evaluated in the EPM starting on P60, PPI of startle on P70 and corticosterone responses to restraint on ≥P95, followed by collection of trunk blood and brain tissue samples.

Only G1 males, but not G1 females, exhibited persistent neurobehavioral deficiencies, exaggerated hypothalamic-pituitary-adrenal (HPA) axis responses to restraint, elevated levels of testosterone and reduced testis weight. Changes in hypothalamic-pituitary-testicular (HPT) axis function and expression of hypothalamic aromatase and estrogen receptors were consistent with a role for systemic testosterone/brain estradiol in G1 sex-specific effects of sevoflurane. Only the male offspring (G2) of exposed parents exhibited neurobehavioral deficiencies, but had unaltered HPA and HPT axis functioning. Finally, down-regulated K⁺-2Cl⁻ (Kcc2) Cl⁻ exporter expression in G1 and G2 male hypothalamus and hippocampus, and hyper-methylated Kcc2 promoter in G1 sperm and ovary and G2 male hypothalamus and hippocampus support the involvement of epigenetic mechanisms in sevoflurane’s intergenerational effects. Repeated exposure of young adult rats to sevoflurane results in sex-specific central and systemic abnormalities, some of which are passed to offspring.

**P196 - Drosha expression and localization during bovine oocyte maturation in vitro.**
Deirdre Stuart, Allison Tscherner, Meritxell Flotats, Leanne Stalker, Jonathan Lamarre

MicroRNAs (miRNA) regulate gene expression post-transcriptionally by binding to messenger RNAs (mRNAs) and causing their degradation or suppressing translation. One critical early step in miRNA synthesis is mediated by a multiprotein complex called the Microprocessor. This complex consists of Drosha (a type III RNase), DGCR8/Pasha, and RNA helicases, which collectively cleave precursor primary transcripts (pri-miRNAs) to pre-miRNAs. The role of Drosha in embryogenesis has previously been shown to be non-essential in mice, due to the utilization of an alternate pathway (endo-siRNAs) involving Dicer for mRNA silencing. However, more recent work in bovine and humans indicate that this may be a species-specific phenomenon and that Drosha may be important for normal early embryo development in those species.

Earlier results in our laboratory demonstrated a marked increase in mRNA and protein levels of Drosha within the bovine oocyte over the course of maturation, continuing after fertilization until the 2-4 cell stage. To further characterize the roles and regulation of Drosha in this context, we examined the expression and localization of Drosha within germinal vesicle (GV) and mature (MII) oocytes using immunofluorescence and confocal microscopy. Results indicated a marked increase in Drosha expression, diffusely distributed throughout the ooplasm, in MII oocytes relative to GV oocytes. A comparable increase in expression was observed after transient (24 h) inhibition of maturation with 100 micromolar butyrolactone-I, suggesting that Drosha expression
is strongly linked with maturation. Collectively these studies support potentially important roles for Drosha in the maturing oocyte and early embryo. Future studies examining microRNA profiles and the effects of Drosha suppression should help further elucidate the importance of this miRNA pathway.

**P197 - Effect of preservation at freezing or supra-zero temperatures on epigenetic modifications and nuclear envelope structure of oocytes’ germinal vesicles in the cat model.**
Pei-Chih Lee, Pierre Comizzoli

The goal for developing female fertility preservation strategies is to retain normal oocyte structure and functionality while ensuring biosafety and cost-effectiveness. Compared to cryopreservation, dry-preservation and storage at supra-zero temperatures is a less detrimental and more economical alternative. Using the domestic cat model, we have previously customized microwave-assisted dehydration for oocytes’ germinal vesicles and demonstrated that the incidence of DNA damage remained low after dehydration and storage at supra-zero temperatures. However, key epigenetic factors and nuclear envelope integrity have not been investigated yet. The objectives of the present study were to investigate the effect of drying and rehydration on the levels of histone H3 trimethylation at lysine 4 (H3K4me3) and at lysine 9 (H3K9me3) as well as the nuclear envelope structure compared to vitrification. For dry-preservation, oocytes were permeabilized and exposed to trehalose (as protectant) before controlled microwave dehydration, and then stored in moisture barrier bags for up to 7 days. For reanimation, samples were either directly rehydrated with the rehydration medium or sequentially rehydrated with gradually decreasing trehalose concentrations. Results showed that H3K4me3 intensity significantly decreased \((P < 0.05)\) to 25% of the level of fresh controls when rehydrated immediately after drying. The intensity was partially recovered (40%) after storage at either 4˚C or ambient temperatures. Similar decreased intensity was observed regardless of rehydration strategies. Contrarily, H3K9me3 intensity was unaffected \((P > 0.05)\) throughout the dehydration, storage and rehydration procedure. Interestingly, similar epigenetic patterns were observed in vitrified oocytes after storage in liquid nitrogen. However, large proportion of vitrified/warmed oocytes (74%) had irregularly-shaped or collapsed nuclear envelope compared to the desiccated/rehydrated counterparts (44%), although both were higher than the fresh controls (16%). In conclusion, microwave-assisted dehydration approach maintains similar levels of epigenetic status and provides better protection to nuclear envelope structure than a well-established vitrification method.

**P198 - Genomic Insights Into IVF Failure; Dysregulated Inflammation In Stimulated Follicles.** Marc-André Sirard, Chloé Fortin

This study aims to understand IVF failure and see if markers could be used to characterize the patient response to the treatment and identify the failure cause. This could provide clues on how the stimulation protocol of subsequent cycles could be modified and personalized to turn unproductive cycles into successful ones. We first performed a microarray analysis to identify the granulosa gene expression profile of women \((n=32)\) that didn’t get pregnant after an IVF cycle and identify potential failure causes. 165 differentially expressed genes (DEGs) were found
between cells from follicles associated with failed and successful IVF cycles. The biological functions significantly affected in the negative patients were mainly related to immune and inflammatory responses. Indeed, many of the DEGs encode pro-inflammatory cytokines (e.g. IL1B and EGR1) or other inflammation-related factors that have been seen transcriptionally active in granulosa cells. Overexpression of several factors, including some acting upstream from VEGF, also indicates increased permeability and vasodilation. Another mechanism that appears more pronounced in the negative group is the recruitment of immune cells to ovarian tissues. Additionally to this sustained pro-inflammatory response, the anti-inflammatory mechanisms normally helping restoration of homeostasis seem to be impaired in the negative group supporting the hypothesis that the ovarian environment created by hormonal stimulation is prone to dysregulation. Using these inflammation markers and genes indicative of follicular status that could also be indicative of failure (e.g. growth phase or over-differentiated follicles) we analyzed an enlarged cohort of negative patients (n=63) by qRT-PCR. A hierarchical cluster analysis showed that the negative patients could cluster into 3 groups. Moreover, these groups mainly differ on the expression of genes indicative of different failure causes. These results highlight the possibility of creating a simple diagnostic tool to identify the probable cause of failure and means to improve the next cycle.

**P199 - Characterization of a novel F-box domain containing gene in the chicken primordial germ cells.** Deivendran Rengaraj, Bo Ram Lee, Jae Yong Han

Primordial germ cells (PGCs) are the precursor cells of male and female gametes that transmits genetic information to the progenies. The characteristic features of PGCs and functions as an early germ cells are tightly regulated by various germ cells-specific genes. When compared to humans and mice, the identification of germ cells-specific genes and characterization of their crucial roles required for maintaining and survival of germ cells is not well-understood in avian species. In our earlier microarray-based study, a novel transcript that highly expressed in the chicken PGCs compared to gonadal stromal cells was found. However, further information about this transcript was not known. Therefore, in the present study, we aimed to extensively characterize the expression and functions of this novel transcript in the chicken PGCs. Based on the BLAST search, this transcript was linked to the chicken chromosome 14. Moreover, the predicted protein sequences of this transcript contain F-box functional domain, and share some sequence conservation with the cyclin F gene. We constructed C-terminally 3xFLAG-tagged expression vectors of this transcript and delivered into chicken DF1 cells. Then, FLAG protein expression was detected in the transfected cells, indicating the translational abilities of this transcript. According to expression analyses, this novel F-box domain containing cyclin F like (CCNFL) gene was strongly detected in the chicken embryonic gonads and PGCs. RNA-interference mediated knockdown of this gene caused DNA double-strand breakage and apoptosis in the chicken PGCs in vitro and in vivo. Moreover, when this gene was knocked down in the chicken PGCs, the expression of several apoptosis-related genes were significantly upregulated, and the anti-apoptotic genes were significantly downregulated. Our findings in this study indicates that the novel F-box domain containing cyclin F like gene is required for the normal functioning of chicken PGCs.
**P200 - Specific endometrial-AKT isoform deletion using PR-Cre mice alters mouse estrous cycle and uterine development.** Laurence Tardif, Dadou Lokengo, Pascal Adam, Sophie Parent, Eric Asselin

The opposition between apoptosis and cell survival factors in the receptive uterus is important during embryo implantation. Akt pathway has a key role during implantation and gestation. Akt (1,2,3) is involved at various times in the ovarian cycle and during implantation. However, their specific roles in the endometrium is unknown. Studies have shown that the expression of Akt isoforms varies during gestation. The objective of this study was to establish the role of Akt isoforms during mouse estrous cycle. The hypothesis is that a dysfunction of Akt isoforms could cause cycling difficulties and inappropriate preparation of the uterus for embryonic implantation. Using PGR-Cre-LoxP system, C57BL/6 mice were knocked out for the three Akt isoforms (and their combination) in the endometrium. Data suggest that in absence of one or more Akt isoforms, female fecundity is reduced. Stroma size is not affected by Akt KO for all phases. However, we show that absence of Akt1-2 decreases the number of endometrial glands. We analyzed, by immunohistochemistry, important proteins involved in Akt pathway such as c-jun, cl-caspase3, 7, PCNA, ERα and PGR. Data suggest that cl-caspase3,7, cl-PARP, p-Bad expression are absent in stroma for all. In the luminal epithelium, cl-casp3,7 are increased at estrus and decreased at metestrus in Akt3, Akt1-2, Akt1-3, Akt2-3 KO mice. C-jun are increased in stroma and endometrial glands at estrus in all genotypes compared to WT. Expression of ERα and PGR reduced at proestrus in Akt1-2 KO mice in stroma while PCNA was decreased at estrus in endometrial glands. This decrease of hormone receptors and PCNA during proestrus and estrus could explain why Akt1-2 KO mice have fewer endometrial glands and a reduced fertility. These results suggest that Akt isoforms, particularly Akt1 and Akt2 are essential signaling molecule involved in the regulation of estrous cycle and endometrial modeling.

**P201 - ERα-binding Super Enhancers Drive Key Mediators that Convey Uterine Responses.** Sylvia Hewitt, Sara Grimm, Kenneth Korach

Estrogen receptor α (ERα) modulates gene expression through interactions with enhancer regions of chromatin that are often distal from transcriptional promoters for estrogen regulated transcripts, and current models incorporate a mechanism of looping between enhancer and promoter regions of chromatin. “Super enhancers” (SE) are enhancers that have especially high enrichment of indicators of active chromatin. These SE often control production of key cell type-determining transcription factors. We sought to define the estrogen-dependent enhancer landscape of the developing mouse uterus by analyzing uterine chromatin isolated from pre-pubertal (21-days old) and ovariectomized adult (10-weeks old) mice. ERα binding enhancers were identified using ERα chromatin immunoprecipitation next-generation sequencing (ChIP seq) data. 4600 ERα binding enhancers were then classified as “typical” or “super” enhancers according to ranked H3K27Ac signal. 368 SE were present in prepubertal samples (pre-formed SE), whereas 281 SE were seen in ovariectomized adult estrogen treated samples (matured tissue SE). The highest ranked SE were seen in regions encoding transcription factors associated with uterine function. Using chromosome conformation capture with high throughput sequencing (HiC) and cohesin subunit SMC1a ChIP seq, we located and analyzed chromatin loops between SE and gene promoters. 263 ERα-binding SE overlapped a loop end; these SE were connected
via 1015 loops. To get an indication of the gene regulation that might occur by looping interaction with the SEs, we determined transcripts that were either within one of the SE, or that were at the other end of a loop that formed from a SE. Altogether, this entailed 1600 genes, and based on RNAseq of uterine RNA, 1160 of these genes are expressed, and 566 transcripts are regulated by estrogen, with both up- and down-regulated transcripts observed. Our observations are consistent with an important role for ERα-binding SE for uterine function.

**P202 - Somatic cell nuclear transfer in early bovine embryo development is associated with changes in small non-coding RNA species.** Jocelyn Cuthbert, Stewart Russell, Qinggang Meng, Irina Polejaeva, Kenneth White, Abby Benninghoff

While a useful artificial reproduction technology, the method of somatic cell nuclear transfer (scNT) is generally inefficient, with successful pregnancy rates typically below 10%. Following nuclear transfer, the cellular machinery of the oocyte must reprogram the somatic DNA for proper embryo development. The maternal to zygotic transition (MZT) is associated with marked changes in control of gene expression, as well as marked class changes in small non-coding RNAs (sncRNAs), as previously identified by our research group in bovine embryos. Failure of these class changes to occur in scNT embryos may drive higher failure rates. The present study is the first to employ a discovery-based, small RNA sequencing approach to determine the population of sncRNAs in bovine scNT embryos as compared to in vitro-fertilized embryos. In all populations of sncRNA surveyed, large-scale population differences between ScNT and IVF embryos were not apparent for miRNAs, piRNAs, or tRNA fragments. However, we did identify a few select miRNAs that were differentially expressed at specific stages of embryo development, including miR-2340-3p and miR-345-5p in the morula-staged embryos, as well as miR-497-5p at the blastocyst-staged embryos. Distinct populations of piRNA-like RNAs (piRNAs) were identified through development of the scNT embryos, and abundance of several species were differentially expressed in scNT versus IVF embryos at the morula and blastocyst stages. Transfer RNA fragments may function in RNA interference pathways similar to miRNA, and we identified 6 tRNAs differentially expressed in scNT and IVF embryos during early embryo development. In conclusion, widespread sncRNA population differences were not observed between IVF and ScNT embryos, suggesting that errors associated with scNT may be somewhat random in nature. However, those sncRNAs that were consistently differentially expressed in scNT embryos may be important contributors to successful genome reprogramming of cloned embryos and these targets warrant further functional analyses.

**P203 - Bovine imprinted gene analysis in Assisted Reproduction Technology.** Simon Lafontaine, Rémí Labrecque, Patrick Blondin, Marc-André Sirard

The production of bovine embryos through in vitro maturation and fertilization is becoming an important tool of the genomic revolution in dairy cows. The molecular analysis of these embryos indicates some differences according to the culture conditions or hormonal stimulation. We hypothesized that some of the methylation patterns are acquired in the last portion of folliculogenesis and could be influenced by the environment created to generate these oocytes. Such differences may not be erased during the first week of culture or sensitive to the conditions
during this time. To test this hypothesis, an in vivo control group consisting of 8 embryos (day 12) obtained from superovulated and artificially inseminated cows were compared to embryos produced with oocytes collected via ovum pick-up (OPU) following ovarian stimulation and cultured in vitro with (8 embryos) or without Fetal Bovine Serum (FBS) (8 embryos). The DNA from both embryonic disk and trophoblast (2 samples per embryo) of these embryos has been extracted and bisulfite converted separately to reveal methylation marks. Then, the methylation status of 5 imprinted genes (H19, MEST, KCNQ1, SNRPN, PEG3) have been assessed by pyrosequencing. For each gene, we observed overall lower methylation levels and greater variability in the two in vitro groups compared to the in vivo control. The individual analysis indicates that some embryos are deviant from the rest and not all are affected. Overall, embryos cultured with FBS showed greater hypomethylation at imprinting sites which is in agreement with previous observation of epigenetic disturbance caused by serum and its link with the Large Offspring Syndrome (LOS). This work will generate markers at the single CpG resolution to assess the required hormonal and culture environment to minimize epigenetic perturbation in bovine embryos generated by assisted reproduction techniques, thus laying the groundwork for the development of a preimplantation epigenetic diagnostic tool.

**P205 - TET1 regulates expression of pluripotency genes in porcine blastocysts by controlling DNA methylation levels.** Kyungjun Uh, Junghyun Ryu, Noah Wax, Kayla Carey, Hannah Miko, Kiho Lee

TET enzymes (1–3) are important regulators of DNA methylation patterns, as they catalyze the oxidation of 5-methylcytosine (5mC) to produce 5-hydroxycytosine (5hmC), an intermediate of DNA demethylation process. In our previous study, targeted disruption of TET1 in porcine blastocysts unexpectedly upregulated NANOG gene expression, potentially due to the compensatory increase of TET3 transcripts. Here we analyzed the expression patterns and DNA methylation status of pluripotency genes under the presence of a TET enzyme inhibitor to expand our understanding of TET enzyme-mediated regulation of pluripotency in porcine blastocysts. Porcine IVF embryos were cultured in the presence of dimethylxallyl glycine (DMOG), a small-molecule inhibitor of 2OG-dependent oxygenases, between day 4-7 post fertilization; embryos exposed to a vehicle were used as a control. Effectiveness of DMOG on porcine embryos was assessed through immunocytochemistry by tracking the level of 5hmc after DMOG treatment. On day 7, ten to fifteen blastocysts were collected and used for bisulfite sequencing and RT-qPCR; all experiments were conducted at least three times. Student’s t-test was used for statistical analysis and p < 0.05 was considered significant. Lower levels of 5hmc were detected from immunocytochemistry in embryos incubated with the DMOG, indicating specificity of the inhibitor towards TET family. Higher levels of DNA methylation were detected on short interspersed nuclear elements, suggesting that DMOG-treated blastocysts possessed higher levels of global DNA methylation. Expression levels of pluripotency genes, including NANOG, were downregulated in DMOG-treated blastocysts compared to that in control blastocysts. The DNA methylation level of NANOG gene (promoter and gene-body) was
increased by the DMOG treatment, suggesting that suppression of TET family led to an increase in the DNA methylation and subsequently downregulated \textit{NANOG} gene transcription. Our study demonstrates that the TET family is a key regulator of pluripotency gene expression by controlling DNA methylation levels in porcine blastocysts.

**P206 - Bloodborne mRNA and miRNA Profiles Discriminate Beef Heifers of Differing Reproductive Success in the First Breeding Season.** Sarah Dickinson, Bailey Walker, Michelle Elmore, Joshua Elmore, Paul Dyce, Soren Rodning, Fernando Biase

The identification of heifers with high fertility potential remains a pivotal challenge in beef cattle production. We hypothesized that bloodborne mRNA or miRNA profiles were distinct in heifers of differing fertility potential. We aimed to profile and analyze mRNAs in peripheral white blood cells (PWBC) and circulating miRNA from plasma collected at the time of artificial insemination. The experimental groups were pubertal beef heifers that became pregnant at first service artificial insemination (AI-pregnant; \( n=6 \)) or failed to become pregnant following a 60-day breeding season with fertile bulls (Not-pregnant; \( n=6 \)). First, we quantified expression of 8,896 genes from PWBC and determined that 145 genes were differentially expressed between AI-pregnant and Not-pregnant heifers. Gene ontology analysis of the differentially expressed genes revealed significant enrichment of biological processes, including: ‘cytokine-mediated signaling pathway’, ‘inflammatory response’, and ‘neutrophil chemotaxis’. Second, we quantified the expression of 282 miRNAs in plasma and identified seven miRNAs differentially abundant between AI-pregnant and Not-pregnant heifers. Third, we examined co-expression between protein-coding genes and miRNAs by Pearson’s correlation (\( r \)) and inferred differential co-expression by contrasting data from AI-pregnant and Not-pregnant heifers. There were 7,244 pairs of miRNAs (\( n=251 \)) and protein-coding genes (\( n=2,212 \)) presenting strong negative correlation (\( r \leq -0.9 \)) in AI-pregnant but near zero correlation in Not-pregnant heifers, which we interpreted as loss of co-expression. Our results suggest that dysregulated miRNA-gene interaction explains the differential expression of some genes associated with heifer fertility. One remarkable example is the gene \textit{TMIGD3}, which was 5.2-fold less abundant in PWBC of AI-pregnant heifers and was negatively correlated with bta-miR-26c and bta-miR-505 (\( r=-0.97 \) and \( r=-0.9 \), respectively) in AI-pregnant heifers only. In conclusion, altered expression signatures of genes related to inflammatory processes may explain low fertility in beef heifers. Circulating miRNA is potentially involved in the regulatory mechanisms of these expression blueprints associated with heifer fertility.

**P207 - Influence of microRNAs from Semen on bovine fertility.** Stephanie Perkins, Brittney Keel, Emmalee Northrop, Tara McDaneld, Robert Cushman, Bo Harstine, Mel DeJarnette, Matthew Utt, George Perry

MicroRNAs (miRNAs) are a family of small RNAs that play a key role in regulating gene expression by binding to complementary mRNA and altering translation. It has been reported that this alteration of specific RNAs plays a role in male fertility. The objective of this study was to compare the miRNAs within sperm cells of bulls considered to have high and low fertility. Bulls were selected and assigned to low and high fertility groups (\( n=11 \) and \( n=12 \), respectively).
respectively) based on being a minimum of 6.5 sire conception rate units apart (average of 13,443 inseminations per sire). Straws of semen that had been collected on two different dates (mean of 5 months apart) were obtained from Select Sires. An equal number of straws from each collection date were pooled, and RNA was extracted separately for each bull. MicroRNAs were extracted and libraries were prepared using the Illumina TruSeq Small RNA preparation kit and sequenced on an Illumina MiSeq. Paired-end reads were merged with PEAR and adaptors were trimmed using Trimmomatic. Resulting reads were then mapped to the bovine genome and quantified using miRDeep2. Differential expression analysis was conducted using the DESeq2 package in R. Of the 516 miRNAs identified, 10 miRNAs were differentially expressed between bulls of high and low fertility ($P < 0.05$). These were bta-miR-9-5p, bta-miR-98, bta-miR-329a, bta-miR-142-5p, bta-miR-449a, bta-miR-126-5p, bta-miR-182, bta-miR-2284y, bta-miR-1839, and bta-miR-296-3p. PCR was performed to validate sequencing results on 3 miRNAs: miR-9-5p ($P=0.76$), miR-2284 ($P=0.05$), and miR-296-3p ($P=0.01$). Micro-RNA-296-3p is regulated by neurofibromatis 2, while miR-2284 and miR-9-5p have been identified in cells associated with immune response. These results support the idea that a small proportion of miRNAs may have a direct impact on fertility, possibly through early embryo development.

**P208 - Peroxiredoxin is a powerful biomarker of male fertility.** Do-Yeal Ryu, Won-Ki Pang, Sarder Arifuzzaman, Won-Hee Song, Md Saidur Rahman, Yoo-Jin Park, Myung-Geol Pang

Conventional semen analysis has limitation to predict male fertility in the livestock industry. Although several studies have been performed to overcome the limitation, the current solution for predicting male fertility is still unsatisfactory. Therefore, a new approach on this topic is required. It is generally accepted that peroxiredoxins (PRDXs) have a critical role in the regulation of male fertility. Here, the study was designed to investigate the relationship between PRDXs and male fertility. The expression of PRDXs was analyzed in spermatozoa collected from 20 individual boars with different litter size (10.3 - 14.2). Five hundred thirty sows were artificially inseminated to determine the litter size. Simultaneously, several sperm functions were evaluated. Our study showed there is a significant positive correlation between litter size and PRDX IV ($r=0.363$ and $p = 0.005$) and hyperactivity ($r=0.2556$ and $p = 0.023$). Conversely, a negative correlation was found between litter size and PRDX III ($r=0.0569$ and $p =0.045$). The accuracy for prediction of boar fertility was determined by the receive operating characteristic (ROC) curves. The ROC analysis showed that PRDX III showed sensitivity (87.5%), specificity (75%), negative predictive value (93.33%), positive predictive value (60%), and overall accuracy (85%) for predicting litter size. In addition, PRDX IV can predict litter size with overall accuracy 95% (sensitivity 93.75%, specificity 100%, negative predictive value 80%, and positive predictive value 100%). Also, hyperactivity showed 75% overall accuracy to predict litter size (sensitivity 75%, specificity 75%, negative predictive value 42.86%, and positive predictive value 92.31%). PRDX III, PRDX IV, and hyperactivity are expected to increase pups (0.51, 1.55, and 0.92, respectively) than average litter size. As far we know no studies have found any
relationship between PRDXs and litter size. Consequently, PRDX III and IV might be powerful biomarkers for diagnosing male fertility in the livestock industry.

**P209 - Functional connections within steroidogenic pathway in primary cultures of bovine granulosa cells investigated with PPI and co-expression networks.** Dariusz Skarzynski, Małgorzta Wieteska, John Hession, Katarzyna K. Piotrowska-Tomala Piotrowska-Tomala, Agnieszka Jonczyk, Paweł Kordowitzki, Karolina Lukasik, Leo Creedon

Bovine granulosa cells of dominant follicles were treated with insulin growth factor-1 (IGF) (25ng/ml, 50ng/ml, 100ng/ml) or with IGF-1 in combination with LH (50ng/ml IGF+2ng/ml LH and 100ng/ml IGF-1+2ng/ml LH). Gene expression of steroidogenic and cell-death pathways, and steroid hormone levels were assessed every 4h or 8h, respectively, during 24-h culture. The values were Log2 transformed. The functional connections between examined genes, 17b-estrogen (E2) and progesterone (P4) were studied using PPI network and co-expression network techniques. Dataset (n=23) analysed was composed of expression values recorded at respective times. To investigate the annotated connections between genes a PPI network was created based on KEGG, Reactome-Pathways and GO-biological processes databases. To overcome species differences (human versus bovine) a co-expression network focusing on direct co-expressions between genes using statistical methods was inferred. Network modularity applied to tested datasets produced only one cluster containing 4 genes (CYP19A1, CYP11A1, HSD3B2, RIPK3). Co-expression networks discard indirect links due to a common strong correlation with a third gene. Those connections were compared to direct connections found in PPI network. The connection between RIPK3 and CYP19A1 was not shown in PPI networks and this connection should be investigated further. The correlation between network topology and relationship between E2 and P4 and respective gene expressions were calculated using weighted correlation coefficients detecting spatial randomness. The p-values were lower for genes in our network than in genes distributed independently from network structure and apart from the connection with RIPK3 (p=0.0539) and HSD3B2 (p=0.1484) were statistically significant. The influence of expression values of each of the tested genes on the relationship between E2 and P4 level were assessed using partial correlation. Statistically significant effects of CYP19A1, BAX, CYP11A1, HSD17B1, RIPK3 and LHR on E2 and P4 relationship were found.

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**P210 - Optimization of spermatozoal mRNA processing for screening male fertility.** Won-Ki Pang, Saehan Kang, Do-Yeal Ryu, Won-Hee Song, Md Saidur Rahman, Yoo-Jin Park, Myung-Geol Pang

Recent years have witnessed that spermatozoal RNA contribute to fertilization in mammalian species. In this regard, spermatozoal RNA has the possibility to be used as a diagnostic marker of male fertility. However, because spermatozoa contain fewer RNA level with the highly fragmented state, the application of transcriptomes as fertility marker remains a subject of debate. Therefore, we tried to optimize the boar fertility screening method with mRNA using.
Equatorin (EQTN) and Peroxiredoxin-4 (PRDX4) as known as porcine fertility marker. To increase fertility predicting the ability of mRNA, we tried to explore the optimized conditions of spermatozoal RNA preparation and qRT-PCR methods. The condition under -80°C stored, snap freezing technique, and oligo dT cDNA synthesis was considered to be the optimized sample preparation method. Using the corresponding methods, we compared the qRT-PCR results on EQTN and PRDX4 and filed litter size from 22 individual porcine. EQTN and PRDX4 were selected as a negative and positive marker of porcine fertility, respectively. Interestingly, Non-DNase treated samples showed a higher correlation with litter size compared to DNase RNA extracted sample. Then, for the elimination of genomic DNA (gDNA) factor, intron sequence primer of markers was used to check the contamination and adjusted with modified ΔΔCt formula. After the elimination of gDNA factor, the result among replicates of qRT-PCR presented unstable results. For conclusion, our optimized method showed consistency and high predictability on porcine male fertility on both negative and positive markers.

**P211 - Identifying genetic factors that contribute to female infertility in humans.** Karen Schindler, Katarzyna Tyc, Warif El Yakoubi, Jessica Landis, Yiping Zhan, Xin Tao, Richard Scott, Jinchuan Xing

Infertility is a reproductive disorder that affects approximately 10% of women. Maternal age is an established contributing factor that correlates with an increased production of aneuploid eggs—a major cause of early miscarriage and infertility. Nevertheless, clinical data report cases of reproductively young (<35y) women producing unusually high percent of aneuploid concepti, and women of advanced maternal age (>35y) producing lower than average percent of aneuploid concepti for their respective age categories. These observations suggest that genetic factors contribute to fertility decline, irrespective of age. To begin to test this hypothesis, we obtained whole-exome sequencing data from over 500 reproductively challenged women, and performed a search for functional mutations that could cause gametogenesis defects. Thus far we identified several candidate genes that could be causative of the increased aneuploidy phenotype. These genes are linked to processes important for proper chromosome packaging and/or cell division. For example, we identified a gene that encodes a condensin complex subunit, whose product is an important player in compacting the genetic material before meiotic divisions. Furthermore, we identified a gene that encodes a protein required to maintain microtubule stability. Using a mouse oocyte model to evaluate meiotic functions, we have validated the functional significance of the identified mutations on regulating chromosome segregation during meiosis I. Importantly, we find that these mutations encode proteins that have dominant negative function and are therefore high-ranking causative candidates. This is the first step in identifying of genes and mutations contributing to the risk of aneuploidy and could allow prescreening of patients that have better chances to benefit from preimplantation genetic testing. This work is supported by a grant from the NIH/NICHD: R01-HD091331.

**P213 - Adenosine Deaminase Acting on RNA (ADAR1) Deletion in Granulosa Cells Causes Dyssynchronous Ovulation and Infertility.** Rikki Nelson, Xiaoman Hong, Pavla Brachova, Lane Christenson
Acuity in post-transcriptional gene regulation contributes to the cell’s ability to maintain or respond to its physiological environment by altering gene expression without transcriptional reprogramming. RNA sequencing has highlighted the abundance of non-encoded polymorphisms in RNA and the potential for RNA editing to contribute to post-transcriptional gene regulation. Adenosine deaminase acting on RNA (ADAR1) specifically catalyzes adenosine to inosine (A-to-I) editing. In complement, the periovulatory period provides a landscape for evaluating the role of post-transcriptional regulation as the somatic cells are undergoing differentiation and integrating signals from the maturing oocyte. A granulosa cell specific Adar depleted model was utilized to evaluate the role of ADAR1 during this process. ADARFL/FL/Aromatase-Cre (n=5) and wild-type control littermate (n=6) female mice were bred to wild-type males for fertility evaluation over a 7-month period. To assess ovulation, ADARFL/FL/Arom-Cre and control females were administered 5IU of PMSG and 46 hours later, 5IU of hCG, eggs were then collected from oviducts 16, 18, and 20 hours post-hCG. Ovarian histology and extracellular matrix distribution, via picrosirius red staining, was evaluated in 35-40-week-old female mice. In the mating trial, control females had 7.0 ± 1.0 pups/litter while only one ADARFL/FL/Arom-Cre female had two litters (4.5±0.5 pups). At 16 hours post-hCG, 44.0±8.0 and 0.7±0.6 eggs were recovered from control and ADARFL/FL/Arom-Cre females. By 18 and 20 hours post-hCG, the ADARFL/FL/Arom-Cre females ovulated 16.0±8.1 and 7.3±1.6 eggs, respectively. Diestrous staged ADARFL/FL/Arom-Cre mice showed inconsistent evidence of luteinization, increased macrophage presence, and increased extracellular matrix staining intensity (166005±36643 pixels/µm²; n=4; p=0.04) compared to controls (45133±14019; n=3). Further elucidation of cellular changes circa ovulation in ADARFL/FL/Aromatase-Cre granulosa cells through gene expression and steroidogenesis analysis is ongoing to establish the foundation for identifying how Adar impacts ovarian function.

P214 - Gene regulatory networks between porcine oocytes and surrounding cumulus cells.
Bailey Walker, Sarah Dickinson, Katelyn Kimble, Fernando Biase

Acquisition of developmental competence by an oocyte during folliculogenesis is profoundly reliant upon bidirectional communication with the surrounding cumulus cells (CCs). Nonetheless, little is known of their co-regulation of gene expression. We hypothesized that interactions between oocytes and CCs lead to interdependencies at the transcript level, which result in the formation of gene regulatory networks between the two compartments of the cumulus oocyte complex (COC). We collected 17 COCs from antral follicles of one gilt (150 days old) and generated single-cell RNA-seq data for the oocytes and RNA-seq data for the corresponding CCs. We quantified the expression of 6,811, 3,593 genes in oocytes and cumulus cells, respectively. In oocytes, we identified genes forming co-expression modules enriched for biological processes, among which, 66 genes associated with ‘regulation of transcription’ were dispersed across three modules. The genes BIRC6, CREBBP, HIF1A, RB1, and RBL1 formed hubs on these regulatory modules. In cumulus cells, there were two modules of co-expressing genes significantly enriched for biological processes, one of which was enriched for ‘translation’ with 63 annotated genes. Next, we identified 297 genes expressed in oocytes highly correlated (Pearson’s r, |r|>0.9) with 481 genes expressed in cumulus cells, forming 885 connections. One example of the functional implication of these interactions is the gene Maternal Embryonic Leucine Zipper Kinase (MELK), expressed in oocytes, which forms correlative connections withRNA.
24 genes expressed in CCs, including Prohibitin 2 (PHB2), which coregulates the function of estrogen receptors. In summary, our analyses revealed complex gene regulatory networks of oocytes, including hundreds of genes associated with the regulation of gene expression, in contrast to the translational control exhibited by cumulus cells. Additionally, several genes expressed in oocytes form regulatory networks with genes expressed in CCs, and those are functionally relevant for oocyte health.

**P215 - Heat Shock Can Influence The Expression Of DROSHA In Bovine Oocytes.** Luiz Camargo, Carolina Quintao, Gustavo Souza, Michele Munk, Vanessa Souza

Heat shock has a negative impact on developmental competence of bovine oocytes but the reasons are not well understood and epigenetic regulation may be involved. This study evaluated the effect of heat shock during in vitro maturation on expression of microRNAs (Bta-miR-21, -148a, -34c, -106a and -103), and DROSHA and DICER1 in oocytes. Oocytes were in vitro matured according to the experimental groups: (control group) 38.8°C for 24 hours and (heat shock group) 41°C for the first 12 hours and 38.8°C for further 12 hours. After in vitro maturation, pools of 80 oocytes were denuded and total RNA extracted (three biological replicates). Reverse transcription was performed with miScript II RT kit (Qiagen, Valencia, CA), and qPCR was performed with miScript SYBR Green PCR Kit (Qiagen), both following the manufacturer's recommendations. Primers sequences were designed based on miRBase (utilizing the mature sequence as forward primers) or GenBank database. Real time PCR values were normalized by let-7a1 for microRNAs and by YWHZA and ACTB for DICER1 and DROSHA. Data was analyzed by REST Software. Oocytes (n=598) were also in vitro fertilized and cultured to evaluate the developmental competence and data was compared by chi-square. There was no effect (P>0.05) of heat shock during in vitro maturation on abundance of miR-103, miR-106, miR-21, miR-148a, miR-34c and DICER1; however, the expression of DROSHA was up-regulated (P<0.05) in heat-shocked oocytes. Heat shock decreased (P<0.01) the development rate of fertilized oocytes toward blastocyst stage (22.2% vs 11.5% for control and heat shock, respectively). In conclusion, heat shock during in vitro maturation can affect the expression of DROSHA, which encodes an endonuclease involved in pre-microRNAs processing, and subsequently disturb the development after fertilization. Financial support: CAPES, CNPq, Fapemig.

**P217 - Developmental Genome-Wide DNA Methylation Asymmetry Between Mouse Placenta and Embryo.** Karine Doiron, Lisa-Marie Legault, Anthony Lemieux, Maxime Caron, Donovan Chan, Natale David R, Flavia Lopes, Daniel Sinnett, Serge McGraw

During early embryonic development, DNA methylation patterns are largely erased and re-established. For unknown reasons, methylation is acquired unequally between the cells of the embryo and the placenta. To better understand this process, we generated high-resolution maps of DNA methylation in mouse embryo and placenta at midgestation (E10.5). Our thorough investigation uncovered specific differentially methylated regions (DMRs) across distinct genomic features, which contribute directly to the developmental genome-wide asymmetry that exists between the mid-gestation mouse embryonic and placental DNA methylation profiles.
Amongst DMRs, we uncovered the presence of particular DMR categories based on their levels of DNA methylation in the embryo and placenta. Importantly, we found that these asymmetric regions are already discernable in E6.5 embryos and are especially driven by Dnmt3b methylation. In addition, most of these regions remained methylated to wild-type levels in Dnmt3a-deficient embryonic and extraembryonic cell lineages. However, at E8.5, lack of Dnmt3a caused a significant reduction in DNA methylation levels for these DMRs. Overall, we show that Dnmt3a and Dnmt3b participate in the establishment of the methylation patterns associated with the various DMR categories in both embryonic and extraembryonic cells, with Dnmt3b being the principal contributor in both cell lineages. Furthermore, our data suggests that for most of these DMR associated tiles, Dnmt3b can compensate almost entirely for Dnmt3a loss in early stages of development, but that Dnmt3a can only partially palliate for the absence of Dnmt3b.

**P218 - Progesterone stimulates histone citrullination to increase Insulin Like Growth Factor Binding Protein 1 (IGFBP1) expression in ovine luminal epithelial cells.** Coleman Young, Amanda Christensen, Brian Cherrington

Peptidylarginine deiminases (PADs) are a family of calcium dependent enzymes that post-translationally convert positively charged arginine amino acids in proteins into neutral citrulline residues. Targets for PAD catalyzed citrullination include arginine residues on histone tails which results in chromatin decondensation and changes in gene expression. PADs were first discovered in the uterus; however, since then no studies have examined their functional significance in this tissue. Our previous work shows that PAD2 and PAD4 are highly expressed in luminal epithelial cells from ovine caruncles and an ovine uterine luminal epithelial (OLE) derived cell line. In OLE cells, inhibiting PAD activity blunts histone citrullination resulting in decreased basal expression of insulin like growth factor binding proteins 1 (IGFBP1). Given that progesterone (P4) is critical to maintain the uterine epithelium during pregnancy and regulates IGFBP1 expression, herein we examined if P4 stimulates PAD catalyzed histone citrullination to regulate IGFBP1 expression in OLE cells. To test this, OLE cells were pretreated for 6 hr with vehicle or 2 µM BB-CLA, a pan PAD inhibitor, and then treated with 100 nM P4 for 60 minutes. Following 60 minutes of P4 treatment, there is a significant increase in citrullination of histone H3 arginine residues 2, 8, and 17 concurrent with increased IGFBP1 mRNA expression. In contrast, cells pretreated with BB-CLA do not show an increase in IGFBP1 mRNA expression despite P4 treatment. We next examined if citrullinated histones are directly associated with the IGFBP1 gene promoter using an anti-H3Cit2,8,17 antibody for chromatin immunoprecipitation. Stimulation of OLE cells with 100 nM P4 results in significant enrichment of the IGFBP1 gene promoter compared to vehicle treated controls. In conclusion, our work suggests that P4 stimulates PAD catalyzed histone citrullination to regulate IGFBP1 expression, which is important for uterine epithelial cell function during pregnancy.

**P219 - Effects of Advanced Maternal Age and Assisted Reproductive Technologies on Genomic Imprinting in Oocytes and Preimplantation Embryos.** Mellissa Mann, Audrey Kindsfater, Catherine Pressimone
During the last four decades, the average age of first-time mothers has steadily risen, leading to the idea that we have now entered an epidemic of age-related infertility. These women often turn to assisted reproductive technologies (ARTs) to improve their chances of conceiving. However, there has been little investigation on the effects of advanced maternal age, with or without ARTs, on genomic imprinting, where expression of specific genes is restricted to either the maternally or paternally-inherited allele. We hypothesize that advanced maternal age alone, or in combination with ARTs, leads to a higher frequency of imprinted methylation errors in oocytes and preimplantation embryos compared to young mothers. To test this hypothesis, retired female breeders, with proven fertility, were categorized as young (2-4 months), middle (6-10 months); or advanced maternal age (10-14 months). Virgin females (~1.5 months) were used as controls.

To access the effects of maternal age on imprinted methylation acquisition, oocytes were recovered from control and aged females (n=3 females; n>10 oocytes). To access the effects of maternal age on imprinted methylation maintenance, control and aged females were each split into 4 treatment groups, no ARTs, superovulation only, embryo culture only, and superovulation plus embryo culture, and blastocysts were collected (n=3 females; n>10 embryos). Imprinted methylation was analyzed at Snrpn, Kcnq1ot1 and H19 genes. We found no change in imprinted methylation acquisition in oocytes from control and maternal age females. For imprinted methylation maintenance, treatment with any ART resulted in a loss of imprinted methylation in blastocysts from both control and maternal age females. However, increasing maternal age with or without ARTs had no additional effect. Thus, in contrast to our hypothesis, these results indicate that advanced maternal age, alone or in combination with ARTs, does not increase the frequency of imprinted methylation errors in oocytes or blastocysts.

**P220 - Impact of chronological age on sperm methylome and its implication on early development.** Oladele Oluwayiose, Haotian Wu, Nicole Brandon, Alexander Suvorov, Rahil Tayyab, Cynthia Sites, Richard Pilsner

Compelling data indicates that advanced paternal age is associated with increased offspring risk of a host of adverse health including neurodevelopmental outcomes such as schizophrenia and autism. However, the precise mechanism of this paternal intergenerational transfer of risk remains unclear. Hence, we investigated the influence of paternal age on sperm DNA methylation as one such potential mechanism of action.

A total of 47 sperm samples were collected from male participants as part of the Sperm Environmental Epigenetics and Development Study (SEEDS). Sperm DNA methylation was assessed with the HumanMethylation 450K array and relevant demographic and lifestyle information were collected via self-reported questionnaire. Individual CpG methylation and differentially methylated regions (DMRs) were analyzed using linear models and corrected for false discover rate (q < 0.05).

Male age ranged from 21-45 years with majority of participants (53%) in their thirties. Male age was found to be inversely associated with fertilization rate (p = 5.67E-05) and life birth (p = 0.001). Adjusting for BMI, smoking and clinical infertility status, age was associated with 1,526 CpG sites (q < 0.05) that were mostly (89%) hypermethylated. Regional analyses revealed 1,090 age-associated DMRs. Moreover, 19 CpGs and 129 DMRs remained significant at level of
Bonferroni correction. Gene ontology analyses of the genes associated with our 1,090 DMRs revealed enrichment for developmental pathways such as spinal cord, muscle structure and forebrain development as well as embryonic organ morphogenesis, WNT ligand biogenesis and behavior.

Our results revealed that male age is associated with sperm DNA methylation profiles at genomic loci enriched in genes that are important for early embryonic development, behavior and signaling. This study implicates sperm epigenetics as a mechanism for the paternal transmission of age on the health and development of subsequent progeny.

**P221 - A-to-I RNA modifications are enriched in oocyte ribosome associated RNA.** Pavla Brachova, Nehemiah Alvarez, Lane Christenson

In growing oocytes, maternal mRNAs are transcribed and stored. Stored transcripts support oocyte meiotic maturation and early embryonic development, and then undergo decay. We previously identified adenosine into inosine (A-to-I) RNA editing as a common RNA epitranscriptome modification in GV oocytes and MII eggs. These A-to-I RNA edits were enriched at the third nucleotide of the codon (wobble position), altering codon optimality, which can potentially impact mRNA stability and translation efficiency during the GV-to-MII transition. Due to the codon-specific nature of RNA editing, we hypothesized that A-to-I RNA editing was associated with translational machinery. To test this, we used a computational approach to examine A-to-I RNA editing dynamics in total RNA-seq and polysomal RNA-seq data during meiotic maturation (GV, MI, and MII stages, n=2/stage). During meiotic maturation, there was no difference in the number of A-to-I RNA edited transcripts in total RNA (GV=1750.5±337.5; MI=999.5±109.5; MII=1132±213; means±SEM). In contrast, ribosome associated RNA exhibited a decrease of A-to-I RNA edits during meiotic maturation (GV=1666±30; MI=850.5±5.5; MII=709.5±6.5; p<0.05 one-way ANOVA). Efficiency of A-to-I RNA editing also decreased at these stages (GV=83%, MI=38%, and MII=32%). Both total RNA and ribosome associated RNA exhibited an enrichment of editing at the wobble position. In ribosome associated RNA, 18 codons were highly edited in GV oocytes, and declined during meiotic maturation. Overall, we show that oocyte meiotic maturation coincides with a reduction in ribosome associated RNA edits, as well as editing efficiency, indicating that A-to-I RNA modifications are involved in RNA clearance during maternal RNA degradation.

**P222 - Aberrant ERα Binding and Excessive Histone H3K27ac Association near Persistently Altered Genes in the Adult Mouse Uterus Following Neonatal DES Exposure.** Wendy Jefferson, Tianyuan Wang, Carmen Williams

Developmental exposure to the estrogenic chemical diethylstilbestrol (DES) induces extensive alterations in ERα dependent histone H3K27ac association at enhancers of differentially expressed genes (DEGs) in neonatal uterine tissue. To determine if these features persist in adults, we used a global epigenomic approach. RNA-seq and ChIP-seq was performed on mouse uteri collected from adult controls at diestrus (CoDi) and estrus (CoE) and adults neonatally exposed to DES, who stay in persistent estrus. There were 4,161 DEGs between CoDi and CoE,
attributable to estrous cycle changes, and 3,330 DEGs between CoE and DES, attributable to the neonatal DES exposure. About 40% of the adult DES-specific uterine DEGs were persistently altered as indicated by their overlap with neonatal uterine DEGs. H3K27ac ChIP-seq in adult uteri revealed 24,721 differentially associated peaks between CoDi and CoE and 24,679 when comparing CoE to DES. Of the 3,330 DES-induced DEGs, 53% had a nearby differential H3K27ac peak. In addition, H3K27ac signal in these regions was much higher than H3K27ac signal in either control group suggesting excessive accumulation of this mark near DES-induced DEGs. ERα ChIP-seq in adult uteri revealed 31,971 differentially associated peaks in CoDi vs CoE and 21,114 between CoE and DES uteri. There was a differential ERα peak nearby 67% of the 3,330 DES-induced DEGs and there was some overlap with H3K27ac peaks uniquely observed in the DES group. The vast majority of these ERα peaks were aberrant as there was little to no ERα binding in these locations in either CoDi or CoE samples. These data suggest that aberrant ERα and excessive H3K27ac binding plays a role in the permanent alteration of gene expression in DES-exposed uteri.

**P223 - Sperm Protamines and Bull Fertility.** Muhammet Ugur, Naseer Kutchy, Erika Menezes, Asma-ul Husna, Holly Evans, Mustafa Hitit, Abdullah Kaya, Arlindo Moura, Erdogan Memili

Bull fertility is vital for cattle reproduction and production. Despite producing abundant sperm with normal motility and morphology, some bulls still have low fertility because of molecular defects to the spermatozoa and its proteins. The objective of this study was to test the hypothesis that sperm nuclear proteins Protamines (PRM) 1 and 2 are associated with bull fertility. Expression levels of PRM 1 and 2 were determined in sperm from 10 bulls with different field fertility using flow cytometry and immunocytochemistry. Differences in sperm chromatin decondensation ability was ascertained using chromatin decondensation test, and computational biology approaches were employed to uncover interactomes of PRM1 and microRNA transcripts targeting PRM transcripts. The results of flow cytometry experiment revealed that expression of PRM1 and PRM2 was not significantly different between the two groups (high and low fertility bulls; p> 0.05). Importantly, the abundance of PRM1 and 2 levels were inversely related to each other (p < 0.0001); which means a given spermatozoa having expressed PRM1 in its chromatin has decreased to no expression of PRM 2 protein. Results of nuclear chromatin decondensation assay showed that the average percentage of decondensed sperm cells in high and low fertility bulls were 25.1± 4.16 and 50.7 ± 9.5, respectively. These values were significantly different in high and low fertility group (p < 0.0001). Pathway analyses revealed that PRM1 and 2 have significant gene ontology terms on post-translational protein modification, chromatin organization, and regulation of transcription from RNA polymerase I promoter. In addition, the PRM1-2 interact with total of 132 genes including CDC42, SPT10, and TAF1. Furthermore, total of 161 miRNAs, targeting bovine PRM 1 and 2 were determined using bioinformatic tools, such as miR-2185, miR-7847, and mir-2673. These findings are significant because they help advance fundamental science of reproductive biotechnology and predict bull fertility.
**P224 - Cross-species transcriptome analysis of oocyte maturation.** Tyler Garner, Francisco Diaz

Oocyte quality governs its fertilization competence and the developmental potential of the resulting embryo. Maturation from the germinal vesicle (GV) oocyte to the metaphase II (MII) stage is a conserved developmental pathway across many species and thus a critical process that determines oocyte quality and fertilization success. Phenotypic differences among species are driven by evolutionary deviations in gene sequence and transcript abundance, and those genes with less variance in physiological processes across species have a higher translational potential. Genes with conserved sequence and expression patterns are likely to be crucial for oocyte maturation and have greater relevance for the treatment of infertility and improving *in vitro* maturation and fertilization success than more divergent genes. The aim of this study was to classify and compare transcript abundance at three distinct stages of oocyte development across four species. RNA-seq datasets from *Homo sapiens*, *Macaca mulatta*, *Mus musculus*, and *Danio rerio* oocytes at the GV, metaphase I (MI), and MII stages were integrated using a computational pipeline standardized for quality control, alignment, and feature quantification. Global principal analysis and hierarchical clustering on protein-coding, one-to-one orthologues annotated across all four species displayed preferential clustering by species. Gene ontology enrichment analysis for each individual species identified a common set of overrepresented biological pathways. When assessing differential expression between GV-MI and MI-MII stage transitions we found a subset of genes with a consistent profile across all species. Analysis on this subset of genes shifted the preferential clustering by species toward by cell type, indicating that these genes may be particularly important for oocyte maturation and thus less susceptible to evolutionary divergence. These data may provide a useful resource when planning, interpreting, and translating experimental data from model organisms to humans and for identifying novel protein-coding genes that are essential in human reproductive processes.

**P225 - Transcriptomic profiling and bioinformatic analysis of endometriosis-associated ovarian clear cell carcinoma.** Kaitlyn Collins, Xiyin Wang, Chi Zhang, Doug Rusch, Aaron Buechlein, Chad Creighton, Shannon Hawkins

Endometriosis, a chronic, inflammatory condition where endometrial-like tissue grows outside the uterus, is a significant risk factor for specific histologic subtypes of ovarian cancer. Women with endometriosis are three times more likely to develop ovarian clear cell carcinoma (OCCC) than women without endometriosis. Although OCCC is a rare histologic subtype, women with OCCC have poor response to platinum-based chemotherapies and worse prognosis at late-stage disease compared to the more common high-grade serous tumors. Previous studies have shown ovarian endometrioid adenocarcinoma to be molecularly distinct in women with endometriosis compared to tumors from women without endometriosis, suggesting an important contribution of the endometriotic tumor microenvironment in ovarian cancer. To study the contributions of the endometriotic tumor microenvironment in OCCC, next-generation sequencing for both RNAs and small RNAs was performed on pathology-proven OCCC with concurrent endometriosis. Analysis of sequencing identified 4799 differentially expressed genes, 2223 upregulated and
2576 downregulated \((P<0.01, \text{log2fold change}>±1)\) and 66 differentially expressed miRNAs, 19 upregulated and 47 downregulated \((P<0.05, \text{foldchange}>±1.2)\) compared to endometriomas. Integrated analysis of dysregulated genes with reciprocally dysregulated miRNA molecules with \textit{in silico} miRNA target prediction algorithms predicted 1908 miRNA-target genes dysregulated, 1009 upregulated and 899 downregulated. MiR-10a was 11-fold upregulated in OCCC with concurrent endometriosis, and Ingenuity Pathway Analysis indicated that miR-10a was a significant upstream regulatory molecule \((P=7.6e-3)\). MiR-10a is predicted to affect 117-target genes dysregulated in OCCC. Functional annotation of these 117 miR-10a target-genes showed enrichment in inflammation, proliferation, and transcription factor regulation. Overall, this data indicates miR-10a as a possible driver and therapeutic target in OCCC with concurrent endometriosis.

\textit{P226 - Reproduction in space: Does prenatal exposure to altered gravity program sex-biased placental expression of stress-related genes and adult outcomes?} Simranjit Kalotia, Molly Heit, Moniece Lowe, Sophie Benson, Yuli Talyansky, Linda Guttman, Candice Tahimic, April Ronca

Interest of space agencies and private entities in extra-terrestrial colonization is growing, emphasizing the importance of reproductive and developmental research in the absence of Earth’s gravity. Maternal stressors can modify offspring development, exerting sex-biased lifespan and crossgenerational changes via prenatal programming. The microgravity of space is stressful, therefore exposure during pregnancy to gravity loads that are either lower or higher than the Earth’s 1g may impact later life outcomes in offspring. Young adult male and female \((F_0)\) Sprague-Dawley rats \((N=10/condition)\) were adapted to continuous 2g exposure for one-week prior to timed-mating using the NASA Ames Research Center 24ft dia centrifuge. Centrifugation was discontinued at birth and neonates \((F_1)\) were fostered to non-manipulated, newly parturient dams until weaning. Same-sex paired were maintained under standard colony conditions. Adult male (but not female) offspring developed 8-15\% increased body mass and elevated anxiety responses relative to their respective 1g controls. We next conducted a gravity dose-response study to analyze expression of stress-related genes in placenta that may mediate the response profiles of males exposed to increased gravity. Timebred female rats \((N=5/condition)\) were exposed fractional increments in gravitational loading across 1, 1.5, 1.75 or 2g to analyze gravity dose-response relationships across increasing g-loads. On G20, placentas were harvested and sex determined using primers for SRY gene. Placentas were analyzed for expression of placental 11ß-hydroxysteroid dehydrogenase type 2 \((\text{HSD11B2})\) that reduces fetal cortisol/corticosterone exposure to 10–20\% of maternal levels, and for DNA methyltransferase \((\text{DNMT3a})\), a placental enzyme that promotes DNA methylation to examine possible mechanistic pathways through which stress-induced epigenetic variation is achieved. Our preliminary findings suggest that a systematic dose-response relationship exists between gravity load and the expression of these stress-related genes, thereby linking exposure to altered gravity during pregnancy with maternal stress and adult male phenotypic outcomes.

\textit{P227 - Using mouse models of LINE-1 to study retrotansposons during development.} Wenfeng An, Partha Saha, Simon Newkirk
Transposable elements (TEs) are the most abundant sequences in the human and mouse genomes. However, often dismissed as junk DNA, their roles in genomic structure and function remain poorly understood. Among all TEs, long interspersed elements type 1 (LINE-1, or L1) are the most prevalent by mass, constituting 17% and 19% of the human and mouse genomes, respectively. In addition, L1s are the sole class of TEs that are both autonomous and active in the human genome. To fully understand the role of L1s during development and disease, it is important to track L1 activities in vivo, but it is technically challenging to monitor the transcriptional and insertional activities of endogenous L1s due to their highly repetitive nature. Toward this goal, our lab has created two types of L1 reporter mouse lines. The first type is for monitoring L1 promoter activities and is composed of independent single-copy LacZ transgenic mouse lines, in which the LacZ gene is regulated by the endogenous mouse L1 promoter. After genomic mapping the transgene in each line, mouse tissues are stained with X-gal to visualize L1 promoter activity at single cell level. Initial screening demonstrated that the frequencies of X-gal signal in each tissue were largely conserved across generations for a specific LacZ mouse line but varied among different lines. Our goal is to comprehensively profile L1 promoter activity in a cell dependent and locus dependent manner. The second type is for monitoring L1 insertional activities. Using single-copy full-length L1 transgene that carries a retrotransposition indicator cassette, we have examined the impact of piRNA pathway on retrotransposition in the male germline. Ongoing effort aims to define the developmental timing of L1 retrotransposition, which is key to our understanding of L1-mediated mutational burden and its contribution to various pathophysiological processes.

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**P228 - TET1 is required for maternal imprint erasure during primordial germ cell development.** Rexxi Prasasya, Marisa Bartolomei

In mammals, a subset of developmentally critical genes is expressed from a single parental allele. The monoallelic expression of these imprinted genes is regulated by differential DNA methylation between the two parental alleles on cis-acting elements known as imprinting control regions (ICRs). Somatic methylation patterns of ICRs are reprogrammed in the primordial germ cells (PGCs) to allow for the acquisition of sex-specific imprinting marks during gametogenesis. Recent work supported the involvement of Tet-Eleven Translocation (TET) family of methylcytosine dioxygenases in the proper establishment of germline imprinting. We previously observed abnormal hypermethylation of ICRs in mature gametes and progeny of Tet1\(^{-/-}\) male and female mice and hypothesized that TET1 is required for methylation erasure of ICRs in PGC development. Through incorporation of Oct4-GFP reporter line, we isolated PGCs from Tet1\(^{-/-}\) and Tet1\(^{+/+}\) mutant mice at embryonic day (E)12.5, E13.5, and E14.5 and characterized methylation levels of representative ICRs using bisulfite mutagenesis, followed by pyrosequencing. Several maternally methylated ICRs fail to fully demethylate in male and female Tet1\(^{-/-}\) PGCs by E14.5. This directly correlates with hypermethylation of maternally methylated ICRs in Tet1\(^{-/-}\) sperm. In contrast, two paternally methylated ICRs, H19/Igf2 and IG-DMR, achieved hypomethylation in Tet1\(^{-/-}\) female and male PGCs. As H19/Igf2 and IG-DMR were previously found to be stochastically hypermethylated in Tet1\(^{-/-}\) oocytes, our result suggests that the lack of TET1 may render paternally methylated ICRs susceptible to ectopic
remethylation in growing oocytes irrespective of the completion of methylation erasure in PGCs. As TET2 is concurrently expressed in demethylating PGCs along with TET1, we are currently generating \textit{Tet1^{-/-};Tet2^{-/-}} embryos to characterize the contribution of TET2 in ICR methylation erasure. Overall, this study demonstrates the differential requirement for TET1 in demethylation of maternal and paternal imprints during PGC development.

\textbf{P229 - Altered reproductive epigenetic profiles in mature IVF-conceived offspring.} Eric Rhon-Calderon, Laren Riesche, Lisa Vrooman, Marisa Bartolomei

Assisted Reproductive Technologies (ART) have helped many couples to overcome infertility problems. \textit{In vitro} fertilization (IVF) is the most common technique used as ART and has recently been refined in order to increase success rate of pregnancies. However, laboratories have shown that IVF is related to health risks for mothers and fetuses including, stillbirth, preterm birth, intrauterine growth restriction, abnormal placentation, and other pregnancy complications. Using our IVF mouse model, we aimed to investigate morphological and epigenetic outcomes of reproductive tissues. For the IVF group, female mice were superovulated, cumulus-egg complexes were collected and IVF was performed with capacitated mature sperm. Fertilized eggs were cultured to blastocyst stage and transferred into pseudo-pregnant females. For the natural group (Nat), females were naturally mated overnight. All pups were delivered via c-section. At 39-weeks of age, the offspring were euthanized and reproductive tissues were fixed and frozen. Global DNA methylation (DNAm) and DNAm of imprinted genes was assessed with luminometric methylation assay (LUMA) and pyrosequencing, respectively. Student’s t-test was used to evaluate differences between groups. IVF females presented a lower ovarian/body weight ratio (p=0.0075) [Nat: 4.50±3.13; IVF: 0.50±0.17], ovaries presented lower global DNAm (p=0.0124) [Nat: 67.2%±3.5; IVF: 57.4%±7.9], lower DNAm at \textit{IG}-DMR (p=0.005) [Nat: 42.3%±1.6; IVF: 36.6%±2.9]; decreased expression of \textit{Dlk1} (p=0.0044) [Nat: 1.0±0.3; IVF: 0.2±0.1] and \textit{Esr1} (p=0.0012) [Nat: 0.8±0.5; IVF: 0.2±0.1]. While IVF males did not show difference in testes/body weight ratio, their testes and sperm showed higher global DNAm (p=0.0234) [Nat: 64.9%±6.0; IVF: 70.6%±2.3] (p=0.0484) [Nat: 66.5%±5.5; IVF: 74.3%±3.1] respectively, higher DNAm at \textit{IG}-DMR (p=0.0458) [Nat: 90.8%±1.6; IVF: 88.0%±2.7] and decreased expression of \textit{AR} (p=0.0368) [Nat: 1.00±0.15; IVF: 0.70±0.20]. Taken together, our results suggest that IVF procedures alter the epigenetic profiles of reproductive tissues in a manner that likely compromises the reproductive function of offspring in both sexes.

\textbf{P230 - Histone lysine beta-hydroxybutyrylation, an epigenetic mark, is induced by the ketone body beta-hydroxybutyrate in cattle cells.} Juliano Sangalli, Maite del Collado, Rafael Sampaio, Juliano da Silveira, Felipe Perecin, Richard Schultz, Pablo Ross, Flávio Meirelles

Beta-hydroxybutyrate (BHB), a ketone body and central for ruminant metabolism and health, can generate a novel histone acylation mark—lysine beta-hydroxybutyrylation (Kbhb)—associated with activating genes regulating lipid metabolism and starvation response. To ascertain whether this modification is present in relevant organs in vivo, we acid-extracted histones from dairy cow ovary, cumulus cells, liver, heart, kidney, brain, skin fibroblasts and mammary gland and
confirmed the presence of Kbhb histones by immunoblotting. We next determined whether BHB supplementation increased Kbhb levels using skin fibroblasts. Fibroblasts were cultured in the absence (control) or 2, 4 and 6 mM BHB for 24 h and the relative fluorescence Kbhb levels determined by laser scanning confocal microscopy using a specific H3K9bhb antibody. BHB treatment increased H3K9bhb levels in fibroblasts in a dose-dependent manner (p<0.0001). Cells treated with 2 mM BHB presented ~4.8-fold higher levels compared to controls. Treatment with 4 mM and 6 mM BHB, concentrations found in cows with clinical and severe clinical ketosis, dramatically increased H3K9bhb levels ~10.7-fold and 23-fold, respectively, compared to untreated cells. We also matured cumulus cell-oocyte complexes (COCs) in the absence (control) or presence of high levels (6 mM) of BHB and assessed the effect on H3K9bhb levels in oocytes and cumulus cells. We failed to detect this epigenetic modification on the metaphase plate in oocytes after staining and confocal analysis but observed a 3-fold increase in cumulus cells relative to controls (p<0.0001), suggesting that cumulus cells may somehow shield the enclosed oocyte from a high BHB concentration. These results lay a foundation for future studies to dissect connections between nutrition, epigenetics and reproduction in cattle. This work was supported by FAPESP grants: 2016/13416-9 and 2018/09552-0.

P231 - Single-blastocyst genome-wide bisulfite sequencing for assessing the impact of in vitro follicle culture, superovulation and age on mouse embryo development. Laura Saucedo-Cuevas, Elena Ivanova, Anamaria Herta, Katy Billooye, Johan Smitz, Gavin Kelsey, Ellen Anckaert

The success of oncology treatments amongst girls and women with cancer has increased the focus on their future life quality, including safeguarding the ability to have biological children. For some patients fertility preservation approaches such as embryo/oocyte freezing or ovarian tissue cryopreservation and autotransplantation are not suitable for safety reasons. An alternative option is using in vitro follicle culture (IFC) systems that support growth of oocytes from early stage follicles. IFC have proven successful in mouse to support the development of follicles from primordial or early pre-antral stages up to preovulatory stage. Although live offspring have been obtained following IFC, there is concern that it might affect normal embryonic development by interfering with the timely acquisition of correct methylation patterns in oocytes and the maintenance of genomic imprinting after fertilization. Recent unpublished data from our group shows that both IFC and superovulation globally preserve the methylation landscape of oocytes and do not affect de novo methylation of imprinted genes. Interestingly, specific and consistent alterations in DNA methylation are found depending on both the age of the animals and the approach used to grow and mature the oocytes. To elucidate the extent to which IFC might alter DNA methylation after fertilization we have conducted whole-genome bisulfite sequencing on single blastocyst using a post-bisulfite adapter tagging approach (PBAT). We have generated genome-wide DNA methylation maps in blastocysts derived from in vitro cultured oocytes collected from pre-pubertal and adult mice and their age-matched superovulated and in vivo controls. Preliminary results suggest a global loss of DNA methylation in IFC derived blastocysts. Detailed analysis of methylation levels at the regulatory regions in imprinted genes, and at other genomic features will define the effect of IFC and superovulation in relation to maternal age on maintenance of methylation and proper reprogramming of blastocyst.
Investigation of polycystic ovary syndrome ancestry-driven comorbidity patterns.
Ky'Era Actkins, Digna Velez Edwards, Melinda Aldrich, Lea Davis

Polycystic ovary syndrome (PCOS) demonstrates a complex genetic architecture and significant clinical heterogeneity affecting metabolic and endocrine processes related to the female reproductive system. Current diagnostic criteria target only a portion of the PCOS phenotypic spectrum and neglect the impact of comorbid conditions such as insulin resistance, type 2 diabetes, and cardiovascular diseases. However, comorbidity due to metabolic conditions is reported to drive ancestry-related differences in PCOS severity. We aim to study ancestry-driven comorbidity patterns in PCOS using electronic health records at Vanderbilt University Medical Center. We developed two broad and two stringent phenotyping algorithms to identify PCOS cases and performed a logistic regression on PCOS case status across the entire medical phenome to examine the relationship between PCOS and its comorbidities across different racial and ancestral groups. We observed that women of European decent consistently had more unique phenotypes associated with PCOS case status than African American women. Through a moderation analysis, we examined the interacting effects of self-reported race on PCOS case status and found six phenotypes with significant (p < 1.56e-3) effects in both European and African American women identified by our best performing phenotyping algorithm. African American women with PCOS had greater odds of being diagnosed with “Ovarian cyst” (p = 2.91e-4, OR = 9.16) and “Pain and other symptoms associated with female genital organs” (p = 3.80e-3, OR = 3.62). European women had greater odds of being diagnosed with “Symptoms involving female genital tract” (p = 9.11e-15, OR = 5.43) and “Vaginitis and vulvovaginitis” (p = 4.36e-17, OR = 3.77). Our findings suggest that PCOS symptomology varies between European and African American women in a large hospital population, although they are exposed to the same diagnostic standards. Future directions include further exploration of metabolic comorbidity networks for PCOS across racial and ethnically diverse populations.

miR-23b of Endometrial Origin: Small, Non-coding Regulator of Trophoblast Transcriptome.
Joanna Najmula, Monika Kaczmarek

MicroRNAs (miRNA) are small, non-coding RNAs important for the regulation of various cellular processes across a wide range of animal species. Recently, we have identified numerous miRNAs in embryos, trophoblasts and endometrium, showing a great importance to affect gene expression at the embryo-maternal interface in pigs. Significance of paracrine interactions in pregnancy success lead us to selected miR-23b of endometrial origin to be tested in porcine trophoblast cells <i>in vitro</i>. A sequence of experiments using lipofection of miR-23b mimic into primary trophoblast cells isolated form 15-16-day pregnant pigs (n=8) was performed in order to assess miR-23b effect on gene expression and cell function (<i>e.g.</i>, proliferation, adhesion). Successful miR-23b mimic delivery to trophoblast cells was assessed with confocal microscopy and qPCR. Screening for potential direct targets of ssc-miR-23b was performed ahead of qPCR using <i>in silico</i> prediction tools (<i>e.g.</i>, TargetScan, MirWalk) and Ingenuity Pathway Analysis to identify relevant functions. Among 13 genes assessed by qPCR, four were downregulated (<i>e.g.</i>, IL6R, p=0.0397; FGFR2, p=0.0301; CFL2, p=0.0301; ANXA2, p<0.0001) and one upregulated (<i>e.g.</i>, STAT3, p<0.0001), while remaining nine genes were not affected (<i>e.g.</i>, IFNG, PGR, TGFB3).
by miR-23b overexpression (n=6-8). Any changes in trophoblast cell proliferation (n=5) and adhesion to fibronectin (n=5) and laminin (n=3) were seen after miR-23b mimic delivery. Our ongoing efforts are aimed to identify crucial miR-23b-mRNA interactions and draw functional networks responsible for proper trophoblast function during pregnancy. We believe miR-23b of endometrial origin is an important element of the early embryo-maternal communication, controlling trophoblast transcriptome plasticity. Funded by Polish National Science Centre 2014/15/B/NZ9/04932.

**P234 - Quiet! They May Be at Rest: Metabolic Characterization and modulation of Paused-Pluripotency.** João Ramalho-Santos, Maria Inês Sousa, Bibiana Correia, Ana Sofia Rodrigues

Embryonic diapause (ED) is a conserved reproductive strategy in which development temporarily arrests to ensure proper conditions are available for embryonic development. Recently mTOR inhibition was proven to reversibly induce ED on mouse blastocysts and a paused-like state on mouse embryonic stem cells (mESCs) culture. The mTOR pathway is a major integrative pathway that responds to a variety of environmental cues to coordinate several cellular processes including metabolism. Additionally, the induction of an in vitro diapause-like state could render a valuable tool to expand the understanding of the cellular and molecular mechanism of ED. Therefore, we first aimed to characterize this paused-pluripotent state, focusing on its glycolytic and oxidative metabolic function. Also, and given that mTOR activity is regulated by nutrient availability, especially amino acids we modulated culture conditions to induce this paused-state by withdrawing specific amino acids from the medium, avoiding pharmacological approaches. Naïve mESCs [were cultured in the presence/absence of the mTOR inhibitor INK128 and different conditions of amino acid availability. Cell proliferation, cell cycle, pluripotency status and glycolytic and oxidative metabolism were assessed. INK-128 significantly decreased mESC culture growth through cell cycle modulation, without inducing apoptosis or altering the pluripotency status of the cells. Absence of specific amino acids was more effective than INK128, reducing cell proliferation while affecting cell cycle progression. Moreover, a reduction in glycolytic and oxidative metabolism was observed, further evidenced by decreased lactate production and differential nutrient uptake. Furthermore, privation of these amino acids reduced mTOR activity-related phosphorylation without affecting pluripotency and the observed effects of amino acid withdrawal were reversible. Altogether, our results suggest that metabolic modulation of culture conditions in mESCs, by amino acid withdrawal were potent inducers of the paused-state.

**P235 - Trophoblastic spheroid (BAP-EB) differentiation from human embryonic stem cells.** Si Yu Tian, Sze Wan Fong, Chao Min Yue, Andy C.H. Chen, Kai-Chuen Lee, William S.B. Yeung, Yin-Lau Lee

The success rate of in vitro fertilization remains low. Implantation failure is one of the major reasons. However, the exact mechanism is still unclear due to lack of proper human implantation model. Our team has established an embryo surrogate (BAP-EB) from human embryonic stem
cells (hESC) for the study of early implantation process. In this study, the effects of hESC culture media on BAP-EB differentiation was compared. The three-dimensional BAP-EB (BAP-EB/3D) was also compared to the two-dimensional (BAP/2D) differentiation protocols. Similar morphologies and pluripotent markers (\textit{NANOG}, \textit{OCT4}) expression were found in hESCs (VAL3) cultured in both mTeSR or E8. VAL3 cultured in the two media were then subjected to BAP-EB differentiations. It was found that mRNA levels of pluripotent markers (\textit{NANOG}, \textit{OCT4}) were decreased significantly from 0h to 96h while the trophoblastic markers (\textit{KRT7}, \textit{SYNCYTIN} and \textit{b-HCG}) were induced significantly from 48h onwards in both groups. Similar protein expressions of pluripotent marker (\textit{OCT4}) and trophoblast markers (\textit{KRT7}, \textit{b-catenin}) were also detected in both groups. However, the attachment rates of BAP-EB-72h differentiated from hESCs cultured in mTeSR was significantly higher than those from E8. The comparison of BAP-EB/3D and BAP/2D was followed. Blastocyst-like structure was observed in BAP-EB/3D derived from both VAL3 and H9. Similar mRNA expression levels of pluripotent markers and early trophoblast markers were also detected in both BAP-EB/3D and BAP/2D. Interestingly, higher syncytiotrophoblast markers (\textit{b-HCG}, \textit{SYNCYTIN}) and extravillous trophoblast marker (HLA-G) expressions were detected in BAP-EB/3D when compared to BAP/2D. In conclusion, BAP-EB cultured in mTeSR had higher potential for the acquisition of attachment competency. Compared to BAP/2D, BAP-EB/3D is more efficient in trophoblast cell differentiation. This work was partly supported by GRF and HMRF from the Research Grants Council of Hong Kong.

\textbf{P236 - Gestational Exposure to Bisphenol A Affects Testicular Tissues and Functions of Spermatogonial Stem Cells in Male Offspring.} Polash Karmakar, Jin Seop Ahn, Yong-Hee Kim, Sang-Eun Jung, Seok-Man Kim, Bang-Jin Kim, Hee-Seok Lee, Young-Hyun Kim, Myung-Geol Pang, Buom-Yong Ryu

Bisphenol A (BPA) is a widely used in industrial materials. It has been proven to have a harmful effect on the reproduction of the male offspring during pregnancy. However, detailed evidence of BPA-induced destruction of genetic embryos and functional characteristics is not well known. The purpose of this study is to investigate the effect of BAP on testicular germ cells during pregnant. In the current study, pregnant mice (F0) were three types of BPA doses (50 \textmu g, 5 mg, and 50 mg/kg; TDI, NOAEL, and LOAEL, respectively) on embryonic days 7 to 14 and were investigated trans-generation in male offspring.

We observed that NOAEL- and LOAEL-exposed F1 offspring have abnormalities in anogenital distances, nipple retention, and pubertal onset (days) together with differences in seminiferous epithelial stages and testis morphology. Also, the ratio of germ cell population was altered, and increased apoptosis rate in germ cells of F1 offspring at LOAEL dose. A reduction in the stemness properties of SSCs in F1 offspring was observed due to LOAEL exposure. However, the total number of spermatogonia was found unchanged in F2 and F3. These effects may be restored to the next generation of F2 and F3.

In conclusion, our study with the evidence of BPA-induced disruption in male germ cells physiology and functions due to gestational exposure which may lead several reproductive health issues and infertility in offspring.
Key word: Bisphenol A, pregnancy, testicular tissues, Spermatogonial stem cell

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P237 - Mobilized Peripheral Blood Mononuclear Cells Combined with Platelet-rich Plasma Accelerates Restoration of Cyclophosphamide-disrupted Ovarian Function in Rats. Yihua Yang, Qiuyan Huang, Bo Liu, Aiping Qin, Dongbao Chen

Premature ovarian insufficiency (POI) is a devastating consequence of cancer chemotherapy treatment in young females. Previous studies have shown that transplantation of hematopoietic peripheral blood mononuclear cells (PBMCs) or platelet rich plasma (PRP) can restore ovarian function. However, the detailed mechanism is largely unknown. This study used a cyclophosphamide (CTX)-induced POI rat model for testing a hypothesis that PBMCs and PRP have a synergistic effect on restoring ovarian function by increasing folliculogenesis via reducing granulosa cell apoptosis and increasing neovascularization. PBMCs and PRP were collected from 12 male rat donors injected with G-CSF to activate PBMCs. Adult POI rats were generated by CTX injection and received transplantation of vehicle, PRP, PBMCs, PBMCs+PRP, and sham treated control (n = 6/group). Serum hormones were measured by ELISA. Ovarian tissue was collected for gene expression analysis by qPCR and protein expression analysis by immunofluorescence/immunochemistry staining. Apoptosis was determined by TUNEL assay. Angiogenesis was determined by CD31 and VEGF stained vessels. Folliculogenesis was accessed by counting numbers of different stage follicles. CTX injection induced typical POI phenotype with increased diestrus, shortened estrous, follicle arrest at all stages, decreased serum levels of E2 and AMH and increased FSH. Male PBMCs were detected in ovarian tissues in the receipts at 5 days and beyond after transplantation by using SRY gene as a probe. CTX treatment resulted in decreased stromal CD34+ and VEGF and granulosa cell AMH and FSHR expression; the beneficial effects in restoring folliculogenesis and angiogenesis were in the rank order of PBMCs+PRP > PBMCs ≈ PRP > vihecle. PBMCs+PRP inhibited granulosa cell apoptosis by downregulating BAX and upregulating BCL-2 expression. Thus, G-CSF mobilized PBMCs in combination with PRP accelerate the restoration of ovarian function in POI rats via increasing ovarian neovascularization, reducing granulosa cell apoptosis, and increasing folliculogenesis.

P238 - unique epigenetic programming distinguishes functional spermatogonial stem cells in the immature mouse testis. Keren Cheng, I-Chung Chen, Christopher Geyer, Jon Oatley, John McCarrey

In the mammalian testis, spermatogonial stem cells (SSCs) sustain steady-state spermatogenesis leading to the production of ~100 million sperm per day by an adult man. SSCs self-renew and also give rise to progenitors that enter the spermatogenic differentiation pathway. The extent to
which SSCs and progenitors represent distinct spermatogonial subtypes, and whether, in addition to SSCs giving rise to progenitors, progenitors may revert back to SSCs, are unresolved questions. The \textit{ld4-egfp} transgenic mouse model facilitates FACS-based isolation of SSC-enriched/progenitor-depleted and SSC-depleted/progenitor-enriched spermatogonial subpopulations, functionally validated by the capacity to seed spermatogenesis following transplantation to a recipient testis. We examined genome-wide patterns of a) gene expression by RNA-seq, b) six histone modifications: H3K4me1-3, H3K9me1, H3K27me3 and H3K27ac – by ChIP-seq, c) chromatin accessibility by ATAC-seq, and d) DNA methylation by MeDIP-seq in SSC-enriched and progenitor-enriched subpopulations from the immature mouse testis. We found consistent differences in epigenetic landscapes associated with consistent differences in gene expression between the two spermatogonial subtypes. Differential enrichment of H3K27me3 or H3K27ac at promoters, and differential enrichment of H3K4me1 or H3K27ac at enhancers of differentially expressed genes, as well as sites of differential methylation (DMRs) in intergenic regions, appear to be the most consistent epigenomic distinctions associated with genes differentially expressed in SSCs and progenitors. Our results suggest that regulated changes in epigenetic landscapes promote the transition from SSCs to progenitors. Motif enrichment analysis of differentially programmed promoter and enhancer regions revealed binding sites for candidates for upstream regulators of spermatogonial subtype-specific epigenetic programming. Our own and previously reported ChIP experiments confirmed that FOXP1, DMRT1, and DMRTB1, are differentially bound at differentially programmed enhancers. Thus, SSCs and progenitors are distinguished by 1) differential developmental potential, 2) differential gene expression, 3) differential epigenetic programming, and 4) differential binding of master regulators.

\textit{P239 - Comparative characterization of mesenchymal stem cells derived from synovial fluid depending on rheumatoid arthritis.} Hyeon-Jeong Lee, Si-Jung Jang, Ji-Sung Park, Yong-Ho Choe, Sang-II Lee, Sung-Lim Lee

Mesenchymal stem cells (MSCs) can be easily obtained from various tissues and are capable to self-renew, differentiate into multiple lineages, and modulate the immune response. All these properties may be influenced by the specific condition of donor and its cellular microenvironment. Hence, it is crucial to understand the patients-specific pathological and physiological status-dependent characteristics of MSCs derived from rheumatoid arthritis (RA) patients, which might be used for autologous cell therapy.

We established the MSCs derived from synovial fluid of healthy donors and rheumatoid arthritis patients, and then comparatively analyzed the characteristics of MSCs such as stemness, proliferation, cellular senescence, \textit{in vitro} differentiation and \textit{in vitro} immunomodulation. For \textit{in vivo} immunomodulation properties of synovial fluid derived MSCs, collagen-induced arthritis (CIA) mice models as the RA mice models were generated and grouped as per the administration of synovial fluid derived MSCs (experimental group) or PBS (control group) by intraperitoneal injection and further evaluated for clinical and histological scores.

In present study, RA patients-derived MSCs showed significantly higher differentiation potential and cellular senescence whereas significantly lower proliferation and expression of pluripotency related genes than healthy donor derived MSCs. Also, synovial fluid derived MSCs possess the
in vitro and in vivo immunomodulatory property, but, in CIA mice model, RA patients-derived MSCs significantly less ameliorated the clinical symptoms than healthy donor derived MSCs. In conclusion, although RA patients-derived MSCs have lower potency than healthy donors-derived MSCs, RA patients-derived MSCs possess differentiation potency and immunomodulatory function and thus, can be used as an alternative cell source to treat RA without the risk of immune related complications.

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**P240 - Odotogenesis of mesenchymal stem cells derived from various dental tissues.** Young-Bum Son, Sung-Lim Lee, Young-Hoon Kang, Si-Jung Jang, Dinesh Bharti, Gyu-jin Rho

Teeth which are an ectodermal organ begin to appear around 6 to 8 months after birth. Specially, dentin is one of the major tissue of tooth with enamel, cementum and pulp which is important for tooth regeneration, and is secreted by the odontoblasts of the dental pulp tissue. Mesenchymal stem cells (MSCs) for tooth regeneration will give significant advantages in odontoblast regeneration. MSCs can be derived from various tissues, the dental tissue has recently gained more attention since MSCs can be isolated without invasive procedures from extracted tooth which is considered to be a biomedical waste in dental care. In this study we investigated the possible role of different dental MSCs for odontoblast differentiation.

MSCs derived from pulp, papilla, follicle, and ligament (DPSCs, SCAPs, DFSCs, and PDLSCs) were isolated and differentiated into mesenchymal lineages such as osteocytes, adipocytes and chondrocytes. All dental MSCs were found to be positive for mesenchymal markers (CD44, CD73, CD90, and CD105) while negative for hematopoietic markers (CD34 and CD45). All four types of dental MSCs were in vitro differentiated into odontoblasts in DMEM supplemented with 10% FBS, 1% penicillin/streptomycin, 10 mM β-glycerophosphate, 50 ug/ml ascorbate-2-phosphate, 10⁻⁷ M dexamethasone and 10 uM 17β-estradiol for 14 days. Odontoblast differentiated cells were assessed for their morphology, expression of odontoblast markers, ALP activity and calcium amount deposition. The DPSCs exhibited higher odontoblast differentiation potential as evaluated by the expression of odontoblast specific markers (ALP, DMP-1 and DSPP) both at mRNA and protein levels and also showed higher ALP activity and calcium content related to dentin than others.

This is the first study in which odontoblast differentiation capacity has been compared from four dental MSCs from a same donor. These findings suggest that DPSCs can be used a suitable cell source for odontoblast regeneration.
**P241 - Regulation of spermatogenic gene network by 8-oxoguanine (8-oxoG) in Korean native striped cattle (Bos namadicus Falconer, Chikso).** Sung Woo Kim, Young In Han, Jongsoo Mok, Eun Seo Kim, Joonghoon Park

8-OxoG is the most common type of oxidative lesion in genomic DNA. Growing body of evidence indicates that 8-oxoG is not only pre-mutagenic, but also plays an essential role in gene regulation. In testis, oxidative stress may be beneficial or harmful in the process of spermatogenesis; however, epigenetic effect of 8-oxoG in spermatogenesis has never been investigated. Here, we identified one infertile Korean native striped cattle at 20 months old (C14) due to asthenozoospermia and teratozoospermia. We compared global concentration of 8-oxoG by reverse-phase liquid chromatography/mass spectrometry (LC/MS), genomic distribution of 8-oxoG by next-generation sequencing (OG-seq), and expression of sperm proteins by 2-dimensional electrophoresis followed by mass spectrometry (2D/MS) in sperms of C14 with those of normal counterpart (C13). C14 had significantly increased concentration of 8-oxoG than C13 (0.0427 ± 0.003% 8-oxoG of total dG in C14 vs. 0.027 ± 0.004% in C13, p = 0.0028). 8-OxoG peaks were identified in 614 loci in C14 and 370 loci in C13, and 8-oxoG was differentially enriched in GC-rich Sp1 binding motif-containing promoter region in C14 (p = 0.066). Genes harboring 8-oxoG in the promoter regions in C14 included ERCC4, TSPY1, TRIP10, GSTA4, DUSP26, ARSG, and TSN, which are associated with sperm production. In contrast, genes with 8-oxoG in promoter in C13 were lack of any evidence in spermatogenesis. We also identified 58 differentially expressed protein spots in C14, including OXCT2, ANXA4, ALB, UQCRCl (up-regulated), ODF2, ACRBP, and SPESP1 (down-regulated), which are associated with sperm morphology and/or sperm motility. Protein-protein interacting network analysis with OG-seq and 2D/MS results demonstrated that genes harboring 8-oxoG in GC-rich promoters interconnected with differentially expressed sperm proteins to regulate sperm part and spermatogenesis in C14. These results indicate that the occurrence of 8-oxoG in GC-rich Sp1 binding sites is important for gene network for spermatogenesis in cattle.

**P242 - Epigenetic Reprogramming in a Dish – An In Vitro Model of Transgenerational Epigenetic Inheritance.** Jake Lehle, I-Chung Chen, John McCarrey

Exposure to endocrine disrupting chemicals (EDCs) can alter the epigenome causing epimutations which can predispose diseases that can be transmitted to subsequent generations through inter- or transgenerational epigenetic inheritance. As EDCs are thought to induce epimutations by disrupting endocrine signaling via receptor mediated signaling, different cell types expressing different endocrine receptors may vary in their susceptibility to EDC-induced epimutagenesis. We used immunofluorescence staining to determine presence or absence of endocrine receptors to predict differential susceptibility of somatic and germ cells to EDC epimutagenesis. We found the somatic cell types (Sertoli and granulosa cells) stained positive for five endocrine receptors (ERα, ERβ, AR, PPARγ, and RXR) known to be disrupted by the EDCs vinclozolin, diethylstilbestrol, and/or tributyltin. Two germ cell types (primordial germ cells and spermatogonial stem cells) were negative for the five endocrine receptors. We hypothesize that somatic cells may therefore be more vulnerable to epimutations induced by EDC exposure than germ cells due to differential presence/absence of relevant endocrine receptors. Inter- or transgenerational transmission of epimutations is difficult to study due to extensive cost, time,
labor, and animal use required to investigate this phenomenon over multiple generations in vivo. We established a quintuple transgenic induced pluripotent stem (iPS) cell system designed to recapitulate in vitro the major transitions in epigenetic reprogramming that occur in the embryo and germ line in vivo. This system is designed to allow us to induce epimutations by exposure to EDCs, and then follow these epimutations through recapitulation of embryonic, germline, and somatic epigenetic programming/reprogramming over the equivalent of multiple generations in vitro. Our goal is to establish this novel in vitro system to facilitate a better understanding of inter- or transgenerational inheritance of epimutations induced by EDC exposure, in a rapid and cost-efficient manner, minimizing the need for whole animal studies.

P243 - Effective and robust protocols for generation of clinically-relevant numbers of porcine mesenchymal stem cells from bone marrow and subcutaneous fat. Maria Sady, Maciej Olszewski, Magdalena Gajewska, Zdzislaw Gajewski

Cervical dysfunction in pigs may lead to contamination of the uterus, impaired fertilization or impeded pregnancy and parturition. Mesenchymal stem cells (MSC) seem to be a promising source for cell-based treatment in regenerative medicine. We hypothesize MSC implantation into dysfunctional cervix may improve its function, as measured by electromyography. We have established protocols for isolation and rapid expansion of clinically-relevant numbers of MSC from bone marrow and subcutaneous fat. Bone marrow was aspirated from the head of the humerus and fat tissue was excised from belly. Following mechanical or enzymatic dispersion, extensive washing and density gradient centrifugation, the crude cellular fraction containing stem cells was collected. These cells were cultured in expansion medium that facilitated the removal of contaminating cells and, at the same time, prevented premature differentiation of the MSC. Multipotency of the obtained population was assessed by trilineage in vitro differentiation. The cells were successfully differentiated into adipogenic, osteogenic and chondrogenic lineages, as demonstrated by histochemical staining with Oil Red O, Alizarin S and Alcian Blue, respectively. The undifferentiated cells were cultured under normoxic or hypoxic conditions and a range of FGF concentrations as culture medium additive was tested. Addition of FGF increased growth of the cells over five-fold, with EC50/EC90 of 188/976 and 197/1009 ng/ml for normoxic and hypoxic culture of bone marrow-derived cells, respectively, and EC50/EC90 of 212/997 and 242/1063 ng/ml for normoxic and hypoxic culture of fat-derived cells, respectively. Independently, hypoxic culture further increased yield by 50%. Data presented here demonstrates an establishment of a robust protocol allowing production of high numbers of porcine bone marrow- and fat-derived MSC. Establishment of the protocol allows proceeding to measurements of the impact of MSC implantation on myoelectric functions as measured by electromyography.
**P244 - The effect of DNA demethylation on the differentiation of a BMP4-induced, in vitro model of trophoblast.** Rowan Karvas, Juliann Leake, Danny Schust, Toshihiko Ezashi, R Roberts, Laura Schulz

DNA methylation may play an important role in the differentiation of the trophoblast lineage. Although the human placenta is somewhat hypomethylated, it becomes progressively more methylated during the first trimester. Changes in DNA methylation associated with trophoblast differentiation into syncytialized or invasive lineages have been found in term trophoblast and in established cell lines, but it is unknown whether active DNA methylation is required for human trophoblast differentiation from pluripotent stem cells. Induced pluripotent stem cells (iPSC) will differentiate into trophoblasts via the addition of BMP4, A83-01 (ACTIVIN/NODAL signaling inhibitor), and PD173074 (FGF2 signaling inhibitor), or BAP treatment. Following two days of BAP treatment, the cells differentiate into KRT7-positive trophoblasts. By 6 days of BAP, colonies terminally differentiate into a heterogeneous mix of trophoblasts expressing markers of cytotrophoblast (KRT7), syncytiotrophoblast (CGA, CGB) and extravillous trophoblast (HLA-G) lineages. Undifferentiated iPSC administered demethylating agent 1µM decitabine for 24h lost viability. Whereas iPSC administered decitabine on day 3 of a 6 day BAP treatment were resistant to this exposure. Immunofluorescent staining showed clear decreases in expression of HLA-G and CGB on day 6 BAP at higher decitabine concentrations (5 and 10 µM). In preliminary qPCR analysis of two iPSC lines, 1µM decitabine treatment decreased expression of differentiation-associated genes such as HLA-G (3.4-fold decrease from control), CGB (-3.2-fold), ERIV-1 (-3.2-fold), and ITGA1 (-2.3-fold) at 6 days of BAP treatment. Decitabine also reduced invasion by BAP-treated iPSC lines by 65.6-86% regardless of decitabine concentration (1, 5, or 10 µM) when compared to controls. In contrast, expression of markers of less differentiated trophoblast, CDX2, HAND1, and ID2, were not reduced by decitabine. These preliminary results demonstrate that differentiation to the syncytiotrophoblast and extravillous trophoblast lineages, but not maintenance of trophoblast viability, requires DNA methyltransferase activity.

Supported by NIH grant R01HD094937

**P245 - The effect of transplantation of autologous bone marrow-derived mesenchymal stem cells into the porcine cervix.** Zdzislaw Gajewski, Maria Sady, Maciej Olszewski, Magdalena Gajewska, Malgorzata Domino, Jaroslaw Olszewski

The purpose of this study was to evaluate the effect of collection, isolation, culturing and transplantation of bone marrow-derived mesenchymal stem cells into the cervix, in the *in vivo* porcine experimental model. The experiment was conducted on mature Polish Landrace sows (n = 12), weighing 90 - 120 kg. The study was divided into five stages: 1. collection of the bone marrow-derived mesenchymal stem cells, isolation, 2. culture and staining *in vivo*, 3. transplantation of the BM-MSCs into the cervix, 4. collection of cervices, 5. analysis of intracervical BM-MSCs transfer. The analysis was conduct in order to determine the presence of the specific, fluorophores-derived signal in the whole explanted cervices based on the two-step protocol applied for assessment of cell transplantation.
effects in large animal models. The analysis of red and yellow fluorescence, referred to DID and PKH26 respectively, in the collected cervixes revealed that 28 days after transplantation, demonstrated that the separate spots with specific fluorescence could be recognized both in the thick and thin slices of the cross-sections of the porcine cervix. The imaging of the thick sections with the IVIS to detect fluorescence from the DID and PKH26 and for quantitative analysis the systematic microscopic analysis of thin sections prepared from examined cervixes' fragments was performed. Based on our results, we may conclude that porcine BM-MSC can be repeatedly isolated and expanded in vitro to obtain a yield sufficient for transplantation within 3 weeks, and transplanted cells survived 28 d, and probably more, after injection into the muscle layer of uterine cervix. Moreover, morphological parameters of porcine cervix are similar to those reported in women, therefore we claim that the pig is an attractive large animal for testing cell therapy procedures of CD treatment.

**P246 - Serotoninergic System on Human Amniotic Epithelial cells (hAEC).** Jessica Romero-Reyes, Jessica López-Jiménez, N. Díaz, Ignacio Camacho-Arroyo

The serotonin (5-HT) and serotoninergic system have a ubiquitous distribution in diverse organisms. The tryptophan hydroxylase, type I (TPH1, found in the peripheral system) and type 2 (TPH2, distributed principally in the central nervous system), the serotonin reuptake protein (SERT), the enzyme monoamine oxidase (MAO) and some receptors like 5HT1D and 5-HT7 are principal components of this system. Thus, 5-HT participates in the regulation of several physiological functions like mammalian embryo development. However, their impact on human embryo has been poorly studied because of ethical issues. On the other hand, pluripotent stem cells are characterized by self-renewal and capacity to differentiate into derivates of the three embryonic layers. Properties that offer an alternative in the establishment of new models in developmental biology. Interestingly, it has been described the presence of pluripotent markers in vivo and in vitro in human amnion. Indeed, the human amniotic epithelial cells (hAEC) are derivate of the epiblast and posses the capacity to differentiate into tissues of the three germinal layers. Therefore, hAEC could be a valuable resource on human development, we characterized the serotoninergic system on hAEC. To list end, we determined the expression of TPH2, TPH1, SERT, MAO, 5-HT1D and 5-HT7 by RT-PCR and immunofluorescence. We found all components of the serotoninergic system on hAEC. Notably, the TPH1, TPH2 and 5-HT1D immunolocalization were cytoplasmic and nuclear. These results suggest that serotoninergic system is present on hAEC. However, further studies are necessaries to elucidate their possible function in hAEC.

**P247 - Regulation of spermatogonial stem cells by H3K27 demethylases.** Sakurako Shima, Tokuko Iwamori, Hiroshi Iida, Naoki Iwamori
Spermatogenesis is continuous process, because spermatogonial stem cells (SSCs) can maintain themselves as well as provide differentiated progenies. The maintenance of SSC compartment is supported by not only self-renewal of stem cells but also fragmentation of differentiating spermatogonia through abscission of intercellular bridges in a random and stochastic manner. The molecular mechanisms that regulate this reversible developmental lineage still remain unclear. We found that histone H3 lysine 27 (H3K27) demethylase, JMJD3 (KDM6B), has some roles in the regulation of SSC compartment. Although lack of JMJD3 in germ cells did not affect differentiation of spermatogonia, JMJD3 null mice have larger testes and sire offspring for a longer period compared to controls, likely secondary to increased and prolonged maintenance of the spermatogonial compartment. The absence of JMJD3 could induce frequent fragmentation of spermatogonial cysts by abscission of intercellular bridges. However, we also found that UTX (KDM6A), which is another H3K27 demethylase and is not detectable in wildtype undifferentiated spermatogonia, was redundantly expressed in JMJD3 null undifferentiated spermatogonia. These results suggest that not only JMJD3 but also UTX may contribute to control the spermatogonial compartment through the regulation of fragmentation of spermatogonial cysts. Now we are analyzing other mouse models and expression profiles to elucidate the role of H3K27 demethylases in the regulation of SSC compartment. Our findings may be involved in maintenance of diverse stem cell niches.

**P248 - Differentiation of Neuro-organoid Derived from Pig Embryonic Stem Cell Line.**
Seon-Ung Hwang, Kiyoung Eun, Mirae Kim, Hyunggee Kim, Sang-Hwan Hyun

Although the brain is an ideal model for studying neuropathology, it is difficult to cultivate or genetically engineer healthy or diseased brain tissue. In this study, we established two porcine embryonic stem cell(pES) lines. It was established through whole seeding of somatic cell nuclear transfer blastocysts. Both transgenic (TG) pES showed a primed form and showed bFGF-dependent properties. These pES lines contained a brain tumor-inducing gene and were confirmed by PCR. These pES lines were used to induce neural differentiation to produce the *in vitro* brain tumor model. pES were cultured using the SFEBq (serum-free floating culture of embryoid-body(EB)-like aggregates with quick reaggregation) method. The SFEBq culture method recreates the process of in situ generation by using the phenomenon (self-organization) that the cell group makes a spontaneous orderly structure. We cultured in *in vitro* until the final 61 days after EB formation, neural induction, neural patterning, and neural expansion. The organoids were sampled at each step and the expression of Dopaminergic neuronal marker (TH) and Mature neuronal marker (MAP2) was confirmed by PCR. In conclusion, we have formed neuronal organoids derived from porcine embryonic stem cells in *in vitro*. Further studies are needed on immunohistochemical staining of neuronal organoids.

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of Korea (NRF) funded by the Ministry of Education, Science and Technology (2017K1A4A3014959)”, Republic of Korea.

**P249 - Autologous canine fetal fibroblasts may be used as feeder layers as well as a source of canine induced pluripotent stem cells.** Mirae Kim, Seon-Ung Hwang, Kiyoun Eun, Yeon Woo Jeong, Sang-Hwan Hyun

Several studies have reported the generation of canine induced pluripotent stem cells (ciPSCs) using retroviral or lentiviral transduction of genes encoding Yamanaka's factors. In this study, we tried to reprogram canine fetal fibroblasts (CFFs) with lentivirus encoding polycistronic human pCDH-CMV-Oct3/4-Klf4-Sox2-puro reprogramming factors (OKS; Oct3/4, Klf4 and Sox2) and co-infected with lentiviral vector expressing human pCDH-CMV-Glis1-CopGFP (G; Glis1) or human pCDH-CMV-L-Myc-Lin28A-CopGFP (2L; L-myc and Lin28A). Primary CFFs were obtained from the Sooam Biotech Research Foundation. CFFs were seeded at 3 x 10⁴ cells per well in a 4 well dish. The next day, CFFs were co-infected with lentivirus expressing Oct3/4, Klf4, Sox2 and Glis1 (OKSG) or Oct3/4, Klf4, Sox2, L-myc and Lin28A (OKS2L). After 72 hours, the transfectants were selected with 0.5 μg/mL of puromycin. At Day 10 post-transfection, transfected cells were passaged onto inactivated autologous feeders and cultured in iPSC medium, consisting of DMEM/F12, 20% KnockOut Serum Replacement medium, 10 ng/mL bFGF and 10 ng/mL murine LIF. At day 15 post induction, putative ciPSC colonies first appeared and the colonies were mechanically isolated and subcultured onto 24-well plate on each autologous feeder cells. They were proliferated and maintained the undifferentiated morphologies on isogenic feeders for at least 3 passages. The isolated putative ciPSC colonies also showed alkaline phosphatase activity. Further studies are needed to analyze the expression of pluripotent marker genes and to optimize cell culture conditions, but this study suggests that autologous fibroblasts may be used as feeder layers as well as a source of ciPSCs.

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**P250 - Characterization of human cerebral organoids derived from induced pluripotent stem cells.** Kyoung-Ha So, Ga-Hey Kim, Young Seok Park, Sang-Hwan Hyun

The advances stem cell research have performed with the isolation of tissue-resident adult stem cells, cellular reprogramming, and mechanism study. Organoid, three-dimensional (3D) culture is a self-organizing culture system which offer several advantages over conventional two-dimensional platforms.

In this study, we had isolated human periodontal ligament fibroblasts (PDLs) from dental patient, reprogrammed hPDLs for induced pluripotent stem cells (iPSCs) by non-integration RNA, and generated cerebral organoid by serum-free floating culture of embryoid body (EB)-like aggregates with quick re-aggregation (SFEBq) method.
Human iPSCs were characterized by Alkaline Phosphatase and immunostained as surface marker TRA-1-60 and TRA-1-81. The other stemness markers (TRA-1-60 and SSEA4) were detected by FACS. Then, human iPSCs were generated to cerebral organoid which induction step measured neural ectoderm, neuroepithelial bud and cerebrum on bright filed. After the expansion step, cerebral organoid like 3D cells were immunostained and detected neuronal progenitors (SOX2, PAX6), neuron-specific cells neurons (TUJ1), apical membrane (N-cadherin) and mature neurons (MAP2) by confocal.

In conclusion, reprogramming of human somatic cell is good source to regeneration study. Also organoid technology maybe great tools for brain development and modelling diseases study.

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Key words: Reprogramming, Periodontal ligament fibroblasts, human iPSCs, Cerebral organoids

P251 - An optimal condition for synthetic mRNA transfection of canine fetal fibroblasts.
Taehye Cho, Miraee Kim, Seon-Ung Hwang, Sang-Hwan Hyun

Synthetic mRNA-transfection-based methods can avoid potential integration problems. Despite these advantages, there has been no study of canine somatic cell reprogramming using mRNA. Prior to somatic cell reprogramming, canine fetal fibroblasts (CFFs) were transfected using a polycistronic and self-replicating Venezuelan equine encephalitis (VEE) RNA expresses mCherry reporter gene in order to evaluate transfection efficiency of CFFs. The VEE replicon has no potential for problems associated with genomic DNA integration because it does not use a DNA intermediate. Because of the strong immune response induced by VEE RNA, B18R RNA was co-transfected to minimize the immune response. We synthesized VEE-mCherry mRNA and B18R mRNA by in vitro transcription (IVT) and then investigated the optimal mRNA concentration that could be used for efficient transfection of CFFs. IVT was performed for 4 hours at 37°C with the RiboMAX Large Scale RNA Production System-T7 Kit. RT PCR analysis was performed to confirm that both mRNAs were properly synthesized. CFFs were co-transfected with various concentrations of VEE-mCherry mRNA and B18R mRNA (ranging from 1 to 6μg of both mRNAs concentrations) for 4 hours to measure which mRNA concentrations were more suitable. 48 hours after co-transfection, mCherry-positive CFFs were analyzed by flow cytometry. The transfection efficiency was highest when 3μg of both mRNAs was used. Taken together, these results suggest that using a 3μg of synthetic mRNA may be possible for an efficient RNA transfection of CFFs.

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**P252 - Increased ROS levels in Sertoli cells and in epididymal fluid are a cause of subfertility in aging Arsa knockout mice.** Nongnuj Tanphaichitr, Kessiri Kongamanas, Arpornrad Saewu, Wongsakorn Kiattiburut, Mark Baker, Kym Faull, Dylan Burger

Arylsulfatase A (ARSA) is a lysosomal enzyme, catalyzing desulfation of sulfogalactosylglycerolipid (SGG), selectively present in testicular germ cells (TGCs) and sperm. We have shown that aging (8-month-old) *Arsa* knockout male mice become subfertile with 50% spermatogenesis rate (Xu H et al., J Lipid Res 2011). Among seminiferous tubule cells, Sertoli cells have the highest ARSA activity, which is likely essential for desulfating SGG of phagocytosed residual bodies and apoptotic germ cells to galactosylglycerolipid-GG. Galactosylceramidase then degalactosylates GG to a neutral lipid, alkylacylglycerol, which likely crosses plasma membranes into TGCs to become a building block for a new round of SGG synthesis. In aging *Arsa* knockout mice, Sertoli cells become markedly swollen, typical of a lysosomal storage disorder, concurrently with intracellular accumulation of SGG (detected by immunofluorescence and quantitative mass-spectrometry-based lipidomic analyses). Our objective is to further understand molecular mechanisms through which spermatogenesis is impaired in aging *Arsa* knockout mice. Since intracellular accumulation of (sulfo)glycolipids may lead to increased production of reactive oxygen species (ROS), we measured levels of superoxide and other ROS by incubating wild type (WT) and *Arsa*<sup>-/-</sup> Sertoli cell cultures with dihydroxyethidium-DHE followed by isolation and HPLC-separation of the DHE products, 2-hydroxyethidium-reporting superoxide anions and ethidium-reporting other ROS. Among 7 pairs of WT and *Arsa*<sup>-/-</sup> Sertoli cell cultures, levels of superoxide anions remained unchanged, but levels of other ROS appeared higher in *Arsa*<sup>-/-</sup> Sertoli cells. Amplex Red assay indicated that H<sub>2</sub>O<sub>2</sub> levels in culture medium of *Arsa*<sup>-/-</sup> Sertoli cells were twice those of WT counterparts. H<sub>2</sub>O<sub>2</sub> levels in epididymal fluid from aging *Arsa*<sup>-/-</sup> mice were also double those from age-matched WTs. These results suggest that increased H<sub>2</sub>O<sub>2</sub> levels in seminiferous tubules and epididymis may be a cause in spermatogenesis impairment in *Arsa*<sup>-/-</sup> mice.

**P253 - Spar1 is a novel transmembrane gene required for acrosome formation in mouse.** Julio Castaneda, Yuhkoh Satouh, Darius Devlin, Martin Matzuk, Masahito Ikawa

The sperm acrosome is a cytoplasmic organelle adjacent to the nucleus that contains receptors that are required for binding to the egg surface to initiate sperm-egg fusion. Failure to form the acrosome leads to globozoospermia (round-headed sperm) and male infertility. Using an *in silico* approach, we have identified a novel mouse gene required for sperm acrosome formation, *Spar1*. Sperm Acrosome Required 1 (*Spar1*), is a transmembrane gene conserved among mammals including human. Using CRISPR/Cas9, we have generated *Spar1* mutant alleles in mice. Males homozygous for a +1-frameshift mutation have sperm that can initiate acrosome formation; however, step 7 round spermatids begin to display acrosome vesicle detachment from the nuclear envelope with eventual loss of the vesicle in late spermiogenesis. Acrosome deficient spermatids also display defects in their nuclear shape, reminiscent of globozoospermia. Using a FLAG
tagged allele of endogenous Spar1, we have localized SPAR1 to the inner leaf of the acrosome vesicle adjacent to the nuclear envelope. SPAR1 localization suggests that SPAR1 functions to anchor the acrosome vesicle to the acroplaxome, a protein complex containing structural proteins located between the acrosome vesicle and nucleus. Interestingly, SPAR1 proteins is not expressed in elongated spermatids and not present in epididymal spermatozoa, indicating that SPAR1’s role in anchoring the acrosome is restricted to the initial stages of spermiogenesis. We have also generated a hypomorphic allele of Spar1. Males homozygous for the hypomorphic allele are also infertile; however, these males display spermiogenesis comparable to wildtype controls. The studies on both the null and hypomorphic allele will be presented.

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**P254** - Autophagy core protein ATG5 is required for sperm individualization and normal male fertility in mice. Qian Huang, Shiyang Zhang, Wei Li, Ling Zhang, Shizheng Song, Rex A Hess, Zhibing Zhang

Spermiogenesis is the longest phase of spermatogenesis, with dramatic morphologic changes and a final step of sperm individualization, when intercellular bridges are dissolved and sperm are released as individual cells during spermiation. Spermiation involves the removal of excess cytoplasm and protein degradation; therefore, we hypothesized that autophagy may play an important catabolic role in these process. To test this hypothesis, we examined the function of ATG5, a core autophagy protein in male germ cell development. Immunofluorescence staining demonstrated an ATG5 signal was present as cytoplasmic vesicles in spermatocytes and round spermatids. Floxed Atg5 and Stra8−iCre mice were crossed to produce conditional inactivation of Atg5 in male germ cells. In mutant mice, testicular expression of the autophagosome marker, microtubule associated protein1 light chain 3-B (LC3-B), was significantly reduced and expression of autophagy receptor p62/SQSTM1 was significantly increased, indicating a decrease in autophagy activity. The mutant mice showed no gross abnormalities and survived to adulthood; however, their fertility was dramatically reduced, with about 80% infertility. Litter size was significantly reduced in the few fertile mutant males associated with reduced sperm count and motility, but testis weight was normal. Histological examination of the mutant testes revealed numerous, large abnormal residual bodies in the lumen of Stage XI seminiferous tubules. The cauda epididymal lumen was filled with numerous sloughed germ cells, large cytoplasmic bodies, and spermatozoa with disorganized heads and tails. Microscopic examination of cauda epididymal sperm revealed misshapen sperm heads, a disconnected accessory structure in the mid-piece and abnormal sperm individualization. Mitochondria sheath and acrosome formation were disrupted. The present study demonstrated ATF5 to be essential for male fertility, particularly important during spermiation and removal of the intercellular bridges and formation of the residual body.

**P255** - C-terminal mutation in DRC1 causes male infertility with multiple morphological abnormalities of sperm flagella. Jintao Zhang, Xiaojin He, Rong Hua, Xin Zhang, Huan Wu, Yunxia Cao, Mingxi Liu
Flagella and cilia are critical cellular organelles used for locomotion and as sensory organelles in bacteria, archaea, and eukaryotes. The central component of a flagellum is the axoneme, which comprises the “9+2” microtubule arrangement, dynein arms, radial spokes, and the nexin-dynein regulatory complex (N-DRC). The N-DRC appears as a thin filament between the outer microtubule doublets. Loss-of-function mutations disrupting a conserved component of N-DRC, dynein regulatory complex subunit 1 (DRC1), result in severe defects in assembly of the N-DRC structure and defective respiratory ciliary movement in humans. Recently, we reported that another conserved component of N-DRC, T-complex-associated-testis-expressed 1 (TCTE1 or DRC5), was essential for mouse spermatozoa motility. In the present study, we investigated the genetic cause of male infertility in a Chinese family with an infertile individual, who was born to a first-cousin marriage, and presented with a typical multiple morphological abnormalities of the sperm flagellum (MMAF) phenotype. We identified a homozygous mutation in this infertile individual, which causes a C-terminal truncation of DRC1. We generated a deletion from exon 2 to exon6 of Drc1 (Drc1Δ) and a C-terminal truncation of DRC1 (Drc1CT) in mice using the CRISPR/Cas9 system. Drc1Δ mice showed pre-weaning developmental retardation and died before gonad maturation. In contrast, Drc1CT/CT mice survived until adulthood but were infertile. C-terminal truncation of DRC1 leads to several severe abnormalities of sperm flagella, including shortening, coiling, absence, and irregular quality. Our results highlight the role for N-DRC integrity in regulating flagellar assembly and provide direct evidence that mutations in DRC genes cause human MMAF.

P256 - PTBP1 contributes to spermatogenesis through regulation of proliferation in spermatogonia. Manabu Ozawa, Manami Senoo, Takashi Takijiri, Takuya Yamamoto, Yasuhiro Yamada, Masahito Ikawa

Polypyrimidine tract-binding protein 1 (PTBP1) is a highly conserved RNA-binding protein that is a well-known regulator of alternative splicing. Testicular tissue is one of the richest tissues with respect to the number of alternative splicing mRNA isoforms, but the molecular role(s) of PTBP1 in the regulation of these isoforms during spermatogenesis is still unclear. Here, we developed a germ cell-specific Ptbp1 conditional knockout (cKO) mouse model to investigate the role of PTBP1 in spermatogenesis. Testis weight in Ptbp1 cKO mice was comparable to that in age-matched controls until 3 weeks of age; at ≥2 months old, testis weight was significantly lighter in cKO mice than in age-matched controls. Seminiferous tubules that exhibited degeneration in spermatogenic function were more evident in the 2-month-old Ptbp1 cKO mice than in controls. In addition, the early neonatal proliferation of spermatogonia, during postnatal days 1–5, was significantly retarded in Ptbp1 cKO mice compared with that in controls. We also compared transcriptome or spliceome in spermatogonia using spermatogonia culture model (germline stem cells, GSCs) by NGS. Interestingly, a group of genes of which expression was significantly different between Ptbp1KO GSCs or control was totally apart from a group of genes of which alternative splicing was changed between the genotypes. Furthermore, mRNA expression of Nanos3, known as an essential gene for primordial germ cell development and sustainable spermatogenesis, was significantly lower in the Ptbp1KO GSCs. We developed Nanos3+/−;Ptbp1+/− mouse (double-hetero mouse) to compare spermatogenesis with either Nanos3+/− or Ptbp1+/− mouse (single-hetero mouse). Strikingly, no significant abnormality in spermatogenesis was observed in each single hetero mouse, whereas double-hetero
mouseshowed $Pthp1cKO$ like abnormal spermatogenesis. These data suggest that PTBP1 contributes for maintaining spermatogonial proliferation through regulation of Nanos3 expression.

**P257 - Co-expression of sperm membrane proteins CMTM2A and CMTM2B is essential for ADAM3 localization and male fertility in mice.** Yoshitaka Fujihara, Asami Oji, Kanako Kita, Tamara Larasati, Masahito Ikawa

Chemokines are signaling proteins that are secreted to induce chemotaxis during an immunological response. However, the functions of transmembrane-type chemokine-like factor (CKLF) and the CMTM (CKLF-like MARVEL transmembrane domain containing) protein family remain to be determined. In this study, we focused on the testis-specific mouse CMTM gene cluster ($Cmtm1$, $Cmtm2a$ and $Cmtm2b$) and generated CRISPR/Cas9-mediated mutant mice to examine their physiological functions. Although $Cmtm1$ mutant mice were fertile, $Cmtm2a$ and $Cmtm2b$ double mutant mice had defects in male fertility due to impaired sperm function. We found that co-expression of sperm membrane proteins CMTM2A and CMTM2B is required for male fertility and affects the localization of the sperm membrane protein ADAM3 in regulating sperm fertilizing ability.

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**P258 - Glycerol kinase 2 is important for proper arrangement of crescent-like mitochondria to form the mitochondrial sheath.** Keisuke Shimada, Haruhiko Miyata, Masahito Ikawa

Sperm mitochondria surround the axoneme and pack tightly to form the mitochondrial sheath. Initially, mitochondria are recruited from the cytoplasm to the flagellum late in spermatogenesis. Recruited mitochondria are spherical but then elongate laterally to become crescent-like in shape. Subsequently, crescent-like mitochondria elongate continuously to coil tightly around the flagellum. Although the formation of the mitochondrial sheath has been well described, the molecular mechanism of mitochondrial sheath formation remains unclear.

Recently, disorganization of the mitochondrial sheath was reported in Glycerol kinase 2 ($Gk2$) disrupted mice. To analyze the disorganization of the mitochondrial sheath further, we generated $Gk2$-deficient mice using the CRISPR/Cas9 system and observed sperm mitochondria in testis using a freeze-fracture method with scanning electron microscopy. $Gk2$-disrupted spermatids show abnormal localization of crescent-like mitochondria, in spite of the initial proper alignment of spherical mitochondria around the flagellum, which causes abnormal mitochondrial sheath formation leading to exposure of the outer dense fibers. These results indicate that GK2 is important for proper arrangement of crescent-like mitochondria to form the mitochondrial sheath during mouse spermatogenesis.
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P259 - Influence of Epididymosome Exposure on the Developmental Potential of Maturing Spermatozoa in the Domestic Cat Model. Tricia Rowlison, Pierre Comizzoli

Spermatozoa undergo critical changes in structure and function during epididymal transit. Previously, we demonstrated that certain proteins are transferred to maturing spermatozoa through exosomes (or epididymosomes) secreted by the epididymal epithelium. Using the domestic cat model, the objective was to better understand the influence of epididymosomes on sperm developmental potential by assessing effects on in vitro fertilization and early embryonic development. Immature spermatozoa from caput epididymidis (n = 5 males) were exposed to epididymosomes (isolated from whole epididymides of 5 males) for 1 hour and 15 minutes then washed prior to incubation with in vitro matured oocytes at 38.5°C. Spermatozoa from caput and cauda epididymidis also were exposed to base medium as controls (n = 20 oocytes per treatment; 5 replicates). Embryonic development was assessed on Days 3 and 7 followed by fixation of oocytes and embryos (2.5% paraformaldehyde) and chromatin staining (Hoechst) to estimate the percentage of fertilization as well as the number of blastomeres in embryos. While there was no significant difference in the percentage of fertilized oocytes (range, 8.6 - 15.9%), proportions of embryos developing to the 4 – 8 cell stage on Day 3 tended to be higher (P = 0.0970) when oocytes were inseminated with caput spermatozoa exposed to epididymosomes (81.3 ± 12.2%) compared to caput spermatozoa control (50.0 ± 15.8%; cauda spermatozoa: 87.8 ± 5.6%). A significant increase (P = 0.0021) in development to morulae - blastocyst stages was observed on Day 7 following epididymosome exposure of caput spermatozoa (89.3 ± 10.7%) compared to caput spermatozoa control (16.7 ± 9.6%; cauda spermatozoa: 71.3 ± 10.9%). Blastomere numbers (range, 56 - 256 blastomeres) in resulting blastocysts were not different across treatments. Results clearly demonstrate that epididymosomes contribute to sperm maturation and subsequent quality of early embryo development.

P260 - Axonemal dynein light intermediate polypeptide 1 forms a complex with PACRG in the manchette for cargo transport. Wei Li, David Williams, Zhibing Zhang

Axonemal dynein light intermediate polypeptide 1 (DNALI1) was originally cloned from Chlamydomonas reinhardtii in an effort to find motor proteins essential for flagellar motility. Earlier studies demonstrated that mouse DNALI1 protein is highly abundant in the testis. During the first wave of spermatogenesis, both Dnali1 mRNA and protein are dramatically increased at the spermiogenesis phase. Immunofluorescence studies demonstrated weak DNALI1 staining in late meiotic germ cells, but strong staining was found in round and elongated spermatids. DNALI1 has been identified as a binding partner of cytoplasmic dynein heavy chain 1 (CDHC1), which directly associates with microtubules. In our studies to investigate the mechanism of parkin co-regulated gene1 (PACRG) in the regulation of spermatogenesis, we identified DNALI1 to be a PACRG binding partner. PACRG and meiosis expressed gene 1 (MEIG1) form
a complex in the manchette, a transient and unique structure only present in the elongating spermatids for normal spermiogenesis. In transfected CHO cells, DNALI1 recruited the PACRG protein. PACRG alone is not stable when expressed in bacteria and transfected mammalian cells; however, co-expression of DNALI1 significantly increased PACRG expression level. When co-expressed in bacteria, non-tagged DNALI1 could be co-purified with His-tagged PACRG, and in the gel filtration assay, the two proteins were present in the same fractions. Immunofluorescence staining on the isolated male germ cells revealed that both DNALI1 and CDHC1 were present in the manchette of elongating spermatids, and DNALI1 and PACRG were co-localized in this structure. In the Pacrg knockout mice, localization of DNALI1 in the manchette was not changed. These observations strongly suggest that DNALI1 and PACRG form a complex in the manchette, with DNALI1 as an upstream molecular. DNALI1/CDHC1 may function as a motor system to drive MEIG1/PACRG complex to carry cargo proteins along the manchette microtubules for sperm flagella formation.

**P261 - HIPK4 is Essential for Murine Spermiogenesis.** J. Aaron Crapster, Paul Rack, Zane Hellmann, Josh Elias, Jennifer Lin, Yanfeng Li, Barry Behr, James Chen

Spermiogenesis is a remarkable cellular transformation in which haploid round spermatids become elongated spermatozoa capable of motility and fertilization. Previous studies have revealed that many cytological changes are associated with this process, including formation of the actin−scaffolded acrosome/acroplaxome complex, nuclear reshaping, and flagellum assembly. However, the signaling pathways that coordinate these steps remain enigmatic. Using a knockout mouse line, we have now demonstrated that an atypical member of the homeodomain−interacting protein kinase family, HIPK4, is essential for spermiogenesis and male fertility. HIPK4 is expressed in round and elongating spermatids. Male Hipk4 knockout mice are infertile, exhibiting oligoasthenoteratozoospermia, with no other overt physiological defects. Sperm from these mice are incompetent for in vitro fertilization, despite retaining the intrinsic ability to capacitate and acrosome react. Viable pups from Hipk4 knockout males can be generated via ICSI. Subtle acrosome/acroplaxome defects arise in elongating Hipk4 mutant spermatids and lead to abnormal head morphologies, revealed by electron microscopy. Loss of Hipk4 does not dramatically alter a transcriptional program, however, HIPK4 overexpression alters actin and Golgi dynamics in cultured somatic cells, and modulates the phosphorylation of known actin regulators as measured by quantitative mass spectrometry. Our findings establish HIPK4 as one of the few known germ cell-specific kinases that are required for spermatid head shaping, and demonstrate its potential as a contraceptive target.

**P263 - Temporal-dynamic single-cell transcriptome analyses identify novel adult spermatogonial stem cell states in mouse.** Brian Hermann

Within the male germline, spermatogonial stem cells (SSCs) sustain the spermatogenic lineage by balancing self-renewal and initiation of differentiation. Recent studies from several groups, including our own, have profiled spermatogenic cells with single-cell RNA-Seq, and confirmed
significant heterogeneity among undifferentiated spermatogonia. Predicting cell state relationships among individual spermatogonia to understand SSC fate, however, is limited by the mature of mRNA level measurements. Thus, we performed RNA velocity analysis integrating spliced and unspliced mRNA levels to infer dynamic relationships among adult mouse spermatogonia. First, we confirmed this approach using data from steady-state mouse spermatogenesis. As expected, vector fields overlaid on tSNE projections demonstrated several spermatogenic phases during which little transcriptional change was evident interspersed with phases of significant transcriptome change. Next, we analyzed single-cell transcriptomes from adult mouse spermatogonia to explore dynamics of SSCs. Transplantable adult SSCs (ID4-EGFP-bright) separated into two discrete states that were related, based on clustering analyses, to distinct progenitor populations via cell cycle activation and bi-directional stochastic transition, respectively. To validate these phenotypes, we co-stained adult mouse testes for markers of undifferentiated spermatogonia (PLZF), progenitor spermatogonia (RARγ), and cell proliferation (Ki67). We defined SSCs as PLZF\textsuperscript{high}/RARγ\textsuperscript{negative} and found that 35.9% were quiescent (Ki67\textsuperscript{negative/low}), while the remainder were proliferative (Ki67\textsuperscript{positive}), aligning with our RNA velocity results. Among PLZF\textsuperscript{high}/RARγ\textsuperscript{low} progenitors, 35.3% were proliferative (Ki67\textsuperscript{positive}), likely relating to the proliferative SSCs. The remaining PLZF\textsuperscript{high}/RARγ\textsuperscript{low/Ki67\textsuperscript{negative/low}} quiescent progenitors likely reflect the quiescent SSC cell cluster. Nearly all (94.5%) of PLZF\textsuperscript{high}/RARγ\textsuperscript{high} advanced progenitors were Ki67\textsuperscript{negative/low}. These results suggest that RARγ levels establish a rheostat for SSC-to-progenitor transition and indicate substantial complexity and possible flexibility in the modes of SSC renewal and differentiation in adult testes, including gradual and bi-directional state transitions.

P264 - Seasonal analysis of gonadal transcriptome in endemic cyprinid Honmoroko (Gnathopogon caerulescens). Tatsuyuki Takada, Shogo Higaki, Reika Kawahara, Noriyoshi Sakai, Akira Hirasawa

Honmoroko (Gnathopogon caerulescens) is a small cyprinid endemic to ancient Lake Biwa in Japan. They breed once a year and repeat this reproductive cycle for 2~3 years throughout their life. In the non-spawning testis at September, spermatogonia was predominant, and little spermatocytes, spermatids, and sperm were observed. Thereafter, spermatogenesis proceeds synchronously, suggesting this can be a good model to study the process and the mechanism of spermatogenesis as well as reproductive cycle. In this study, we investigated seasonal changes of testicular cell types and the global gene expression profile of the testes by histological and transcriptome analysis, respectively, to understand the regulation of the entire process of spermatogenesis and regeneration of the spermatogenic cells. More than five male fishes were sampled every month and seasonal changes of gonadosomatic index (GSI), together with the testicular histology and their transcriptome were examined. GSI showed clear seasonal change from < 1 at August and September to > 10 at April and May, and roughly divided into 3 groups. Morphological and histological analysis of testis indicated that reproductive cycle was classified into the four phases; Spawning capable, Regressing, Regenerating, and Developing phases. Cluster analysis and primary component analysis revealed that annual testicular transcriptome was classified into four distinct groups at least, and implied that August may need to be distinguished from these four groups, independently. In addition, transcription do not appear to change gradually but transit in a stepwise manner from one phase to the other. GO
analysis successfully extracted characteristic GOs in each group such as translation (winter), cilium assembly (spring), cell adhesion (summer), and DNA replication (autumn). These results suggest that transcriptome analysis of testes is valuable to define the steps of reproductive cycle and provide deeper insight of germ cell differentiation in endemic fish.

**P265 - Cep76 is a centriole-related gene with an essential role in sperm development.**
Brendan Houston, Richard Burke, Liina Nagirnaja, Alexandra Lopes, Don Conrad, Moira O'Bryan

Male infertility is a common disease with implications beyond an inability to conceive a child. It affects approximately 7% of men in the Western world, and its etiology is unknown in the majority of cases. It is, however, estimated that at least 50% of cases are genetic in origin. Whole exome screening performed on infertile men has identified a potential pathogenic mutation in the centriole-related protein gene *CEP76*, a gene that has been implicated in the prevention of over-duplication of centrioles in cell lines. In order to define its role in male fertility, we generated a knockout mouse model for *Cep76*. We observed that male *Cep76* knockout mice are infertile, due to a combination of sperm structural defects and an almost complete failure of sperm motility. As such, *Cep76*−/− males mated normally, but no pregnancies resulted. *Cep76*−/− sperm are almost completely immotile and are unable to manifest forward progressive motility (p < 0.0001). Approximately 40% of the sperm also contain head defects (p < 0.0001). Further investigation is underway to decipher the centriole content in male germ cells of *Cep76*−/− mice and the precise cause of, and relationship between, the head and tail defects in these sperm. This research further evidences the roles of centriole function in establishing male fertility and the potential of these targets for the development of novel contraceptives.

**P266 - Simplified pipelines for genetic engineering of mammalian embryos by CRISPR-Cas9 electroporation.**
Deqiang Miao, Mariana Giassetti, Michela Ciccarelli, Blanca Lopez-Biladeau, Jon Oatley

Gene editing technologies, such as CRISPR-Cas9, have important applications in mammalian embryos to generate novel animal models for biomedical research and lines of livestock with enhanced production traits. However, the lack of methods for efficient introduction of gene editing reagents into zygotes and the need for surgical embryo transfer in mice have been technical barriers of widespread use. Here, we described methodologies that overcome these limitations for embryos of mice, cattle, and pigs. Using mutation of the *Nanos2* gene as a readout, we refined electroporation parameters with preassembled sgRNA-Cas9 RNPs for zygotes of all three species without the need for zona pellucida dissolution that led to high efficiency INDEL edits. In addition, we optimized culture conditions to support maturation from zygote to blastocyst stage for all three species that generates embryos ready for transfer to produce gene edited animals. Moreover, for mice, we devised a non-surgical embryo transfer method that yields offspring at an efficiency comparable to conventional surgical approaches. Collectively, outcomes of these studies provide simplified pipelines for CRISPR-Cas9 based gene editing in mammalian embryos that can be used to generate novel animal models for biomedical research and enhanced livestock production.
Tiger-nut (Cyperus esculentus) is a crop that belongs to family Cyperaceae which produces rhizome and tuber that are small spherical in shape. The tubers have aphrodisiac, carminative, diuretics and several health benefits. The aim of the present study was to investigate the influence of methanol extract of tiger nut on the male reproductive pathology of chronic lead poisoning in red Sokoto goat. The tiger nut was extracted using 95% methanol by cold extraction. Twelve adults red Sokoto bucks (16.50 ± 1.406 to 21.92 ± 0.97 kg) were randomly grouped into four (n=4). Group I was administered distilled water (200mg/kg). Group II was administered lead acetate (200mg/kg) only. Group III was administered tiger nut (150mg/kg) and lead acetate (200mg/kg). Group IV was administered tiger nut (200mg/kg) only. Microscopic evaluation of semen characteristics (semen motility, semen concentration, ejaculate volume and semen pH) were determined by standard method. The serum testosterone changes were determined using commercial kits. The lead acetate (200mg/kg) group II showed decreased semen characteristics parameters while tiger-nut (LA + TN) group III showed improved values of the semen characteristics. However, the semen characteristics values on the distilled water (DW) group I and sole tiger nut (TN) group IV showed a good semen characteristics values. In conclusion the methanol extract of tiger nut contained aphrodisiac ingredient, antioxidant phytochemicals and macro nutrient that significantly influence a protective role in ameliorating the pathological effect of lead poisoning in male reproductive pathology in red Sokoto goat.

**P268 - Effects of Sertoli cell secreted C-Peptide on high glucose induced endothelial cell damage.** Jannette Dufour, Karl Mueller, Tanir Moreno, Gurvinder Kaur

Testicular Sertoli cells (SC) are immune-privileged cells that have the ability to protect germ cells from an immune response and to survive long-term without the use of immunosuppressive drugs when transplanted as allografts or xenografts. The ability of SC to survive transplantation when most other cells are immunologically rejected suggests SC could be engineered as a vehicle for gene therapy. Previously we engineered SC to express insulin and C-peptide as a potential treatment for diabetes. These SC stably produced biologically active insulin at levels that transiently decreased blood glucose levels (BGL) in diabetic mice. Cardiovascular disease is the leading cause of death among people with diabetes. C-peptide, a coproduct of the insulin gene, has been shown to have important biological functions relevant to treatment of endothelial cell damage. However, C-peptide is not present in current insulin replacement therapies. Thus, transduced SC expressing insulin and C-peptide have the potential to not only normalize BGL but also treat diabetes associated cardiovascular complications. An adenoviral vector containing the insulin gene was used to engineer neonatal porcine SC to express insulin and C-peptide. The efficacy of our engineered SC (ESC) vs C-peptide or SC media alone was tested on various endothelial cell genes in vitro, using high glucose and normal glucose controls. High glucose increased vasoactive molecules, adhesion molecules, and oxidative stress. Treatment of endothelial cells exposed to high glucose with C-peptide or ESC improved vasoregulation (decreased vasoconstrictor endothelin 1 and prothombotic factors PAI-1 and vWF, and increased eNOS), decreased adhesion molecules (ICAM and VCAM) and decreased oxidative stress.
(decreased ROS and superoxide and increased antioxidant genes). This demonstrates the beneficial effects of C-peptide generated by SC on endothelial damage related molecules in vitro and the potential of this treatment for diabetic cardiovascular disease.

P269 - Spontaneous calcium signaling within the mouse seminiferous epithelium. Justine Fischoeder, Naofumi Uesaka, David Fleck, Jennifer Spehr, Marc Spehr

While representing a fundamental process in male reproduction, most mechanisms that control spermatogenesis still remain elusive. The seminiferous tubules, building blocks of the mammalian testis and functional units of spermatogenesis, harbor three cell types that ensure spermatogenesis – i.e., peritubular, Sertoli, and germ cells. Here, we investigate spontaneous Ca²⁺ signaling events in all three cell types using live-cell imaging. Acute slice preparations of mouse seminiferous tubules enable in situ analysis of signaling patterns, while preserving tissue architecture. Genetically modified mice that conditionally express the Ca²⁺ indicator protein GCaMP6f allow cell-specific dissection of spontaneous Ca²⁺ activity. Our analysis reveals that signal kinetics differ between cell types. In addition, independent of cell type, signal shape is both modulated by extracellular Ca²⁺ and dependent on intracellular Ca²⁺ stores. Moreover, we report changes in Ca²⁺ signaling as a function of developmental / endocrine state and we observe age-related effects. Strikingly, we record spontaneous Ca²⁺ signals that display intrinsic periodicity. Cross-correlation analysis indicates that distinct testicular networks appear to coordinate ensemble activity. Additional in vivo Ca²⁺ imaging confirms the existence of such Ca²⁺ signaling networks within the testis. Together, our results will provide deeper insight into male reproductive physiology.

P271 - Acyl-CoA synthetase 6 (ACSL6)-mediated activation of DHA is required for normal spermatogenesis and fertility in mice. Benjamin Hale, Regina Fernandez, Jessica Ellis, Chris Geyer

Docosahexaenoic acid (DHA) is an essential omega-3 polyunsaturated fatty acid critical for male fertility, but the mechanistic function of DHA within the developing germ cell remains unclear. Studies in other cell types have shown that DHA can affect both membrane fluidity and mitochondrial energetics. To define the role of DHA in spermatogenesis we have focused our studies on ‘long-chain acyl-CoA synthetase 6’ (Acs16), which encodes an enzymatic activator of DHA prior to its incorporation into cell or mitochondrial membranes. Interestingly, ACSL6 protein is expressed predominantly in the central nervous system and the testis. We therefore hypothesized that Acs16 deletion would cause DHA deficiency and major defects in spermatogenesis, resulting in male infertility. To determine the requirement for DHA activation in spermatogenesis, we generated a novel whole-body Acs16 knockout (Acs16⁻/⁻) mouse. Lipidomic analyses on whole testes showed a marked decrease in DHA-containing phospholipids in Acs16⁻/⁻ mice compared to littermate controls. Although female Acs16⁻/⁻ mice were fertile and appeared normal, we made the exciting discovery that Acs16⁻/⁻ males were highly subfertile (6-month breeding trial: n = 4 pups from Acs16⁻/⁻ males vs 86 pups from control males). Acs16⁻/⁻ mice had decreased testis weights at 6 and 18 months of age compared to controls, as well as decreased cauda epididymal sperm counts. While there was no difference in the number of Sertoli cells, Acs16⁻/⁻ testes had decreased numbers of undifferentiated spermatogonia and meiotic spermatocytes. In addition, there was a general
disorganization of the stages of the seminiferous epithelium; spermatogenic cell types were uncharacteristically coincident in stages of the seminiferous epithelium, suggesting disrupted timing of spermatogenesis. Taken together, these results reveal a requirement for ACSL6-mediated DHA enrichment in normal spermatogenesis and male fertility.

**P272 - TDRKH scaffolding function controlling different steps in pachytene piRNA biogenesis is essential for transposon silencing and adult spermatogenesis.** Deqiang Ding, Chen Chen

PIWI-interacting RNAs (piRNAs) are a major class of small regulatory RNAs that play evolutionarily conserved roles in the animal germline to suppress harmful transposons and promote germ cell development. In mice, three PIWI proteins (MIWI, MILI and MIWI2) associate with two distinct developmental stage-specific piRNA populations. In fetal/neonatal germ cells, MILI and MIWI2 associate with transposon sequence-rich fetal piRNAs (pre-pachytene piRNA). In postnatal germ cells, MIWI and MILI associate instead with transposon sequence-poor pachytene piRNAs that are expressed beginning in the pachytene stage of meiosis. piRNA biogenesis occurs near the mitochondrial surface, and involves mitochondrial membrane-anchored factors. In mice, TDRKH is a mitochondrial membrane protein required for pre-pachytene piRNA biogenesis. To study TDRKH function in pachytene piRNA biogenesis and function, we generate Stra8-Cre; Tdrkh conditional knockout (TdrkhcKO) mice. We show that mitochondria-anchored TDRKH controls multiple steps of pachytene piRNA biogenesis in mice. In TdrkhcKO mice, MILI-piRNAs are extended at 3’ end, indicating that TDRKH is required for pachytene piRNA 3’ end trimming. Strikingly, in TdrkhcKO mice, MIWI did not associate with any detectable piRNAs in TdrkhcKO testes. TDRKH specifically recruits MIWI, but not MILI, to mitochondrial surface and is required for the production of the entire MIWI-bound piRNA population. We further uncover that the failure to recruit MIWI to the mitochondrial surface with TDRKH deficiency in spermatocytes results in loss of MIWI in the chromatoid body in round spermatids, leading to spermiogenic arrest and piRNA-independent retrotransposon LINE1 derepression in round spermatids. These results reveal a mitochondrial surface-based scaffolding mechanism that couples specific PIWI protein recruitment and piRNA trimming essential for separable downstream effector functions of different PIWI proteins. These findings also suggest species diversity in mitochondrial TDRKH-mediated assembly of piRNA processing machinery.

**P273 - Effect of Recombinant Albumin as Serum Replacement on the Cryopreservation of Spermatogonial Stem Cells.** Ju-Hee Jin, Sang-Eun Jung, Jin Seop Ahn, Seok-Man Kim, Buom-Yong Ryu

Spermatogonial stem cell (SSCs), known as adult germline stem cells, plays important roles of foundation of spermatogenesis through self-renewal and differentiation. It is essential to preserve for long-term because SSCs could be used in various field like male reproduction and stem cell biology. Cryopreservation is the most effective technique for stable preservation of SSCs. In most research, serum has been used as cryoprotectant because it acts as a buffer to prevent osmotic shock and damage of membrane from crystallization and recrystallization during freezing and thawing. However, serum will be resulted in variation and cross-contamination
among species because the components of serum are not defined exactly due to complex mixture and different serum batches. Thus, the purpose of our research is to verify the most effective serum replacement by using recombinant albumin, dextran, methylcellulose, and polyvinylpyrrolidone. We carried out recovery rate after thawing, proliferation capacity by BrdU for DNA synthesis, proliferation rate by 1-week culture and apoptosis by FACS with Annexin V to verify the serum replacements. To determine characterization and functional test of SSCs, we performed qRT-PCR, immunofluorescence, and transplantation. We indicate that cryopreservation using recombinant albumin leads to increase of recovery rate, decrease of apoptosis, maintenance of self-renewal, and normal function of SSC. In conclusion, stable cryopreservation of SSCs using recombinant albumin will contribute to production of transgenic animal, stem cell biology, preservation of endangered species, and male infertility treatment.

**Key words:** Spermatogonial stem cell, cryopreservation, serum replacement, recombinant albumin, cryoprotectant

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**P274 - Effects of Trehalose on Cryopreservation of Spermatogonial Stem Cell by Equilibration Time and Temperature.** Sang-Eun Jung, Myongzun Kim, Jin Seop Ahn, Ju-Hee Jin, Seok-Man Kim, Joong-Hyuck Auh, Buom-Yong Ryu

Spermatogonial stem cells (SSCs), known as male germline stem cells, are able to transfer genetic information to the next generation by spermatogenesis through self-renewal and differentiation. It was reported that trehalose has beneficial effects on cryopreservation of SSCs for long-term. However, there has been a few attempts to study proper equilibration time and temperature in cryopreservation using trehalose for SSCs. The purpose of this study is to determine standardized freezing protocol for SSCs. To verify effects of trehalose during cryopreservation, we carried out the relationship between proliferation capacity and quantification of permeant trehalose. Proliferation capacity of SSCs was resulted from equilibration time and temperature with trehalose 200mM. Proliferation rate was gradually increased by 20-minute and then decrease after 40-minute at room temperature (RT). Meanwhile, proliferation rate was maintained constantly after 20-minute at 4°C. Furthermore, it was determined that SSCs after thawing were maintained their self-renewal by characterization of SSCs using immunofluorescence and qRT-PCR. In conclusion, improved methodology from our research would be contributed to standardized freezing protocol for SSCs and thereby to effective application of SSCs such as male infertility treatment and preservation of male species.

**Key words:** Spermatogonial stem cell, cryopreservation, trehalose, equilibration, male infertility
This research was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (NRF-2017R1A2B4003798), Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2018R01A6A1A03025159), and the Bio & Medical Technology Development Program of the NRF funded by the Korean government (MSIP) (NRF-2018M3A9H1023139)

P275 - RNA binding protein TRIM71 is a novel regulator for murine spermatogonial stem/progenitor cell development. Xin Wu, Guihua Du, Xinrui Wang, Lufan Li, Weiya Xu

RNA-binding proteins (RBPs) control the fate of RNAs and tissue regeneration and homeostasis depend on tissue-specific stem cells, but the scope of RBP involvement in these processes remains largely unknown. Here, we have identified the RBP repertoire of undifferentiated spermatogonial population that consists of stem cells and transit amplifying progenitors in mouse testes. Proteins bound to polyadenylated RNAs are captured via oligo (dT)-conjugated beads after UV crosslinking and identified by proteomics. Of these, we find TRIM71, the member of Trim-NHL protein family, marks mouse testis germline progenitors. Tissue-specific deletion of TRIM71 in the male germline induces significant loss of undifferentiated spermatogonia and blocks spermatogenesis. Through immunoprecipitation, proteins including SCML2, EWSR1, CSNK2A2 and ACOT8 are identified as the partners of TRIM71 to regulate germline differentiation. Collectively, our results provide novel clues for dissecting the fate regulation of spermatogonial stem/progenitor cell as well as other tissue-specific stem cells.

P276 - DRC7 is required for sperm flagellum formation and male fertility in mice. Haruhiko Miyata, Akane Morohoshi, Keisuke Shimada, Kaori Nozawa, Takafumi Matsumura, Masahito Ikawa

Flagella and cilia are evolutionarily conserved cellular organelles. Abnormal ciliary motility in humans causes several syndromic diseases termed ciliopathies. In several of these ciliopathies, male infertility due to immotile spermatozoa often occurs. The central component of flagella and cilia is the axoneme that is composed of nine doublet microtubules, a central pair of microtubules, dynein arms, radial spokes, and the Nexin-Dynein Regulatory Complex (N-DRC). The N-DRC is localized between doublet microtubules and has been extensively studied in the unicellular flagellate *Chlamydomonas*. In *Chlamydomonas*, 11 proteins have been identified that comprise the N-DRC and thought to regulate the motor activity of dynein. In contrast, their functions in mammals have yet to be fully elucidated. Mouse DRC7 is the ortholog of *Chlamydomonas* DRC7 that is one of the components of the N-DRC. Using RT-PCR analysis, we found that *Drc7* expression is testes-enriched in mice, suggesting that DRC7 may play an important role in male fertility. To clarify its function *in vivo*, we generated *Drc7* knockout mice. Male homozygous mutant mice were viable, but no offspring were obtained from these mice when mated to wild-type females. Further analysis showed a significant decrease of spermatozoa in the seminiferous tubules and epididymis. Spermatozoa obtained from the cauda epididymis possessed short tails and were immotile. Further, when we observed *Drc7* KO spermatozoa using electron microscopy, axonemal structures were difficult to detect. These results indicate that *Drc7* is essential for the assembly of sperm flagella and male fertility in mice.
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**P277 - Mitochondria-eating protein is essential for sperm function, but not oocyte quality, in in vitro fertilization.** Makoto Orisaka, Yasuyuki Nakamura, Hirofumi Arakawa, Yoshio Yoshida

Mitochondria-eating protein (Mieap) is a key regulator of mitochondrial quality control system in cells. Although Mieap plays a critical role in maintaining healthy mitochondria in various pathophysiological states, its role in reproduction is unknown. In the present study, in vitro fertilization (IVF) was conducted by using sperm from male wild type (WT-sperm) and Mieap-knockout (Mieap-KO-sperm) mice, and oocytes from female WT (WT-oocytes) and Mieap-KO (Mieap-KO-oocytes) mice. The developed blastocysts were transferred into the uterus of pseudopregnant female mice. Data expressed as percentages were analyzed using Fisher’s exact test. Mieap-KO-sperm exhibited reduced motility and increased morphological abnormalities. The number of Mieap-KO-oocytes after ovarian stimulation was identical to that of WT-oocytes. The fertilization rate and percentage of 4-cell embryos/2-cell embryos were significantly lower between Mieap-KO-sperm and WT-oocytes and Mieap-KO-sperm and Mieap-KO-oocytes, than between WT-sperm and WT-oocytes and WT-sperm and Mieap-KO-oocytes. 2-cell embryos fertilized by Mieap-KO-sperm and WT-oocytes exhibited elevated levels of cytoplasmic oxidative stress as compared to 2-cell embryos fertilized by WT-sperm and WT-oocytes. Live birth rate after blastocyst transfer was comparable among embryos from WT-sperm and WT-oocytes, WT-sperm and Mieap-KO-oocytes, Mieap-KO-sperm and WT-oocytes, and Mieap-KO-sperm and Mieap-KO-oocytes. In conclusion, Mieap, a key regulator of mitochondrial quality control, is required for sperm motility and structural integrity. Mieap-KO-sperm increased 2-cell stage block of embryos suggesting that the down-regulation of Mieap in sperm induces oxidative stress in embryos during IVF. Mieap is not essential for oocyte quality nor implantation of blastocysts.

**P278 - The interaction of PHB with AKT protein plays a key role in sperm motility.** Hong CHEN, Xiaohui LI, Ranran CHAI, Guowu CHEN, Wenjing Tantai, Lingfei ZHANG, Wai Sum O, Patricia Martin-DeLeon

Phosphoinositide 3-kinase (PI3K) activity has been reported to be critical for sperm motility and mitochondrial reactive oxygen species (mROS) generation. It has also been shown that AKT phosphorylates the mitochondrial membrane protein, Prohibitin (PHB), to regulate somatic cell function. Our recent findings showed that sperm PHB protein is involved in human sperm motility loss associated with increased generation of mROS at mitochondrial complex I. Therefore, the objective of this study was to test if PHB is associated with the PI3K/AKT pathway in sperm motility. After obtaining ethics approval and informed consent, semen samples from male subjects between 30–40 years old attempting ICSI/IVF were collected and identified as poor sperm motility (asthenospermia, A) and/or low sperm concentrations (oligoasthenospermia, OA) according to 2010 WHO standards. Using SDS-PAGE and Western
blot analysis, the phosphorylated levels of PI3K (P85 subunit Tyr199 and Tyr467) and AKT (Ser473 and Thr308) proteins were found to be significantly lower in sperm from A and OA subjects compared to normal subjects. Interestingly, the phosphorylated PHB (Thr258) protein was also significantly decreased. Co-immunoprecipitation (co-IP) and immunofluorescence staining analysis confirmed that both PHB and phospho-PHB (Thr258) proteins interacted and co-localized with AKT and phospho-AKT (Thr308) proteins in mature sperm. After blocking AKT activity using PI3K inhibitor wortmannin, mature sperm showed significant decreases in the level of phosphorylation of PHB (Thr258) protein and total and progressive motility. Collectively, our observations suggest that the PI3K/AKT pathway is critical for sperm motility and a key role is played by phosphorylated PHB protein via AKT protein activity. (This project was supported by National Natural Science Foundation of China, Grant No. 81270738 and the Major State Basic Research Development Program of China, Grant No. 2014CB943100).

**P279** - Mitofusin2 is required for male fertility. Xiaoli Wang, Yujiao Wen, Jin Zhang, Shuiqiao Yuan

Mitofusins (Mfn2) are critical regulators for the mitochondrial fusion processes in mammalian cells. However, their roles in male germ cell development and spermatogenesis are largely unknown. Here, we report that mitofusin2 (Mfn2) is required for spermatogenesis and cooperates with PIWI proteins in postnatal male germ cells. Conditional loss of Mfn2 in postnatal germ cells causes mitochondrial dysfunction, the activation of a DNA damage response, oligo-teratozoospermia, and aberrant spermatogenesis, leading to complete male sterility. Meanwhile, we show that Mfn2 interplays with PIWI associated proteins, such as DDX4, MIWI, GASZ, and TDRKH in mouse testes. Ablation of Mfn2 in postnatal male germ cells leads to mitochondrial fragmentation, abnormal mitochondria-ER contacts and decreased protein levels of DDX4 and MIWI in chromatoid body, decreased expression of GASZ not only in pachytene spermatocytes but also in the round spermatids. In addition, whole-transcriptome sequencing analysis reveals that Mfn2 may regulate splicing events and translation efficiency in the meiotic and post-meiotic stage of spermatogenesis. In sum, our data demonstrate that Mfn2 is essential for spermatogenesis, and identify a novel role of Mfn2 in postnatal male germ development, providing additional layers of the mechanism of Mfn2 in controlling male fertility.

**P280** - Loss of hnRNP F in Sertoli cell impairs blood-testes barrier and leads to male infertility in mice. Hui Wen, Mengneng Xiong, Shuiqiao Yuan

In mammalian testes, Sertoli cell as the major type of somatic cells functions the continual production of sperm, and blood-testis barrier (BTB) structural integrity is essential for normal spermatogenesis. However, the exact role of Sertoli cell in spermatogenesis and the underlying mechanism of BTB structural integrity maintenance are largely unknown. Here, we report that hnRNP F, one member of heterogeneous nuclear ribonucleoproteins family, control Sertoli cell function and maintain BTB structural integrity in mice. Conventional knockout hnRNP F in mice leads to post-implantation embryonic lethality phenotype, which characterized by homozygous mutants died at embryonic day 9.5 (E9.5). Specific ablation of hnRNP F in Sertoli cell causes
BTB structural integrity and tight junction disruption, testis weight reduction, and spermatogenesis arrest leading to complete male sterility. Furthermore, we show that hnRNP F is involved in mRNA stability and splicing regulation in Sertoli cell in vivo and in vitro, which may respond to maintain the BTB structural integrity and regulate Sertoli cell differentiation. Collectively, our data suggest that hnRNP F is required for maintenance of BTB structural integrity and male fertility in mice.

**P281 - CRISPR interference for in vivo gene knockdown in mammalian male germ cells using lentivirus injection.** Naseer Kutchy, Zhicong Liao, Bluma Lesch

Germ cells carry genetic information precisely saved to the next generation. Along with DNA, germ cells are loaded with chromatin associated proteins which carry regulatory information across generations. The perturbation of these regulatory proteins can result in transgenerational transmission of diseases and cause male infertility. During the process of spermatogenesis, many developmental genes are marked by post translational histone modifications of histone H3. The loci where both the activating mark of H3 lysine 4 trimethylation (H3K4me3) and the repressive mark H3K27me3 are present are said to be poised. Genes which are under the control of poising have been reported to have profound roles in somatic development. However, how chromatin poising is regulated and how poising in germ cells influences somatic development in the embryo at fertilization is not well explained. The objectives of this study are to develop a method for in vivo lentiviral transduction in male germ line cells and use it to test the effect of germ line specific knockdown of chromatin regulators of poising by CRISPR interference (CRISPRi). We have developed a surgical procedure to deliver lentiviral constructs to the interstitial space of mouse testes. We used this approach to deliver vectors encoding components of the CRISPRi system directly to testes. We first tested the integration and expression of lentivirus encoding green fluorescent protein (GFP) in spermatogenic cells. We found that GFP was effectively expressed, and the integrated transgene was inherited to the F2 generation. Importantly, lentivirus expression did not affect fertility. As a proof of principle, we targeted protamine 1 (Prm1) in mouse germ cells and found reduced Prm1 expression in injected vs un-injected testis. This study will provide a novel means of characterizing the roles of chromatin regulators and will allow identification of regulators of poising in male germ cells.

**P282 - Soy-isoflavones Regulates Steroidogenic Capacity in Testes of Male Rats at all Ages.** Bamidele Jeminiwa, Rachel Knight, Erica Molina, John Fischer, Samantha Bradley, Benson Akingbemi

Soybeans contain the isoflavones genistin and daidzin, which are hydrolyzed in the digestive tract to their aglycones genistein and daidzein. We performed experiments to determine 1) influence of timing of exposure to isoflavones on testicular androgen secretion, and 2) whether the effects of soy-based diets on testicular function are due to genistein, daidzein or both. Male Long-Evans rats were maintained on control and soy-based (SBM) diets or the control diet supplemented with genistein and daidzein aglycones (G+D) for 14 days: postnatal days (PND) 21-35 (n = 45), 35-49 (n = 36) and 75-90 (n = 18). In the second experiment, pubertal animals were maintained on a control diet or the control diet supplemented with daidzein (D), genistein
(G) or both isoflavones (G+D) for 14 days \((n = 48)\). At sacrifice, i.e., within 24 h of terminating isoflavone exposure, blood was collected to obtain serum which were analyzed by radioimmunoassay (RIA) to measure testosterone (T) concentrations. Testicular explants and Leydig cells were incubated in DMEM/F12 culture medium with and without 100 ng/ml LH for 3 h. Spent media were analyzed by RIA to measure T production. Expression of FSHβ and LHβ subunit protein were analyzed in western blots of pituitary glands collected at sacrifice. Results showed that feeding of both SBM and G+D diets decreased serum and testicular T concentrations across all three stages of development \((P<0.05)\), but this effect was most profound with adult exposures. Pituitary FSHβ and LHβ subunit protein were greater in rats maintained on SBM and G+D diets than in control animal \((P<0.05)\). The G+D diet caused the most inhibition of Leydig cell T production \((P<0.05)\) Altogether, data showed that testicular steroidogenic capacity in male rats is sensitive to regulation by soy isoflavones, regardless of age.

This study was supported in part by NIH grant ES 158882-02.

**P283 - Impact of glycosylation on the functions of sperm during maturation in rhesus monkey (Macaca mulatta).** Ram Singh, Abhishek Chandra, Archana Srivastav, Sukanta Mondal

Glycosylation plays a critical role in spermatogenesis, extracellular quality control of sperm, and early embryo development through the building and remodeling of glycosylated cytosolic factors. The aim of the present experiment was to study post-translational modifications in the glycosylation status during epididymal passage and significance in fertility of a 33 kDa glycoprotein of rhesus monkey \((Macaca mulatta)\), known as MEF3 (monkey epididymal fluid protein 3). MEF3 exhibited staining for N-linked alpha-D-mannose groups and O-linked N-Ac-galactosamine linkages in epididymal fluids and faint or staining for N-Ac-glucosaminylated (wheat germ agglutinin), fucosylated (Tetragonolotus purpurea), and N-Ac-galactosamine (peanut agglutinin) residues on more mature corpus and caudal spermatozoa in a maturation-dependent manner on Western blots probed with specific biotinylated lectins. Polyclonal antiserum raised against affinity-purified MEF3 from caudal epididymal fluid (CEF) cross-reacted specifically with CEF and caudal sperm membrane of macaque and with Triton X-100 extract of ejaculated human spermatozoa, suggesting the existence of antigenically related components in both species. The tangled agglutination caused by anti-33 kDa serum of human spermatozoa, along with localization of MEF3 on entire sperm surface of epididymal and testicular sperm of monkey and human spermatozoa, suggest the significance of MEF3 in sperm function. The 100% inhibition of fertility of immunized female rabbits with this protein in vivo and inhibition of human sperm penetration in zona-free hamster eggs in vitro suggests the functional significance of MEF3 in fertility. Together, these results clearly indicate that MEF3 has potential significance as a target for antibodies that inhibit sperm function and fertility.

**P284 - Mrnlp is critical for male meiosis and fertility.** Renata Prunskaitė-Hyyrylainen, Julio Castañeda, Samina Kazi, Kaori Nozawa, Zhifeng Yu, Ramiro Ramirez-Solis, Martin Matzuk
Globally, 9% of men are infertile with 15% of these infertile cases due to genetic reasons. For most infertile men, the genetic cause underlying infertility has yet to be identified. In the search for new genetic factors affecting male fecundity, we have identified MRN complex interacting protein (Mrnip). Mrnip is highly conserved and has orthologues in nearly all eukaryotic lineages, including human. Our analysis has demonstrated that Mrnip is ubiquitously expressed in multiple tissues with the strongest expression in testis, kidney, and brain. We have obtained Mrnip knock-out mice (Mrnip\textsuperscript{\textit{tm1a} (EUCOMM)Wtsi}) from the knock-out mouse consortium and have shown that Mrnip knock-out males were infertile while female fertility was not altered. Analysis of juvenile Mrnip KO mice at P15 revealed no changes in testes morphology; however, adult mouse testes weight was significantly reduced in Mrnip knock-out mice as compared to heterozygous controls. The number of apoptotic cells in Mrnip seminiferous tubules was substantially increased compared to controls, and there was no mature sperm in testis and epididymis in adult knock-out mice. Antibody staining indicated that Mrnip is present in spermatocytes undergoing meiosis at mid-pachytene and diplotene stages. Expression analysis of meiosis-specific genes showed a reduction in Mrnip knock-out adult mouse testis, but not at P15 as demonstrated by qRT-PCR. MNRIP immunoprecipitation (IP) from testis lysate and mass spectrometry has identified 127 proteins enriched over control IPs from Mrnip knock-out testis lysate. Thus far, our data suggests that MRNIP functions in meiosis, and the Mrnip knock-out male infertility is likely due to defective progression through meiosis.

**P285 - Mechanistic insights into testicular granulosa cell tumor development.** Xin Fang, Nan Ni, Ivan Ivanov, Qinglei Li

Transforming growth factor beta (TGFβ) superfamily members are associated with GCT development. Little is known about the mechanisms regulating testicular GCT development. We recently created a testicular GCT mouse model by conditionally overactivating TGFβ receptor 1 in the testis (TGFBR1-CA). The objective of this study is to determine mechanisms that underpin testicular GCT development. Testis samples were collected from 14-day-old control and TGFBR1-CA mice (n = 4 per group) for RNA sequencing. Differentially expressed genes were identified using R-Studio and related regulatory pathways revealed by Ingenuity Pathway Analysis. Results showed dysregulated genes in TGFβ signaling, integrin signaling, cancer development, Sertoli cell development, and tight junction signaling. Interestingly, the expression of mature Sertoli cell marker, reproductive homeobox 5 (Rhox5), was reduced in TGRBR1-CA testes. This result indicates impaired maturation of Sertoli cells and is in agreement with our previous finding of loss of doublesex and mab-3 related transcription factor 1 (DMRT1, a protein that maintains testis identity) but gain of forkhead box L2 (FOXL2; a granulosa cell lineage maker) expression in the TGFBR1-CA testes. Consistent with the increased tumorigenic potential, dysregulation of cancer-related genes including jun proto-oncogene (Jun), Smad7, integrin alpha 3 (Itga3), cyclin dependent kinase 14 (Cdk14), and cadherin 1 (Cdh1) was found in the TGFBR1-CA testes. Upstream analysis predicted activation of catenin (cadherin associated protein) beta 1 (CTNNB1), an effector of WNT signaling that plays an important role in GCT development. Furthermore, we discovered alteration of gonadal differentiation-related genes such as sex determining region Y (SRY)-box 4 (Sox4) and GATA binding protein 4 (Gata4) in TGFBR1-CA testes. In summary, our results suggest that dysregulation of TGFβ...
signaling promotes testicular GCT development via altering the identity and promoting the trasdifferentiation of Sertoli cells.

**P286 - Centrosome inheritance during spermatocyte meiosis in C. elegans nematodes.** Mara Schvarzstein, Anthony James, Katherine Rivera Gomez

In male meiosis centrosomes organize the bipolar spindle that partitions chromosomes. Thus, precise centrosome inheritance is key to preventing aneuploidy in sperm and embryos. Centrosome inheritance is also crucial for sperm motility, as the microtubule-based centriole component of the centrosome forms the basal body of the sperm’s flagellum. Orthogonally engaged centrioles (⊥) disengage (|) in late mitosis and this licenses centriole duplication starting in G1/S phase of the cell cycle. However, in male meiosis, two nuclear divisions (MI and MII) and two centrosome duplications take place after a single genome replication (S phase). It is not known how the centrosome and cell cycle are coordinated in MII. The centrosome cycle in MII is unique because it takes place in the absence of an S phase and centrioles do not disengage at the end of this division. Live imaging of meiotic mutant spermatocyte divisions in *C. elegans* provided evidence that the cell and centrosome cycles in MII are coordinated differently in male meiosis. In some meiotic mutants, centrosome duplication progresses normally into MII despite not undergoing chromosome segregation in MI. Uncoupling of the chromosome and centrosome cycles in these mutants will be instrumental to uncover mechanisms regulating centrosome inheritance in male meiosis. In addition, high-resolution imaging revealed a new role for conserved chromosomal meiotic HORMA proteins in preventing centriole disengagement at the end of MII, possibly by preventing the Separase protease from disengaging centrioles. Mutant analysis provides evidence that REC-8 cohesin and SGO-1/Shugoshin are also required to keep the centrioles engaged at the end of MII. We will present queries of the mechanisms of 1. centrosome duplication in the absence of an S phase, and 2. maintenance of centriole engagement at the end of MII.

**P287 - NANOS2 knockout pigs as a model to devise strategies for treating male infertility.** Mariana Giassetti, Michela Ciccarelli, Ki-Eun Park, Bhanu Telugu, Jon Oatley

Approximately 10-15% of adult men suffer from infertility or subfertility with ~1% being diagnosed with idiopathic non-obstructive azoospermia. The similarities in organ anatomy and physiology make the domestic pig an excellent experimental model to study the etiology of human fertility disorders and devise therapeutic strategies. With advances in gene editing tools such as CRISPR/Cas9, the sophistication of generating pig models of human genetic diseases has improved greatly. To this end, we have been exploring the role of NANOS2 in spermatogenesis using pig models. In previous studies, we used CRISPR/Cas9 technology to generate pigs with inactivating mutations in the *NANOS2* gene and discovered that they phenocopy knockout mice with male specific sterility due to germline ablation. In the current study, we found that seminiferous tubules of *NANOS2* deficient male pigs are able to harbor regeneration of spermatogenesis following homologous transplantation with wild-type spermatogonial stem cells. At ~90 days post-transplantation, we detected presumptive round spermatids in the
ejaculate and motile spermatozoa were observed at ~100 days post-transplantation. Importantly, sperm production persisted for several months and the outcomes of genotyping analysis confirmed that the sperm were of donor origin. These findings demonstrate that the testicular soma is intact in males that lack germline due to NANOS2 deficiency. Moreover, the germline ablation phenotype but intact soma and testicular architecture of NANOS2 knockout boars provides an outstanding model for refining spermatogonial stem cell transplantation methodology that could be applicable to humans.

**P288 - Proteomic Characterization of Poor Sperm Chromatin Compaction Suggests Nuclear Retention.** Jacob Netherton, Mark Baker

Spermatogenesis is an extremely specialised process that generates a cell capable of the protection and delivery of the paternal genome to the oocyte. During the development of a spermatozoon, the basic chromatin structure of DNA bound to histones is drastically altered, and nuclear volume is greatly reduced. Importantly poor chromatin compaction has been associated with interruption of the histone/protamine 1/protamine 2 ratios in infertile men, however it has been shown these ratios are subject to change over time and to environmental stresses.

To better understand the process of sperm nuclear condensation, we isolated the sperm nuclei from cells with markers of good and poor compaction from an ejaculate. Comparative proteomics was performed on the nuclear proteins, using the quantitative SWATH platform on the Sciex 6600 TripleToF. We confidently identified 342 proteins, and of these proteins 20 were found to be more abundant in the sperm possessing poor chromatin compaction, many of which are associated with nucleoplasm. Immunoblots using an antibody against TOP2A and ODFP2 confirmed the proteomic analysis. Unexpectedly, no changes were observed in any of the identified histone peptides (H4, H3.3, H1T, H2A/B), nor for protamine 2. Our data suggests an alternate explanation for poor chromatin compaction. Rather than changes in histone or protamine content, it appears that retained or excess nucleoplasm is more prevalent in poorly compacted nuclei.

**P289 - Changes in epigenetic chromatin modification enzymes, chromatin remodelling factors and ubiquitination enzymes caused due to Dcaf17 mutation in mouse testis.** Thuraya Alharbi, Bhavesh Mistry, Maha Alanazi, Mohamed Rajab, Junaid Kashir, Fowzan Alkuraya, Abdullah Assiri

The DDB1– and CUL4–associated factor 17 (Dcaf17) is a member of DCAF family genes that encode putative substrate receptors for Cullin-4 (CUL4) based E3 ubiquitin ligases (CRLs) and regulate selective ubiquitylation of proteins. CRLs play critical roles in regulation of many cellular processes including spermatogenesis. Recently, it has been shown that deletion of Dcaf17 in mice caused male infertility due to defective spermatogenesis. The Dcaf17 mutant mice produced low number of sperm with abnormal shape and significantly low motility. Testis of Dcaf17 knockout mice displayed defects in nuclear compaction, acrosome biogenesis, and manchette assembly in
spermatids and mature sperm. The aim of this study is to decipher the molecular function of DCAF17 during normal sperm development. Using quantitative real-time PCR, Western blot and immunofluorescence techniques, we show abnormal expression pattern of several epigenetic chromatin modification enzymes, chromatin remodelling factors and ubiquitylation enzymes in the Dcaf17mutant mouse testis which may contribute to the increased levels of histone 2B and ubiquityl H2B in the KO testis and sperms. The aberrant expression of the different chromatin modification enzymes, chromatin remodelling factors and ubiquitylation enzymes due to the loss of DCAF17 suggests that DCAF17 may play crucial role(s) in regulation of ubiquitlyation, chromatin compaction and chromatin remodelling, important aspects of normal sperm biogenesis.

**P290 - Mining and mRNA expression profiling of WD-40 family genes including DDB1- and CUL4- associated factor genes in the mouse and human testes.** Bhavesh Mistry, Maha Alanazi, Hana Fitwi, Olfat AlHarazi, Mohamed Rajab, Abdullah Altorbag, Dilek Colak, Falah Almohanna, Abdullah Assiri

The DDB1- and CUL4- associated factors (DCAFs) are member of WD-40 family genes and important component of the cullin-RING E3 ligase complexes that are involved in a wide range of cellular processes including spermatogenesis. In this study we sought to characterize the mRNA expression of potential DCAF genes in testis and other major tissues using publically available transcriptomics dataset and the molecular biology techniques. In addition, several bioinformatics tools were employed to identify enriched functions and pathways. We identified more than 350 putative WD-40 family genes in each of the mouse and human genomes with 324 conserved genes between the two species. Analysis of RNA-sequencing dataset showed that around 130 and 89 WD-40 genes were highly or specifically expressed in the testes of mouse and human, respectively. Among the highly or specifically expressed WD-40 genes, 22% and 27% of the genes belonged to DCAF subfamily in mouse and human, respectively. Furthermore, the data from RNA-sequencing of testis at different developmental stages from embryonic day 13 to adult revealed that the genes were expressed differentially and showed clear clustering of the genes with some specifically expressed during embryonic stages and others expressed during post-natal developmental stages. To examine mRNA expression by q/RT-PCR, we selected a sub-group of 56 genes that encode for putative DDB1-CUL4 associated factors (DCAFs) and function as substrate receptors for multi-protein E3 ubiquitin ligase complexes. The q/RT-PCR results showed that many of the selected genes were predominantly expressed in the testis. Furthermore, the predominantly expressed DCAF genes showed differential gene expression during post-natal development of testis. Collectively, our data suggest that DCAF family proteins might perform diverse and important functions in mammalian spermatogenesis.

**P291 - Exploring role of DNA damage-binding 1 (DDB1) protein in mouse spermatogenesis.** Raed Abu-Dawud, Maha Alanazi, Mohamed Rajab, Hala Ahmed, Nadya Alyacoub, Falah Almohanna, Bhavesh Mistry, Abdullah Assiri

Spermatogenesis is an array of complex and orchestrated processes, which requires ubiquitination during germ cell development for successful sperm production. Here, we are addressing the role of Ddb1, an E3-ligase protein that is involved in proteins’ proteolytic and non-proteolytic
ubiquitination, in mouse spermatogenic cell development. To elucidate the role occupied by Ddb1 in spermatogenesis, we conducted expression analysis in different tissues, different testis developmental stages and in different testicular cell lines; TM3, TM4 and F9 using qPCR, Western blotting and immunohistochemistry (IHC). Expression analysis using qPCR showed highest expression of Ddb1 in testis, followed by ovary, brain, oviduct, kidney, heart and epididymis. Further, qPCR showed that Ddb1 mRNA transcript levels increase during post-natal testis development whereby 56 PND exhibited more than 10fold increase in comparison to 5 PND. Moreover, Western blotting analysis showed that Cul4a, Cul4b & Ddb1 proteins (components of CRL3 complex) are expressed in cell lysates of TM3, TM4 and F9 cells as well as tissue from brain and testis. Then, we examined Ddb1 expression in murine testicular sections at 5, 14, 23, 32 and 56 PND. The Ddb1 protein is differentially expressed in the different cell types of the seminiferous tubules, namely in the spermatogonial stem cells, spermatocytes, round spermatids and at appreciable levels of the elongated spermatids. Moreover, the expression is dynamic in nature and appears to correlate with the waves of spermatogenesis. In addition, we found Ddb1 to be expressed in Leydig cells. Currently, we are conducting Ddb1 pull down experiments and generating Ddb1 germ cells Ddb1 knock-out mice for functional analysis. Our obtained data and on-going experiments will shed more light on the role(s) of Ddb1 gene in spermatogenesis.

P292 - Short-term treatment with mTORC1 inhibitors rapamycin and Everolimus negatively impact male germ cell differentiation. Oleksandr Kirsanov, Randall Renegar, Nicholas Serra, Christopher Geyer

Rapamycin (sirolimus) and its closely related analog (rapalog), Everolimus, inhibit the ‘mechanistic target of rapamycin complex 1’ (mTORC1), a master regulator of multiple cellular functions. Both rapamycin and Everolimus are FDA-approved to slow the growth of aggressive cancers and to prevent organ transplant rejection in human patients. Case reports have suggested reversible male infertility is an unintended side-effect of chronic rapamycin treatment. This correlates with recent findings from our lab and others, which employed genetic approaches and acute high dosages of rapamycin in mice to uncover a requirement for mTORC1 activation during spermatogonial differentiation. Here, we test the hypothesis that chronic Everolimus treatments approximating the lower human therapeutic dosages exert dose-dependent detrimental, yet reversible, effects on reproductive health of young and adult mice similar to rapamycin. Juvenile (P20) or adult (P60) mice received daily injections of rapamycin (10 µg/g) or Everolimus (5 µg/g) for 30 days, after which tissues were recovered immediately or following a recovery period. We found that rapalog treatment reduced body and testis weights, testis weight/body weight ratios, cauda epididymal sperm counts, and seminal vesicle weights in young and older males. In addition, rapalog treatment increased the numbers of undifferentiated ZBTB16/PLZF+ spermatogonia while reducing differentiating KIT+ spermatogonia. Additional groups of adult mice received dosages of Everolimus similar to those used to treat cancer (0.17 µg/g) or for immunosuppression following organ transplantation (0.035 µg/g) to more faithfully model current clinical human rapalog utilization. In these animals, only testis weights, testis/body weight ratios, and tubule diameters were reduced. Return to control values was dependent upon the rapalog dosage and length of the recovery period. Together, these data indicate that Everolimus treatment significantly impairs spermatogenesis by blocking spermatogonial differentiation, and results in incomplete recovery of reproductive parameters in young and adult mice.
**P293 - Protective effect of alpha-tocopherol against arsenic induced-toxicity in testes.**
Manuel Sanchez-Gutiérrez, Pedro Becerra-Fajardo, Jeannett Izquierdo-Vega, Eduardo Madrigal-Santillán, Luz Del Razo-Jiménez, Valeria Lagunas-Ortiz, Kevin Flores-Elizalde

Arsenic is a natural metalloid found in abundance in the environment. Contamination of water with arsenic has been evidenced in many countries as India, Taiwan, China, Chile, Argentina, Mexico, and the United States. Chronic arsenic exposure via contaminated drinking water is a global environmental health problem associated with many serious systematics disorders including the male reproduction system. Epidemiological studies in population expose have been associated with infertility. Arsenic-induced the production of reactive oxygen species (ROS), causing oxidative damage. The aim of this study was to evaluate the protective effects of alpha-tocopherol against arsenic toxicity on testes. Twenty-four male CD1 mice were randomly divided into four groups. Mice were exposed with distilled water or 0.75 mg/kg of NaAsO2, for 65 days. Group co-exposed was pretreated with alpha-tocopherol (100 mg/kg) by gavage for 25 days before and during arsenic exposure. The control group of alpha-tocopherol was exposed for 90 days. We evaluate several parameters in testes in the four groups: i) Glutathione (GSH) concentration, ii) Protein carbonyl content and iii) TBARS concentration. Data were analyzed by an ANOVA (p <0.05), followed by the Tukey post hoc test, using GraphPad version 7. In testis, the arsenic exposure caused a significant increased in the concentration of TBARS (p <0.001), and protein carbonyl concentrations (p<0.001), accompanied by a significant decreased in the concentration of GSH in comparison with control group. Co-exposure of arsenite with alpha-tocopherol caused a decrease a significant in TBARS concentration (p>0.001), and protein carbonyl concentrations (p<0.001), and also by a significant increase in the concentration of GSH in comparison with the arsenic group. Co-exposure of alpha-tocopherol before and along with arsenic resulted in reduces oxidative damage in testes.

**P294 - Effect of induced-obesity by hyperlipidic diet on toxicity testicular by subchronic exposure to fluoride in Wistar rats.** Jeannett Izquierdo-Vega, Itziar Hernández-Martínez, Manuel Sanchez-Gutiérrez, Araceli Hernández-Zavala, Eduardo Madrigal-Santillán, Valeria Lagunas-Ortiz, Kevin Flores-Elizalde

Globally, fluoride is known as an environmental pollutant. There is a high fluoride content in the groundwater of diverse geographic areas of Mexico, reaching concentrations above the maximum permissible limit for drinking water (1.2 mg/L). Obesity has reached epidemic proportions worldwide, is a complex metabolic disorder that is linked to numerous serious health complications with high morbidity. It is well known that fluoride and obesity induce stress and damage oxidative. The aim of this study was to evaluate the effect of subchronic-exposure to fluoride on the oxidative stress in testes in a model of obesity induced by hyperlipidic diet in rats. Twenty-four male Wistar rats were randomly divided into four groups, which were administrated by gavage with distilled water containing 0 and 10 mg/kg of NaF, respectively for 90 days. During exposure to fluoride, half of the groups were fed with a diet containing 50% kcal of lipids and the other half with a control diet. We evaluated several parameters in the four groups, after 90 days of exposure, it was observed in the induced-obesity group increased of the adiposity index (p<0.001),
and increased the epididymal fat content (p<0.001) vs control group. In testis, causes a significant increase in the concentration of TBARS (p<0.001), compared with the group exposed to fluoride. The co-exposure to fluoride and hyperlipidic diet increased the adiposity index (p<0.05) however, decreased the epididymal fat content (p<0.05) caused by hyperlipidic diet. Likewise, in the testes, the co-exposure significantly decreased the concentration of GSH (p<0.001), in comparison with both control groups; and cause a significant increased in the concentration of TBARS (p<0.001), in comparison with the control group. The co-exposure to fluoride and hyperlipidic diet cause oxidative damage in testes and modulation in the lipid metabolism.

**P295 - Vitrification of testicular tissue from adult Spix’s yellow-toothed cavies’ (Galea spixii ) using different cryoprotectants.** Andrea Silva, Ana Pereira, Erika Camila Praxedes, Samara Sandy Moreira, Moacir Oliveira, Pierre Comizzoli, Alexandre Silva

The objective was to assess different cryoprotectants for the vitrification of testicular tissue from the Spix’s yellow-toothed cavy, a wild rodent from the Caatinga biome. Testes from six adults were recovered and dissected in small fragments (3 mm³). Fresh tissues were evaluated for morphology according to scores (3 – adequate; 2 regular; 1 – poor) using the following criteria: separation of the basal membrane, structure integrity, cell swelling, cell loss, and rupture. Moreover, fresh controls were evaluated for proliferative activity by the Ag-NOR technique. Other fragments were immersed in a MEM-based solution with 10% of fetal bovine serum plus 0.25M sucrose for 5 min before exposure to either 3 or 6 M of dimethyl-sulfoxide (DMSO) or ethylene glycol (EG) for 5 min. Tissues then were cryopreserved using a solid-surface vitrification technique. After two weeks of storage in liquid nitrogen, samples were warmed at 37°C, washed in decreasing sucrose concentrations, and evaluated as described previously.

Scores of fresh samples were: 2.97 ± 0.02 membrane separation, 2.49 ± 0.04 structure integrity, 2.81 ± 0.04 swelling, 2.97 ± 0.01 cell loss, and 2.97 ± 0.01 rupture. After warming, scores of 3M EG groups were similar to controls (P > 0.05) for cell swelling (2.71 ± 0.04), cell loss (2.98 ± 0.01) and membrane rupture (2.98 ± 0.01). The 3M DMSO group was only efficient to avoid cell swelling (2.72 ± 0.03). Regarding proliferative activity, fresh control presented 3.73 ± 0.09 nucleolar organizing regions for spermatogonia, 3.72 ± 0.11 for spermatocytes, 1.68 ± 0.08 for spermatids, 2.55 ± 0.11 for Leydig and 3.89 ± 0.11 for Sertoli cells, which was efficiently preserved after thawing using all cryoprotectants. Collective results suggest that 3M EG is optimal for testicular tissue vitrification in adult Spix’s cavies. Research supported by CAPES (Financial Code 01), Brazil.

**P296 - Acute effects of estradiol on Sertoli cell numbers in intact and hemicastrated boars.** Jennifer Jankovitz, Barbara Jean Nitta - Oda, Trish Berger

Sperm production capacity is largely influenced by Sertoli cells numbers. The current objective was to assess the acute effects of exogenous estradiol on Sertoli cell numbers in intact and hemicastrated pubertal boars. Each replicate contained four littermate boars. Two boars were hemicastrated at day eight of age while the other two remained intact. At week 18.5, one hemicastrated boar and one intact boar received a silastic implant containing estradiol in their left testis and the remaining boars received a blank implant. One week later, tissues were collected
from three locations on the left testis of the hemicastrated and intact boars with additional tissue collected from the right testis of intact boars. Tissue samples were fixed, dehydrated, and embedded in paraffin. Sertoli cell density was determined after labeling Sertoli cells with an antibody to GATA-4 and total Sertoli cell number calculated. The number of Sertoli cells per testis in hemicastrated boars (estradiol and vehicle-treated) was approximately twice the number present in intact littermates, a significant increase (P < 0.001). Testis weight was also approximately doubled by hemicastration (P < 0.001) meaning that one testis weighed approximately the same as the two testes present in intact littermates. Exogenous estradiol had no detectable acute effect on Sertoli cell numbers or on testis weight in intact or hemicastrated boars. The ability of exogenous estradiol to decrease Sertoli cell numbers in a population previously enlarged by reducing endogenous estradiol may be unique to this population or a prolonged response. This study enhances current understanding of estradiol’s acute effects on Sertoli cell numbers and mechanisms affecting the maintenance of Sertoli cells. (Supported by W3171 MSP, a W.K. Kellogg Endowment, the infrastructure support of the Department of Animal Science, College of Agricultural and Environmental Sciences, and the California Agricultural Experiment Station of the University of California–Davis.)

**P297 - Deletion of the mouse Prame gene affects DDX4 expression in seminiferous tubules during the first wave of spermatogenesis.** Mingyao Yang, Weber Feitosa, Wan-sheng Liu

The preferentially expressed antigen in melanoma (PRAME) constitutes a large gene family in Eutherian mammals, in which Prame and Pramel1 are predominantly expressed in testis. However, their function in spermatogenesis is unclear. To explore the functional role of PRAME and PRAMEL1 in testis, we have generated a conditional Prame knockout (cKO) mouse. We previously reported that the Prame cKO mice undergo seminiferous tubule degeneration at postnatal day 21 (P21) and P35, resulting in a Sertoli-cell-only syndrome (SCOS) pattern in the effected seminiferous tubules and a smaller testis (compared to the control) in adult mice. To study the molecular mechanism underlying the degeneration of the seminiferous tubules at P21 and P35, we investigated the number of Sertoli cells and germ cells in the Prame cKO testis using anti-Sox9 and anti-DDX4 antibodies. No difference was observed in the Sertoli cell numbers between the Prame cKO and control mice as well as between the normal and degenerated seminiferous tubules in the Prame cKO testis. However, we observed three different expression patterns, high, low and no expression (DDX4-negative) for DDX4. At P21, 24% of seminiferoustubules in the Prame cKO testis were DDX4-negative, significantly higher than that (12%) in the control mice (P<0.001). In contrast, the number of seminiferoustubules with the high-DDX4-expression was significantly decreased in the Prame cKO mice (47%) than control mice (60%) (P=0.005). At P35, DDX4 was expressed in all seminiferoustubules except for those that have been degenerated in the Prame cKO mice. In addition, we have also generated a global Pramel1 KO mouse, which is fertile with a smaller testis and is being characterized. In conclusion, degeneration of seminiferous tubules in the Prame cKO mice is independent of Sertoli cells and is at least in part mediated by changes in the DDX4 expression.

**P298 - Patterns of the PRAMEY expression in the bovine testis and epididymis.** Weber Feitosa, Chandlar Kern, Wan-sheng Liu
The Y-linked preferentially expressed antigen in melanoma (PRAMEY) gene subfamily is a cancer/testis antigen expressed predominantly in spermatogenic cells, playing an important role during bovine spermatogenesis. To better understand the PRAMEY function, we studied its protein dynamics in spermatozoa, fluid and tissues from testis and caput and cauda epididymis by western blot with a PRAMEY-specific antibody. Results from 3-5 bulls were analyzed by Image J and compared by one-way ANOVA with post-hoc Tukey test. Testicle sperm expressed the 58 kDa protein, which is the predicted molecular weight for the intact PRAMEY and the 30 kDa protein isoform. As spermatozoa migrate to epididymis, two new isoforms of 26 and 13 kDa were detected, in which the 13 kDa expression was higher in cauda spermatozoa. Similar to testis sperm, we observed only the 58 kDa and the 30 kDa isoforms in the testis fluid. Compared to testis fluid, no difference in the PRAMEY patterns was observed in caput fluid. However, fluid from the cauda epididymis showed the 13 kDa isoform. Tissue evaluation indicated that the 58 kDa isoform had significantly higher expression in caput tissue and the 13 kDa isoform had higher expression in cauda tissue, while the 30kDa kept the same level between caput and cauda tissue. Interestingly, the 26 kDa isoform was not observed in epididymal fluid and tissue. In summary, our results showed that testis and testicular sperm and fluid expressed solely the 58 kDa and 30 kDa PRAMEY isoform, suggesting their involvement in spermatogenesis. In contrast, the 26 kDa was an epididymal sperm-specific isoform and the 13 kDa isoform was marked in sperm, fluid and tissue from the cauda segment of the epididymis, suggesting their involvement in sperm maturation.

**P299 - Impact of maintenance intake in testis parameters and sperm volume in young bucks.** Ciro Torres, Marco Novaes, Palloma Almeida, Domingos Netto, Victor Carvalho, João Lovatti, Mariana Neves

Little is known regarding how maintenance intake can impact testis parameters. We observed the effect of the maintenance versus *ad libitum* intake of young Alpine bucks in testicular biometry, ejaculated sperm volume, oxidative stress markers in the testis, and hystomorphometry of the seminiferous tubules. Ten healthy Alpine bucks were used, with initial age between five and six months, with five animals per treatment. The animals were randomly assigned to one of the treatments, in a completely randomized design. The treatments were: i) maintenance intake (meeting the nutritional requirements for the category, according to AFRC, 1998); ii) *ad libitum* intake. The scrotal perimeter, and the testicular length and width were measured and recorded. The activities of superoxide dismutase (SOD), catalase (CAT), and glutathione S-transferase (GST), and total protein level were evaluated. The testis were completely immersed in Bouin solution for 24 h, and testicular fragments were selected and embedded in histological paraffin. Subsequently, 7 μm thickness samples were stained with hematoxylin and eosin, and seminiferous tubule and luminal diameters as well as epithelium height, were recorded. The ejaculates were collected and the sperm volume was recorded. Scrotal circumference and volume of the left and right testicles were higher in the *ad libitum* fed bucks. The oxidative stress markers were similar for animals of both treatments, demonstrating that diet does not interfere in the metabolism of the other antioxidant enzymes or in carboxylated and total proteins in young Alpine bucks. The tubular and luminal diameter and epithelium height were higher in *ad libitum* fed bucks than in the animals receiving maintenance diet. Finally, sperm volume was higher in the *ad libitum* fed bucks. These data suggest that the animals fed *ad libitum* produce larger ejaculates when
compared with maintenance fed animals, although there are no differences in oxidative stress markers animals profiles.

**P300 - Morphological and ultrastructural evaluation of jaguar (Panthera onca) sperm cryopreserved in different extenders.** Herlon Silva, Thalles Nunes, Lívia Campos, Andréia Silva, Alexandre Silva, Lúcia Silva

In order to contribute to the formation of biobanks, we aimed to verify the effect of different extenders on the morphology and ultrastructure of frozen-thawed sperm of jaguars (<i>Panthera onca</i>). Semen from five adult males was obtained through electroejaculation, totaling 8 semen samples. Samples were frozen in extenders based on Tris (plus 20% egg yolk) or coconut water (ACP-117C; plus 10% egg yolk), both supplemented with 6% glycerol, stored in liquid nitrogen and thawed at 37 °C after two weeks. Fresh and frozen samples were evaluated for morphology through a Rose Bengal stained smear at counting 200 cells. To a detailed ultrastructural evaluation, samples were processed for transmission electron microscopy. Fresh samples presented 80.0 ± 3.5% normal morphological cells, a value higher (P < 0.05) than those verified for samples cryopreserved in Tris (71.1 ± 3.1%) or ACP-117C (64.0 ± 3.9%). Moreover, a significant (P < 0.05) increase on acrosome damage was observed for samples frozen both in Tris (5.9 ± 1.3%) or ACP-117C (8.4 ± 1.8%) in comparison to fresh samples (0.8 ± 0.5%). Regarding other damage types, fresh and frozen-thawed samples were similar. For ultrastructure, a great amount of electrolucent points was verified into the sperm nucleus of samples preserved in ACP-117C, but they were not evident in fresh samples or those frozen in Tris. Moreover, the mitochondria of sperm preserved in ACP-117C were unorganized and presented large amounts of vacuolization, which were not observed in fresh samples or those frozen in Tris. In conclusion, we recommend the use of a Tris-based extender for the cryopreservation of jaguar semen, proving less ultrastructural damages after thawing than ACP. This research was supported by CAPES (Financial Code 01), Brazil.

**P301 - Rapid response to oestrogen blocks SOX9 in human testis cells.** Melanie Stewart, Deidre Mattiske, Andrew Pask

SOX9 is a transcription factor essential for testicular development. SOX9 protein is sequestered in the cytoplasm of gonadal cells by binding to stabilised microtubules. In males, SOX9 dissociates from the tubulin and translocates from the cytoplasm to the nucleus to activate Sertoli cell development. This pathway can be blocked by exposure to exogenous oestrogen which causes cytoplasmic retention of SOX9, resulting in gonadal cells taking on an ovarian fate. Alterations to the MAPK pathway can also tilt the balance between testis and ovarian genes in somatic cells by affecting aspects of this pathway that are known to be oestrogen responsive. Furthermore, MAPK signalling, and in particular ERK1/2, is known to affect the microtubule network. Therefore, we hypothesised that oestrogen could cause cytoplasmic retention of SOX9 by stabilising microtubules via activation of ERK1/2. To test this hypothesis, we treated the human testis cell line NT2/D1 with oestrogen for 30 minutes in the presence or absence of the ERK1/2 inhibitor U0126 and examined the distribution of SOX9, microtubules and phosphorylated ERK1/2 within the cell by immunofluorescence. Oestrogen rapidly blocked the
nuclear translocation of SOX9 in NT2/D1 cells and the microtubule network was stabilised. Phosphorylated ERK1/2 was more abundant and localised in the nucleus following oestrogen treatment. The effect of oestrogen was reduced upon pre-treatment with the ERK1/2 inhibitor U0126, demonstrating that oestrogen requires active ERK1/2 to inhibit SOX9. Together, these data suggest that oestrogen can rapidly activate ERK1/2 to stabilise microtubules and cause cytoplasmic retention of SOX9. We have revealed a previously unknown mechanism for oestrogen in impacting the function and differentiation of human testis cells.

**P302** - Steroidogenesis during prenatal testicular development in Spix cavies (Galea spixii).
Amilton Santos, Alan Conley, Moacir Oliveira, Antonio Assis Neto

Spix cavies are potentially good experimental models for research on reproductive biology and sexual development. The aim of the present study was to evaluate the ontogeny of immunolocalization of the steroidogenic enzymes involved in testicular androgen synthesis during prenatal development compared with adult testes. Testes were investigated at 25, 30, 40 and > 50 DG (days of gestation) (n = 4 males each DG and adult). Immunological techniques (immuno-histochemistry and immuno-blotting) were performed to establish the site and relative amount of androgenic enzymes including 5α-reductase, 17β-HSDI and 3β-HSDII throughout prenatal development together with routine hematoxylin-eosin staining. The testicular parenchyma began to organize at the 25 DG with the development of recognizable testicular cords. The mesonephros was established after the 25 DG and differentiated to form the epididymis, as testicular cords were beginning to proliferate and the interstitium to organize by 30 DG, continuing thereafter. The androgen synthetic enzymes 5α-reductase, 17β-HSDI and 3β-HSDII were evident in Leydig cells as they differentiated at all subsequent gestational ages studied. In addition, immunoblotting showed an increase in the immunoreactivity of the enzymes at 30 and 40 DG (p <0.05) and a decrease at 50 DG (p <0.05). It is concluded that the increase in the presence of androgenic enzymes coincides with the functional differentiation of the testes, and with the stabilization and differentiation of the mesonephros forming the epididymis.

**P303** - Anatomy and functional ultrastructure of the pathways spermatids in the scorpion mud turtle (Kirosternon scorpioides).
Vinicius Godoy, Alana Sousa, Antonio Assis Neto

The scorpion mud turtle is found in South America. Although it is not endangered species, its population has been decreasing considerably in Amazonia Region of Brazil because of deforestation and hunting. The aim of the work was to investigate in detail the morphology of the testes, epididymis and vas deferent using macroscopics descriptions, histological and ultrastructural analyses. Adults animals were collected in the Maranhao States (Northeast Brazil), which is one of the States belonging to the Legal and International Amazonia. Samples from 11 adults were fixed in 2.5% glutaraldehyde, 4.5% paraformaldehyde and bouin solutions. The tissues were used to histological, scanning, transmission microscopy analyses. All procedures were performed at the Advance Center for Diagnostic Imaging of the University of Sao Paulo (CADIMFVZ-USP). Males had on average: 296.43g (body weigh); 13.53cm
(carapace length); 8.4cm (wide carapace); 11.8cm (length plastron); 5.9cm (plastron width) and 4.56cm (size). The seminiferous tubules were filled with sperm in their lumen. TEM revealed light and dark spermatogonia, and spermatids cell with morphology well-defined and nuclei rounded. The epididymis has no distinction in head and tail, the cells had rounded nuclei and few chromatins inside. The vas deferent were completely filled with spermatozoa. The sperm had its morphology preserved. In this way, a head an acrosome with vesicles, mitochondria and flagellum were identified. In conclusion, the animal presented a good capacity to produce sperm and, therefore reproductive activity, along the spermatic pathway.

**P304 - Examination of centriole marker in spermatozoa separated by density gradient.**

Mariam Asadullah, Emily Fishman, Ahmed Hussain, Andrew Gerts, Tariq Shah, Puneet Sindhwani, Tomer Avidor-Reiss

The sperm is the sole contributor of centrioles to the zygote, and these centrioles are thought to be essential for fertility. Sperm centrioles are unique in that male germ cells undergo “Centriole Remodeling,” which results in highly modified centrioles. In flies, defects in this process lead to embryonic developmental defects. The implications of a defect in this process are unknown in humans. As such, the objective of this study was to detect a molecular marker that identifies abnormal sperm centrioles in humans and to determine their relationship to infertility. Once such a marker is identified, it may be possible to diagnose sperm centriole abnormalities in infertile males. We compared normal and abnormal sperm from five infertile men. Semen were separated based on density, yielding a pellet of sperm that is denser than an interface of sperm. The less dense sperm in the interface is thought to be morphologically abnormal or immature. The interface and pellet sperm were stained with fluorescent antibodies against the centriole-specific protein POC1B. We used photon counting confocal microscopy to determine intensities in both centrioles and the axoneme. We found that POC1B immunostaining was significantly enriched (P=0.008) in the axoneme of the interface (26±56) when compared to the pellet (12±22) in infertile males (n=5). Therefore, POC1B, which is normally restricted to the centrioles, is found unexpectedly in the axoneme in the interface sperm, suggesting that elevated POC1B levels in the axoneme is a feature of abnormal sperm. POC1B could be the first centriole-specific marker that has differential staining between normal and abnormal sperm, which is an important first step towards using a centriolar marker to determine sperm quality. Future studies will be directed to investigate if the presence of POC1B in the axoneme is due to a remodeling defect.

**P305 - The perforatorium and postacrosomal sheath of mouse spermatozoa share common developmental origins and protein constituents.**

Nicole Protopapas, Lauren Hamilton, Morgan Lion, Wei Xu, Sutovsky Peter, Richard Oko

The perinuclear theca (PT) is a cytosolic protein capsule that surrounds the nucleus of eutherian spermatozoa. It can be divided into three regions in falciform spermatozoa: the subacrosomal layer (SAL), the postacrosomal sheath (PAS), and the perforatorium. The SAL forms early in spermiogenesis in concert with acrosome formation, while the PAS and perforatorium form later,
during the appearance of the microtubular manchette. PAS- and perforatorial-bound proteins are proposed to make use of the manchette for transport to their subcellular destination. Recently, we have demonstrated in the rat that the common developmental mechanism between the formation of the PAS and perforatorium resulted in proteins being shared between these two regions of the PT. The goal of the present study was to further validate these findings in the mouse. Immunodetection analysis revealed that in addition to its major endemic protein constituent, PERF15, the murine perforatorium also contains proteins found in the PAS. These include GSTO2, PAWP/WBP2NL, WBP2, and the core histones. Immunofluorescence indicates that these proteins were localised to the manchette in developing spermatids prior to their deposition in the PAS and perforatorium. The shared mechanistic assembly and compositional homogeny of the PAS and perforatorium is thus likely a conserved feature of murid spermatozoa.

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**P306 - The molecular mechanism mediating the formation of atypical distal centriole (DC) in mammalian spermatozoa.** Sushil Khanal, Katerina Turner, Emily Fishman, Kebron Assefa, Mohamad Nawras, Matthew Stojsavljevic, Tomer Avidor-Reiss

The spermatozoa of most mammals have only one recognizable centriole, the proximal centriole, and the spermatozoa of murine have no recognizable centrioles. Recently, we discovered in human and other mammals that the spermatozoon distal centriole is remodeled during spermiogenesis to have an atypical structure, which we refer as the spermatozoon distal centriole (SDC). The SDC in human consists of 9-fold doublet microtubules splayed outward making a funnel shape structure, instead of triplet microtubules and barrel-shaped structure in the typical centriole. More importantly, the SDC consists of a unique organization of centriole lumen proteins, POC1B, POC5, and CETN1/2 into 2-3 rod structures, which flank the microtubules. The mechanistic detail forming the atypical centriole during spermiogenesis remains an enigma. Recently, we identified a new protein, FAM161A in the SDC. This protein binds to both microtubules and SDC rod proteins (POC1B, and POC5), suggesting that it may function as a linker between the SDC rods and microtubules. The FAM161A enriches along with other rod proteins in the round spermatid stage during remodeling process, and it gets incorporated in the distal centriole (DC) at a later stage of remodeling process in rabbit and bovine. This suggests that the FAM161A is required after rods have already started to form. However, in mice, the FAM161A is present in spermatids but it does not incorporate into DC at any stage of spermiogenesis. Unlike other mammals, in mice, the rods proteins level in the DC start to decline at about the stage when fAM161A incorporates into the DC, suggesting that FAM161A stabilizes the SDC rods. Altogether, this study gives a first mechanistic clue on how the sperm atypical centriole forms. This work was supported by grant HD092700 from Eunice Kennedy Shriver National Institute of Child Health & Human Development (NICHD).
**P307 - Mitochondrial Dynamic and Acrosomal Reaction are Disturbed In Spermatozoa from Stressed Adult Rats.** Silvana Andric, Isidora Starovlah, Sava Radovic, Tatjana Kostic

Although psychophysical stress is the most common stress in human society and the major contributor to wide variety of pathological conditions, the molecular events in spermatozoa from stressed males were not described well. The aim of this study was to determine the functionality and molecular adaptation of spermatozoa from stressed rat using in vivo and in vitro approach. For in vivo experimental model, psychophysiological stress by immobilization (IMO) was performed for 3 hours in different time during the day (ZT3, ZT11, ZT23), for one (1xIMO) or ten (10xIMO) consecutive days. For in vitro approach, epididymal spermatozoa from undisturbed rats were stimulated with stress hormones adrenaline and cortisol. Results showed that number of spermatozoa significantly decreased in 10xIMO-rats comparing to control. Acrosomal status (response to acrosome-reaction-inducer progesterone) significantly decreased in spermatozoa from 1xIMO and 10xIMO rats comparing to control. The same effect was observed in spermatozoa stimulated in vitro with stress hormones. RQ-PCR results revealed that transcription of the main mitochondrial biogenesis markers Ppargc1a, Nrf1 and Nrf2 increased in spermatozoa from 10xIMO rats in ZT3 time point. In the same spermatozoa samples, transcription of main markers of mitochondrial architecture Opal, Mfn1 and Mfn2 increased in ZT3 while decreased in ZT11 time point. Incubation of spermatozoa with adrenaline decreased level of Ppargc1a and Nrf2a transcripts, while cortisol decreased expression of mitochondrial transcription factor TFAM. In summary, repeated psychophysical stress decreased the number and functionality of spermatozoa and disturbed transcriptional profile of their mitochondrial biogenesis and architecture markers and some of the effects were mediated by stress-mimetics.

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**P308 - The Caenorhabditis elegans ortholog of human T-complex protein 11 (TCP11), M05D6.2, is necessary for sperm production and fertility.** Danielle Cooley, Emily Lopes, Amber Jacob, Matthew Marcello

Human t−complex protein 11 (TCP11) is a testis−specific gene product that is hypothesized to be necessary for proper sperm capacitation, acrosome reaction, and sperm morphology. M05D6.2 is the Caenorhabditis elegans ortholog of human TCP11. Our goal is to use the investigation of M05D6.2 gene function to understand the role of TCP11 in human reproduction. C. elegans have two sexes: hermaphrodite and male. Sperm from both hermaphrodites and males must undergo proper sperm activation, which includes processes similar to sperm capacitation and acrosome reaction in mammals, in order to migrate to and fertilize the egg. We have used RNA interference (RNAi) to disrupt the gene function of M05D6.2 in C. elegans. Hermaphrodites subject to M05D6.2 RNAi−treatment show no reduction in fertility. However, when male C. elegans are subject to M05D6.2 RNAi−treatment they have a significant decrease in fertility, despite making a normal number of sperm. We have generated three transgenic C. elegans strains using CRISPR/Cas9 genome editing (a deletion mutant, a mutant mimicking
mutations found in infertile male patients, and a GFP−tagged version of the protein) to further characterize M05D6.2 function and localization. Our preliminary results indicate that M05D6.2 is expressed in sperm and is necessary for proper sperm production.

**P309 - Comparative Computer Assisted Semen Analysis in Nonhuman Primates.** Katherine Mean, Thaddeus Golos, Jenna Kropp Schmidt

Biomedical research has recently focused on implementing germline and somatic cell genome editing strategies in nonhuman primates (NHPs) to generate gene mutations associated with human disease conditions. These approaches rely on semen collection to perform *in vitro* fertilization for germline-based editing approaches in embryos, and also subsequently to evaluate the fertility in the genome edited offspring. Thus, there is a need for establish methods for evaluating and selecting semen quality. Our objective was to establish sperm motility kinematic parameters for rhesus macaques, cynomologus macaques, and marmosets as well as evaluate whether season impacts rhesus semen quality in Primate Center indoor housing under constant environmental conditions. Utilizing a Hamilton-Thorne IVOS-II system, computer assisted semen analysis (CASA) was performed for 3 males of each species by assessing 6 fields in 2 chambers of a Leja 20 µm depth chamber with a minimum analysis of 200 sperm per chamber. Macaque ejaculates were incubated for 20 minutes to allow for liquification prior to being analyzed, whereas to analyze marmoset ejaculates, medium was added followed by a 90 minute swim-up incubation. Mean percent of motile sperm cells for rhesus, cynomolgus and marmosets were 68.93 ± 15.35 SD, 78.5 ± 7.98 SD, 69.23 ± 22.73 SD respectively, and progressive motility was decreased by ~12-19% relative to total motility. Kinematic parameters including amplitude of lateral head displacement, straightness and beat cross frequency parameters were significantly different between NHP species (Kruskal-Wallis test, *P* < 0.05). Despite being indoor-housed, rhesus macaque sperm total motility and progressive motility appeared to be impacted by season. However, kinematic parameters also varied greatly between individual males. Overall, these data establish a baseline for CASA kinematic parameters for three captive NHP species, in which implementation of CASA may serve as tool to evaluate NHP semen quality.

**P311 - Does miRNA378 have a role in modulating testicular aromatase activity?** Tana Jo Almand, Barbara Jean Nitta-Oda, Trish Berger

Testicular aromatase activity remained suppressed months after treatment with an aromatase inhibitor ceased in a previous study; this suggested reprogramming of testicular aromatase. Since continued suppression of estradiol synthesis appeared to influence maintenance of an enlarged Sertoli cell population, this study was designed to explore mechanisms of that reprogramming. Littermate pairs of boars were orally treated with letrozole (0.1mg/kg bw) or with canola oil vehicle from 1-16 weeks of age. Testicular tissue was collected from post pubertal boars and aliquots were flash frozen. Leydig cells were isolated from aliquots of testicular tissue at the time of collection and stored at -80°C prior to further analysis. As previously observed, aromatase activity remained suppressed (an approximate 35% reduction) in letrozole-treated boars compared with vehicle-treated littermates at 40 weeks of age (*P* < 0.05).
Although qPCR analysis indicated gene expression was numerically lower in letrozole-treated boars, this was not significant. These observations led to the hypothesis that miRNA378 suppressed synthesis of aromatase protein and consequently aromatase enzymatic activity in testes from letrozole-treated boars. This role for miRNA378 would be analogous to its role in porcine cumulus-oocyte complexes and granulosa cells. Expression of miRNA378 was evaluated in isolated Leydig cells from postpubertal boars and in testicular tissue from the same boars using endogenous miRNA103 as a reference. Expression of miRNA378 was increased in testes of letrozole-treated animals but decreased in Leydig cells from these same animals (P < 0.05). In preliminary results, additional miRNAs thought to interact with aromatase (miRNA423 and miRNA106) were not altered in Leydig cells from these same boars. The miRNA378 may modulate aromatase in porcine Leydig cells or may be altered in response to altered aromatase activity. (Supported in part by W3171 MSP, a W.K. Kellogg Endowment, and Jastro-Shields Graduate Research Funds.)

P312 - Testis-specific MAGE genes evolved to protect mammalian male germ cells against genotoxic and metabolic stress. Klementina Fon Tacer, Melissa Oatley, Tessa Lord, Jonathon Klein, Heather Tillman, P. Potts

The basic processes of spermatogenesis are surprisingly similar in even very distinct species, and the genes responsible are highly conserved. However, in addition to a set of conserved genes, a majority of reproductive genes evolve faster than their non-reproductive counterparts and drive lineage-specific evolution. One of the evolutionarily most intriguing classes is testis-specific genes that provide species-specific benefits to male germline. Their function, in particular in mammals, is still poorly understood. Here, we determine the function of a family of genes called melanoma antigens (MAGEs) that evolved in eutherian mammals and are normally restricted to the expression in testis but are often aberrantly activated in cancer. We showed that depletion of Mage-a genes in mice disrupted spermatogonial stem cell maintenance in culture and impaired repopulation efficiency in vivo. Furthermore, we showed that Mage-a genes confer resistance of male germline to genotoxic stress or long-term starvation that mimics famine in nature. Namely, Mage-a ko mice had impaired recovery after stress and reduced fertility. Furthermore, human MAGE-As, that are activated in many cancers, also confer resistance to metabolic stress and promote the growth of cancer cells. Our results suggest that mammalian-specific MAGE-A genes evolved to protect the male germ cells against diverse stressors, ensure reproductive success under non-optimal conditions, and are hijacked by cancer cells. Furthermore, understanding the physiological function of the testis-specific gene has clinical implication in cancer therapy development and in the fertility preservation of childhood cancer survivors.

P313 - Proper axonemal assembly requires wampa, an essential dynein for male fertility. Elisabeth Bauerly, Matthew Gibson
Axonemal dyneins are cytoskeletal motor proteins that form the inner and outer dynein arms of the axoneme for cilia and flagella. The molecular structure of motile axonemes are highly conserved across eukaryotes and are composed of bundles of microtubules that are arranged in a 9+2 manner. The microtubules in the outer 9 doublets contain inner and outer dynein arms that are required for proper movement of the flagellum. Defects in the dynein arms are the leading cause of primary ciliary dyskinesia (PCD), which is a type of ciliopathy that is characterized by chronic respiratory infections, situs inversus, and sterility. Despite current understanding of pathological features associated with PCD, many of their causative genes still remain elusive. Here, we analyzed a previously uncharacterized component of the outer dynein arm, *wampa (wam)*, that when mutated results in complete male sterility. Transmission electron microscopy revealed that wam homozygous mutants lack outer dynein arms (ODA) along the axoneme, which leads to a complete loss of flagellar motility within the sperm. Surprisingly, we also uncovered a multifaceted role for this gene throughout spermiogenesis, including in shaping of the nuclear head and in mitochondrial remodeling. The diverse phenotypes displayed in this mutant emphasizes the critical role that dyneins play during spermatogenesis and, interestingly, are reminiscent of phenotypes observed in mammalian counterparts that may suggest spermatogenesis between insects and mammalians is more similar than previously recognized. Due to the conserved nature of axonemal dyneins and their essential role in both PCD and fertility, our study will aid in the understanding of the functional role of dyneins throughout spermatogenesis and during axonemal formation.

**P314 - Effect of κ-Carrageenan on the Cryopreserved Sperm Quality of Canine Semen.**
Nabeel Talha, YuByeol Jeon, Il-Jeong Yu

Assisted reproduction techniques have been tried to solve the problems like infertility, preserving the species. For the successful reproduction, superb sperms are required. Therefore, the cryopreservation for storing high-quality sperm and to facilitating the handling of sperm is actively being studied. κ-Carrageenan, a polysaccharide, is obtained by heat extraction from the irish moss of the red algae. In this study, we conducted to find out the effect of κ-carrageenan on the canine sperm when it was added to the semen extender. Extender basically was composed of Trizma base, citric acid, glucose, penicillin G, streptomycin sulfate. Extender 1 was supplemented with 0.1%, 0.2%, 0.3%, and 0.5% κ-carrageenan, while extender 2 was supplemented with glycerol. After freezing-thawing, the motility, viability, acrosome integrity, apoptosis, and reactive oxygen species of sperm were measured to analyze the effects of κ-carrageenan in cryoprotectant. Motility was measured by the CASA system after thawing in 37°C water. Group of 0.2% κ-carrageenan was significantly higher total motility than control (p<0.05). Rapid progressive motility of 0.1% and 0.2% κ-carrageenan groups were significantly higher than the other groups (p<0.05). Acrosome integrity was assayed by stain of PSA-FITC with an optical microscope. Normal acrosome ratio in 0.5% κ-carrageenan was higher than the other groups (p<0.05). Apoptosis was measured with a FACSalibur. Treated groups of 0.1%, 0.2% κ-carrageenan were significantly higher (p<0.05). SYBER/PI staining was used for determination of viability and DCF staining was used for evaluation of ROS. Viability and ROS of treated groups were not significantly different from other groups. In conclusion, supplementation of κ-carrageenan to semen extender in canine cryopreservation improved sperm motility, acrosome integrity and prevented apoptosis.
We previously described a process termed sperm chromatin fragmentation (SCF) by which mouse sperm chromatin is degraded into roughly 25 kb fragments by incubation with MnCl₂. We proposed that a topoisomerase II–like enzyme either alone or in combination with a nuclease introduced double-stranded DNA breaks at the toroid linker regions (TRLs) in sperm chromatin. TRLs are the small stretches of nuclease-sensitive DNA that link protamine toroids (which are the major structural component of sperm chromatin, and are largely nuclease insensitive) and contain about 25 kb of DNA. Vas deferens sperm (Vas-sperm) fragmented their DNA to a much greater extent than epididymal sperm (Epi-sperm). However, in these previous studies, Vas-sperm, and occasionally Epi-sperm, degraded their DNA to fragments much smaller than the 25 kb under certain circumstances. Here, we demonstrate that this degradation to smaller DNA occurs only when the sperm preparations are digested with digestion buffer (DB) which contains SDS and DTT in preparation for gel electrophoresis. We show that SCF can be completely inhibited by preincubation with proteinase K, but this inhibition requires the presence of the non-ionic detergent Triton X-100, suggesting that the nuclease or activating factor for SCF is a protein sequestered behind a plasma membrane, either within the sperm cell, or in the surrounding vesicles, or both. SCF can also be inhibited by SDS. We are currently testing if EDTA can reverse the SCF induced DNA double stranded breaks. These data suggest that SCF degradation is limited to the TRL regions, thereby limiting the break sizes to around 25 kb. They also suggest that a nuclease is available to the sperm cell that aggressively digests DNA in the first few minutes of DB incubation as the protamines are removed. This work has implications for storing and using sperm in ART.

P316 - Nuclear DNA Damage is Clearly Reflected by Changes in the Human Sperm Proteome. Taylor Pini, Monika Dzieciatkowska, Jason Parks, Kirk Hansen, William Schoolcraft, Mandy Katz-Jaffe

Sperm nuclear DNA damage has been linked to lower fertilization rates, poor embryo quality, reduced implantation, and increased spontaneous pregnancy loss. However, DNA damage is not always correlated with other semen parameters (e.g. motility, morphology). In addition, even ejaculates with a significant proportion of DNA damaged spermatozoa can still be capable of establishing pregnancy. This raises the question of what the overall impact of DNA damage is on sperm physiology, including direct responses to the damage, as well as any secondary effects with potential consequences for reproductive success. To investigate these impacts on a molecular level, we performed a proteomic comparison by LC-MS/MS of human semen with low (0-4%, n = 7) or high (≥16%, n = 6) proportions of spermatozoa with DNA damage as assessed by TUNEL. A quantitative comparison was performed using normalized weighted spectra (NWS), compared by Student’s t-test. A total of 2511 proteins were confidently identified, with 78 significantly (p<0.05, fold change ≥ 1.5) differentially abundant proteins (30
decreased, 48 increased) in high versus low DNA damage samples. Proteins involved in DNA damage repair by nucleotide excision repair (DDB1), homologous recombination (PRMT5) and non-homologous end joining (TSNAX) were significantly increased in ejaculates with high DNA damage, suggesting an active transcriptional response to damage during spermatogenesis. Proteins with roles in apoptosis (ATP5IF1, GRHPR) and oxidative stress (PGD, FTH1) were also more abundant, confirming the dynamic interaction of these processes with DNA damage. Other notable impacted pathways included protein breakdown (26s subunits, NAGK), transcription and translation (aaRs, PRMT5, NME1) and iron homeostasis (FTH1, FDXR). Interestingly, several key sperm functional proteins (ENO4, KCNU1, LIPE, CEP70, CFAP100) were significantly decreased in ejaculates with high DNA damage, potentially contributing to poor reproductive performance. These results confirm the significant impact of DNA damage on sperm physiology, reflected by substantial protein-based changes.

**P317 - Suitability of different techniques of measuring sperm DNA damage and sperm preservation for remote labs: A comparative study.** Yasmine Issa, Uwe Paasch, Sonja Grunewald, Amira Eid

Evaluation of DNA damage in ejaculated spermatozoa is important as it is a potential cause of male infertility, as well as fear of introduction of a defective sperm to the oocyte when assisted reproduction techniques are used. Various techniques have been adopted to assess DNA damage. Of the widely accepted techniques are Acridine orange staining, SCSA (sperm chromatin structure assay) and TUNEL (Terminal deoxynucleotidyl nick end labelling). The first two techniques measure the susceptibility of sperm DNA to fragmentation after acid treatment while the later measures single and double strand DNA breaks. As the importance of measuring DNA damage is increasing, the need of some remote labs with lack of facilities to send the semen samples to a central lab has aroused. Our aim was to find out a suitable, sensitive feasible technique of measuring sperm DNA fragmentation as well as evaluating the best preservation method that would help send semen samples from remote labs to a central one, with the least compromise possible to the sperm DNA status. 20 patients and 20 donors were asked to donate their semen samples. Samples were divided into neat, rapid cryopreserved and slow cryopreserved aliquots. In each of the aliquots, DNA fragmentation was measured using Acridine orange, SCSA and TUNEL. Results showed higher DNA fragmentation in patients than in donors using both TUNEL and SCSA techniques while AO failed to detect such difference. Rapid cryopreservation showed the least When DNA damage in sperms. TUNEL and SCSA results correlated significantly while AO didn’t correlate with either of them in the corresponding aliquots. Rapid cryopreservation in Tris HCL-NaCl-EDTA (TNE) seems to be the most convenient method of transferring samples from remote to central labs while SCSA seems to be the most sensitive, time and cost saving technique.

**P318 - Metformin and Nigella sativa seed oil extract improves male reproductive parameters and histology following diet induced obesity in Wistar rats.** Kristian Leisegang, Walid Almaghrawi, Ralf Henkel
Excess adiposity causes testicular dysfunction and infertility. *Nigella sativa* (Ns) seed oil and metformin have demonstrated potential positive effect on obesity induced male infertility parameters. Wistar rats (n=54) were divided into six groups: normal chow (NC), high sugar diet (HSD) only, HSD and saline (S), HSD and metformin (75 mg/Kg/day) (Met), HSD and low dose Ns (200 mg/Kg/day) (NS200) and HSD and high dose Ns (400 mg/Kg/day) (NS400). HSD consisted of 33% NC, 33% sucrose and 7% sugar dissolved in 27% water for 14 weeks. Intervention was force fed for the last 8 weeks. Following euthanasia, total body weight (TBW), prostate, testes and epididymis weights, sperm concentration, motility, vitality and mitochondrial membrane potential (MMP), serum testosterone and lumen size of seminiferous tubule (ST) and cauda epididymis (CE) were compared. The HSD compared to NC group showed significant increases in TBW and organ weights, with reduced sperm concentration, progressive motility and testosterone. Compared to saline, metformin improved all organ weights, sperm concentration, MMP and testosterone. NS200 improved TBW and epididymis weight, and NS400 improved TBW, prostate and epididymis weight, sperm concentration, vitality, MMP and testosterone. HSD reduced ST and CE lumen size and corresponding epithelial cell height. Metformin, NS200 and NS400 all showed significant increases in ST and EC lumen size in obesity. Consistent with literature, an obesogenic diet has negative effects on TBW, reproductive organ weights, semen parameters and ST and CE lumen size. Metformin may be useful in obesity-induced infertility, without evidence of detrimental effects. This was similar for Ns, particularly at the high dose, supporting traditional claims and a positive effect on male fertility reported in the literature. This is likely mediated through antioxidant and immune mediating properties. Current evidence suggests Met and Ns require further investigation in the consideration of infertility management in obese males.

**P319 - Identification and in vivo Evaluation of Bioactive Flavonoids Isolated from Typha capensis Rhizome Extract on Leydig and Prostate Cancer Cells.** Kristian Leisegang, Abdulkarem Ilfergane, Edith Antunes, Ralf Henkel

*Typha capensis* (Tc), a Southern African medicinal plant, is traditionally used to treat male reproductive problems. Anecdotal evidence and limited studies suggest a beneficial effect on male reproduction. This study aimed to isolate and identify bioactive flavonoids from Tc rhizomes, and investigate their action on TM3 Leydig cells and LNCaP prostate cancer cells. Flavonoids were extracted from Tc rhizomes harvested during four different seasons. HPLC showed the F1 summer fractionation to have highest flavonoid concentration. After purification, NMR spectroscopy (1D and 2D NMR) revealed quercetin and naringenin to be the bioactive compounds. TM3 and LNCaP cells were subsequently exposed to quercetin and naringenin (0, 0.025, 0.05, 0.075, 0.1, 0.125, 0.15, 0.175, and 0.2 µmol/L). Exposures were classified as acute (24 h and 96 h single exposure) and chronic (96 h repeated exposure daily). Outcomes included cell viability (MTT), testosterone concentration and DNA fragmentation (TUNEL). In TM3 cells, quercetin, naringenin and combined exposures, did not affect viability for acute exposures. However, for chronic exposure, viability decreased for almost all concentrations, peaking at the mid-range. TM3 cells exposed to quercetin showed no acute effect on DNA fragmentation, with a dose-dependent increase for chronic exposures. In LNCaP cells, quercetin, naringenin and combined exposures showed a dose-dependent, negative effect on cell viability at all concentrations for all time-points. A significant and dose-dependent increase in the percentage of
cells with DNA fragmentation was observed at all concentrations of quercetin exposure. The summer harvest and the aqueous Tc extract have the highest concentration of flavonoids. Isolated quercetin and naringenin have a beneficial effect on testosterone production in Leydig cells, but may show toxicity with chronic exposure. The isolates further induce cellular stress and apoptosis in prostate cancer cells. These results suggest potential benefit in male hypogonadism and male prostate cancer patients that require further investigation.

P320 - Characteristics of Sperm Binding to the Mouse Oviduct. Kankanit Doungkamchan, David Miller

A wide variety of animals store sperm between mating and ovulation. While being stored in a reservoir, sperm are quiescent and their lifespan is lengthened. In some species, sperm are retained in the oviduct to form the reservoir by binding to the oviduct epithelium. Many studies have examined sperm adherence to isolated oviduct cell aggregates, an approach that ignores the complex architecture of the oviduct. Herein, we studied sperm binding to intact mouse oviducts ex vivo. Sperm were allowed to bind oviducts that had a single longitudinal slit to allow sperm entry. First, binding specificity was determined using the isthmus, ampulla, and trachea, another ciliated mucus-covered epithelium (n = 4). There was no difference in binding between isthmus and trachea, suggesting that sperm receptors are not uniquely found in the oviduct but binding to the ampulla was 22% less than the isthmus (P < 0.05). Sperm prior to and after capacitation were examined (n = 3). Following capacitation, the number of sperm bound to the isthmus was reduced by 78% (P < 0.05). Oviducts from females older than 20 months bound the same number of sperm as those younger than 3 months (P < 0.05), despite the reported increased stiffness of oviduct cilia from aged females. The number of bound sperm differed in oviducts collected from animals at different stages of the estrous cycle (P < 0.05); diestrus oviducts bound the most sperm (n = 3). Sperm binding was species-restricted because 93% fewer porcine sperm bound to the mouse isthmus (n =3). In conclusion, sperm adhesion ex vivo was not completely specific to oviduct epithelial cells or dependent on the age of the female providing the oviduct but was species-restricted, capacitation-dependent, and dependent on the stage of the estrous cycle.

P321 - Testicular macrophages: local control of phenotype, function and turnover. Sudhanshu Bhushan, Ming Wang, Britta Klein, Monika Fijak, Andreas Meinhardt

Infection and inflammation of the male reproductive tract are significant, and potentially curable, causes of male factor infertility. The defined clinical entities comprise urethritis, prostatitis, seminal vesiculitis and epididymo-orchitis. Testicular macrophages (TM) are heterogeneous and comprise the largest immune cell population in the male gonad. TM are located in the testicular interstitial space and play an essential role in maintaining normal organ functions, i.e. steroidogenesis and spermatogenesis. TM contribute to the establishment of testicular immune privilege by displaying an immunoregulatory M2 macrophage phenotype. In this regard, TM are characterized by the low expression of pro-inflammatory genes such as IL-6 and TNFa with concomitant high expression levels of a large number of M2 macrophage phenotype genes such as
IL-10 and CD163. It was long assumed that the testicular interstitial fluid includes factors that determine TM function and phenotype. We show that the testicular interstitial fluid shifted GM-CSF–induced M1 toward the M2 macrophage phenotype. IF-polarized M2 macrophages mimic the properties of TM, such as increased expression of CD163, high secretion of IL-10, and low secretion of TNF-α. Of note, among the testicular cells, TM produce significant amounts of corticosterone that acts glucocorticoid-receptor-dependent in an autocrine manner to sustain the M2 phenotype of macrophages. Further data obtained with parabiotic (CD45.1/CD45.2) and Ccr2<sup>−/−</sup> mice suggest that circulatory monocytes contribute minimally to the TM pool in normal adult mice. However, under inflammatory conditions, circulatory monocytes derived macrophages infiltrate the testis, and as a consequence can impair spermatogenesis, possibly by abrogating immune privilege.

**P322 - Male mice housed in the International Space Station sire healthy offspring.** Taichi Noda, TakaFumi Matsumura, Masafumi Muratani, Risa Okada, Mutsumi Yamane, Ayako Isotani, Takashi Kudo, Satoru Takahashi, Masahito Ikawa

The era of commercial space flight and tourism is fast approaching, but the impact of space on the physiological function of the human body remains unclear. To address this concern, the Japan Aerospace Exploration Agency developed mouse cages that can be installed in the centrifuge equipped Biological Experiment Facility in the International Space Station (ISS). These cages housed 12 male mice under artificial gravity (≈ 1 gravity) (AG) and microgravity (MG) in the ISS for 35 days, afterwards all mice were safely returned to earth (Shiba et al., Sci Rep, 7, 10837, 2017). Mice caged on the earth during the 35 days served as a ground control (GC).

Here, we characterized the effects of space environment on the male reproductive system on these mice after their return to earth. Specifically, the physiological function of male reproductive organs (testis, epididymis, and accessory glands) was examined by histology and gene expression analysis using RNA-seq. The sperm fertilizing ability and developmental rates of fertilized eggs from AG and MG mice were compared with GC mice. The results of organ function, expression analysis, fertility rates, and viability of the offspring will be presented.

This work was supported by prioritized research selected for life sciences commissioned by the Japan Aerospace Exploration Agency (14YPTK-005512).

**P323 - Incorporation of cystine via Soluble Carrier Family 7 Member 11 (SLC7A11) is part of the redox regulatory mechanism in spermatozoa.** Fernando Peña, Jose Ortiz- Rodríguez, Francisco Martin-Cano, Cristina Ortega Ferrusola

Oxidative stress is considered as a major mechanism causing sperm damage during cryopreservation and storage, but also behind male-factor infertility. At present, oxidative stress is no longer considered as caused only by overproduction of reactive oxygen species, but rather by the deregulation of redox signaling and control. With this concept in mind we hereby describe
for the first time the presence of the xCT antiporter which exchanges extracellular cystine (Cyss) for intracellular glutamate in stallion spermatozoa as well as its impact on sperm function and investigated using the specific inhibitor sulfasalazine. Spermatozoa incubated in the presence of cystine increased intracellular GSH content with respect to controls \( (P<0.01) \) by 50% in fresh extended stallion spermatozoa and by 30% in frozen-thawed spermatozoa; an effect which was prevented by sulfasalazine in the media. Cyss supplementation also reduced the oxidation-reduction potential of spermatozoa, with sulfasalazine only preventing this effect in fresh spermatozoa incubated for three hours at 37°C but not in frozen-thawed spermatozoa. While sulfasalazine reduced sperm motility in frozen-thawed spermatozoa, it increased motility in fresh samples. The present findings provide new and relevant data on how spermatozoa regulate their redox status, suggesting a different redox regulation in cryopreserved spermatozoa thus providing new clues to improve current cryopreservation technologies and for the treatment of male-factor infertility.

**P324 - Localization and expression of the vacuolar ATPase and cytokeratin 5 during postnatal development in the pig epididymis.** Bongki Kim, Yun-Jae Park, Yu-Da Jeong, Gye-Woong Kim, Ji-Hyuk Kim, Hack-Youn Kim, Hee-Bok Park, Sun-Young Baek, Hak-Jae Chung

An elaborate intercellular communication network among several major cell types such as principal (PCs), clear (CCs), and basal cells (BCs), is essential to create an optimum luminal environment in the epididymis. The aim of this study was to examine the localization and expression of V-ATPase and cytokeratin 5 (KRT5), which are known as the markers for CCs and BCs, respectively. Immunofluorescence labeling for V-ATPase showed that CCs were present in all epididymal regions except for the proximal caput at birth. At 5 days old epididymis, the localization of CCs continued moving forward to the efferent duct (ED) as well as the proximal caput of the epididymis. The CCs were identified with a goblet-shaped body along the entire epididymis and did not reveal obvious morphological differences among regions of the epididymis until 4 months of age, unlike in the rodent. In addition, we observed that the CCs labeled by V-ATPase disappeared from corpus to cauda, and only maintained in the caput epididymis at adults. On the other hand, BCs labeled by KRT5 were present in distal cauda and vas deferens (VD) at birth, and in all regions of the epididymis at 2 weeks of age. BCs were localized at the base of the epithelium and no BCs with a luminal-reaching body extension were observed in all regions of the epididymis. In summary, 1) both CCs and BCs progressively appear in a retrograde manner from the VD and cauda epididymis to the caput and ED. 2) the plasticity of CCs and BCs was not observed as mouse epididymis. 3) Interestingly, CCs disappear from corpus to cauda, and only maintain in the caput epididymis at adults, raising questions about the possibility of the presence of other mechanisms to maintain the acidity of the luminal fluid in the epididymis.

**P325 - Investigation of Regulatory Mechanism of Quiescin Sulphydryl Oxidase 2 (QSOX2) in the Epididymis.** Tse-En Wang, Shiori Minabe, Hiroko Tsukamura, Matsuda Fuko, Pei-Shiue Tsai
Quiescin Sulphydryl Oxidase 2 (QSOX2) was characterized as a Golgi-associated protein in proximal region of the epididymis where initial sperm maturation occurs; moreover, the secreted QSOX2 is able to attach at specific sperm surface via epididymosomes. These findings suggest that QSOX2 might be delineated as a critical protein contributing to sperm maturation processes temporally and spatially. However, the underlying regulatory mechanism remains to be elucidated. Sex hormone modulation is known to be important to the integrity of epididymal structure and function during postnatal organ development. Thus, we harvested and analyzed the epididymal protein sample from different developmental stages (20, 30, 40, 50, 60, 80 postpartum day). The results showed that QSOX2 protein expression was in synteny with testosterone surge during development. Furthermore, we applied Kiss1 knock-out rats that are deficient in sex hormone production and subcutaneously administrated compensated testosterone for further investigation. In contrast to Kiss1 knock-out control rats, epididymal QSOX2 was profoundly rescued by testosterone stimulation. Intriguingly, we didn’t observe the same phenomenon in other male reproductive organs, such as seminal vesicle or testis. This suggests that upregulation of QSOX2 by testosterone is in epididymis-specific manner. In conclusion, our observations not only indicate potential involvement of epididymal QSOX2 in sperm maturation, but also provide new insight in the participation of testosterone in the regulation of epididymal QSOX2 protein synthesis.

**P326 - Calcium and sperm motility in Drosophila melanogaster.** Halli Weiner, Frances Sunga, Mollie Manier

Calcium is required for proper sperm function in mammals. However, the drivers of sperm motility in insects remain a mystery. Unlike most mammals, female insects store sperm in specialized storage organs for extended periods. In *Drosophila melanogaster*, this takes place in the seminal receptacle (SR). Sperm have been observed swimming in head- and tail-leading directions within the SR, and sperm with slower motility outcompete faster sperm from rival males. A calcium channel (Pkd2) has been implicated in regulating waveform movements in sperm, but we asked whether ambient calcium levels could also influence sperm motility. Males with sperm tails expressing GFP and sperm heads expressing RFP were mated to wild type females. Female reproductive tracts were dissected into 50 µL of (1) Grace’s Insect Medium, 1.0 g/L CaCl$_2$ (n=20) (GIM, control), (2) Grace’s Insect Medium, 1.5 g/L CaCl$_2$ (n=20) (GIM(+)), (3) Schneider’s Insect Medium, 0.6 g/L CaCl$_2$ (n=20) (SIM(+)), and (4) Schneider’s Insect Medium, 0.0 g/L CaCl$_2$ (n=20) (SIM(-)). Motility (%) was measured at 0, 5, 10, 15, 30, 45, and 60min following dissection. For all treatments over time, the proportion of non-motile sperm increased, and highly-motile sperm decreased, but not in a calcium-dependent manner. At 30min, the proportion of highly-motile sperm was significantly decreased in SIM(-) (5.5% ± 3.3 SEM) compared to SIM(+) (21.5% ± 8.1 SEM), GIM (22.6% ± 7.8 SEM), and GIM(+) (22.5% ± 8.6 SEM). Medium type (i.e. SIM vs. GIM) affected the proportion of slowly-motile sperm at 5min, 45min, and 60min, but not in a calcium-dependent manner. Furthermore, sperm directionality does not appear to be influenced by constant ambient calcium levels. Further investigation into sperm metabolism during storage and naturally occurring changes in calcium levels is necessary to understand the cellular processes and molecular mechanisms behind this phenomenon.
**P327 - Splicing up your sex life; Why men are failing to produce in the bedroom.**  Mark Baker, Jacob Netherton, Gary Hime

Male infertility is a very common condition, with reports suggesting that one in 15-20 men of reproductive age are affected. Understanding why or how men produce defective sperm is a question that has remained elusive. We have used proteomic screens to identify mechanisms responsible for building defective sperm in men. Significantly, we have found regulators of alternate splicing appear to be a major key; being more abundant within infertile spermatozoa.

To understand this, we overexpressed specific alternate-splicing regulators within *Drosophilia*. Amazingly, our data show that sperm overexpression RNA-splicing regulators produced typical patterns of “male-factor” infertility, including (i) decreased amounts of sperm production, (ii) head morphology defects and (iii) poor sperm motility. Furthermore, fertility data demonstrate changes to alternate splicing have dramatic consequences. Fly strains ranged from completely infertile to extremely subfertile. This data strongly suggest that aberrant alternate splicing is likely to play a major role when it comes to the production of poor quality spermatozoa, and male factor infertility.

**P328 - The effect of STM paste® on the viability of bovine cryopreserved spermatozoa with egg yolk plasma and native phospho caseinate containing Triladyl diluent.**  Sung Woo Kim, Yeun Hye Yu, Chan-Lan Kim, Seung Rye Choe, Namtae Kim, Yeoung-Gyu Ko

Equex STM paste was a well-known mild detergent and has been used for sperm cryopreservation. However, it is hard to purchase because the termination of production was done by the company. So, substitute materials needed to get the same result and methodological procedures must be prepared for standard cryopreservation processes. We have found one substitute from mini tube, STM paste, and it was used for egg yolk plasma preparation for standard procedures. Egg yolk from Amerucana and Korean native gray line was mixed with 140 mM NaCl solution with 0.2, 0.4, 0.8 or 1.6% STM paste for 1 hr and was centrifuged 10,000 g for 1 hr with two times. 10% of clear egg yolk plasma was added in Triladyl diluent with 3% native phosphor caseinate (NPC). The NPC was precipitated by ultracentrifugation with 30,000 g for 1hr from UHT treated milk. When the semen from Korean native bulls were cryopreserved, the survival rates of 0.2, 0.4, 0.8 or 1.6% STM paste containing diluent were 55.5±1.8, 91.3±4.3, 69.9±4.3 and 50.6±1.0. The egg yolk containing Triladyl diluents from 2 lines were also compared with 0.4% STM paste treated egg yolk plasma Triladyl diluents. Interestingly, G line egg yolk containing diluent showed higher motile sperm ratio than Amerucana egg yolk one (72.8±1.5 vs 68.1, \( P < 0.05 \)). However, STM paste treated egg yolk plasma from Amerucana egg yolk showed higher viability of cryopreserved and thawed spermatozoa than G lines egg yolk plasma (89.5±7.5 vs 86.1±1.5).

**P329 - Sperm Tail Mitochondrial Sheath Length Correlates with Bull Fertility Outcomes.**  Grace Wiley, Eriklis Nogueira, Camile Sanches, Karl Kerns, Peter Sutovsky

The ability to predict male fertility is desirable for bulls used in artificial insemination (AI). It is known that sperm tail mitochondrial sheath (MS) length correlates with reproductive traits of
mice, fish, and birds. However, when examined in higher species, such as the boar, unpublished data from our lab shows that as the average sperm tail midpiece increased, so did the percent abnormal morphology. The purpose of this study is to examine if the MS length will correlate with bull fertility, mirroring the findings in boar. It was hypothesized that as the mitochondrial sheath lengthens, the increased energy production/metabolism of the spermatozoa will impact bulls’ AI fertility. Sperm samples fixed in formaldehyde were stained with the epifluorescent probes/dyes lectin PNA (Arachis hypogaea/peanut agglutinin), aggresome (AGG), and diamidino-2-phenylindole (DAPI) then analyzed with Metamorph software on a Nikon Eclipse E800 with the 60x objective. Measurements of the sperm MS were made and results were statistically analyzed with SAS program for correlation between the known fertility rates of 71 bulls. The pregnancy/artificial insemination (P/AI) data was collected from 43’231 Nelore cows inseminated by timed AI (TAI) with basic protocol using frozen-thawed semen from 21 Nelore and 50 Angus bulls. The pregnancy rates were evaluated by transrectal ultrasonography 30-45 days after TAI. Differences in P/AI were found between bulls (P<0.001) and between breeds – Nelore: 54.44%, and Angus: 49.23% (P<0.001). Also, a difference was found in MS length between breeds (Nelore: 9.74±0.31 μm vs Angus= 9.35±0.28 μm, P<.0001). We found a positive correlation (0.38, P=0.0006) between the MS length and fertility rates between bulls, which demonstrates that mitochondrial sheath structure influences fertility. The correlation discovered here could contribute to improving bull selection in AI, aiding overall productivity within the cattle industry. Supported by NIH 1R01HD084353 (PS) and MU F21C Program (PS).


The purpose of this study was to determine subcellular localization of the Y-linked PRAME (PRAMEY) protein during sperm maturation. Expression of PRAMEY in the head, tail, and mitochondria of bovine caput and cauda sperm were examined by western blot with a PRAMEY-specific antibody. Results from 3 bulls were analyzed by Image J and data was compared by one-way ANOVA with post-hoc Tukey test. There have been 4 isoforms identified for PRAMEY (58, 30, 26, and 13 kDa), but the 58 kDa isoform was weakly expressed in this experiment, therefore it was not evaluated. When comparing the 30, 26, and 13 kDa isoforms of PRAMEY individually, we found a remarkable decrease of 10.9, 5.4, and 3.8 fold respectively, in protein expression from the caput to cauda epididymis in sperm heads, but there was a small decrease in the expression for both the 30 and 26 kDa isoforms in sperm tails (1.9 and 1.2 fold respectively). In contrast, the 13 kDa isoform increased 4 fold in sperm tails from caput to cauda, suggesting this isoform may have a significant role in tail function, but is likely not as important in sperm head function of cauda spermatozoa. When expression levels of all isoforms were combined, PRAMEY expression was nearly equivalent for both caput sperm head and tail. Alternatively, cauda sperm tails have an expression 6 fold higher than cauda sperm heads, suggesting the role of PRAMEY in tail function. We also evaluated PRAMEY in isolated mitochondria from caput and cauda sperm. Similar to the PRAMEY dynamic observed in sperm tail during maturation, the 30 and 26 kDa isoforms decreased in sperm mitochondria from caput to cauda, whereas the 13 kDa isoform increased. In conclusion, the subcellular localization during sperm maturation suggests the involvement of PRAMEY, especially the 13 kDa isoform, in sperm motility.
P331 - Evaluation of the G Protein-Coupled Receptor 56 (GPR56) in Mice: Phenotypic Effects in the Testis and Epididymis, and Localization in Spermatogenic Cells and Sperm. Madeleine Purcell, Jolene Davis, Steven Neal, Maria Battistone, Sylvie Breton, James Foster

GPR56 is a member of the adhesion family of GPCRs and is involved in several important developmental processes (e.g. cerebral cortex) as well as in several pathologies (e.g. melanoma, polymicrogyria). A knockout mouse model has shown that GPR56 is necessary for normal testis development and male fertility (Chen et al, Dev Dyn. 2010) and our further analysis has confirmed and extended this. Westerns showed that GPR56 is expressed at similar levels in the caput, corpus, and cauda epididymis. In the Gpr56-/- testes, a small percentage of seminiferous tubules were normal and a higher percentage were mildly to severely deformed. The Gpr56-/- cauda epididymides appeared somewhat distended and less firm than the wild-type, and also the epithelium was significantly thinner and the lumen wider than the wildtype. There were few sperm and many residual bodies in the lumen. The low numbers of sperm are likely due to developmental abnormalities in the testis and the appearance of excess residual bodies suggest a role for GPR56 in residual body uptake in the testis. Immunofluorescence microscopy (IF) of the entire epididymis showed that the aquaporin 9 (AQP9) protein expression was reduced in the Gpr56-/- mice. Areas of wavy, slightly disorganized basement membrane were seen in Gpr56-/- epididymides, which is similar to that seen in other tissues. By IF we found that the GPR56 protein in germ cells and sperm colocalized with the acrosomal marker ZP3R and was not detected on the cell surface, which means that in order for it to function as a canonical GPCR it would have to relocate to the cell surface at a later stage in the female reproductive tract. Overall, our results confirm that GPR56 is involved in multiple steps of the male reproductive tract. Supported by R-MC SURF, and the Jeffress Memorial Trust.

P332 - Proteins of The Seminal Plasma Of Romosinuan Bulls And Its Relationship With The Spermal Quality In Times Of Rain And Drought, Under The Conditions Of The Valle Del Sinú. Natalia Herrera

With the objective of establishing the effect of the climatic period on the expression of seminal plasma proteins and seminal quality variables of Romosinuano bulls. We worked with PS obtained from 10 RS bulls, both in the rainy season and in the dry season. Performing the andrological examination and evaluation of motility sperm characteristics (M%), vitality (V%), morphology and sperm concentration (spz / mL) by traditional methodologies, in addition the response to the hypoosmotic test (Host) and thermoresistance test (TTR). Analyzing the expression of PS proteins in the dry and rainy season, by means of one-dimensional and two-dimensional electrophoresis, identifying qualitative and quantitative changes derived from possible differences in the climatic period. Additionally, correlation tests were performed among seminal quality parameters: MIRP, Sperm concentration, Host test, viability and the relative amount of each protein point. The results obtained show that there are differences in the values of the variables analyzed between the study periods, with the seminal quality being better in dry season compared to the rainy season. The results of the one-dimensional electrophoresis suggest that the most abundant proteins in it PS, present throughout the year are 12kDa and 14kDa. The
analysis of the relative amounts of each PS protein suggests that there is a positive effect of the dry season on protein expression and an increase of proteins in the dry season, while MIRP and Host Test parameters showed a positive statistical correlation with 26 and 30 proteins respectively. The positive effect of the dry season on PS protein expression could be due to thermal stability during this time. The results obtained show a behavior of the Romosinuano race against the agroclimatic conditions of the region and that the most propitious time for semen collection is dry.

**P333 - Free Fatty Acid Release from Cauda-isolated Mouse Sperm.** Theodore Chauvin, Kenneth Roberts

At the end of spermiogenesis, testicular sperm have acquired much of their mature morphology. With the exception of their mitochondria, sperm lose most of their histologically recognizable organelles critical to cellular function during spermiogenesis. As a result of these morphological changes, sperm have been viewed as unable to carry out many of the metabolic pathways common to somatic cells. Using published mouse sperm proteomes, we have learned that sperm contain all of the enzymes necessary for liberation of free fatty acids (FFA) from a triacylglycerol (TAG) pool, that they transport these acyl lipids to the mitochondria, and they break down these FFA via beta-oxidation.

To investigate whether there is a TAG pool, we utilized Nile Red, a lipophilic stain that stains intracellular lipid droplets, to stain spermatozoa isolated from the cauda. The stain localized to the midpiece region, overlying the area where mitochondria are located. We believe the proximity of these lipid deposits is due to the sperm’s utilization of fatty acids stored as a source of energy through Beta-oxidation. We observed a significant decrease in TAG in sperm that were allowed to swim for an hour. We also observed a four-fold increase of FFA released from sperm when sperm swam in capacitating medium conditions for one hour.

Lipid droplets are usually surrounded by perilipins and, using immunocytochemistry, we have localized Perilipin 3 (Plin3) to the midpiece in spermatozoa. Furthermore, we also localized hormone-sensitive lipase (Lipe) to the acrosome region of sperm. Lipe is a lipase that can liberate FFA from TAG.

Our preliminary data suggests that sperm may be utilizing TAG as an energy source. This may be important for providing energy during storage of sperm in the epididymis. It may also serve to provide a store of energy when sperm are released into the female tract.

**P334 - Derivation of Organoids and Gene Expression in Epididymal Columnar Cells.** Daniel Cyr, Tifen el Belaidi, Sylvie Pinto, Laurie Pinel, Julie Dufresne, Mary Gregory

Differentiated epididymal cells (principal, apical, clear and basal) are derived from undifferentiated small columnar cells during postnatal development. There is, however, little
information regarding columnar cells. The objectives of this study were: (1) to assess gene expression profiles in epididymides from 7 day old versus adult rats, in order to identify signaling pathways implicated in the differentiation of columnar cells; and (2) to determine whether or not these cells can form organoids and differentiate in vitro. Total RNA was isolated from epididymides of one week old and adult (60 days) rats. Microarray analyses revealed a differential expression of over 3000 genes in epididymides of immature versus adult animals. Multiple signaling pathways were primarily associated with columnar cells of young rats, including the androgen receptor pathway, EGFR pathway, IGF2 pathway, and WNT pathway. Interestingly, genes implicated in the estrogen receptor pathway was expressed only in adults. To assess whether or not columnar cells displayed stem cell properties, cells were subjected to 3-dimensional culture (3D). Single cell cultures rapidly formed robust organoids. Organoids cultured for 7 days expressed connexin 26 (GJB2) and the stem cell marker LGR5. Furthermore, some of the cells within organoids expressed the basal cell marker p63. By 14 days of culture, some of the organoids expressed the principal cell marker AQP9. To further characterize columnar cell organoids, cells were purified by magnetic separation using an antibody against integrin-alpha-6 (ITGA6). Organoids were derived from ITGA6+ cells, while no organoids were developed by ITGA6- cells. Together these data indicate that columnar cells represent an epididymal stem/progenitor cell population. Supported by NSERC and CIHR.

**P335 - Female reproductive life span is determined by cytoplasmic enrichment during oocyte differentiation in mice.** Nafisa Nuzhat, Kanoko Ikami, Haley Abbot, Allan Spradling, Lei Lei

Mammalian females are endowed with a finite pool of primary oocytes in fetal/neonatal ovaries. The number of primary oocytes that can develop to mature eggs determines ovarian function and thus female reproductive life span. Our previous study in mice revealed that during oocyte differentiation in mouse fetal ovaries, germ cells derived from one primordial germ cell are connected via intercellular bridges, forming germline cysts. Within germline cysts, 20% of the germ cells collect cytoplasmic content from sister germ cells and differentiate into primary oocytes. The remaining germ cells undergo apoptosis after donate their cytoplasm. In the present study, based on the defects in germline cyst formation, cyst fragmentation, and cytoplasmic enrichment characterized in *Tex14* mutant ovaries, we uncovered a new mechanism underlying female reproductive life span. In *Tex14*⁻/⁻ fetal ovaries, germ cells divide to form cysts, in which sister germ cells are connected in syncytia or due to fragmented cell membrane. The female-specific mitotic defect led to smaller cysts and a significant reduction in primary oocyte number in postnatal day 4 (P4) ovary. However, in the absence of bridges in *Tex14*⁻/⁻ cysts, primary oocytes form with precious cytoplasmic enrichment and larger volume. By contrast, cysts in *Tex14*⁺/⁺ mutant were in larger size. The *Tex14*⁺/⁺ P4 ovary contained more primary oocytes but in smaller cell volume. Comparable numbers of developing oocytes and ovulated oocytes were found in young adult wildtype and mutant ovaries. Interestingly, 2 times shorter half-life of primary oocytes resulted in a significantly smaller primary oocytes pool in *Tex14*⁺/⁺ by 8 months, which was comparable to that of *Tex14*⁻/⁻ ovaries. In summary, the present study revealed that adult ovarian function is not determined by the size of primary oocyte pool, instead, the proportion of primary oocytes with enriched cytoplasm during oocyte differentiation.
**P336 - Maternal RNAs methylation profiles in oocyte and somatic cells.** Karine Dubuc, Isabelle Gilbert, Alexandre Bastien, Géraldine Delbès, Claude Robert

During oogenesis, majority of the resources needed to support early embryonic development are stored. These reserves are used in a coordinated manner to support first cells divisions until embryonic genome activation. Amongst these reserves are stored mRNAs that are used to support protein synthesis in absence of nuclear transcription. These stored transcripts exhibit an extended half-life that can be estimated in days rather than in minutes as generally seen in somatic cells. So far little is known about the stabilization process and mechanisms associated with the sequential recruitment from storage to translation. We hypothesized that in addition to the protection offered by protein complexes, post-transcriptional modifications are playing a role in stabilizing and managing maternal mRNAs. So far, more than a hundred distinct chemical modifications can be found on RNA molecules including methylation. The function of the epitranscriptome is starting to be explored and is mainly unknown. The project’s global aim is to characterize the potential of RNA methylation in governing protein production.

Similarity to the common concept of epigenetics, writers, readers and erasers of RNA methylation have been detected by microscopy in mouse, porcine and bovine oocytes and tissue sections. Targeted writers were DNMT2 for r5mC and METTL3 for rN6mA whereas the candidate reader was MBD and candidate erasers were FTO and ALKHB5. Results demonstrate subcortical localization of these proteins. We also measured the abundance of modified nucleotides such as r1mA, rN6mA, r5mC and r7mG by mass spectrometry from total somatic tissues RNA that was contrasted with maternal RNAs from oocytes. Oocyte had significantly lower abundance of r1mA and higher rN6mA than liver RNAs used as reference. The presence of RNA post-transcriptional modifications in oocyte suggests the existence of a layer of epigenetic regulation at the RNA level.

**P338 - Dynamic TAF Expression and Function in Establishing the Ovarian Reserve.** Megan Gura, Kimberly Seymour, Richard Freiman

Proper embryonic female germ cell development is critical for the healthy establishment of the adult ovarian reserve. TBP-Associated Factor 4B (TAF4B) plays a crucial role as an embryonic transcription regulator in the development of the ovarian reserve. TAF4B is a subunit of the general transcription factor TFIID complex, which is required for RNA Polymerase II recruitment in gonadal tissues. TAF4B is the paralog of TAF4A, which is broadly expressed in most somatic tissues. Taf4b-deficient female mice are completely infertile, and the etiology of this infertility is seen during germline embryonic development. We have re-processed publicly available RNA-seq datasets and examined Oct4-GFP embryonic ovaries to understand in what ovarian cell types Taf4b mRNA expression can be found and how this expression may change over time. We find that Taf4b mRNA and protein are consistently enriched in the germ cells of the embryonic ovary (E9.5-E18.5) and Taf4b mRNA expression significantly increases soon after meiotic initiation. Furthermore, we find that Taf4b, but not Taf4a, mRNA is significantly reduced in Dazl- and Stra8-KO E14.5 ovaries. Using RNA-seq on sorted GFP+ germ cells, we have identified a number of
critical oogenesis genes whose expression is dependent on TAF4B. To our surprise, Taf4b was not the lone TAF with these characteristics during early female germ cell differentiation and regulation by Dazl/Stra8. Several other notable TAFs such as Taf7l and Taf9b also display similarly dynamic germ cell expression patterns. Together these data suggest that specialized sub-complexes of TFIID subunits uniquely integrate transcriptional programs required to correctly establish the initial mammalian ovarian reserve.

**P339 - Non-canonical activity of retinoic acid stimulates ERK1/2 pathway to regulate meiotic initiation in mouse fetal germ cells.** Yukiko Yamazaki, Toshifumi Yokoyama, Ferhat Ulu, Sung-Min Kim

In mouse germ cells, retinoic acid (RA) is an extrinsic cue for meiotic initiation that stimulates transcriptional activation of the Stimulated by retinoic acid gene 8 (Stra8), which is required for entry of germ cells into meiosis. In general, RA molecules move into the nucleus and bind to nuclear RA receptors for their biological activities (canonical activity of RA). Importantly, recent studies in somatic cells revealed that RA quickly activates signal transduction pathways in the cytoplasm to regulate multiple cellular processes (non-canonical activity of RA). In this study, we examined the non-canonical activity of RA in fetal germ cells. Using a germ cell culture system, we investigated (1) whether RA treatment activates any mitogen-activated protein kinase (MAPK) pathways in fetal germ cells at the time of sex differentiation, and (2) if this is the case, whether the corresponding RA-stimulated signaling pathway regulates Stra8 and meiotic marker (Rec8, Dmc1, Syce3, Spo11) gene expressions for entry into meiosis. To determine the effect of RA on the activities of intracellular signal transduction pathways, XX fetal germ cells at E12.5 were cultured with RA and then subjected to western blot. Interestingly, RA treatment appears to predominantly stimulate the extracellular-signal-regulated kinase (ERK)1/2 pathway in the germ cells. ERK1/2 inhibitor (U0126) treatment suppressed RA-induced Stra8 expression at both the mRNA and protein levels, even in the presence of RA. U0126 treatment also dramatically suppressed meiotic marker expressions and reduced numbers of γH2AX-positive meiotic cells among cultured XX fetal germ cells. Finally, we also observed similar results in XY fetal germ cells at E13.5 cultured with RA and U0126. Taken together, our results suggest the novel concept that the RA rapidly functions by stimulating the ERK1/2 pathway and that this activity is critical for Stra8 expression and meiotic initiation in fetal germ cells.

**P340 - Cumulus oocyte complexes can be recovered from small antral follicles following processing of pediatric ovarian tissue for cryopreservation.** Luhan Zhou, Jordan Machlin, Farners Amargant, Courtney Harris, Erin Rowell, Monica Laronda, Francesca Duncan

Life preserving cancer treatments may compromise reproductive function. For young girls, ovarian tissue cryopreservation (OTC) may be the only option for fertility preservation. This procedure involves removing ovarian tissue, isolating the cortex from the medulla, and cryopreserving the cortex containing primordial follicles. During this process cumulus oocyte complexes (COCs) are released from small antral follicles into the media, which is typically
discarded. The recovery of COCs from this media may provide an additional fertility preservation option for pediatric girls. However, our understanding of the yield and quality of these recovered COCs is minimal in this population. Therefore, our goal was to analyze the tissue processing media for COCs from pediatric girls undergoing OTC through IRB-approved protocols. Over six months, we received media from 16 participants between 0.5 – 22 years old (average 9.4 ± 1.8 years) and Tanner Stage from 1 – 5 (50% stage 1, 0% stage 2, 6% stage 3, 31% stage 4, and 13% stage 5). OTC cases were performed in individuals with malignant (94%) and non-malignant conditions (6%). Half of the participants had received chemotherapy, radiation, or combination of both prior to OTC. From the media we recovered intact COCs, denuded and degenerate oocytes. An average of 5.5 ± 1.8 intact COCs were recovered per participant. A history of previous treatment was associated with a reduced yield of COCs (average 1.4 ± 0.7 intact COCs; P = 0.01). COCs contained oocytes with diameters ranging from 78.8 um to 125.8 um (average 105.7 um ± 1.1). We previously demonstrated that oocytes within COCs from an adult population must be >112 um in diameter to resume meiosis and produce mature gametes. Thus, the majority of oocytes within this pediatric population may lack meiotic competence, necessitating specific methods to mature these oocytes in vitro, which are currently under investigation.

**P341 - Poorly-controlled Severe Type 1 Diabetes Mellitus Impairs LH-LHCGR signaling in Ovary and Decreases Female Fertility in Mice.** Jaewang Lee, Jihyun Kim, Wontae Kim, Jung Won Choi, Jin Hyun Jun, Teresa Woodruff

Purpose: The aim of this study was to investigate how the type I diabetic condition affects the development of oocytes and folliculogenesis—from follicle growth to embryonic development

Materials and methods: A comparative animal study was carried out by using two different mouse models of T1D: a genetic AKITA model and a streptozotocin-induced model

Results: Both T1D model animals definitely increased blood glucose level while only STZ-injected mice reduced body weight. Also, folliculogenesis, oogenesis and even preimplantation embryogenesis were impaired by an induction of T1D in mice. With respect to the body weight and blood glucose, streptozotocin-induced mouse showed more severe T1D compared with AKITA mouse. Interestingly, the mice caused T1D by an exogenous streptozotocin injection dramatically decreased ovary size, LHCGR expression in the ovary, the number of corpus luteum per ovary, oocyte maturation and even serum progesterone which might be correlated with LH-LHCGR interaction. However, there were no significant differences in number of oocyte retrieved after superovulation, serum hormones level (17 b-estradiol, AMH and testosterone) and oocyte microorganelles between STZ-injected and AKITA mouse. Both T1D model animals significantly reduced the pre-implantation embryo quality. However, there was no significant difference between STZ-injected and AKITA mouse.

Conclusion: These results suggested that severe T1D might be involved in the regulation of folliculogenesis, oogenesis and embryogenesis in mice. However, the exact physiological roles of diabetic condition need to be further elucidated and it may even contribute to provide insights to overcome metabolic syndrome-related reproductive complications.
**P342 - Follicle-stimulating hormone receptor is expressed in bovine preantral follicles from primary to full secondary stage of development.** Juliana Candelaria, Anna Denicol

Ovarian follicular development is foundational to successful conception, pregnancy, and postnatal life. However, our understanding of the mechanisms controlling early (preantral) follicular development is still limited. Evidence points at a role of follicle-stimulating hormone (FSH) to promote preantral follicle survival; moreover, follicles may be responsive to FSH at early stages. We hypothesized that bovine preantral follicles express receptors for FSH (FSHR) as early as the primary stage. Our objectives were to characterize isolated bovine preantral follicles as primary, early and full secondary stages according to morphology, diameter, and cell number, and to determine expression of FSHR. Preantral follicles were isolated from ovaries by tissue homogenization followed by filtration. Follicles were evaluated for viability using trypan blue and only viable follicles were used. In experiment 1, follicles (n = 151) were imaged for measurement and morphological classification and then incubated with Hoeschst 33342 for nuclear staining and cell counting. In experiment 2, follicles (n=71) were subjected to immunocytochemistry to detect FSHR and also with Hoeschst 33342. Intensity of FSHR staining was measured in arbitrary units using ImageJ software and groups were compared by one-way ANOVA (SAS 9.4). Diameter and cell number were 62.2 ± 2.2 µm and 62.4 ± 5.13 cells for primary; 83.7 ± 0.88 µm and 103.0 ± 3.6 cells for early secondary, and 124.9 ± 3.4 µm and 172.2 ± 5.2 cells for full secondary. There was no difference in FSHR staining intensity between follicle stages (p= 0.2249). However, primary follicles exhibited a larger variation in level of FSHR expression compared to the other categories. In conclusion, we have established an accurate classification of bovine preantral follicle stages based on diameter and cell number. FSHR was expressed as early as the primary stage of follicular development, and its variable level may suggest early follicular selection.

**P343 - The peculiar translational machinery of the bovine oocyte.** Mallorie Trottier-Lavoie, Isabelle Gilbert, Alexandre Bastien, Frédéric Guénard, Claude Robert

Protein synthesis is one of the most essential biological processes whereby cells generate new protein with the assembly of amino acids by ribosomes. In somatic cells, ribosomes are found within the endoplasmic reticulum, attached to the nuclear envelope and some are believed to be free in the cytoplasm. In addition, mitochondria have their own contingent of ribosomes. Despite, being in a state of transcriptional silence, bovine fully grown oocytes and early embryos have an intense need for protein synthesis to support the first steps of development. In these cells, we observed atypical ribosomal RNAs (rRNAs) profiles that only regain normalcy following embryonic genome activation. We hypothesized that oocytes and early embryos utilize a different type of ribosomes to sustain protein synthesis. To describe the rRNA population, transcript quantification in bovine, mouse and pig oocytes was performed and demonstrated different relative abundances in rRNA subunits compared to somatic cells. Using different staining, localization of ribosomes showed a close relationship between the translation machinery, mitochondria and the endoplasmic reticulum (ER). The odd shaped mitochondria and its intimate relationship with the ER has been documented more than 40 years ago but never
further characterized. Identification of active translation concomitant to the specific inhibition of different types of ribosomes is underway. In parallel, localization of known anchoring proteins that are tethering the interactions between the ER and the mitochondrial wall is ongoing. This work is conducted to provide new insights on the peculiar translational machinery of the female gamete and early blastomeres.

**P344 - SWI/SNF chromatin remodeling subunit SMARCA4/BRG1 is essential for female fertility.** David Landry, Atefeh Abedini, Ashna Parbhakar, Reza Salehi, Barbara Vanderhyden

Mammalian folliculogenesis is a complex process that involves regulation of gene expression and meiotic recombination, both of which require the modulation of chromatin structure. The complex SWI/SNF is a chromatin remodeler using either BRG1 or BRM (encoded by *Smarca4* and *Smarca2*) as its catalytic subunit; however, its function during folliculogenesis remains poorly understood. In this study we are defining the phenotype of BRG1 mutant mice to better understand its role in female fertility. Conditional knockout mouse models generated by deletion of *Brg1* in granulosa cells and oocytes were used to analyze the function of BRG1 during folliculogenesis in vivo. Conditional deletion of *Brg1* in the granulosa cells of *Brg1*(*floxflox*);*Amhr2*(cre/+) mice caused sterility, while conditional deletion of *Brg1* in the oocytes of *Brg1*(*floxflox*);*Gdf9*(cre/+) mice resulted in sub-fertility. Comparative histopathological analysis revealed that mutants ovaries had significantly higher numbers of primary and secondary follicles at 2 months of age and more atretic antral follicles at 8 months of age. Recovery of oocytes from the oviducts showed no significant difference in the *Brg1*(f/f);*Amhr2*(cre/+) mutant and significantly fewer oocytes in the *Brg1*(f/f);*Gdf9*(cre/+) mutant, which may account for the sub-fertility. Interestingly, the evaluation of oocyte developmental competence by in vitro culture of 2-cell embryos indicated that oocytes originating from the *Brg1*(f/f);*Amhr2*(cre/+) mice do not reach the blastocyst stage. Current investigations are assessing the differences in gene expression profiles of granulosa cells and oocytes by RNA-seq in both mutant animals to elucidate the mechanisms by which loss of BRG1 impairs fertility. Together, these results indicate that BRG1 plays an important role in fertility by regulating both granulosa and oocyte functions during follicle growth and the acquisition of oocyte developmental competence.

**P345 - Single-cell RNA-Seq reveals a highly coordinated transcriptional program in mouse germ cells during primordial follicle formation.** Jing Li, Yuanlin He, Tinghe Wu

In mammals, the assembly of primordial follicles represents one of the most critical events in ovarian biology. It has been characterized by the dramatic remodeling of the ovarian tissue to form the basic unit of the ovary, follicle. The process directly affects the number of oocytes available to a female throughout her reproductive life. Premature depletion of primordial follicles contributes to the ovarian pathology, premature ovarian insufficiency (POI), which affects 1–2% women before 40 years old. In human, primordial follicle formation begins during mid-gestation, while in mouse; it starts from E17.5 (17.5dpc) and occurs approximately within the first three
days after birth. The process includes meiosis initiation, cyst breakdown and follicle assembly which happens in the ovary in a temporally and spatially asynchronous manner. The asynchronies on follicle assembly are represented by the heterogeneity of germ cells, all three kinds of germ cells (germ cells in cysts, germ cells undergoing cyst breakdown and germ cells in follicles) being found coexisted in the newborn ovary (P0.5). To better delineate the developmental transitions during follicle formation, we sequenced the transcriptome of total 118 single germ cells collected from P0.5 ovaries. Our data clearly showed the differential gene expressions among germ cells. Monocle analysis then revealed the unidirectional development of germ cells along the pseudo-time line. Our study not only confirmed prior knowledge, but also identified the transcriptional factor portfolios and demonstrated how TFs and target genes coordinated the state transitions during follicle assembly. Moreover, with the help of siRNA knockdown experiments and genetic mouse models, we found the differentially regulatory mechanisms of transcriptional regulators Id1 and Id2 that were pivotal in the process.

**P346** - **DPAGT1 is essential for oocyte and follicle development in mice.** You-Qiang Su, Hui Li, Lanying Shi

N-glycosylation is an important type of protein posttranslational modification, which is crucial for many life processes. However, the exact contribution of protein N-glycosylation to mammalian female reproduction remains largely undefined. Here, we identified that DPAGT1, an enzyme that catalyzes the rate-limiting step of protein N-glycosylation, is indispensable for oocyte and follicle development in mice. A missense point mutation of Dpagt1 caused female subfertility owing to defects in oocyte and follicle development. Dpagt1 mutant females ovulated fewer eggs, which was attributed to the defective development of follicles beyond the late secondary stage. The mutant ovulated oocytes had a thin and fragile zona pellucida, but were able to be fertilized in vitro with even a slightly increased rate of formation of zygotes. However, the mutant zygotes had poor competence of preimplantation development, with fewer of them forming normal and competent blastocysts. Moreover, completion of the first meiosis, as demonstrated by emission of the first polar bodies, was significantly accelerated in Dpagt1 mutant oocytes although the resumption of meiosis was normal. In accord with these defects observed in Dpagt1 mutant oocytes, transcriptomic analysis revealed the downregulation of a number of transcripts essential for oocyte meiotic progression and preimplantation development (e.g., Pttgt1, Esco2, Orc6, and Npm2) in Dpagt1 mutant oocytes, which could be responsible for the mutant oocyte incompetence. Conditional knockout (cKO) of Dpagt1 in oocytes resulted in the similar oocyte defects as the whole body mutants, but barely affected the follicle. In contrast, granulosa cell cKO of Dpagt1 only caused severe follicular defects without affecting much of the oocytes. Collectively, these data indicate that protein N-glycosylation in the germinal and somatic compartments of the follicles is essential for female fertility in mammals by specific control of the development of oocytes and follicles, respectively.

**P347** - **The influence of FSH during bovine IVM on lipid storage and metabolism.** Maite del Collado, Gabriella Andrade, Alessandra Bridi, Ana Clara Ávila, Flávio Meirelles, Juliano da Silveira, Felipe Perecin
Cumulus-oocyte complexes (COC) under metabolically altered environments, such as the *in vitro* maturation (IVM), accumulate excessive amounts of lipids. Here, we aimed to investigate the role of FSH supplementation during IVM on lipid storage and lipid metabolism in bovine COCs. To this aim we performed IVM supplemented with high (0.01 UI/mL; HIGH group) or low (0.000875 UI/mL; LOW group) FSH concentrations. Cumulus cells from COC after 12 h of IVM and cumulus cells from matured COCs (in Metaphase II, MII) after 24 h were used to access gene expression of lipid metabolism related genes. Furthermore, matured oocyte (in MII; n=41 and 46/group, respectively) were submitted to lipid quantification using Bodipy 493/503 and confocal microscopy to determine ratio between lipid droplets area and total area. Five pools (n=5) of cumulus cells per group, formed by cells retrieved from 10 or 20 COCs each, were submitted to total RNA extraction using TRizol, DNase treatment and cDNA synthesis by High Capacity cDNA Reverse Transcription Kit. The relative gene expression of FABP3, PLIN2 and SREBP1 were determined using the reference genes PPIA, RPL15 and YWHAZ. After 24 h of IVM nuclear maturation rates were similar between groups. We found an increase in lipid droplets area in the oocytes from HIGH group (0.0956 ± 0.0498) compared to LOW group (0.0781 ± 0.0375). Moreover, after 12 and 24 h of IVM, higher levels of FSH induce an increase of FABP3 and PLIN2 gene expression in cumulus cells. After 24 h, but not after 12 h, the SREBP1 expression was higher in cumulus cells from HIGH group when compared to LOW group. In conclusion, high levels of FSH during bovine IVM lead to the activation of lipogenesis pathway in cumulus cells, resulting in higher lipid content in matured oocytes. Financial support FAPESP (2013/08135-2, 2014/22887-0, 2017/19825-0, 2018/01431-9, 2018/13155-6).

**P348 - Analysis of oocyte-specific multi-copy gene, Oog1 using CRISPR/Cas9 system.** Yuri Kunimoto, Yuka Miki, Satoshi Tsukamoto, Takuro Horii, Izuho Hatada, Naojiro Minami

It is well known that sex-specific genes in germ cells are necessary for the proper germ cell differentiation. Oog1 is an oocyte-specific gene and seems to function as a transcription factor during meiosis. Oog1-knock-down (Oog1-KD) experiments reveal that Oog1 suppresses genes involved in spermatogenesis and activates oocyte-specific genes in oocytes. In Oog1-KD oocytes, spermatogenesis related genes such as Tektin2 (Tekt2), Tudor Domain Containing 6 (Tdrd6), Kelch Like Family Member 5 (Klhl5) and Transition Protein 2 (Tnp2) are upregulated and oocyte-specific linker histone H1foo is downregulated. Significant defect in oogenesis, however, is not observed in Oog1-KD mice. Therefore, generation of Oog1-knockout mice using CRISPR/Cas9 system was performed, although it was challenging because Oog1 has 5 copies on two chromosomes. The two copies are located on chromosome 4 (Copy1, 2) and the others (Copy3, 4, 5) are on chromosome 12. The gRNA, the sequence of which are homologous among all 5 copies, and the Cas9 protein were injected into the embryos and genetically modified (GM) pups were obtained by embryo transfer. In one GM mouse line (#1), Copy1 on chromosome 4 was largely defected enough to be recognized by the size of DNA amplified by PCR, and Copy2 was shortly defected on the same chromosome. In another GM mouse line (#2), Copy3 on chromosome 12 was largely defected, and Copy5 was shortly defected on the same chromosome. Since Copy4 was not attacked in this mouse line, we performed the second CRISPR/Cas9 experiment using the same gRNA, on the embryos whose father is a heterozygous knockout male mouse obtained by crossing between #1 and #2 mouse lines. As a result, new mutations on Copy2, 3, 4 and 5 in addition to the conventional large defects of Copy1 and 3 were detected.
**P349 - Igf3, a new key player in fish ovary.** Jianzhen Li, Christopher Cheng

Female fertility requires the precise regulation of ovary development. We have identified a gonad-specific Igf subtype (Igf3) which possesses pivotal functional roles on reproduction in fish. Using zebrafish as a model, we have systematically investigated the gene expression, regulation and the action of Igf3 in the ovary. The expression of igf3 mRNA gradually increases during folliculogenesis. Both the mRNA and protein of this growth factor is confined to the follicular cells of the ovarian follicles. The expression of igf3 could be up-regulated by LH signaling through a cAMP pathway in the follicular cell layer. Recombinant zebrafish Igf3 could induce oocyte maturation and ovulation via Igf type 1 receptors (Igf1rs). Moreover, we have demonstrated that Igf3 or hCG treatment could stimulate Igf1rs phosphorylation and this hCG-induced oocyte maturation and ovulation could be blocked by Igf1r inhibitors or Igf3 antiserum *in vivo*, indicating that Igf3 plays a crucial role in mediating the action for LH on oocyte maturation and ovulation. These results clearly demonstrated that Igf3 acts as an important mediator of LH action on oocyte maturation and ovulation in zebrafish. Furthermore, we have recently generated the zebrafish Igf3 mutant line using TALENs, and found that the homozygous mutant develop exclusively as males, indicating the essentiality of Igf3 on ovarian development in zebrafish. All these evidence indicates that Igf3 severs as a new key player in fish ovary development.

**P351 - GDF9 - BMP15 Enhance HAS2, PTGS2, TNFAIP6 Gene Expression in Canine Cumulus Oocyte Complexes.** Monica De los Reyes George Ramirez

GDF9 and BMP15 have been involved in cumulus expansion in many species. Our previous studies in canine follicles have shown that these paracrine proteins decreased before ovulation. In dogs, the cumulus undergoes partial mucification because the *corona radiata* cells remain attached to the oocyte after ovulation. It has been reported that the low concentrations of these paracrine factors cannot stimulate the enzymes: HAS2, TNFAIP6 and PTGS2, that regulate cumulus expansion. Therefore, this study aimed to investigate the effect of these paracrine factors on the HAS2, TNFAIP6, PTGS2 gene expression of in vitro matured (IVM) canine cumulus oocytes complex (COCs).

COCs obtained from antral follicles were in vitro matured with or without (control) recombinant GDF9 and BMP15 (200 ng/mL). After 72 h culture, cumulus cells were removed from enclosed oocytes by pipetting and then submitted to q-PCR analysis in order to evaluate the level HAS2, TNFAIP6, PTGS2 transcripts. Primers for these target genes were designed using the annotated genomes of Canis familiaris. Relative mRNA levels were compared by ANOVA and Duncans’ test. Supplementation with these two growth factors during IVM positively influenced (P<0.01) the levels of mRNA transcript of cumulus cells. The gene expression of HAS2 was three times greater in comparison to those from COCs matured in control medium, and TNFAIP6 and PTGS2 four times greater than control. This study provides evidence that BMP15 and GDF9 in dogs stimulate the expression of genes involved in cumulus cell expansion cascade. Therefore, the addition of BMP15 and GDF9 to the maturation medium would be beneficial for this purpose.
Mitochondrial function, largely regulated by the dynamics of this organelle, is inextricably linked to oocyte health. Mitochondrial dynamics are determined by the opposing processes of fusion and fission that regulate organelle activity, transport and degradation. While the proteins that modulate mitochondrial fusion, Mitofusin 1 (MFN1) and 2 (MFN2), are required for embryogenesis, their role in oocyte development remains unclear. To address the role of Mfn1 and Mfn2 in growing oocytes, conditional knockout females were generated through the use of floxed and Zp3-Cre mice. We found that the oocyte-specific deletion of Mfn1, but not Mfn2, results in a complete loss of oocyte growth and ovulation due to a block in folliculogenesis at the preantral-to-antral follicle transition. Moreover, Mfn1-null oocytes were smaller, lacked cumulus cells and presented evidence of mitochondrial dysfunction. We pinpoint these defects to disrupted oocyte-somatic cell communication. More specifically, Mfn1-null oocytes were deficient for the production of somatic cell signaling factors, including GDF9. Additionally, supplementation with recombinant GDF9 partially rescued growth of Mfn1-null oocytes in vitro. Unexpectedly, the double loss of Mfn1 and Mfn2 mitigated the effects on oocyte growth, cumulus cells proliferation and ovulation. This was explained by a partial rescue of oocyte-somatic cell communication and folliculogenesis. Yet, females lacking both Mfn1 and Mfn2 were infertile due to ovulation of germinal vesicle-arrested oocytes. We can conclude that the oocyte relies on Mfn1, but not Mfn2, for growth and ovulation. Importantly, this work reframes the role of mitochondria in oocyte biology by identifying a specific role for a mitochondrial protein in intercellular signaling during oocyte growth. Also, it suggests that the balanced expression of Mfn1 and Mfn2 is determinant for the gamete.

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Performance of brazilian Senepol donors after OPU and IVC: oocyte recovery rates.
Aline Camargos, Ana Paula Pires, Graziela Tarôco, José Renato Chiari, Roberta Silva, Bruna Cirilo

This study aimed to evaluate the performance of brazilian Senepol donors after OPU and IVC by oocyte recovery rates. It was performed with a data collection from Senepol donors during the year. These donors were attended by the Reproduction Biotechnology Laboratory of Samvet embryos and OPU was performed on the Alge Senepol farm, located at Goiás, Brazil. Data from 33 Senepol donors were analyzed in 604 OPU. 3-10 years-old Senepol females were maintained in a semi-confinement system with pasture feeding and supplementation. During the months of the year, laboratory data of OPU and IVC were collected. The descriptive statistics for the analyzed characteristics and evaluation of the significance of the effects (months and donors) by Analysis of Variance and Tukey's test were determined (statistical significance of 5%). It was
observed per donor after OPU 27.13±14.38 total oocytes, 3.69±2.28 non-viable oocytes, 23.17±12.94 viable oocytes, 2.07±2.76 oocytes of degree I, 10.13±6.12 oocytes of degree II, 10.61±7.76 oocytes of degree III and 23.24±13.14% of viable oocytes after in vitro culture. The minimum number of oocytes recovered after OPU was zero and the maximum were 75 total oocytes and 66 viable oocytes recovered after only one OPU. The influence of the month-of-year effects and donors' individual factor on the analyzed characteristics was significant (p>0.05). Improved rates of recovery of oocytes in the months of February, March and April were observed. These months correspond to the period of high rainfall in the region. In the month of June, the highest rate of grade I oocytes after OPU (4.69 oocytes per donor) was observed. The recovery rates of oocytes from Senepol donors showed satisfactory results, with significant variation between donors and between months of the year.

P354 - Pharmacological inhibition of CHK2 signaling to protect ovarian reserve from genotoxic cancer treatments. Chihiro Emori, Ryan Kurtz, Ewelina Bolcun-Filas

Genotoxic cancer treatments can deplete ovarian reserve in women cancer survivors and put them at higher risk of primary ovarian failure or infertility. Therefore, there is a critical need to develop strategies to protect the ovarian reserve during cancer treatments in young patients. Previous study from our group found that Checkpoint Kinase 2 (CHK2) has a critical role in eliminating oocytes in primordial follicles after radiation damage. We further showed that Chk2 deficient oocytes survive treatments with chemotherapy drugs such as cisplatin or mafosfamide. This suggests that inhibition of CHK2 signaling during genotoxic cancer treatments could be used as a therapeutic strategy to protect ovarian reserve of immature eggs. The aim of this study was to identify potent and safe ovario-protective agents among known pharmacological inhibitors of CHK2 signaling. We tested the ability of CHK2 inhibitors that have not yet been tested to prevent oocyte loss caused by radiation or chemo-drugs using ex-vivo organ culture system. Ovaries from 7-day-old mice were exposed to radiation or chemo-drugs with or without inhibitors and oocyte survival was analyzed after 7-day culture. From 4 inhibitors tested, we found that 20 hours of AZD7762 treatment significantly improved oocyte survival after radiation, while 48 hours of AZD7762 concomitant treatment with chemo-drug (cisplatin or mafosfamide) prevented oocyte loss. However, despite protective effects on meiotically arrested oocytes, AZD7762 treatment itself induced DNA damage and apoptosis in somatic cells resulting in retarded growth of the ovarian explant. Many CHK2 inhibitors can also inhibit a related kinase CHK1, which is essential for cell cycle regulation in all cells, and therefore can exhibit a cyto-toxic side effect. Overall, our study further confirms feasibility of CHK2 inhibition as ovario-protective treatment and emphasizes the need for development of more specific CHK2 inhibitors.

P355 - AMH promotes maturation of nude oocytes but inhibits FSH-induced COC maturation and cumulus expansion. Liping Hua, Zan Li, Tong Qiao, Guohua Hua, Liguo Yang, John S. Davis, Aixin Liang

Anti-Mullerian hormone (AMH), a member of the transforming growth factor β (TGF-β) superfamily, is secreted by the ovaries of female animals and exerts its biological effects through
the type II receptor (AMHR2). AMH regulates follicular growth by inhibiting recruitment of primordial follicles and reducing the sensitivity of antral follicles to FSH. AMH is used as a marker to evaluate ovarian reserve and predict superovulation. Despite considerable research on the actions of AMH on mural granulosa cells, the role of AMH on in vitro maturation of oocytes and the maturation and expansion of cumulus oocyte complexes (COC) remains largely unknown. In the current study, we found that AMH is expressed by murine cumulus cells, and AMHR2 is present in cumulus granulosa cells and oocytes. Expression of AMHR2 decreased gradually with the maturation of the oocyte. AMHR2 expression in GV oocytes was significantly higher than that in MI oocytes (P<0.05). Treatment with increasing concentrations of rh-AMH (1-100 ng/ml) resulted in dose-dependent increases in the first polar body extrusion (PBE) rate of nude oocytes, with 100 ng/ml AMH stimulating the highest maturation rate in vitro (76.8%). Furthermore, AMH promoted the expression of BMP15, increased the content of MPF and decreased levels of cAMP. However, AMH (100ng/ml) had no significant effect on the nuclear maturation of COC, and inhibited the stimulatory effects of FSH on COC nuclear maturation and cumulus expansion (P<0.05). AMH treatment also effectively inhibited the stimulatory effect of FSH on the expression of Has2, PTX3 and Tnfaip6 transcripts in COC. Taken together, the results indicate that AMH can improve the in vitro maturation of nude oocytes, while inhibiting the effects of FSH on COC maturation and cumulus expansion.

P356 - Discovery of Large Quantity of Acanthocytes in Ovarian Follicular Fluids of the Infertility Patients. Ping Xia

One in six couples suffers from infertility. Its etiology in majority female patients is still not clear. Our research proposal was approved by the Johns Hopkins Medicine Institutional Review Board (IRB00185243). The cells in the ovarian follicular aspirates were observed under inverted microscope in more than 200 patients who underwent in vitro fertilization procedures. To our great surprise, acanthocytes were found to be present in different quantities in the follicular fluids of the infertility patients, especially in the patients diagnosed as endometriosis. The morphology of acanthocytes were further confirmed by the scanning electron microscope. The oocyte from the fragmented acanthocytes presented a degenerated morphology. These findings will shed light in understanding the etiology of endometriosis and unexplained infertility. To our knowledge, this is the first report in the field of assisted reproductive technologies. The results have indicated ovarian vascular microenvironment plays an important role in maintaining healthy follicular development. Future therapies focusing on improving blood supplies to the follicles would potentially improve oocyte quality for the purpose of ensuring healthy babies born using in vitro fertilization approaches.

P357 - Investigating the effect of allelic diversity on the establishment of the ovarian reserve. Ruby Boateng, Ewelina Bolcun-Filas

A substantial gap exists in our knowledge regarding how genes and allelic variations influence fertility and reproductive health of women. Addressing this gap will contribute toward personalized treatment options for patients experiencing infertility. A major determinant of female fertility and reproductive lifespan is the size and quality of the Ovarian Reserve (OR).
Since infertility affects genetically heterogeneous individuals in human population, it is important to understand how allelic variants influence the female’s OR. Our goal is to identify allelic variants and their combinations which could be utilized to predict the OR size and quality.

To accomplish this goal, we are investigating the early stages of female germ cell development in diverse inbred strains of mice and in a novel panel of recombinant strains, the Collaborative Cross (CC). We analyzed germ cell proliferation, fetal oocyte atresia, meiotic recombination and oocyte cyst breakdown in multiple strains. We quantified germ cells numbers at specific prenatal and postnatal developmental time points and analyzed meiotic chromosomal abnormalities in late prophase I oocytes. We found interesting and clinically relevant reproductive characteristics among lesser known inbred strains and genetically diverse mice. For example, females from the NOD/ShiLtJ inbred strain have a significantly higher number of immature oocytes and larger litters compared to more widely used C57BL/6J strain. Further, NOD/ShiLtJ oocytes in late meiotic prophase exhibit fewer meiotic abnormalities than seen in other strains, which may contribute to increased oocyte survival and better quality, contributing to the larger OR and litter sizes compared to other analyzed strains. Interestingly, there was a higher incidence of primordial and growing follicles with multiple oocytes in NOD/ShiLtJ females, which does not seem to compromise their fertility. These and other findings from this study will help us reveal potentially predictive genetic markers of infertility for personalized medicine approaches in human patients.

**P358 - Cytoplasm lipid can be modulated by CAY10499 through HSL pathway and related with mitochondrial function in porcine in vitro matured oocytes.** Qingrui Zhuan, Haojia Ma, Jing Chen, Yuxi Luo, Yan Luo, Lei Gao, Yunpeng Hou, Shien Zhu, Xiangwei Fu

The intracellular lipid provides energy source for oocytes maturation and development. Triglyceride is the main component of cytoplasm lipid droplets and it’s hydrolysis requires different steps through the action of lipases. In this study, porcine oocytes were cultured with the optimum concentration of 5mg/L Isoprenaline (ISO, β-adrenoceptor agonist) and 20mg/L CAY10499 (HSL inhibitor). The results revealed that 5 mg/L ISO improve the normal meiotic progression by remarkably increase the rate of polar body extrusion (P<0.01). On the other hand, polar body extrusion, cleavage and blastocyst formation were compromised when lipases HSL were blocked as the IVM medium supplement with CAY10499 (P<0.01).Further, the triglyceride content was increased in CAY10499 group (P<0.05) and expression of lipases genes HSL was decreased in matured oocytes (P<0.05). Besides, CAY10499 could decrease the ATP content in porcine oocytes. We also use a novel temperature sensitive fluorescent probe named Mito Thermo Yellow (MTY) to detect the intracellular mitochondrial temperature. When mitochondria are in an active state, the internal temperature will be higher than that of basal state, accompanied by a declined fluorescence intensity. Results showed that CAY10499 could significantly increase intracellular mitochondrial temperature (P<0.01) which consistent with the change of lipid metabolism. In conclusion, the intracellular lipid metabolism of in vitro matured porcine oocytes can be modulated by CAY10499 through HSL pathway and has close relationship with mitochondrial function.
P359 - Comparison of brazilian Gir and Holstein-Gir oocyte recovery rates after OPU.
Aline Camargos, Larissa Cruvinel, Graziela Tarôco, José Renato Chiari, Roberta Silva, Bruna Cirilo

This study aimed to compare the performance of brazilian Gir and Holstein-Gir donors after OPU by oocyte recovery rates. It was performed with a data collection from Gir and Holstein-Gir donors during the years 2013, 2014, 2015. These donors were attended by the Reproduction Biotechnology Laboratory of Samvet embryos located at São Caetano farm, Morrinhos, Goiás, Brazil. Data from 41 Gir and 72 Holstein-Gir donors were analyzed in 645 OPU. 3-12 years old Gir and Holstein-Gir females were maintained in a semi-confinment system with pasture feeding and supplementation. During the years, laboratory data of OPU were collected. The descriptive statistics for the analyzed characteristics and evaluation of the significance of the breed effect by Analysis of Variance were determined (statistical significance of 5%). It was observed per donor after OPU: 23.28±13.22 Gir and 32,30±14,51 Holstein-Gir total oocytes, 3.98±2.90 Gir and 4,97±3,60 Holstein-Gir non-viable oocytes, 21.31±11.94 Gir and 26,91±12,37 Holstein-Gir viable oocytes. Holstein-Gir showed significant better results of total oocytes, viable oocytes and non-viable oocytes recovered than Gir donors (p>0.05). The minimum number of oocytes recovered after OPU was 2 for Gir and 5 for Holstein-Gir. The maximum were 80 for Gir and 78 for Holstein-Gir total oocytes recovered in one donor. 64 for Gir and 70 for Holstein-Gir viable oocytes recovered after only one OPU. The recovery rates of oocytes from Holstein-Gir donors showed better results. Acknowledgements to IF Goiano and FAPEG. Key-words: cattle; total oocytes; viable oocytes.

P360 - Oocyte competence acquisition during folliculogenesis in the domestic cat model: a role for the cholesterol trafficking protein SCP2 and the transcriptional regulator BRD2.
Daniela Chavez, Pierre Comizzoli

We previously identified nuclear proteins associated with the acquisition of oocyte developmental competence from the preantral to the antral follicular stage. The objective of the present study was to specifically investigate the roles of two candidate factors: sterol-carrier protein 2 (SCP2) and bromodomain containing protein 2 (BRD2). SCP2 is a cholesterol trafficking protein with unknown roles in the nucleus that has not been studied in oocytes. BRD2 is a MAP kinase and transcriptional regulator implicated in folliculogenesis, yet its function remains unclear. Oocytes from preantral and antral follicular stages were isolated from adult cat ovaries and immunostained for SCP2 (n=16 preantral; n=26 antral; from 20 ovaries) or BRD2 (n=22 preantral; n=24 antral; from 41 ovaries) and fluorescence intensity was quantified. We confirmed that SCP2 and BRD2 were localized in oocyte germinal vesicles.
(GV). We found that both proteins localized to the GV from pre-antral and antral oocytes. Additionally SCP2 and BRD2 were upregulated from the pre-antral to the antral stage by 1.44 and 1.71 fold, respectively. To characterize the involvement of SCP2 and BRD2 in developmental competence, we inhibited proteins by transfecting anti-SCP2 or anti-BRD2 antibodies in antral oocytes followed by in vitro maturation. The ability of transfected oocytes to reach metaphase II was reduced (10% with anti-SCP2 and 36% with anti-BRD2) compared to 51% of mock transfected controls. In ongoing and future experiments, we are further characterizing the mechanism by which SCP2 and BRD2 contribute to oocyte developmental competence by analyzing cellular lipids (for SCP2) and assessing the effects of BRD2 inhibition of predicted interacting proteins.

**P361 - ZIM2, a KRAB domain-containing zinc finger protein, is abundantly expressed in bovine oocytes and early embryos.** Mingxiang Zhang, Jaelyn Current, Jianbo Yao

ZIM2 (zinc finger imprinted 2) is located immediately downstream of PEG3 (paternally expressed gene 3), a known imprinted gene in mammals. In humans, ZIM2 gene is paternally imprinted, while bovine ZIM2 is biallelically expressed in testis. As a zinc finger transcription factor, ZIM2 protein contains a Krüppel-associated box domain (KRAB), which is a strong DNA binding dependent transcriptional repression module. To date, the role of ZIM2 has not been reported in any species. Analysis of RNA-Seq data from a bovine oocyte cDNA library revealed that ZIM2 is abundantly expressed in bovine oocytes. The objectives of this study were to characterize the expression of bovine ZIM2 during oocyte maturation and early embryogenesis, and to identify potential ZIM2 binding elements in the regulatory regions of its target genes. RT-PCR analysis revealed that ZIM2 is only expressed in testis and fetal ovary but not in a panel of somatic tissues examined. Expression of ZIM2 mRNA is detected in GV and MII stage oocytes but not in granulosa and theca cells, indicating that ZIM2 expression in the ovary is oocyte-specific. Further analysis of ZIM2 expression in early stage embryos revealed that ZIM2 mRNA is abundantly present in 2-cell, 4-cell and 8-cell stage embryos but drops to undetectable levels in 16-cell and morula stage embryos. ZIM2 mRNA is detected again in embryos collected at blastocyst stage. A cyclic amplification and selection of targets (CASTing) assay was performed using recombinant bovine ZIM2 protein and a random oligonucleotide library. A potential ZIM2 binding element (ACGTTACCCT) was identified. Further identification of the specific genes regulated by ZIM2 will help understanding its functional contribution to oocyte and early embryonic development in cattle.

**P362 - Morphological and Molecular Assessment of Cryopreserved Bovine Ovarian Tissue.** Paula Cornally, James O'Connor-Moneley, Goerga Longhurst, Lynne O'Shea

Ovarian tissue cryopreservation is gaining considerable interest as a potential option for fertility preservation, particularly in female cancer patients. It is highly relevant to patients with time and hormone sensitive malignancies and is the only viable option for prepubertal girls. Since 2004, over 100 live births and pregnancies have been reported using this technique. The aim of this study was to determine the effect of bovine ovarian tissue cryopreservation on follicle
morphological integrity and key hormonal signalling pathways. Bovine ovarian cortical sections were randomly distributed into two cohorts of fresh and cryo-thawed tissue for parallel analytical comparison (n=7). Cryopreserved tissue was supplemented with cryoprotectant and slow-frozen in a Planner Cryo-freezer. Tissue was thawed in media containing decreasing concentrations of ethylene glycol. Fresh and cryo-thawed tissue was cultured for 14 days, with culture media and tissue samples analysed for regulation of key hormonal signalling pathways using ELISA, immunoblotting and immunohistochemistry. Histological examination showed that cryopreservation of bovine ovarian tissue had a significant effect on follicle viability (p<0.05). In addition, cryo-thaw of ovarian tissue caused a significant increase (P<0.05) in progesterone secretion versus the fresh control; however, it had no effect on 17β-estradiol levels. Molecular analysis shows that nuclear progesterone receptor and AVEN protein expression is dynamically regulated in ovarian tissue following cryopreservation. In the present study we determined that cryo-thawing of ovarian tissue has a significant effect on follicle morphology. This work was supported by UCD Foundation. The authors have no financial disclosures.

**P363 - Cell Signaling Mediated by the KIT Receptor is Important for the Formation of the Ovarian Reserve.** Joshua Burton, Melissa Pepling

Signaling mediated by the KIT receptor is important for many aspects of ovarian development, but its role in establishing the ovarian reserve is not well characterized. Using an organ culture system, we have previously shown that KIT promotes cyst breakdown and primordial follicle formation in the mouse ovary, and now aim to characterize the activity of the downstream signaling pathways that facilitate these processes. Immunoblots with extracts from neonatal ovaries cultured for 24 hours in media supplemented with 100ng/ml of KIT ligand show increased activation of the MAPK pathway relative to controls. Conversely, MAPK activity is downregulated in the presence of ACK2 (a KIT function blocking antibody). PI3K and STAT3 activation also appear to be modulated when KIT ligand or ACK2 are added to culture media. To assess the role of these signaling pathways in oocyte development, ovaries were cultured either for four or five days with a daily dose of media supplemented with either 10µM of U0126 (a MEK inhibitor), 10µM of Stattic (a STAT3 inhibitor), 20nM of JAK inhibitor I, or DMSO vehicle controls of 0.05%, 0.04%, or 0.1% respectively (n ≥ 5 ovaries per group). After culture, ovaries were labelled with a germ cell marker for confocal imaging and histological assessment. No significant differences in cyst breakdown or follicle development were observed between any group of control and treated ovaries, but additional replicates and varied doses remain to be analyzed. We deduce that KIT may be working through the PI3K pathway or that there might be crosstalk between the MAPK and JAK/STAT pathways that facilitates compensation when one is blocked. These observations are important as they point to the potential molecular mechanisms that regulate oocyte development and may serve to better elucidate the etiology of reproductive disorders like primary ovarian insufficiency. Research supported by NIH R15 075257

**P364 - Cumulus-oocyte communications and partnership: roles played by the Fragile-X related proteins.** Karen Nenonene, Alexandre Bastien, Jennifer McKey, Blanche Capel, Isabelle Gilbert, Edward Khandjian, Robert Viger, Claude Robert
The ovarian follicle is a complex syncytium that involves a profound interdependence between cell types. Numerous signaling pathways have been identified to communicate between the follicular compartments and synchronize folliculogenesis and oogenesis. Cumulus cells adjacent to the zona pellucidae have an intimate relationship with the oocyte by harboring cellular projections that reach through the glycoprotein shell to contact with the oolema. We have shown that these processes can transport large cargoes in the form of riboprotein granules which can be delivered to the oocyte. Our hypothesis is that transzonal projections (TZPs) function in a neuron-like fashion. To characterize the mechanism by which large cargoes are shuttled along the TZPs, we studied the expression of the three members of the Fragile X related proteins (FXRPs) in cow, pig and mouse. The three RNA binding proteins are structurally similar and absence of FMRP (product of Fmr1), leads to Fragile X syndrome in human, characterized by mental retardation and autism. We found that mRNA and protein for Fmr1, and its homologues Fxr1 and Fxr2, vary in abundance during folliculogenesis. FMRP and FXR2 are highly abundant in preantral follicles. Protein localization and quantification of FXRPs within the TZPs show that although all three FXRPs are present, FXR1 is the most abundant. This suggests different expression patterns during TZP formation compared to late folliculogenesis where FXRPs expression are modulated according to the state of maturity of the follicle. In Fmr1-KO mice, the TZP network differs from its wild type counterparts and it seems that FXR2 compensates for the loss of FMRP. Our data support a model where FMRP is involved in the establishment of the TZP network in early folliculogenesis but where FXR1 is the most abundant FXR involved in active transport of mRNA granules from the cumulus cells to the oocyte.

**P365 - Oocyte-specific deletion of BAF250a affects oocyte epigenetic modifications and embryonic development.** Zhenbo Wang, Qian Zhou

BAF250a is a component of the SWI/SNF ATP-dependent chromatin remodeling complex, which has been shown to control chromatin structure and transcription. BAF250a has been reported to be a key component of the gene regulatory machinery in ES cells controlling self-renewal, differentiation, and cell lineage decisions. Here we constructed Baf250a<sup>F/F</sup>;Gdf9-cre (Baf250a<sup>CKO</sup>) mice to specifically delete BAF250a in oocytes to investigate the role of maternal BAF250a in female germ cells and embryo development. Our results showed that BAF250a deletion did not affect folliculogenesis, ovulation and fertilization, but it caused late embryonic death. We showed that BAF250a contributed to the correct establishment of maternal imprinting genes and covalent histone modifications. The methylation level of Igf2r DMR was decreased in Baf250a<sup>CKO</sup> oocytes. The expression level of DNMTs was also decreased, which might be the cause for impaired imprinting establishment. In addition, covalent histone modifications such as H3K27me3 was also decreased significantly in oocytes, which may reduce oocyte quality and lead to birth defects. In conclusion, our results demonstrate that BAF250a plays an important role in oocyte epigenetic modifications and embryo development.

**P366 - Anethole and robinin improves follicular morphology during vitrification of ovine ovarian tissue.** Maria Santos, Daniele Brito, Yago Silva, Renato Silva, Francielli Cibin, Claudio
An alternative for reducing follicular damages caused by reactive oxygen species (ROS) produced during vitrification procedures of ovarian tissue is the addition of natural antioxidants to the vitrification solution. Here we investigated the effect of anethole and robinin addition in the vitrification solution, and the in vitro incubation medium of ovine ovarian tissue. Ovarian fragments were vitrified without antioxidant (VWA) or with different concentrations of anethole (30, 300 and 2000 μg/mL) or robinin (0.125, 0.25 and 0.50 mg/mL), followed by in vitro incubation. Histological analyses showed that the percentage of morphologically normal preantral follicles in 2000 μg/mL of anethole did not differ from 0.125 mg/mL of robinin or fresh ovarian tissue. Subsequently, ovarian fragments were vitrified in the presence of 2000 μg/mL of anethole and 0.125 mg/mL of robinin followed by in vitro incubation without or with (anethole 2000 μg/mL and robinin 0.125 mg/mL) the same antioxidants. The stroma cell density in all the vitrified fragments was significantly lower than the fresh control. However, in the anethole 2000 μg/mL and robinin 0.125 mg/mL this parameter was significantly higher when compared to the vitrified group without antioxidants. The addition of anethole 2000 μg/mL during vitrification as well as during in vitro incubation significantly reduced the reactive oxygen species in the ovarian cortex in comparison with the control while the intracellular ROS levels of this group and fresh control were similar. The total antioxidant capacity (TAC) in robinin 0.125 mg/mL group was significantly higher than that of VWA and anethole 2000 μg/mL. These data suggested that the use of antioxidants only in the vitrification solution of ovine ovarian tissue is recommended, due to their better preservation of the stromal cells. Moreover, the addition of 2000 μg/mL of anethole best maintains the follicular morphology, while 0.125 mg/mL of robinin has a high TAC.

P367 - Preantral Follicle Numbers and Size in Heifers Carrying the Bovine High Fecundity Trio Allele. James Constantino, Christopher Premanandan, Brian Kirkpatrick, Milo Wiltbank, Alvaro Garcia-Guerra

The bovine high fecundity allele, Trio, results in overexpression of SMAD6 and a 3-fold increase in ovulation rate. Trio carriers have similar number of antral follicles; however, antral follicles develop at slower growth rate. The present study was designed to test the following hypotheses: 1) Trio carrier cattle have similar preantral follicle number as non-carriers; and 2) preantral follicles of Trio carriers are smaller in size than non-carriers. Ovarian tissue from Trio carrier (n=8) and non-carrier (n=8) heifers were obtained by laparotomy and a 1x1 cm section was fixed and paraffin-embedded. Sixty consecutive sections (6 µm) were obtained and every tenth (6 total) mounted and stained with hematoxylin-eosin. Follicle numbers were determined for each stage of development (primordial, primary, and secondary) using a 5x5 grid overlay. Follicle dimensions were determined from 10 random follicles of each stage and heifer using ImageJ. Differences between genotypes were assessed by t-test or Wilcoxon’s rank test. Number of primordial and secondary follicles were not different between genotypes (P>0.6). Trio carriers had greater (P<0.01) number of primary follicles per square mm (0.23±0.02) than non-carriers (0.12±0.02). Primordial follicle and oocyte volume were not different between genotypes (P>0.2). Primary follicle and oocyte volume were 1.5-fold and 1.4-fold larger in Trio carrier than
non-carriers (P<0.05). Secondary follicle volume was not different (P>0.6), however, oocyte volume was 1.7-fold larger in Trio carriers than non-carriers (P<0.05). Granulosa cell number per cross section was not different between genotypes at any stage (P>0.1). In conclusion, follicle numbers were similar for most preantral follicle stages, however, Trio carriers had greater number of primary follicles. In addition, primary follicle and oocyte and secondary oocyte size were greater in Trio carriers. These results suggest that, once activated, Trio carrier follicles, have reduced progression through the primary stage, hence the larger oocyte and greater number.

**P368 - Effects of vitrification of bovine oocytes on mitochondrial number and function.** Hayley Benham, Rolando Pasquariello, Jennifer Barfield, Rebecca Krisher, James Graham

Vitrification of bovine oocytes is still a challenge due to high lipid content in the ooplasm. The objective of this work was to determine whether CryoTop Method® vitrification of bovine oocytes affects mitochondrial DNA (mtDNA) copy number and membrane potential (MMP), along with gene expression. Bovine oocytes were collected from slaughterhouse ovaries and processed in four groups: fresh GV control, vitrified GV, fresh in vitro matured MII control, and vitrified MII. After warming, vitrified oocytes were immediately processed for analysis. mtDNA copy number in single oocytes was determined by real-time quantitative PCR (RT-qPCR) using an absolute quantification assay (n=20 oocytes/group). Mitochondrial membrane potential (MMP) was determined using MitoTracker® Orange CMTMRos staining, and imaged using confocal microscopy (n>20 oocytes/group). Expression of oxidative stress response and lipid metabolism related genes (GCLC, GPX1, NRF2, KEAP1, SOD2, PARAα, SREBP1, and CPT2) was analyzed by RT-qPCR in three biological replicates (10 pooled oocytes per replicate). Statistical analysis of data was performed using one-way ANOVA and student’s t test. mtDNA copy number did not differ significantly between fresh and vitrified GV and MII oocytes. Active mitochondria were distributed in the cortical region in all groups of oocytes. Cortical MMP was reduced in MII compared to GV oocytes (p<0.0001), and was increased in vitrified GV oocytes compared to fresh (p=0.018). Expression of GCLC decreased in vitrified GV oocytes, but was unchanged in fresh and vitrified MII oocytes. Vitrification of bovine oocytes did not significantly effect mitochondrial DNA copy number, but did effect MMP in GV stage oocytes. Reduced GCLC expression in vitrified GV oocytes may indicate a compromised antioxidative stress response. While vitrification effects are more evident in immature oocytes, future studies will investigate if anti-oxidant supplementation during vitrification could be beneficial for both immature and mature oocytes.

**P369 - Zinc biology in early mammalian ovarian follicle development.** Yu-Ying Chen, Thomas O'Halloran, Teresa Woodruff

Zinc is widely used as an enzymatic cofactor and structural element in proteins. It acts as an inorganic signaling mediator through ionic fluxes, similar to calcium transients, as well as by forming covalent bonds with target proteins, similar to phosphorylation. Studies from our lab
have shown the pivotal roles of zinc in oocyte maturation, egg-to-zygote transition, and embryonic mitotic divisions.

To investigate zinc biology in folliculogenesis, we compared zinc and other metal ion concentrations and distributions in mouse oocytes of different stages by X-ray fluorescence microscopy, radioactive zinc uptake, and immunofluorescence staining.

We discovered zinc to be the most abundant transition metal across all stages of oocytes and that total zinc amount increases throughout development. Cellular staining for free zinc revealed different zinc concentrations in different primordial oocytes, as well as a diversity in zinc distribution, as primordial oocytes exhibit punctate structure while GV oocytes show a dispersed staining pattern. A radioactive zinc isotope uptake assay showed a much lower zinc uptake per follicle, in primordials (2 billion atoms in 24 hours) than primaries (10 billion atoms in 2.5 hours). These results indicate the existence of zinc fluxes and a change in zinc physiology during follicle development. Particularly, we observed that in vitro treatment of zinc on post-natal day 6 ovaries increases markers of follicle progression, including p-AKT.

In light of this finding, we plan to examine whether zinc fluxes can trigger follicle activation through interacting with the PI3K antagonist PTEN in future studies. This research is supported by P50 HD076188 National Institutes of Health's Eunice Kennedy Shriver National Institute of Child Health and Human Development.

P370 - Distinct Spatial Localization of the Filopodial Regulator, MYO10, Suggests a Role in the Formation of the Transzonal Projections Coupling Granulosa Cells to the Growing Oocyte. Sibat Anam, Sofia Granados Apirici, Qin Yang, Flora Crozet, Marie-Émilie Terret, Hugh Clarke

Communication between the oocyte and the somatic granulosa cells surrounding it is essential for oocyte growth. Throughout growth, granulosa cells and the oocyte are separated by a thick extra-cellular layer termed the zona pellucida. In order to maintain communication between the two, granulosa cells establish physical contact with the oocyte via actin-containing filopodia-like structures known as transzonal projections (TZPs). These projections allow the direct transfer of nutrients and regulatory signals from the granulosa cells to the oocyte. Given that myosin 10 (MYO10) is a key regulator of filopodial formation in other cell types, we investigated the potential role of MYO10 in TZP dynamics. Using immunofluorescence, populations of MYO10 foci were observed at three discrete locations in follicles throughout oocyte growth. Small foci were detected at the cortex of the oocyte and within the zona pellucida. The foci within the zona pellucida typically co-localized with TZPs as detected using phalloidin. A smaller number of much larger foci were found at the oocyte-facing side of the innermost layer of granulosa cells. These large foci were more abundant among granulosa cells surrounding growing oocytes than among those surrounding fully grown oocytes. RNAi-mediated depletion of Myo10 in the oocyte led to a decrease in the number of small foci at the oocyte cortex and in the zona pellucida. These results suggest that the two populations of small foci originate from the oocyte. Future studies will be directed toward understanding the role of these three populations of MYO10 foci in the generation or function of TZPs.
P371 - The effect of genetic merit for fertility and lactational status on oocyte quality and the follicular microenvironment in dairy cows. Charlotte Reed, Susanne Meier, Chris Burke, Janet Pitman

Genetic merit for fertility and lactational status affect the reproductive performance of dairy cattle. Genetic merit for fertility was defined as the fertility breeding value (FBV) used in the New Zealand national breeding objective. Our objectives were to characterize the follicular microenvironment and oocyte quality in non-lactating nulliparous heifers and lactating primiparous cows with divergent genetic merit for fertility. Cumulus cell-oocyte complexes and follicular fluid were recovered by ovum pickup from preovulatory follicles in 12.4 ± 0.5 month old non-lactating heifers and 25.0 ± 0.6 month old lactating cows (62 ± 17 days postpartum) with either high (+5%) or low (-5%) FBV. Multiplex quantitative PCR was used to quantify molecular markers of oocyte quality in both cumulus cell masses and oocytes. High performance liquid chromatography was used to measure amino acid concentrations in follicular fluid and plasma. There were clear differences in oocyte quality and the follicular microenvironment between high and low FBV lactating cows. Compared with low FBV cows, oocytes from high FBV lactating cows had greater expression of gene markers of oocyte quality (VCAN, PDE8A; P<0.05). The follicular microenvironment was also different, with lower concentrations of serine, proline, methionine, and isoleucine relative to cows with low FBV (P<0.05). We interpreted these data to indicate a higher metabolic rate of preovulatory follicles in high versus low FBV cows. In comparison, there were no differences in follicular-derived amino acid concentrations or molecular biomarkers of oocyte quality between non-lactating heifers with high or low FBV. One exception was greater HSP90B1 gene expression in oocytes from low FBV heifers, indicating potential protein-folding differences associated with FBV earlier in life. These results indicate that variations in reproductive performance due to genetics and lactational state are partly explained by differences in functional development of the preovulatory follicle and cumulus cell-oocyte-complex.

P372 - Regulation of the porcine oocyte maturation by RSPO2-WNT signaling. Dong Jin Oh, Seon-Ung Hwang, Junchul Yoon, Mirae Kim, Sang-Hwan Hyun

R-spondin2 (RSPO2) derived from oocytes is a paracrine factor essential for primary follicle development. Here, we investigated the correlation between RSPO2 and WNT pathway in vitro maturation of porcine oocytes. We confirmed the expression of genes related to the RSPO2-WNT signaling pathways (CTNNB1, LGR5, EGFR, RSPO2), cell proliferation (PCNA), cumulus cell expansion (PTX3), pluripotency marker (POU5F1) and apoptosis-related gene (BAX, BCL2) by treatment with RSPO2, WNT inhibitor or activator during IVM. In cumulus cells, expression of CTNNB1, LGR5, and EGFR were significantly (p<0.05) increased in the LiCl treatment group compared to all other treatment groups (Control, RSPO2 and Dkk1 treatment group). PTX3 was significantly (p<0.05) decreased in dkk1 and LiCl treatment group compared to control group. This suggests that RSPO2 has a pathway that increases the expression of EGFR without going through the WNT pathway. In oocytes, expression of CTNNB1, RSPO2, PCNA, POU5F1, BAX,
and BCl2 were significantly (p<0.05) increased in the Dkk1 treatment group compared to all other treatment groups. Inhibition of the WNT signal via Dkk1 increased expression of RSPO2, EGFR, PCNA, and POU5F1. However, it was found that the Bax/Bcl2 level was higher in the DKK1 treatment group and that apoptosis was relatively more occurred than the RSPO2 treatment group. On the other hand, the RSPO2 treatment group showed a tendency to increase the expression of EGFR, and the Bax/Bcl2 level was significantly lower than the Dkk1 treatment group. This suggests that treatment with RSPO2 reduced apoptosis. Our results did not exclude the role of other factors in controlling EGFR expression. Further experiments are needed to observe only the interaction between RSPO2 and cumulus cells, excluding the role of other factors in the IVM process.

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**P373 - In vitro growth of early preantral follicles by two culture protocols.** Kanako Morohaku, Tomohiro Kohama

To increase efficiency in the usage of mammalian eggs, *in vitro* follicle growth has been studied in livestock and endangered animals and also humans. In this study, we investigated an application of our recent protocol developed (Morohaku et al., 2016 PNAS) to cultivate early preantral follicles. Early preantral follicles were collected from ovaries of mouse pups at 6 to 8 days postpartum by dissecting. The size of the follicles isolated ranged between 50 and 80 µm, and the average diameter of the oocytes included was 38.1±6.82 µm. *In vitro* culture of the follicles was performed by Millicell membrane or by hanging droplets using 5% FBS and 0.1 IU/ml FSH in alpha MEM under 5% CO2 and 95% air at 37˚C for 22 days. After the culture, the diameter of the oocyte within cumulus cell-oocyte complex was measured by a software with Olympus microscopy. As a result, the survival rates in the follicles cultured on Millicell membranes and by hanging droplets were 8.6 and 35.3%, respectively. On the other hand, the resultant sizes of the oocytes showed increment compared to those at the start of the culture; 83.8±6.2 and 76.5±2.9 µm, respectively. Some oocytes grown were subjected to *in vitro* maturation, resulting that they reached to the stage of metaphase II. The present study suggests that our protocol may be useful for culturing early preantral follicles that seem to be classified as primary to early secondary follicle developmental stages.

**P374 - Role of origin recognition complex subunit 4 (ORC4) protein in erythroblast enucleation on Murine erythroleukemia (MEL).** Anna Ung, William Ward

The Origin Replication Complex subunit 4 (ORC4) is one in six subunits of the Origin Replication Complexes (ORCs) which is essential for initiating licensing at DNA replication origins and recruiting adaptor molecules necessary for various cellular processes. Previously, we reported that ORC4 plays a vital role in polar body extrusion (PBE) in addition to DNA licensing. To test whether ORC4 played a broader role in chromatin elimination we tested its role in enucleation during the development of erythrocytes. Murine erythroleukemia (MEL) cells can
be propagated in culture indefinitely, and can be induced to enucleate their DNA by treatment with Vacuolin-1, thereby mimicking normal erythrocyte enucleation. We found that ORC4 appeared around the nuclei of the MEL cells with Vacuolin-1 treatment, gradually increasing in thickness before enucleation. We then tested whether ORC4 was required for MEL enucleation by down regulating ORC4 with siRNA-ORC4 during Vacuolin-1 treatment and found that this prevented MEL enucleation. These data are consistent with the model that ORC4 is required for erythroblast enucleation just as it is for oocyte PBE.

**P375 - CITED2 Participates in Mouse Oocyte Meiosis and Histone Modification.** Juan Lin, Yuan Fang, Shenming Zeng

The exact role and the underlying mechanism by which CBP/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2 (CITED2) affecting mouse oocyte maturation is unknown. Our immunohistochemical staining for mouse ovaries at Day 0, 4, 8, 14, 24, 42 and 56 demonstrated that CITED2 mainly located in the germinal vesicle (GV) of oocyte. CITED2 levels were increased from Day 0 to Day 14, and slowly decreased from Day 24 to D 56. Western blot results showed that CITED2 was expressed throughout meiosis both in vivo and in vitro, and the level in GV was higher than that in metaphase II (MII) oocyte. Microinjecting Cited2-specific siRNA into the GV oocyte promoted its reaching MII at 18h after maturation in vitro (62.0% for mimic siRNA (n=144) vs. 78.6% for Cited2 siRNA (n=149)) while microinjecting green fluorescent protein (GFP)-tagged Cited2 mRNA into the GV oocyte remarkably inhibited its reaching MII at 18h after maturation in vitro (72.3% for GFP mRNA (n=146) vs. 36.5% for GFP-Cited2 mRNA (n=153)). Co-immunofluorescence staining for CITED2 with respectively CREB-binding protein (CREBBP), E1A binding protein p300 (EP300) and lysine acetyltransferase 2B (KAT2B) demonstrated that the expressions of these four proteins were consistent in time and space, which was higher in non-surrounded-nucleolus (NSN) than surrounded-nucleolus (SN) GV oocyte and mainly restricted to GV. When Cited2-specific siRNA was injected into the GV oocytes (30 per group), the level of H4K12 acetylation of SN oocytes was increased by 13.4 %, while GFP-Cited2 mRNA injection (30 per group) decreased H4K12 acetylation of NSN oocytes by 13.3% and SN oocytes by 35.3%. In summary, our results imply that CITED2 is necessary for oocyte GV maintenance, and it interacts with CBP/P300/PCAF leading to lower the level of H4K12 acetylation. Research supported by the National Key Technology Research and Development Program of China (2017YFC1002003).

**P376 - Novel splice variant of Slo1 channel represents a calcium-sensitive regulatory subunit of KSper channel in human sperm.** Lenka Vylicka, Benjamin Slobodnik, Polina Lishko

Potassium (K⁺) channels play a critical role in regulating membrane potential via K⁺ efflux leading to hyperpolarization which is essential for sperm maturation and successful fertilization. Although the principal K⁺ channel in human sperm, KSper, is essential for sperm function, its full molecular identity has yet to be elucidated. Interestingly, human KSper is activated by
intracellular calcium, which makes it different from mouse KSper. The latter is represented by Slo3 and is highly pH-sensitive and calcium-insensitive. While human sperm cells also express Slo3 channel, as well as calcium-sensitive K+ channel Slo1, their physiological and pharmacological properties do not fully match either Slo3 or Slo1 alone, suggesting a possible presence of a heteromeric complex. Here, we report the identification of a splicing variant of Slo1 (sSlo1) that was cloned from human sperm. sSlo1 is a truncated version of Slo1 channel, a cytoplasmic protein containing the functional calcium-binding sites of Slo1 and can thus represent the regulatory domain of the Slo3 channel that gives the KSper channel calcium sensitivity. To test their interaction, we used the HEK293T and insect cells expressions systems where we expressed fluorescently tagged sSlo1-GFP and C-terminus of Slo3: mCherry-Slo3-CT. Based on our pull-down experiments we show that sSlo1 and Slo3 form a complex in the presence of calcium ions. Regulatory role of sSlo1 in K+ current through Slo3 channel was also supported by electrophysiological experiments. Our data proof that sSlo1 and Slo3 indeed interact and thus, Slo3-sSlo1 complex is the long-thought molecular identity of human KSper. This work was supported by NIH R01GM111802, Pew Biomedical Scholars Award, and Packer Wentz Endowment Will to P.V.L.


Integrins are transmembrane cell receptors involved in two crucial mechanisms for successful fertilization namely, mammalian intracellular signalling and cell adhesion. Integrins α6β4, α3β1 and α6β1 are three major laminin receptors expressed on the surface of mammalian cells including gametes and the presence of individual integrin subunits α3, α6, β1 and β4 have been previously located in mammalian sperm. However, to date the proof of existence of individual heterodimer pairs in sperm and their detailed localization has been missing. The major conclusion of this study is the evidence that the β4 integrin subunit is expressed in mouse sperm and it pairs with subunit α6, additionally, there is a detailed identification of integrin heterodimer pairs across individual membranes in an intact mouse sperm head. We also demonstrate the existence of β4 integrin mRNAs in round spermatids and spermatagonia by q-RT-PCR, which was further supported by sequencing the PCR products. Using super-resolution microscopy, accompanied by colocalization analysis, we located integrin subunits as follows: α6/β4 – inner apical acrosomal membrane and equatorial segment; α3, α6/β1, β4 - plasma membrane overlaying the apical acrosome; α3/β1 - outer acrosomal membrane. The existence of α6β4, α3β1 and α6β1 heterodimers was further confirmed by proximity ligation assay (PLA). In conclusion, we delivered the detailed characterization of α3, α6, β1 and β4 integrin subunits showing their presence in distinct compartments of the intact mouse sperm head. Moreover, we identified sperm-specific localization for heterodimers α6β4, α3β1 and α6β1, and their membrane compartmentalization and the presented data show a complexity of membranes overlaying specialized microdomain structures in the sperm head. Their different protein composition may correspond with a specialized role of these individual membrane rafts based on their involvement in sperm-epithelium and sperm-egg interaction.
**P378 - Zinc exocytosis during egg activation is dependent on myosin light chain activity in the mouse and human.**  Hoi Chang Lee, Maxwell Edmonds, Francesca Duncan, Thomas O’Halloran, Teresa Woodruff

During egg fertilization and activation, the egg releases billions of zinc atoms (Zn\(^{2+}\)) in an exocytotic event termed the “zinc spark.” The zinc spark has been observed in eggs from multiple mammalian species, however, its importance and the mechanisms responsible for its zinc movements are ill-defined. During the meiotic transition of the oocyte from prophase I arrest, to metaphase II arrest, intracellular zinc changes from a symmetrical to a polarized distribution across the cell cortex. This shift mirrors that observed for cortical granules (CGs), structures observed to contain zinc. Thus, we hypothesized that the zinc responsible for the zinc spark is primarily stored in CGs, and should be dependent upon the myosin-actin motor system (MAMS) for its transport and exocytosis. To test this hypothesis, we treated eggs with ML-7, a selective myosin light chain kinase inhibitor, to examine the effects of MAMS-blockade on zinc localization and exocytosis. We used fluorescently labelled *Lens culinaris* agglutinin, a CG marker, to track the location of CGs, and ZincBY-1, a zinc-sensitive fluorophore, to detect intracellular zinc. ML-7 treated eggs had a 80% reduction in zinc spark intensity compared to untreated controls and a 49% reduction in intracellular zinc following activation with ionomycin. In the cortical region, the intensity of labile zinc and the number of CGs were significantly decreased in ML-7-treated eggs activated with ionomycin. During fertilization via intracytoplasmic sperm injection, ML-7 treated eggs demonstrated a tandem movement of both labile zinc and CGs from the cortex to the central cytosol. Moreover, ML-7 treated eggs had a significantly increased rate of polyspermy when fertilized by *in vitro* fertilization. These results support our understanding that CG exocytosis is necessary for the zinc spark, and that the MAMS is necessary and causal in zinc trafficking during oocyte maturation and exocytosis upon egg activation.

**P379 - Prenatal Exposure to Di-(2-ethylhexyl) Phthalate and High-fat Diet Synergistically Disrupts Mouse Fetal Oogenesis and Affects Folliculogenesis.**  Huanyu Qiao, Supipi Mirihagalle, Tianming You, Lois Sue, Chin Tan, Liying Gao, Saniya Rattan

Di-(2-ethylhexyl) phthalate (DEHP) is a chemical that is widely used as a plasticizer. Exposure to DEHP has been shown to alter ovarian function in humans. Additionally, foods high in fat content, regularly found in the western diet, have been shown to be another potential disruptor of fetal ovarian function. Due to DEHP’s lipophilicity, high-fat foods can be easily contaminated. Therefore, exposure to DEHP and a high-fat diet are both health concerns, especially in pregnant women, and the effects of these exposures on fetal oocyte quality and quantity should be elucidated. In this study, our goal was to determine if there are synergistic effects of DEHP exposure at an environmentally relevant level (20 µg/kg body weight/day) and high-fat diet on oogenesis and folliculogenesis. Dams were fed with a high-fat diet (45 kcal% fat) or a control diet (10 kcal% fat) one week before mating and during pregnancy and lactation. The pregnant mice were dosed with DEHP (20 µg/kg body weight/day) or vehicle control from E10.5 to litter birth. We discovered that treatment with an environmentally relevant dosage of DEHP and consumption of high-fat diet significantly increases synapsis defects in meiosis and accelerates affects folliculogenesis in the F1 generation.
**P380 - Cyclin B2 is required for progression through meiosis in mouse oocytes.** Enrico Maria Daldello, Xuan Luong, Cai-Rong Yang, Jonathan Kuhn, Marco Conti

The M-phase-promoting factor (MPF) is composed of a cyclin associated with CDK1, and its activation is essential for progression through both mitosis and meiosis. Three members of the cyclin B family (CCNB1, CCNB2 and CCNB3) are expressed in mice and humans. However, previous genetic studies concluded that CCNB2 is dispensable for cell cycle progression. We have shown that CcnB2 mRNA is translated at a high rate in prophase-arrested oocytes compared to CcnB1, a finding that prompted us to reevaluate the role of this protein during meiosis. CCNB2 concentration in GV-arrested oocytes is five time higher than CCNB1. A 50% decrease in CDK activity is present in GV oocytes from CcnB2−/− mice, demonstrating that CCNB2 contributes to pre-MPF. CcnB2−/− oocytes undergo delayed germinal vesicle breakdown and defective progression through meiosis I, due decreased Cdk1 activity, compromised CcnB1 and Mos mRNA translation, and delayed spindle assembly. Furthermore, in the absence of CCNB2, the spindle-assembly-checkpoint (SAC) cannot be completely satisfied, resulting in reduced APC activity. Given these defects, a significant percentage of CcnB2−/− oocytes fails to complete meiosis I. More importantly, oocytes that complete meiosis I display increased aneuploidy when compared to WT oocytes. In vivo, CCNB2 depletion impairs the oocyte ability to undergo nuclear maturation leading to decreased female fecundity. These findings demonstrate that CCNB2 is required to assemble sufficient MPF for timely meiotic reentry and progression. Although other endogenous cyclins cannot compensate in CcnB2−/− oocytes, overexpression of CCNB1/2 rescues the meiotic phenotypes, demonstrating that the two share similar molecular properties. Therefore, divergent modes of translational regulation distinguishes the function of these two cyclins. Supported by NIH R01 GM116926 and GM097165.

**P381 - Mito-TEMPO as a superoxide scavenger assists meiotic maturation through reduction of mitochondrial derived superoxide during porcine oocyte maturation in vitro.** Seul-Gi Yang, Hyo-Jin Park, Jin-Woo Kim, Min-Ji Kim, In-Su Kim, Ho-Guen Jegal, Deog-Bon Koo

Morphology of cumulus-oocyte complexes (COCs) in the germinal vesicle (GV) stage plays important roles on meiotic maturation for further embryonic development during in vitro maturation (IVM) of porcine oocyte. In addition, various antioxidant enzymes from cumulus cells of COCs regulate the intracellular levels of reactive oxygen species (ROS) in porcine oocyte during in vitro maturation. Recently, we showed that Mito-TEMPO improves porcine embryo development competence through reducing superoxide. However, antioxidant effects of Mito-TEMPO on meiotic maturation of porcine oocyte have not been reported. First, we divided into two groups (Grade 1: G1, high cumulus cells; Grade 2: G2, low cumulus cells) according to number of cumulus cells (CCs) in porcine COCs of GV stage. Meiotic maturation rate of G2 significantly decreased (p < 0.05) in porcine oocyte at 44 h of IVM compared to G1. Next, to investigate the superoxide production and antioxidant enzymes expression in porcine COCs during IVM, we performed Mito-SOX staining and RT-PCR analysis. Expression of Mito-SOX of G2 COCs was higher (p < 0.05) than that of G1 in 44 h of IVM. Also, mRNA levels of mitochondria related antioxidant enzymes (SOD1, SOD2 and PRDX3) decreased (p < 0.05) in G2 COCs compared to G1 in 44 h of IVM. Finally, we confirmed meiotic maturation and
expression of mitochondria related antioxidant enzymes of COCs after Mito-TEMPO treatment during IVM. Interestingly, meiotic maturation rate and mRNA levels of mitochondria related antioxidant enzymes of G2 COCs increased (p < 0.05) in Mito-TEMPO (0.1 μM) treatment G2 compared with non-treated G2. Based on these results, we confirmed that Mito-TEMPO enhances meiotic maturation and mRNA expression of mitochondria related antioxidant enzymes which regulates ROS during porcine oocyte maturation. Therefore, we suggest that regulation of mitochondrial superoxide is important in improvement of meiotic maturation during IVM of porcine oocyte.

P382 - Sestrin2 plays important roles in porcine oocyte maturation and embryonic development in vitro as a stress regulator protein against endoplasmic reticulum stress. In-Su Kim, Hyo-Jin Park, Jin-Woo Kim, Seul-Gi Yang, Ho-Guen Jegal, Min-Ji Kim, Deog-Bon Koo

Activation of unfolded protein response (UPR) signaling in response to endoplasmic reticulum (ER) stress is important in porcine oocyte maturation during in vitro. Sestrin2, a stress-metabolic protein, is known for reduction of reactive oxygen species (ROS) and regulation of UPR signaling activation which upregulates ER chaperone functions against ER stress. However, the effects of SESN2 in conjunction with ER stress during porcine oocyte maturation and early embryonic development in vitro have not been reported. Here, we investigated the expression pattern of SESN2 protein by using Western blot analysis in porcine cumulus-oocyte-complexes (COCs), denuded oocytes (DOs), and cumulus cells (CCs) for 22 h and 44 h after in vitro maturation (IVM). As expected, SESN2 protein level significantly increased in porcine COCs at 44 h of IVM. Meanwhile, protein levels of PERK signal markers (Grp78/Bip, p-eIF2α and ATF4) of COCs increased at 44 h of IVM. We also confirmed changes in protein expression of UPR signaling and SESN2 on embryonic development of pigs. SESN2 protein expression continuously increased from zygote stage until cleaved stage. Protein levels of PERK signal pathways (p-eIF2α, eIF2α, ATF4 and GADD34) increased until cleavage stage. Interestingly, SESN2 protein level significantly increased in blastocyst after tunicamycin treatment as an ER stress inducer during in vitro culture of porcine embryos. Based on these findings, we confirmed that the increase of SESN2 protein level is in conformity with UPR signal activation during oocyte maturation and early embryonic developmental stages. We also observed the activation of SESN2 protein in response to ER stress during porcine embryonic development. Therefore, we suggest that SESN2 protein may play important roles in porcine oocyte maturation and embryonic development in vitro.

P383 - The RNA binding protein Dazl functions as repressor and activator of maternal mRNA translation during mouse oocyte maturation. Cairong Yang, Gabriel Rajkovic, Enrico Daldello, Xuan Luong, Marco Conti

Deleted in azoospermia like (Dazl) is an RNA binding protein with critical function during gamete development. Our previous genome-wide analysis using RiboTag IP/RNA-Seq indicated that Dazl depletion causes both decreased and increased ribosome loading onto maternal mRNAs. This
finding opens the possibility that Dazl may function as both activator and repressor of translation in oocytes. Here we have further investigated this dual function of Dazl during oocyte maturation. The interaction of Dazl with both repressed and activated targets is consistent with the Dazl IP/Rip-Chip data showing Dazl interaction with the transcripts whose translation increases or decreases during meiosis. Using YFP reporters fused with the 3'UTR of the Dazl targets Oosp1 or Obox5, we found that Dazl depletion causes increased reporter translation in GV-arrested oocytes. This finding is in agreement with the increased translation efficiency of these two mRNAs calculated from the RiboTag IP/RNA-Seq data. During oocyte maturation, Dazl depletion is associated with decreased accumulation in Oosp1 and Obox5 reporter and confirmed by a decrease in Oosp1 and Obox5 translation rates. Conversely, translation of a reporter for cyclin B1, which is not a Dazl target, was not affected by Dazl depletion. Injection of a recombinant Dazl protein in Dazl-depleted oocytes completely rescue the reporter translation as well as maturation to MII, indicating that the depletion of Dazl protein with the specific MO is the sole cause of the decreased translation. A single mutation in the DAZL binding sites in either Oosp1 or Obox5 3’UTR is sufficient to significantly decrease the rate of reporter accumulation during meiotic resumption. Mutation of these DAZL binding sites also causes an increase translation in GV-arrested oocytes. In conclusion, our findings demonstrate that Dazl functions both as translational repressor and activator for regulating maternal RNAs translation during mouse oocytes maturation.

P384 - A Shotgun Proteomic Approach to Study Mitochondrial Inheritance. Dalen Zuidema, Peter Sutovsky, Michal Zigo, Won-Hee Song

The uniparental inheritance of mitochondria from the maternal lineage, well documented in mammals and other taxa, is referred to as the “Mitochondrial Eve Paradigm.” The contribution of sperm mitochondria, resulting in heteroplasmy is rarely observed, due to the post-fertilization degradation of sperm mitochondria, hitherto referred to as sperm mitophagy. The mechanisms that contribute to sperm mitophagy are not fully understood. Insight into cofactors and substrates involved must be gained, to better understand this crucial fertilization event. Through the use of our novel mammalian cell-free system, with the addition of biotin as a protein tracer molecule, we have created a proteomic shotgun protocol to observe and study early fertilization protein interactions. This cell-free system recapitulates the early post-fertilization mitophagic events which take place in a zygote and allows for the observation of thousands of spermatozoa interacting with oocyte proteins. After co-incubation, the sperm and oocyte proteins can be separated and those oocyte proteins which have bound to the sperm structures can be visualized and identified. To observe these ooplasmic protein interactions, we utilized biotinylation protocols. Allowing for the observation of oocyte proteins which bind to the sperm mitochondria and other sperm structures during co-incubation with the oocyte extract. Biotinylated oocyte proteins have been observed through western blot detection and immunofluorescent imaging. Using immunofluorescent imaging, we have observed oocyte proteins localizing on the acrosome and midpiece of the spermatozoa, which may implicate oocyte protein interactions in the processes of mitophagy and possibly the processing of sperm acrosomal structures that remain intact after acrosomal exocytosis. Further work includes quantification of such presumed pro-autophagic, sperm-bound proteins by imaged based flow cytometry and their proteomic identification and quantitation by Tandem Mass Tagging system. Proteins of interest are being
further studied using IVF protocols to fully understand the roles which they play in sperm mitophagy.

**P385 - Genetic Regulatory Mechanisms of Mammalian Spermatogonial and Spermatocyte Populations During Postnatal Testis Maturation Revealed by Single-Cell Sequencing.**
Kathryn Grive, Yang Hu, Eileen Shu, Andrew Grimson, Olivier Elemento, Jennifer Grenier, Paula Cohen

Spermatogenesis is the process by which male gametes are formed from a self-renewing population of spermatogonial stem cells residing in the testis. Once spermatogonial differentiation has occurred, the newly formed and highly-proliferative spermatogonia must then enter the meiotic program. While much is known about the critical cellular processes that take place during this specialized cell division, much less is known about how the spermatocytes in the “first-wave” compare to those that contribute to “steady-state” spermatogenesis. First-wave spermatocytes are, however, known to exhibit several unique, and some detrimental, characteristics, including reduced recombination rate and greater incidence of chromosome mis-segregation. These features result in first-wave-spermatooza which are often much less reproductively successful than those which will arise from the self-renewing SSC population later in life, though the mechanisms underlying these effects are not well-understood.

Given this strictly-defined developmental process, this study was aimed at exploring the transcriptional profiles of developmental cell stages over the age of the animal. Using a combination of comprehensive germ cell sampling with 10X Genomics single-cell-mRNA-sequencing, we have generated a reference dataset of germ cell gene expression. We show that discrete developmental stages possess significant differences in their transcriptional profiles. We also show differential utilization of many biological pathways with age in both spermatogonia and spermatocytes, demonstrating significantly different underlying gene regulatory programs over the course of testis development and spermatogenic waves. Not only does this analysis reveal previously unknown transcriptional dynamics of highly transitional cell populations, it has also begun to reveal critical differences in biological pathway utilization in developing spermatogonia and spermatocytes, including response to DNA damage and double-strand breaks. These studies not only provide us with incredibly fine temporal and developmental resolution of the transcriptomic changes during spermatogenesis, but it also helps us better understand differential gene expression during first-wave-spermatogenesis versus steady-state-spermatogenesis.

**P386 - Sycp2 is essential for the synaptonemal complex assembly and early meiotic recombination in zebrafish spermatocytes.** Noriyoshi Sakai, Yukiko Imai

Meiotic recombination is essential for faithful segregation of homologous chromosomes during gametogenesis. Progression of recombination is associated with dynamic changes in meiotic chromatin structures. However, whether structural components of chromosome are required for initiation of meiotic recombination is still unclear in vertebrates. Here, we describe that assembly of the synaptonemal complex comprising Sycp2, Sycp3 and Sycp1 is initiated at telomeres
depending on SyCP2, and that SyCP2 is essential for meiotic DSB formation evaluated by gammaH2AX signals and Dmc1 and Rpa focus formation by using syCP2 mutant zebrafish. A meiotic mutant zebrafish line, ietsugu (its), generated by ENU mutagenesis, harbors a T to A substitution in intron 8 of the syCP2 gene that generates an aberrant splice site leading to a 5-bp insertion containing a premature stop codon in its syCP2 mRNAs. The its mutation did not complement the sterility of a syCP2 knockout, generated by CRISPR mutagenesis. The its mutation appeared to be a hypomorphic, by comparison with the syCP2 knockout that causes defects in the synaptonemal complex assembly and chromosome pairing, indicated by drastically increased numbers of telomere focus in spermatocytes. Kinetic analyses using these mutant lines revealed that assembly of the synaptonemal complex comprising axial SyCP2 and SyCP3 and transversal SyCP1 is initiated at telomeres depending on SyCP2. Interestingly, in wildtype spermatocytes, we found that majority of the meiotic recombinase Dmc1 foci localize on axial elements at leptonema to early zygonema, where assembly of SyCP2 and SyCP3 begins at telomeres. Strikingly, Rpa and Dmc1 foci were not detectable and gammaH2AX signals were strongly diminished in syCP2 knockout spermatocytes. Taken together, our data suggest that SyCP2 plays a critical role in assembly of axial elements from telomeres, and in meiotic DSB formation and/or early repair events that preferentially occurs near chromosomal ends in zebrafish males.

**P387 - Ovarian synchronization by follicle aspiration and moderate FSH treatment improves oocyte quality and the efficiency of in vitro embryo production in cattle.** Ana Caroline Soares, Kelly Marques, Luiz Gustavo Martignoni, Valentina Lodde, Alberto Luciano, Jose Buratini

Oocytes obtained by ovum pick-up (OPU) from cows are heterogeneous with regard to chromatin compaction, and those with an intermediate degree have higher developmental competence. We have developed a protocol combining follicle aspiration and FSH treatment that increases the percentage of oocytes with intermediate chromatin compaction at OPU. In this study, we assessed the effect of this protocol on in vitro embryo production following OPU. Eighteen non-lactating Holstein cows had all follicles larger than 3mm aspirated and a progesterone intravaginal device inserted on a random day (day 0). Four IM injections of FSH (Folltropin; 40/40/20/20 mg) were administered 12h apart on days 2 and 3. On day 5, the progesterone device was removed and OPU was performed. The control group consisted of seventeen non-lactating Holstein cows submitted to OPU on a random day. After OPU, cumulus-oocyte complexes (COC) underwent in vitro maturation (IVM) for 24h in a medium containing 0.5 mg/mL FSH and 10% bovine fetal serum, followed by in vitro fertilization and in vitro (IVC) culture of the presumptive zygotes. After seven days of IVC, embryo production was assessed to determine the percentage of blastocysts in relation to the number of oocytes subjected to IVM, and percentage of viable embryos (morphologically selected for freezing and later transfer) in relation to total blastocysts. Data were arcsine transformed and groups compared with the Student’s t test. Blastocyst rate was higher in the treatment group (37.91 ± 6.48%; 79/206) in relation to the control (20.98 ± 2.62%; 78/348; P<0.05). Control and treatment groups did not differ for percentage of viable embryos (65.19 ± 8% and 64.89 ± 11.18%, respectively). These data indicate that protocols combining antral follicle removal and FSH treatment to homogenize
the oocyte population for IVM can improve the efficiency of embryo in vitro production in cattle. Supported by FAPESP 2016/21671-9.

**P388 - Sperm mitochondrial DNA biomarkers and couple fecundity: The Longitudinal Investigation of Fertility and the Environment (LIFE) Study.** Allyson Rosati, Nicole Brandon, SL Mumford, EF Schisterman, Brian Whitcomb, Richard Pilsner

**Introduction.** Recent studies have linked mitochondrial biomarkers, DNA copy number (mtDNACn) and DNA deletions (mtDNAdel), with poor semen parameters and fertilization rates. However, no study has investigated the associations between sperm mtDNACn and mtDNAdel with conception probability among couples from the general population.

**Methods.** As part of the Longitudinal Investigation of Fertility in the Environment (LIFE) Study, sperm were isolated from 386 semen samples via a one-step gradient to remove somatic cell contamination. Time-to-pregnancy (TTP), determined as the number of menstrual cycles to occur before a human chorionic gonadotropin confirmed pregnancy, was used to measure fecundability (cycle-specific probability of conception). A triplex probe-based qPCR method quantified sperm mtDNACn and mtDNAdel. Cox proportional hazards models were used to estimate fecundability odds ratios (FOR) relating TTP with mtDNA biomarkers and to generate survival curves. Analyses were run using continuous mtDNA biomarkers as well as in quartiles to allow for non-linear relations, and were adjusted for male age and BMI, race and ethnicity, site of sample collection, cotinine, and qPCR batch.

**Results.** In multivariable Cox models, sperm mtDNACn was associated with lower fecundability and longer TTP (FOR: 0.34; 95% confidence interval [CI]: 0.20, 0.58; p=0.0001). This trend was observed in analyses including mtDNA measures in quartiles; compared to the first quartile as the referent, the FOR for the second quartile was 0.81 (95% CI: 0.53, 1.24), 0.65 (95% CI: 0.43, 0.98) for the third, and lowest in the fourth (FOR: 0.52, 95% CI 0.34, 0.72). Sperm mtDNACn and mtDNAdel were correlated (r=0.45, p<0.0001); however, no meaningful association was observed between sperm mtDNAdel and TTP, and model estimates were not statistically significant.

**Conclusions.** Sperm mtDNACn was associated with a two-fold lower odds of cycle-specific conception and thus longer TTP, suggesting the utility of sperm mtDNACn as a biomarker to assess couples’ fecundity.

**P389 - Melatonin rescues the aneuploidy in mice vitrified oocytes by decreasing mitochondrial heat product.** Lei Gao, Shenming Zeng, Yunpeng Hou, Shien Zhu, Xiangwei Fu

Vitrification of germinal vesicle (GV) stage oocytes has been shown to be closely associated with decreased rates of meiosis maturation and increased rates of aneuploidy. However, little is known about the effects of melatonin on these events in mice vitrified GV oocytes. In this study, the effects of melatonin on meiosis maturation potential and the incidence rate of aneuploidy in
mouse vitrified oocytes were analyzed by supplementing IVM solution with melatonin at different concentrations. This study, for the first time, showed that the balance of the mitochondrial energy conversion has been broken by vitrification through significantly decreasing ATP level (0.88 vs. 1.13 pmol, P < 0.05) and markedly increasing mitochondrial heat production (P < 0.05), which resulted in compromising the first polar body extrusion (PBE) of oocytes (85.1% vs. 73.3%, P < 0.05). RNA-Seq results showed the Differentially express genes (DEGs) were significantly enriched in “mitochondrial respiratory chain complex I”. However, 10^{-11} mol/L melatonin could significantly decrease mitochondrial heat production and ROS level (9.1 vs. 12.0 pixels, P < 0.05), meanwhile increase ATP level (1.1 vs. 0.88 pmol, P < 0.05) and mtDNA copies (107438 vs. 67869, P < 0.05), which rescued the abnormal chromosome alignment (32% vs. 69%, P < 0.05) and the incidence of aneuploidy (15.6% vs. 38.5%, P < 0.05) in vitrified oocytes. The meiosis maturation ability of vitrified oocytes with melatonin supplementation was similar to that of fresh ones (83.4% vs. 85.1%, P >0.05). Collectively, our data revealed that melatonin has a protective action against vitrification-induced unbalance of oocytes mitochondrial function.

**P390 - Membrane Raft-associated Src Family Kinases (SFK) Regulate Sperm Acrosome Reaction in Chickens.** Chathura Priyadarshana, Rangga Setiawan, Naoto Ishikawa, Atsushi Tajima, Alexander Travis, Atsushi Asano

Acrosome reaction (AR) is an exocytotic event which is pre-requisite for sperm to penetrate into ovum. Despite that several signaling molecules have been found to regulate AR induction, it still remains unclear how signaling pathways are governed to function when they need. Membrane rafts (MR) are sterol-enriched membrane domains and play an important role in diverse cellular processes. Recently, we have reported in avian sperm that MR regulate AR induction via activation of cAMP/PKA pathway. However, the molecular mechanism connecting PKA activation to AR induction is not understood yet. It has been shown in mammalian sperm that phosphorylation of src family kinases (SFK) are a downstream of PKA activation, consequently leading to elevation of acrosomal responsiveness. These encouraged us to characterize the functional roles of SFK in chicken sperm AR. Immunoblots for c-src and phosphorylated form of SFK (p-SFK) showed their expression at a predicted molecular weight (60kDa). Localization experiments found the presence of c-src in the sperm head with multiple focal enrichments. Using two specific SFK inhibitors (SKI606,SU6656), we found that SFK inhibition stimulated PKA activity and AR induction although no difference was seen in motility profiles. To better understand the relationship between PKA and SFK, we examined change in p-SFK under supplementation of PKA inhibitor, demonstrating that SFK phosphorylation is not downstream of PKA in chicken sperm. In addition, [Ca^{2+}]i and membrane potential assays indicated that increases in p-PKAs and AR in response to SFK inhibition resulted from membrane hyperpolarization. To seek regulation mechanism for SFK phosphorylation, changes in p-SFK in response to MR disruption was examined using 2OHCD and showed the involvement of MR in regulation of SFK phosphorylation. Taken together, our results demonstrate that SFK is involved in AR induction by regulating PKA pathway, providing new insight into the functional roles of MR in avian sperm.
**P391 - New sub-cellular compartmentalization of cAMP-specific phosphodiesterase 8A in ovarian follicular cells.** Amel Lounas, Nathalie Vernoux, Marc Germain, Marie-Eve Tremblay, François J Richard

Cyclic adenosine monophosphate (cAMP) is a ubiquitous secondary messenger that plays a central role in endocrine tissue function, particularly the synthesis of steroid hormones. The intracellular concentration of cAMP is regulated through its synthesis by cyclases and its degradation by cyclic nucleotide phosphodiesterases (PDEs). Although the expression and activity of PDEs impact the specificity and amplitude of the cAMP response, it is becoming increasingly clear that the sub-cellular localization of PDE determines the spatial regulation of the signalling processes that are essential for normal cellular function. We first examined the expression and activity of PDE8A in porcine ovarian cells, as well as its influence on progesterone production and mitochondrial status during the in-vitro maturation of cumulus-oocyte complexes. PDE8A is functionally expressed in granulosa cells, cumulus cells and oocytes. Using an inhibitor specific to PDE8 (PF-04957325), we observed a significant increase (P < 0.05) in progesterone secretion with follicle-stimulating hormone (FSH). This supports the notion that FSH-stimulated progesterone secretion is regulated by PDE8. In addition, mitochondrial activity measured in cumulus cells by fluorescence intensity after labelling with mitoTracker orange CMTM Ros increased (P < 0.05) under FSH stimulation and PDE8 specific inhibition compared to control. Second, we assessed the mitochondrial sub-cellular localization of PDE8A. Using western blotting with isolated mitochondrial fractions from granulosa cells revealed immuno-reactive bands. PDE assay of isolated mitochondrial fractions from granulosa cells measured PF-04957325-sensitive cAMP-PDE activity. The immune-reactive PDE8A signal and MitoTracker labelling co-localized supporting mitochondrial sub-cellular localization of PDE8A, which was confirmed using immuno-electron microscopy. In conclusion, we propose the occurrence of mitochondrial sub-cellular localization of PDE8A in porcine granulosa cells and cumulus cells. This suggests that there is potential for new strategies for ovarian stimulation and artificial reproductive technologies, as well as the possibility for using new media to improve the quality of oocytes.

**P392 - Evaluation of the Variability among Bulls for CD9 and SERPINA5 on the Bovine Sperm Head.** Saulo Menegatti Zoca, Jerica Rich, Kaitlin Epperson, George Perry

Even among bulls that successfully pass a breeding soundness exam; there are differences in fertility. Cell to cell interactions are a critical factor in formation of the sperm reservoir and in binding to the oocyte. Both CD9 and SERPINA5 are proteins associated with cell to cell interaction and are homologous in several mammalian species. To serve as a potential marker of fertility there must be variability in protein expression among animals. Therefore, the objective of this study was to characterize the variability of CD9 and SERPINA5 protein expression on sperm among bulls. Semen from 17 bulls of three different breeds (Angus, Simmental and Hereford) were fixed and evaluated for presence of CD9 and SERPINA5. All samples passed quality control for frozen-thawed semen (Total and progressive post-thaw motility were 32.6±12.5% and 19.0±8.3%, respectively). Aliquots containing ~500,000 sperm were incubated with anti-CD9 or anti-SERPINA5, and fluorescence intensity (FI) was evaluated on a minimum of 100 sperm per bull. Data were analyzed using the GLM procedure in SAS with bull as a fixed
effect to determine if the variance in both proteins was greater between bulls compared to within a bull. Both CD9 and SERPINA5 were localized to the sperm head. However, SERPINA5 was also detected on the proximal region of the sperm tail among all bulls (33.5±4.1% of sperm; range 4% to 61%). Variation in FI on the sperm head was greater for both CD9 \((P<0.001; \text{ FI range 14.1±0.17 to 19.9±0.18})\) and SERPINA5 \((P<0.001; \text{ FI range 12.9±0.35 to 19.0±0.36})\) among bulls (variance of 5.96 and 15.14, respectively) compared to within bulls (variance of 3.32 and 13.03, respectively). In summary, there was significant variation among bulls in CD9 and SERPINA5 (proteins associated with cell to cell interaction) and these sperm proteins might serve as a marker for fertility.

**P393 - Dynamics of the protein modification during sperm capacitation in differential male fertility.** Yoo-Jin Park, Won-Ki Pang, Do-Yeal Ryu, Ji-Hyun Son, Md Saidur Rahman, Won-Hee Song, Myung-Geol Pang

Mature spermatozoa can acquire functionality through capacitation, as a post-translational modification, because the spermatozoa are transcriptionally and translationally silent. While some studies have reported that strongly advocate the activation of sperm translation though mitochondrial mRNA (mt-mRNA) during capacitation, transcriptional and translational activations are still debated. Moreover, these studies were interested in evaluating mRNA translation in spermatozoa during capacitation, regardless of understanding the status of male fertility. Given the possible importance of translation in capacitation, we reasoned that this phenomenon may play a key role in male fertility. In this study, to investigate the physiological and pathological role of proteins during capacitation in fertility, we tried to elucidate the translational modification of these proteins during bovine sperm capacitation associated with the fertility status. We observed the translational modifications in actin-related protein T2 (ACTRT2), voltage dependent anion channel 2, and phospholipid hydroperoxide glutathione peroxidase (GPx4) during capacitation in below-normal fertility. However, enolase1 in below-normal fertility decreased during capacitation while there was no change in normal fertility. Based on these findings, we hypothesized that this modification is a consequence of mt-mRNA translation. To elucidate our hypothesis, we observed translational modifications of these proteins in sperm mitochondrial fraction. GPx4 protein expression was increased in mitochondrial fraction from below-normal fertility, while it was stationary in normal fertility spermatozoa. Moreover, the ACTRT2 expression was increased in head and mitochondrial regions during capacitation only in below-normal fertility. To the best of our knowledge, this study is the first study to support the dynamics translation in spermatozoa during capacitation associated with male fertility. These findings may shed light on the understanding of mechanisms regulating the male fertility during capacitation, fertilization and beyond.

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**P394 - Identification of a rare, fertility affecting mutation in bovine Eml5.** Michal Zigo, Eriklis Nogueira, Karl Kerns, Miriam Sutovsky, JaeWoo Kim, Filip Tirpak, Thomas Spencer, Jeremy Taylor, Robert Schnabel, Peter Sutovsky

The goal of this project is to identify rare, deleterious mutations that adversely affect sperm quality and the ability to convey high developmental potential to embryos and increased fertility to progeny genomes. We sequenced the genomes of 85 Holstein, Jersey and Angus bulls with acceptable but varied artificial insemination (AI) fertility and with indications of unusual sperm phenotypes. A rare homozygous mutation in the Eml5 gene encoding a microtubule associated protein that is highly expressed in testis and brain was identified in an Angus sire used extensively in artificial insemination (AI) in USA and internationally due to outstanding progeny production traits. The sire’s fertility was low in cross-breeding timed AI (Pregnancy/TAI= 25.2%; n=222) and in intra-breed AI (41%; n=822). His spermiogram displayed prominent abnormal sperm phenotypes including piriform/tapered heads and knobbed acrosomes, the latter also prevalent in a heterozygous carrier sire from a different Angus lineage. The mutant allele was not detected in the sequenced Holstein or Jersey bulls. Confirmed by PCR, this non-synonymous, amino acid substituting mutation is within one repeat of the WD40 signaling domain of the EML5 protein and appears to be orthologous to a human Eml5 mutation associated with cancer. In both the homozygous and the heterozygous Angus bulls, increased retention of EML5 was observed in the sperm head though traces of EML5, likely a carryover from spermiogenesis, were also detected in the sperm heads of bulls that were homozygous wild type. Work is under way to re-examine the field AI fertility of carrier bulls and to characterize the role of EML5 in spermatogenesis. Supported by NIH 1R01HD084353 (PS, JT, RS), MU F21C Program (PS), and USDA-NIFA grants 2013-68004-20364, 2016-67015-24923, 2017-67015-26760 (JT, RS) and 2013-68004-20365 (TS). In-kind contributions provided by Genex Cooperative and Select Sires.

**P395 - Nek5 regulates cell cycle progression during mouse oocyte maturation.** Zhiming Han, Yuanyuan Li, Lei Guo, Hui Li, Shengsheng Lu

Mammalian oocytes arrest at prophase of meiosis I is the result of low activity of maturation promoting factor (MPF). MPF is a complex of a catalytic subunit cyclin dependent kinase 1 (CDK1 or Cdc2) and its regulatory subunit cyclin B1. CDK1 plays a critical role in the resumption of meiosis during oocyte maturation and Wee1B is a key CDK1 inhibitory kinase that phosphorylates CDK1 at Tyr15 in mouse oocytes. Nek 5, a member of the NIMA-related kinase family, plays an important role in timely centrosome separation and bipolar spindle formation in mitosis. In this study, we investigated the expression characteristic, localization and function of Nek5 during mouse oocytes maturation. The results of western blot showed that NEK5 was expressed from germinal vesicle (GV) to metaphase II (MII) stages with the highest level of expression at GV stage. Immunofluorescence analysis revealed that NEK5 localized in cytoplasm at GV stage, concentrated around chromatin at germinal vesicle breakdown (GVBD) stage, and localized to the entire spindle at prometaphase I (pro-MI), MI and MII stages. To investigate the function of Nek5, the Nek5 knockdown was performed by the siRNA-mediated Nek5 depletion at GV stage and the effects were examined by western blot, immunofluorescence, quantitative real-time PCR, etc. with at least three replicates per experiment. The results showed that the protein level of p-Cdc2 (Tyr15) significantly increased...
and oocyte meiotic resumption was severely blocked in the Nek5 depletion groups compared with the control group, which can be rescued by Wee1B depletion. This study demonstrated for the first time that Nek5 regulates meiotic cell cycle progression during mouse oocyte maturation. This study was supported by the National Natural Science Foundation of China (31572226, 31372144) to Z.H.

**P396 - Microtubule-dependent cytoplasmic MTOCs are necessary for spindle anchoring and positioning during oocyte meiosis.** Ahmed Balboula

Female meiosis is a notoriously error-prone process, especially with the advancement of age. One of the unique features during oocyte meiosis is the asymmetrical position of the spindle to extrude a small polar body, yet F-actin is believed to be the only player regulating this function. Interestingly, mammalian oocytes also do not contain classic centrosomes. Therefore, spindle assembly depends on the clustering of numerous microtubule organizing centers (MTOCs) to form spindle poles. Whether MTOCs have different functions during oocyte meiosis has remained largely unknown.

We fluorescently labeled MTOCs using AURKA-GFP/Cep192-GFP followed by time-lapse confocal microscopy. Unexpectedly, we observed two distinct pools of MTOCs: those that cluster to form spindle poles (polar; pMTOCs) and those that circulate in the cytoplasm (cyMTOCs). We confirmed that these fluorescently-labeled structures are indeed MTOCs as evidenced by their co-immunostaining with gamma-tubulin and their ability to nucleate microtubules (MTs) when exposed to taxol. Further analysis showed that pMTOCs have similar kinetic and directional behaviors to the cyMTOCs; suggesting the latter might have a unique role in anchoring pMTOCs to regulate spindle positioning. To confirm our hypothesis, we conducted a laser ablation experiment to ablate cyMTOCs using 2-photon laser microscopy. Strikingly, ablation of cyMTOCs resulted in severe defects in spindle positioning. Importantly, using super-resolution microscopy, we found that cyMTOCs are connected to both the oocyte cortex and pMTOCs through MT connections. Taken together, our results suggest a model where astral MTs emanating from pMTOCs attach to cyMTOCs; which in turn act as amplifying sites for MT nucleation, anchoring pMTOCs to the cell cortex. Furthermore, we found that MT-dependent cyMTOCs and F-actin exhibit opposing forces necessary for spindle positioning to prevent premature spindle migration, challenging the current way of thinking that F-actin is the only player executing this function. This research is supported by Marie Curie, Horizon 2020,706170.

**P397 - Pannexin 1 hemichannels and their effect on bovine oocyte maturation and development.** Zachary Dye, Paul Dyce

Cellular communication between the oocyte and its surrounding cells and environment is critical for oocyte growth and embryo development. It is well demonstrated that communication between the oocyte and its surrounding cells is facilitated by gap junctions. However, less is known about the hemichannel forming family of membrane proteins called pannexins, which facilitate small molecule exchange between the cell and the extracellular environment. While
pannexin 1 (PANX1) has been found to be expressed in many human and mouse tissues and facilitates many processes including ATP release, apoptosis, and immune response, little is known on its potential role in reproductive tissues. To characterize PANX1 in bovine reproductive tissues, we looked at its expression and function in bovine cumulus-oocyte complexes (COCs). We found expression of PANX1 in COCs particularly in the cumulus surrounding the oocyte using western blotting and immunochemistry. Maturation processes and development stages were measured during in vitro maturation (IVM) where COCs were treated with or without the PANX1 channel inhibitor 10Panx (100µM). We found that cumulus expansion was significantly greater in the control group (186.3±6.3µm) when compared to the 10Panx treated group (135.0±7.6µm, p<0.05). It was also shown that during nuclear maturation, the PANX1 inhibited group had delayed maturation (60.0±6.7% being at the GV stage) compared to the control (36.7±3.4%, p<0.05) following 6 hours of maturation. Furthermore, we found that the PANX1 inhibited group had significantly higher blastocyst development rates (42.9±1.7% vs. 20.2±2.9%, p<0.05) following in vitro fertilization and embryo culture.

Preliminary results show PANX1 appears to play a role during oocyte maturation. Studies aiming at further elucidating that role are ongoing. This project was supported by the Alabama Agricultural Experiment Station and the Hatch program of the National Institute of Food and Agriculture, U.S. Department of Agriculture.

P398 - MIR21 inhibition influences porcine oocyte maturation through regulation of metabolic pathways. Yunsheng Li, Malavika Adur, Steven Lonergan, Aileen Keating, Jason Ross

MicroRNA (miRNA), small non-coding RNA molecules critical for regulating cellular function, are abundant in the maturing pig oocyte and developing embryo. MicroRNA21 (miR21) is highly abundant in mature porcine oocytes and cumulus cells. However, the mechanism by which miR21 regulates porcine oocyte maturation remains unclear. The objective of this study was to assess the function of miR21 during pig oocyte maturation. Anti-miR21 peptide nucleic acids (miR21-I) were designed to specifically bind to and prevent miR21 activity during oocyte maturation. Inhibition of miR21 activity during in vitro maturation decreased oocyte ability to achieve metaphase II arrest compared to NC-PNA and NC groups (P < 0.05). Following parthenogenetic activation, cleavage rate at 48 h in the miR21-I group was decreased (P < 0.05) relative as compared with NC-PNA and NC groups. miR21-I during IVM did not affect early apoptosis rate in oocytes, nor did it impact mRNA encoding, apoptosis, or autophagy related genes expression in oocytes as compared with the NC-PNA group. miR21-I treatment increased (P = 0.06) reactive oxygen species ROS production (46.4 %) in oocytes compared with the NC-PNA group. Additionally, liquid chromatography-mass spectrometry (LC-MS/MS) was employed to evaluate changes in the oocyte and cumulus cell proteome of oocytes and cumulus cells following miR21-I during IVM. In total, the abundance of 110 proteins in cumulus cells and 30 proteins in oocytes were identified to be differentially abundant between miR21-I and NC-PNA groups. Functionally, important signaling pathway analyses of differentially abundant proteins identified primary involvement of metabolic pathways affected. These data suggest that miR21 potentially influences porcine oocyte maturation through regulation of metabolic pathways in cumulus oocyte complexes (COCs). This project was supported by Agriculture and
Food Research Initiative Competitive Grant no. 2017-67015-26459 from the USDA National Institute of Food and Agriculture.

**P399 - Na/K-ATPase and fertility of breeding boars.** Muhammad Imran, Murray Pettitt, Mary Buhr

The fertility of animal sperm that passes normal function and morphology tests is still hard to predict. The transmembrane protein Na/K-ATPase in the head plasma membrane (HPM) of sperm induces capacitation through ion transport and/or signal transduction, and could correlate with in vivo fertility. Na/K-ATPase consists of paired alpha (A) and beta (B) subunits, each of which has multiple isoforms. We hypothesized that the isoform(s) most critical to capacitation would specifically correlate with fertility. Our objective was to characterize and quantify the Na/K-ATPase isoforms (A1, A2, A3; B1, B2, B3) in sperm from boars of known fertility.

Fertility was determined by Direct Boar Effect (DBE) for farrowing rate and litter size from >20 inseminations for 12 boars (6 high and 6 low fertility). Motility and morphology did not differ between fertility groups (p>0.5). Immunofluorescence of sperm from each ejaculate found distribution of specific isoforms differed with fertility (p<0.05). The HPM isolated from those ejaculates was subjected to Western immunoblotting to detect the molecular weight (MW) banding pattern of each isoform, and that of a standard which monitored gel-to-gel variation. Image Quant analysis assessed the volume (amount) of each band in each isoform, which was corrected with the standard. Randomized Complete Block Design tested for fertility differences. Most isoforms were similar, but total amount of the isoform A3, and that of 7 individual A3 bands, were significantly greater in the high fertility boars (p<0.05). Linear regression confirmed a highly significant (r²=0.90; p<0.0001) relationship of total amount of A3 with farrowing rate. In low fertility boars, several MW bands were missing from isoforms B1 and B2, and total amount of B2 tended to be lower in low fertility boars (p=0.08). These findings suggest that specific isoforms of Na/K-ATPase in the sperm head are correlated to boar in vivo fertility.

**P400 - Sperm decondensation following ICSI in bovine: effect of activation protocols and interspecies ICSI.** Mariana de Macedo, Karina Gutierrez, Werner Glanzner, Vitor Rissi, Luke Currin, Naomi Dicks, Hernan Baldassarre, Vilceu Bordignon

Intracytoplasmic sperm injection (ICSI) is widely used in humans and mice. There is interest in using ICSI in species like cattle and pigs to produce more offspring from expensive semen, improve fertility of semen with compromised motility and to prevent polyspermy. However, bovine embryo production using ICSI remains characterized by low efficiency. Some reasons behind this are defective oocyte activation and sperm decondensation. The objectives of this study were to assess the efficiency of different activation protocols on pronuclear (PN) formation after bovine ICSI and evaluate the effect of interspecies ICSI (bovine-swine) on sperm decondensation. Abattoir-sourced in vitro matured oocytes were used in the experiments and oocytes were analyzed for PN formation 15h post-activation. First, ICSI was performed and oocytes were either activated with Ionomycin for 5min (Ion-5µM/ml) followed by a zinc chelator for 20min (TPEN-100µM/ml) or not activated (CT). The rate of male and female pronuclei formation (2PN) was higher in Ion+TPEN than in CT (29.5% vs 14.3% p<0.05). Second,
following ICSI, oocytes were activated by injecting recombinant bovine phospholipase C zeta mRNA (rbPLCζ) or water (CT). Higher rate of 2PN was obtained in the rbPLCζ compared with the CT group (49.2% vs 7.4% p<0.05). Lastly, ICSI was performed in pig oocytes using bovine sperm. Oocytes were activated with Ion+TPEN, rbPLCζ or not activated (CT). No difference was observed in 2PN formation rates between oocytes activated with Ion+TPEN (56.2%), rbPLCζ (44.4%) or CT (41%). These results revealed that: i) chemical (Ion-TPEN) and physiological (rbPLCζ) activation following ICSI increases the rate of 2PN formation in bovine; and ii) bovine sperm decondense at higher rate when injected in porcine oocytes, even without an activation treatment. This suggests that in vitro matured bovine oocytes lack the required factors to promote sperm decondensation, which explains the poor results following homologous bovine ICSI without chemical activation.

**P401 - The origin and characterization of surface-borne glutathione-s-transferase omega 2 within mouse and boar capacitation.** Lauren Hamilton, Wei Xu, Michal Zigo, Jiude Mao, Peter Sutovsky, Richard Oko

In our pursuit to characterize the newly identified perinuclear theca residents, glutathione-s-transferase omega 2 (GSTO2) within mammalian spermatozoa, our findings revealed a secondary localization of the enzyme on the surface of the sperm plasmalemma. This novel localization of surface-borne GSTO2 led our group to investigate its origins and possible role during sperm capacitation, the morphological and physiological priming of mammalian spermatozoa that enables them to acquire the ability to fertilize the oocyte. Through surface protein biotinylation and indirect immunofluorescence, GSTO2 was identified on the plasmalemma of both mouse and boar spermatozoa. The immunohistochemical localization of GSTO2 in the epididymal epithelium suggests GSTO2 may be secreted into the epididymal lumen and transferred onto the plasma membrane. Functional inhibition studies demonstrate a dampening of tyrosine phosphorylation during in vitro capacitation and a significant decrease in the ability of spermatozoa to undergo the induced acrosome exocytosis reaction when the active site of GSTO2 is inhibited. Furthermore, a decreased fertilization ability of GSTO-impaired spermatozoa during in vitro fertilization lends further support to a possible role for GSTO2 during the capacitation process. Overall, whilst the specific role of GSTO2 within capacitation is not fully understood, our findings suggest that it may participate in a regulatory role as mammalian spermatozoa acquire the ability to fertilize the oocyte.

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**P402 - Destruction dynamic of securin in mammalian meiosis I.** Lenka Radonova, Michal Skultety, Martin Anger
It is known that chromosome segregation in mammalian female meiosis I is prone to errors. In consequence, the metaphase II arrested oocytes are frequently aneuploid. This status leads into termination of further development or to mental or developmental disorders. Chromosome segregation in meiosis I at the molecular level is controlled by multiple pathways, including Spindle Assembly Checkpoint (SAC) and Anaphase Promoting Complex/Cyclosome (APC/C). SAC remains active until all chromosomes are properly connected to the spindle apparatus and its activity blocks the activation of APC/C. This ensures that the oocytes will not enter anaphase unprepared for division. The activation of APC/C leads into proteolytic destruction of various substrate molecules essential for maintaining metaphase, including cyclins and securin. In this study we focused on proteolytic degradation of securin, which is required for controlling the activity of separase. We used microinjection and live cell imaging and our goal was to determine how the degradation of securin is spatially and temporarily synchronized in a relatively large cytoplasm of the mammalian oocyte. Our results showed that the dynamics of securin degradation is affected by its proximity to spindle and chromosomes, which is important for the accurate timing of chromosome segregation. Our project was supported by CSF projects 17-20405S and 19-24528S and by MEYS CR project CEITEC 2020 (LQ1601).

**P403 - Cumulus Oocyte Complex Secretions can Induce Porcine Sperm Release from Oviduct Epithelial Cell Aggregates.** Lantana Grub, David Miller

In many mammals, sperm are stored in the lower oviduct (isthmus) after successful mating for a finite time prior to release from the reservoir for fertilization. This storage period lasts 24-36 hr in pigs and is crucial when ovulation is not tightly coupled to semen deposition. Currently, there are large gaps in knowledge of how sperm are released from oviduct epithelial cells. We hypothesized that the release of sperm from oviduct epithelial cells can be triggered by the secretions of cumulus oocyte complexes. To test this, oocytes were aspirated from the ovaries of gilts. Immature oocytes were cultured in vivo for 45 hr in TCM-199-based porcine oocyte maturation medium. After 45 hr this medium was collected and centrifuged to remove cells and debris. The supernatant was termed Cumulus Oocyte Complex Conditioned Medium (COC-CM). Sperm were introduced to oviduct epithelial cell aggregates at 1x10^6 sperm/ml, sperm were allowed to bind for 45 min, and then COC-CM was introduced at 50% final volume. TCM-199-based porcine oocyte maturation medium was used as a control. The number of sperm bound to oviduct epithelial cells was enumerated just after COC-CM addition (0 hr), and 0.5 hr, 1 hr, and 1.5 hr after COC-CM addition. One hr after COC-CM addition, 19% fewer sperm were bound compared to 0.5 hr after addition and 23% fewer sperm were bound to aggregates compared to medium lacking COC-CM. At 1.5 hr 34% fewer sperm were bound to aggregates compared to control (p<0.05). This demonstrated that, indeed, the secretions of cumulus oocyte complexes can trigger the release of sperm from oviduct epithelial cells. This project was supported by a USDA MINDS in Ag Fellowship, Agriculture and Food Research Initiative Competitive Grant no. 2015-67015-23228 from the USDA National Institute of Food and Agriculture and HD095841 from the NIH.

**P405 - Manipulation of an inactive form of cofilin as proof-of-principle for auxin-inducible protein degradation in oocytes.** Nicole Camlin, Janice Evans
Auxin-inducible protein depletion is an exciting method that allows for specific, temporally-controlled depletion of proteins in cells. We recently demonstrated the utility of this method in mouse oocytes, depleting EGFP tagged with the auxin-inducible degron (AID). We next sought to use this system to degrade an exogenously expressed functional protein, a phosphomimetic version of the actin-binding protein cofilin. Cofilin drives depolymerization of F-actin filaments to monomeric G-actin, with unphosphorylated cofilin being the active form. Previous studies have found that cofilin phosphorylation is perturbed in oocytes when the RhoA-ROCK-LIMK1/2 pathway is altered, and that overexpression of constitutively active cofilin (cofilin-S3A), decreases actin filaments in metaphase I oocytes. Additionally, preliminary data from our lab found that oocytes expressing inactive cofilin (cofilin-S3E) showed impaired migration of DNA to the cortex at metaphase I. The goal of this study was two-pronged: (1) to validate auxin-inducible degradation in oocytes with a functional protein; and (2) to characterize the effects of expression of inactive phosphomimetic cofilin in mouse oocytes. We found the following: (1) Addition of the AID-tag to cofilin-S3E (cofilin-S3E-AID) had no impact on protein localization when compared to untagged cofilin-S3E. (2) Treatment of oocytes with auxin induced degradation of cofilin-S3E-AID, which was not observed in vehicle-treated oocytes. (3) Expression of cofilin-S3E or cofilin-S3E-AID had no impact on meiosis I resumption or on metaphase I plate positioning, as assessed with oocytes fixed at 5 or 7 hours after release from prophase-I arrest, or in live-cell imaging analyses of oocytes. Additionally, F-actin staining with phalloidin was similar in all groups. Taken together, this work establishes that overexpression of inactive cofilin has no impact on meiosis I, but that auxin-inducible degradation can induce loss of an exogenously expressed functional protein in oocytes. Future work will access phenotypic rescue in AID tagged cofilin-S3A expressing oocytes.

**P406 - Role of the Xlr3 Gene Family in Meiotic Sex Chromosome Inactivation in Mice.**
Michael O'Neill, Natali Naveh, Anne Czechanski, Laura Reinholdt, Robert Foley

In mammalian spermatogenesis, synapsis of the heterologous X and Y chromosomes is delayed compared to autosomes and occurs only in the short Pseudoautosomal Regions (PARs). Consequently, the sex chromosomes remain largely asynapsed and are sequestered in a nuclear compartment known as the XY body where they are transcriptionally silenced in a process called Meiotic Sex Chromosome Inactivation (MSCI). MSCI initiates in pachynema and persists through meiosis and into spermiogenesis where the transcriptional repressed state is referred to as Post-Meiotic Sex Chromatin (PMSC). The sequestration of asynapsed chromatin co-occurs with the assembly of the Synaptonemal Complex and sensing of DNA double-strand breaks (DSB) by components of the DNA Damage Response (DDR), which recruit factors to effect silencing. While many of the proteins and their sequential recruitment in MSCI are known, precisely how asynapsed chromatin is initially detected in meiocytes is still poorly understood. Here, we show that deficiency of *X-linked lymphocyte regulated 3 (Xlr3)* in mice leads to spermatogenic defects and a skewed sex ratio that can be traced to MSCI breakdown due to a failure to recruit DDR factors to the XY body.
**P407 - SUMOylation is essential for proper meiotic maturation and progression in mouse oocytes.** Amanda Rodriguez, Alexandra Andrieux, Anne Dejean, Sean Hartig, Stephanie Pangas

The pool of non-growing, prophase I arrested oocytes is present at the time of birth and remains arrested until the LH surge induces meiotic resumption immediately prior to ovulation. Once follicles are recruited for folliculogenesis, increased transcriptional activity prepares the oocyte for the resumption of meiosis and early stages of embryogenesis. Defects in the maturation process lead to meiotic defects, aneuploid oocytes, or follicular atresia. SUMOylation, a dynamic post-translational modification (PTM), is essential for proper formation of the meiotic spindle and proper chromosome segregation in mouse oocytes *in vitro*; inhibition of SUMOylation in fully-grown germinal vesicle stage (GV) oocytes leads to meiotic arrest, abnormal spindles, and aneuploid MII oocytes. However, the role of this PTM has not been studied in oocyte development during folliculogenesis. Our lab developed two oocyte-specific deletions of Ubc9, the only E2 ligase in the SUMOylation pathway. By crossing *Ubc9*<sup>flox/flox</sup> mice to Gdf9-iCre (UG cKO) or Zp3-Cre (UZ cKO) mice, we deleted *Ubc9* in oocytes within primordial follicles or primary follicles, respectively. **Loss of Ubc9 leads to complete female sterility in both models.** UG cKO mice have premature loss of the ovarian reserve while UZ cKO mice retain a normal number of follicles as late as eight months. Loss of *Ubc9* impaired oocyte maturation, leading to defects in the resumption and progression of meiosis. Our results differ from the published *in vitro* studies as UG cKO and UZ cKO oocytes that undergo GVBD subsequently arrest at metaphase I with a morphologically normal spindle and fail to complete meiosis I. **We suspect that SUMOylation regulates key events in the meiotic maturation of oocytes.** Our *Ubc9* knockout models provide insight into the regulation of meiotic maturation, as well as the process of meiosis itself. Support from by NIH/NICHD R01 HD085994 (to S.A.P.).

**P408 - Live-cell imaging analysis of the effects of manipulating expression of the actin-binding protein nexilin on meiotic maturation in mouse oocytes.** Amber Martin, Nicole Camlin, Janice Evans

The actin cytoskeleton plays a critical role in oocytes during meiotic maturation. Actin function is regulated by numerous actin-associated proteins that help to control the formation and stability of actin filaments. The work here examined the actin-binding protein nexilin, which is an essential protein in Z-discs in cardiac muscle, with clinically relevant mutations observed in patients with certain cardiomyopathies. Data from our lab has shown that nexilin is abundantly expressed in mouse oocytes and that nexilin knockdown causes metaphase I arrest. These results prompted our further examination of nexilin in oocytes, using live-cell imaging to analyze meiotic maturation in oocytes expressing exogenous nexilin and in oocytes depleted of nexilin. To accomplish this, prophase I-arrested oocytes were injected with either cRNA encoding *Nexn-GFP* (for expression of exogenous, GFP-tagged nexilin), or with *Nexn*-targeting siRNA (for RNAi-mediated knockdown). Expression of exogenous nexilin-GFP does not affect the oocyte's ability to progress through meiosis. Nexilin-GFP is localized in the oocyte cortex at prophase I and through prometaphase and metaphase I. This cortical signal becomes enriched over the polar body emission site as meiosis I progresses. Prior to polar body emission, the signal becomes less prominent in the cortex and more detectable in the cytoplasm, then returns to the cortex after polar body emission. In nexilin-deficient oocytes (i.e., siRNA-mediated depletion),
germinal vesicle breakdown occurred with the same timing as in control oocytes, but nexilin-deficient oocytes failed in metaphase I, either with the meiotic spindle failing to migrate to the oocyte periphery or with membrane blebbing occurring in the absence of polar body emission. These data indicate that nexilin is one of the actin-binding proteins that plays a role in spindle positioning and/or in cytokinesis in meiosis I. Future studies will evaluate the mechanism by which nexilin impacts these processes in oocytes.

**P409 - The Addition of Conjugated Linoleic Acid During in Vitro Maturation Modulates the Oocyte Gene Expression of Genes Related to Lipid Metabolism in Bovine.** Jennifer Couto, Gabriela Soriano, Adriano Mendes, Anthony Castilho, Ines Giometti, Lauren Schaffer, Caliê Castilho

In vitro maturation (IVM) is the crucial step for the in vitro production of embryos (PIVE). It is at this moment that the oocytes undergo fundamental transformations that assure oocyte competence, thus being able to be fertilized and later develop as embryos. The aim of this study was to evaluate the effect of the addition of conjugated linoleic acid (CLA) on IVM medium and, in particular, to investigate the expression of major genes related to lipid metabolism. The oocytes were collected from cattle in slaughterhouse and submitted to the aspiration, selection and maturation process; were separated into two experimental groups: MIV and MIV + CLA. After the maturation period, the gene expression patterns (FADS2, GREM1, SCD, SREBP1, AREG, COX2) were investigated in oocytes and cumulus cells. For statistical analysis between groups, we used Student's t-test. The data were log-transformed as needed to fit the normal distribution and all data showed a normal distribution. The analyzes were performed using SAS. The results showed that the enzyme stearoyl-CoA desaturase encoded by the SCD gene showed a significant difference (P <0.05) in oocytes matured with CLA supplementation (0.63±0.12) comparing with the MIV group (1.04±0.12). We conclude that the addition of CLA in culture media diminishes the genetic expression of the SCD gene which is related to the lipid metabolism in bovine oocytes.

**P410 - Control of Meiotic Prophase One Progression in the Perinatal Mouse Ovary.** Margaret McCoy, Dr. Melissa Pepling

Infertility is linked to a depletion of the primordial follicle pool consisting of individual oocytes arrested at the diplotene stage of meiotic prophase I surrounded by granulosa cells. Signaling through the KIT receptor, promotes primordial follicle formation. Meiotic errors can also cause infertility and aneuploidy. Normal oocyte meiotic progression includes progress through four stages of prophase I: leptotene (L), zygotene (Z), pachytene (P), and subsequent arrest in an extended diplotene (D) stage until ovulation. Diplotene arrest is critical as this ensures that oocytes have undergone synapsis and are in the most stable chromatin configuration. KIT
signaling accelerates primordial follicle formation therefore we hypothesize that it may also affect meiotic progression. We are investigating mechanisms that regulate early meiotic progression using the meiotic surface spread technique with perinatal mouse ovaries. Meiotic oocyte nuclei were labeled for Synaptonemal Complex Protein 3 and DAPI, a nuclear marker. We determined the percent of nuclei at each stage of prophase one throughout follicle formation from 16.5 days post coitum (dpc) to post-natal day (PND) 4. The base timeline distribution is as follows: 16.5 dpc: 27% L, 70% Z, 3% P, 0% D – 17.5 dpc: 2% L, 81% Z, 15% P, 2% D – 18.5 dpc: 0% L, 60% Z, 39% P, 1% D – PND1: 0% L, 7% Z, 44% P, 49% D – PND2: 0% L, 8% Z, 4% P, 88% D – PND3: 0% L, 0% Z, 0% P, 100% D – PND4: 0% L, 0% Z, 0% P, 100% D. Preliminary trials using ovaries cultured with ACK2, a KIT function blocking antibody, suggest a trend of meiotic delay. Here, we developed a timeline of meiotic progression in the perinatal ovary to further investigate the role of cell signaling on meiotic progression using the meiotic surface spread technique.

P411 - Effect of melatonin on MPF protein amount and cAMP levels during bovine oocyte in vitro maturation. Hugo Fernandes, Leticia Schefer, Daniela Paschoal, Fernanda De Castro, Claudia Leal

The aim of this study was to assess the effect of melatonin (MLT) during in vitro maturation (IVM) on MPF protein amount and cAMP levels in bovine oocytes. Cumulus-oocyte complexes were submitted to IVM in TCM 199 supplemented with 3 mg/mL BSA, 11 µg/mL sodium pyruvate, 10 µg/mL gentamicin with FSH (0.5 µg/mL, control) or MLT (10⁻⁹M). To evaluate the MPF protein amount, oocytes (50/group) were collected at 0, 6, 9, 12h IVM and evaluated for total CDK1 (p34cdc2) protein. An additional 24h IVM group (0.5 µg/mL FSH and 10% FCS) was included. For cAMP levels, oocytes (15/group) were collected at 0, 1, 2, 3, 6, 9h IVM and evaluated by enzyme immunoassay. Data were analyzed by ANOVA: two-way and Bonferroni (p<0.05; GraphPad Prism software) to compare effects of treatments on MPF protein amount and cAMP levels (3-4 replicates/treatment). At 0 and 24h IVM obtained higher (4.7±2.0) and lower (2.1±0.5) relative quantification for total CDK1 protein (p> 0.05), respectively. FSH treatment (2.8±1.2, 3.5±1.5 and 4.3±1.3) did not differ of the total CDK1 protein when compared to MLT (2.3±0.8, 2.1±0.8 and 2.9±0.8) at 6, 9, 12h IVM (p> 0.05). Variation profile of cAMP levels during early hours of IVM was similar (p> 0.05) with 0.0098±0.002 pmoL/oocyte for both groups analyzed at 0h. FSH treatment showed higher values (0.0108±0.002, 0.0099±0.002, 0.0095±0.002, 0.0089±0.002, 0.0152±0.003 pmoL/oocyte for 1, 2, 3, 6, 9h IVM, respectively) when compared with MLT treatment (0.0096±0.002, 0.0077±0.002, 0.0056±0.001, 0.0039±0.001, 0.0055±0.001 pmoL/oocyte for 1, 2, 3, 6, 9h IVM, respectively), but did not differ between treatments and times used (p>0.05). In conclusion, MLT alone had total CDK1 and maintained cAMP levels similar to FSH in the first hours of IVM. However, further studies are necessary to investigate the MLT role in early maturation and its signaling pathways.

P412 - Cyclin N-Terminal Domain-Containing 1 (CNTD1) coordinates meiotic crossover formation with cell cycle progression in a cyclin-independent manner. Paula Cohen, Emerson Santiago, Joshua Chappie, Stephen Gray Gray
During meiotic prophase I, programmed DNA double-strand breaks (DSB) repair as non-crossovers (NCO) or crossovers (CO), the latter ensuring accurate segregation at the first meiotic division. Critical to crossover designation are components of the ZMM/MutSγ/MutLγ/Class I crossover pathway, and the distant cyclin family member, Cyclin N-terminal Domain-Containing 1 (CNTD1). CNTD1 is thought to orchestrate the designation of class I COs by regulating the process by which a finite and fixed subset of MutSγ-defined DSB repair intermediates will received MutLγ (consisting of MLH1 and MLH3), the ultimate marker of class I events. How CNTD1 achieves this CO designation is unclear, but its cyclin domain has been postulated to be important for this role. To investigate CNTD1 function, we generated an epitope-tagged mouse line of Cntd1, named Cntd1-FH (Flag-HA tagged). Surprisingly, mammalian CNTD1 was smaller than predicted, lacking an N-terminal region containing one of three cyclin homology domains, resulting in a protein incapable of binding to any meiotic cyclin-dependent kinases. BLAST sequence analysis revealed variable N-termini across and within species. Mass spectrometry revealed interactions between CNTD1 and two pathways to orchestrate prophase I regulation: through Replication Factor C (RFC) complex to drive crossover formation and through the Skp1-Cullin1-FBox (SCF) ubiquitylation pathway regulating WEE1 degradation and controlling cell cycle progression. Thus, CNTD1 functions as a stop/go regulator, coordinating the processes of crossover formation and cell cycle regulation. Importantly, however, CNTD1 integrates these two aspects of prophase I progression without directly interacting with any meiotic CDK proteins, and without directly interacting with the class I CO machinery.

P413 - Copper supplementation during in vitro maturation of porcine oocytes improves nuclear and cytoplasmic maturation. Hyerin Choi, Sang-Hwan Hyun

Copper acts as an essential cofactor for antioxidative enzyme and reductase enzyme, and also as an electron transfer intermediate in redox reaction. In this study, we investigated the effect of copper on development of porcine oocytes during in vitro maturation (IVM). It was supplemented to maturation media (TCM199-PVA) during IVM and concentrations were 0, 0.7, 1.4, and 2.8ug/ml each group. Porcine cumulus oocytes complexes (COCs) were treated with or without copper for 40-42h. After 40-42 h of IVM, we evaluated the effect of copper on nuclear and cytoplasmic maturation of porcine oocytes. There was a significant difference in the nuclear maturation rates between control group and other groups (0.7ug/ml, 2.8ug/ml Cu). The rates of oocyte in metaphase II stage were 78.9±1.2 (C grade + 0ug/ml Cu), 88.9±1.0 (C grade + 0.7ug/ml Cu), and 86.7±0.7(C grade + 2.8ug/ml Cu) respectively. The copper treatment groups improved metaphase II rate of C grade oocyte up to that of B grade’s (89.5±0.8). Copper treatment did not increase intracellular glutathione (GSH) levels because there was no significant differences between control group and other treatment groups. On the other hand, copper supplementation significantly reduced intracellular ROS levels. The ROS levels were 1±0.039 (control group), 0.8653±0.050 (0.7ug.ml Cu), 0.8517±0.048 (1.4ug/ml Cu), and 0.6621±0.053 (2.8ug/ml Cu) pixels/oocyte, respectively. Further studies are required to investigate subsequent embryonic development potential of in vitro fertilization (IVF) and parthenogenetic activation, copper treatment during IVM improves porcine oocyte nuclear maturation rate and reduced intracellular ROS levels.
P414 - Follicular environment of endometriosis patients alters chromosomal alignment and spindle structure in a mouse IVM model. Sergio Romero, Ingrid Zorrilla, Paola Berrío, Ricardo Pella, Francisco Escudero, Ygor Pérez, Mario García, Carla Gonzáles, Patricia Orihuela

Around 20% of patients requiring Assisted Reproductive Technologies (ART) suffer from endometriosis. Unfortunately, there exists no treatment that ensures good quality oocytes/embryos from such patients.

Our goal was to evaluate the effect of the follicular environment (follicular fluids, FF) of endometriosis patients on In Vitro Maturation (IVM) of mouse oocytes and their potential rescue by cotreatment with Coenzyme Q10 (CoQ10).

Fourty-six F1 female mice (C57xBalb-c), aged 23-25-days, were primed for 48h with eCG. Following collection of cumulus-oocytes complexes (COCs), COCs were subjected to IVM for a period of 18h at 37°C, 5%CO2 in air. COCs were randomly distributed into 3 treatment groups: 20% FF of oocyte donors (Control) or Endometriosis patients with/without supplementation with CoQ10.

Meiotic resumption was assessed by disappearance of the germinal vesicle and maturation completion (MII) by assessing the presence of the first polar body. Additionally, chromosomal alignment and spindle defects were analyzed in a subset of oocytes resuming meiosis.

The percentage of mature oocytes (MII) was significantly affected by exposure to endometriosis FF, decreasing from 71.8±10.1% (Control) to 15.3±30.6%. Supplementation of media with CoQ10 did not improve the maturation rate of oocytes exposed to endometriosis FF (28.4±27.4%) (p<0.05; mean±SD).

Additionally, it was observed 100% of aligned chromosomes in Control or Endometriosis + CoQ10 groups. However, Endometriosis FF produced only 66.7% (p=0.003). Similarly, spindle defects were observed in a minority of oocytes cultured in Control or Endometriosis + CoQ10 groups (3.2% and 0%, respectively), while 45.5% of oocytes exposed to endometriosis FF displayed aberrant spindles (p<0.0001).

Our findings suggest that follicular environment of patients with endometriosis greatly affects oocyte quality. However, addition of CoQ10 to our mouse IVM model seems to protect oocytes from the effects on chromosomal alignment and spindle formation. The implications of our findings for our fertility patients remains to be evaluated.
**P415 - Paternal genome rescues mouse preimplantation embryo development in the absence of maternally-recruited EZH2 activity.** Erika Paulson, Huili Wang, Libing Ma, Pablo Ross, Richard Schultz

Enhancer of zeste homolog 2 (EZH2), a component of the PRC2 complex, trimethylates H3K27, a transcriptionally repressive histone mark, and $Ezh2^{-/-}$ embryos die post-implantation. A dormant maternal mRNA encodes EZH2, but the role of EZH2 synthesized during oocyte maturation is unknown. A combined siRNA/morpholino approach inhibited the maturation-associated increase in EZH2, as determined by immunofluorescence and immunoblotting, did not impair progression to and arrest at MII, but did inhibit development of diploidized parthenotes to the blastocyst stage. Furthermore, a significant decrease in fluorescence intensity for H3K27me3, but not H3K9me3, was observed in 2-cell diploidized parthenotes. Similar results on development and fluorescence intensity of H3K27me3/H3K9me were also obtained by inhibiting EZH2’s enzymatic activity with GSK343, a small molecule, reversible inhibitor. We defined a critical window for GSK343’s inhibitory effect on preimplantation development to be during the first 6 h of egg activation. Strikingly, when fertilized eggs that give rise to biparental embryos were exposed to GSK343, there was no effect on development to the blastocyst stage, despite a reduction in the fluorescent signal intensity of H3K27me3 in 2-cell embryos. GSK343 treatment also resulted in a global decrease in transcription, as determined by EU incorporation, of 2-cell parthenotes but not 2-cell biparental embryos. RNAseq analysis of 2-cell parthenotes and biparental embryos revealed the relative abundance of ~100 zygotically-expressed transcripts was decreased by GSK343 treatment in parthenotes, but not in biparental embryos, with many of the affected transcripts encoding proteins involved in transcription. A previous study found that parthenotes deficient in maternal $Ezh2$ readily develop to the blastocyst stage. To reconcile these differences and why biparental embryo development is not compromised by GSK343 treatment, we propose that the H3K27me3 state present in the zygote needs to be faithfully propagated following DNA replication in at least one parental PN, otherwise development is compromised.

**P416 - Hippo signaling pathway disruption during Bovine preimplantation embryo development.** Jyoti Sharma, Pavneesh Madan

One of the most important milestones during preimplantation embryo development is the formation of a blastocyst. Two distinct cell lineages namely trophectoderm and inner cell mass develop during blastocyst formation. Recent literature illustrates that a specific cell signaling pathway, known as the Hippo signaling pathway is responsible for lineage segregation during blastocyst formation in the murine model. For the first time, our lab has recently established the presence of core cascade components of the Hippo signaling pathway (MST1, MST2, YAP1 and TAZ) during all stages of bovine preimplantation embryogenesis. However, the role of these cell signaling pathway components during early bovine embryogenesis requires further investigation. Therefore, in this study, we hypothesize that inhibition of Hippo signaling pathway components disrupt lineage segregation and henceforth the formation of the bovine blastocyst. To elucidate the role of YAP1/TAZ in bovine embryogenesis, presumptive bovine zygotes were treated with different concentrations (0.5, 1 and 5 M) of Atorvastatin or Lovastatin, the known chemical inhibitors of the Hippo signaling pathway. After treatment, qRT-PCR and laser confocal
microscopy methods were used to quantify the differences in gene expression and protein localization of Hippo signaling pathway components, respectively. A significant decrease was observed in the cleavage and blastocyst rates of bovine embryos. Additionally, following treatment with Atorvastatin (5mM) or Lovastatin (0.5mM) a decrease was observed in nuclear localization of YAP1/TAZ, thereby inactivating the Hippo signaling pathway. Overall, these findings affirm the role of Hippo signaling pathway components during bovine blastocyst formation. Further studies will be performed to establish the effect of this chemical inhibition on other components (MST1/2, LATS1/2 and TEAD4) of the pathway. Elucidating the molecular mechanism of Hippo signaling during blastocyst formation and cell lineage determination will help in improving embryo developmental procedures and practices in bovine and other species.

Funding source: NSERC, Canada

**P417 - Interleukin-6 Increases Inner Cell Mass and Hypoblast Cell Numbers in Bovine Blastocysts.** Lydia Wooldridge, Sally Johnson, Alan Ealy

This research tested the hypothesis that interleukin-6 (IL6), but not leukemia inhibitory factor (LIF), promotes inner cell mass (ICM) development in bovine preimplantation embryos through the JAK/STAT3 pathway. Supplementing 100 ng/ml recombinant bovine IL6 did not affect the total cell number of day 7 morulae but shifted more cells towards the ICM lineage in early blastocysts. In regular and advanced blastocysts, IL6 increased ICM cell numbers without affecting the trophectoderm. Similarly, IL6 increased ICM cell numbers in day 8 and 9 blastocysts. In day 9 blastocysts, IL6 increased the number of hypoblast cells (GATA6⁺:NANOG⁻) and cells containing both lineage markers (noncommitted, GATA6⁺:NANOG⁺). We used immunofluorescence to examine IL6’s ability to activate STAT3. Treatment with IL6 for 30 minutes stimulated nuclear translocation of pSTAT3 in all blastomeres in day 5 embryos, while controls lacked nuclear STAT3. In day 8 blastocysts, controls had minimal nuclear STAT3 solely in ICM cells, and 30 minutes exposure to IL6 greatly increased staining intensity. Chemical inhibition of JAK activity from day 5 to 8 produced blastocysts with pronounced reductions in ICM cell numbers, regardless of IL6 supplementation. We completed a RNAseq analysis to define IL6 family ligand and receptor expression profiles in bovine blastocysts. Other family members were lowly expressed or not detectable. We selected LIF for further examination because its receptor subunit was detected and LIF has known embryonic actions in other species. While LIF succeeded in stimulating nuclear translocation of STAT3 in day 5 morulae, it failed to do so in day 8 blastocysts and did not increase ICM cell numbers. These results indicate that IL6, and not LIF, increases ICM cell numbers potentially through the JAK/STAT3 pathway, maintains ICM cells in a noncommitted state, and increases hypoblast cell numbers in bovine blastocysts.

**P418 - Expression pattern and role of miRNAs during early development in the cow.** Erika Paulson, Pablo Ross, Richard Schultz

miRNAs (miRNAs) are small, noncoding RNAs that regulate gene expression post-transcriptionally. Maternal miRNAs are a relatively minor small-RNA population in mouse
oocytes but become dominant towards the end of preimplantation development. The role of miRNAs in preimplantation development, particularly in larger species, e.g., cattle, is poorly understood. To gain a better understanding of the role of miRNAs in bovine preimplantation development we characterized their expression profile. Pools of ~20 oocytes or embryos were harvested at different developmental stages following culture in vitro in the absence or presence of a-amanitin (to identify zygotically-expressed miRNAs) and used to prepare miRNA libraries. After sequencing, ~9.5 million reads per stage were used to map the sequences to miRBase version 22. A total of 292 known Bos taurus miRNAs were found, with ~160 miRNAs at each developmental stage. To identify miRNAs not yet included in the current bta-miRNA database, unmapped reads were then mapped to the current human miRNA database. An additional 77 human miRNAs were found not yet identified in the bovine genome, but are likely to be conserved. Of the miRNAs found, a small proportion appears oocyte-specific, with the majority being embryo-specific. To ascertain whether miRNAs regulate preimplantation development, a combined siRNA/morpholino approach was used to knockdown DGCR8; knockdown was confirmed by qPCR. DGCR8, a component of the microprocessor complex, is essential for the biogenesis of all canonical miRNAs. Control (scrambled) siRNA/morpholino injected embryos developed to the blastocyst stage at an incidence similar to uninjected embryos, both with an average of 24%. In contrast, preimplantation development in DGCR8-knockdown embryos was compromised, with many arresting around the 16-cell and morula stages, and none reaching the blastocyst stage. These results implicate a necessity for canonical biogenesis of new miRNAs for successful development of bovine embryos to the blastocyst stage.

P419 - Effect of fatty acids on early development of bovine preimplantation embryos.
Natsuko Emura, Shiho Kusanagi, Yuriko Saito, Ruri Miura, Ken Sawai

Fatty acids are included in lipid contents, and the lipids serve as an energy source in mammalian cells. Although the bovine and porcine preimplantation embryos contain more lipid droplets than murine and human embryos, the functions of fatty acids during early development are still unclear. In this study, we evaluated the composition of fatty acids in bovine oocytes and embryos and attempted to elucidate the roles of fatty acids during the early development of bovine embryos. The fatty acid compositions of matured oocytes and blastocyst stage embryos were analyzed by gas chromatography. 1-cell stage embryos were centrifugated and then removed massive of lipid droplets (Delipated). Some embryos were untreated (Untreatment), treated only centrifugation (Centrifugated) or removing part of cytoplasm (Sham-operated). To clarify the fatty acid functions, Delipated embryos were cultured in in vitro culture (IVC) medium containing 0, 100, 200 or 300 µM oleic acid. Furthermore, SCD1 and SCD5, which contribute to fatty acids synthesis, mRNA levels in preimplantation embryos were evaluated. In both matured oocytes and blastocyst stage embryos, palmitic acid was the most abundant, followed by stearic and oleic acids. Linoleic and Linolenic acids were not detected. No difference in the amount of each fatty acid was observed between matured oocytes and blastocyst stage embryos. The blastocyst developmental rate of Delipated embryos was significantly (P<0.05) lower than those of the other treated embryos. Supplementation of oleic acid in IVC medium did not affect developmental competence of Delipated embryos. SCD1 and SCD5 mRNA levels in the 2-cell stage embryos were higher (P<0.05) than that in the other embryos. SCD1 transcript level
dramatically dropped at the 8-cell stage, and again increased at the blastocyst stage. These findings suggest fatty acids are essential for blastocyst formation of bovine embryos.

**P420 - Involvement of linker histone variants in mouse oogenesis.** Satoshi Funaya, Yuría Kawabata, Fugaku Aoki

Oogenesis involves specialized processes of cell division and gene expression. Chromatin structure changes dramatically during oogenesis, which is thought to play important roles in the regulation of these processes. Although the structure loosens in growing oocytes, it becomes condensed into a so-called ‘surrounded nucleolus-type’ when the oocytes are fully grown. As linker histones are involved in the formation of higher-order chromatin structure, we investigated the function of linker histone variants during oogenesis in mice. We analyzed the expression pattern of all 11 linker histone variants in oocytes using RNAseq data, and found that only H1foo was highly expressed in growing and fully-grown oocytes, leading us to investigate the involvement of this protein in the regulation of oogenesis. When H1foo was knocked down by injecting siRNA into growing oocytes, they grew fully, completed maturation, and fertilized normally. As H1foo has less ability to condense chromatin than other variants and linker histone variants compensate for each other, it is possible that other linker histone variant(s) with a weak ability to condense chromatin might compensate for H1foo in oocytes injected with H1foo siRNA. Therefore, we overexpressed several variants in growing oocytes. Overexpressing H1b or H1d, which have strong abilities to condense chromatin, but not H1a, which has a relatively weak ability, had a detrimental effect on maturation. These results suggest that H1foo and other linker histone variants function in a complementary manner during oogenesis and that the loosened chromatin structure in growing oocytes is crucial for oogenesis.

**P421 - Functional analysis of Pwp1 during early embryogenesis in the mouse.** Atsushi Takasu Naojiro Minami

The development of mouse embryos immediately after fertilization is regulated by maternal mRNAs and proteins. After that, the major gene expression occurs from the embryo's own genome at the 2-cell stage. However, it is not fully understood what kind of mechanisms control this progression. One of the regulatory mechanisms of early embryogenesis is histone H4 trimethyl Lys20 (H4K20me3) modification, which is associated with repression of transcription. Overexpression of the lysine methyltransferases SUV420H2, which catalyze trimethylation of H4K20 leads to sustained levels of H4K20me3 and developmental arrest at the 2-cell stage in mouse embryo. It is reported that periodic tryptophan protein 1 (PWP1), a WD-40 repeat-containing protein associated with histone H4 modification, is co-localized with H4K20me3 in a genomic region in mouse embryonic stem cells (mESCs). Additionally, the suppression of Pwp1 in mESCs decreases the level of H4K20me3 and impairs normal differentiation to three germ layers. However, there is no report analyzing the function of Pwp1 during mouse embryogenesis. The purpose of this study is to investigate the function of Pwp1 in mouse preimplantation development. Embryos were microinjected with small interfering RNA targeting Pwp1 (siPwp1) within 2-4 hours after insemination. The development of embryos injected with siPwp1 was arrested at the morula stage. Furthermore, it is also reported that the suppression of the
expression of retrotransposons in the process of early embryogenesis is essential for protecting genomic information and it is usually controlled by histone methylation. Therefore, the expression levels of retrotransposon Line1 in siPwp1 injected embryos were examined by quantitative RT-PCR. As a result, it is revealed that the expression of Line1 in siPwp1 injected embryos is upregulated, suggesting that Pwp1 is necessary for early embryogenesis and is involved in suppressing the expression of retrotransposon Line1.

P422 - Effects of phytohemagglutinin on aggregation efficiency and developmental competence of parthenogenetic embryos in pigs. Joohyeong Lee, Eunsong Lee

Aggregation of blastomeres is a promising method to improve the developmental competence of blastocysts, and may be useful for producing chimeric animals and establishing embryonic stem cell lines by increasing cell numbers in the inner cell mass. In this study, we used early-stage parthenogenesis (PA) embryos to generate high quality blastocysts by applying the aggregation method using phytohemagglutinin-L (PHA). PA embryos were produced by the general procedure followed at our laboratory. On Day 0-2 after PA, the zona pellucida of embryos were removed by treatment with 0.5% (w/v) pronase solution. The 3x zona-free blastomere (ZFB) were randomly distributed in each of the following treatments for aggregation. In Experiment 1, effects of 150 ug/ml PHA treatment on blastomere aggregation and developmental competence of embryos were investigated. The ZFB cultured with PHA showed a higher (P<0.05) aggregation (57.9% vs. 17.5%) and blastocyst formation (83.4% vs. 66.7%) than untreated control ZFB. In Experiment 2, we investigated the effects of various embryonic stages on the aggregation efficiency and developmental competence, up to the blastocyst stage. The ZFB of 2-cell embryos treated with PHA showed a higher (P<0.05) aggregation (75.2%) than zygotes and 4-cell embryos (43.6 and 57.5%, respectively). In Experiment 3, we investigated the optimal PHA concentration and treatment time to improve the aggregation efficiency and developmental competence of ZFB. We observed that ZFB cultured with 15 ug/ml PHA for 144 hr showed a higher (P<0.05) blastocyst formation (100.0%) than ZFB cultured for 20 min with 15 ug/ml and 150 ug/ml PHA (72.4% and 80.4%, respectively). Our results demonstrate that in pigs, PHA treatment during aggregation culture of ZFB is a useful technique for producing blastocysts having increased cell numbers. *This research was supported by Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education (NRF-2018R1D1A1B07042803)

P423 - Histone Lysine Methyltransferase SETD8 Controls Cell Cycle in Mouse Preimplantation Development. Takuto Yamamoto, Daiki Shikata, Naojiro Minami

Mouse development proceeds with the change of gene expression regulated by the modification of amino acid on chromatin. Among these epigenetic regulations, histone post-translational modifications (PTMs) affect dynamically the structure of chromatin and regulate various biological processes. In this study, we investigated the role of SETD8, one of the histone lysine (K) methyltransferase (KMT) enzymes that mono-methylates H4K20 on mouse preimplantation development. It is reported that, SETD8 regulates cell cycle not only through methylating H4K20, but also through methylating non-histone substrates such as the tumor suppressor p53,
the protein NUMB and proliferating cell nuclear antigen (PCNA). However, the role of SETD8 in mouse preimplantation embryo has not yet been elucidated. In the present study, the effects of UNC0379, SETD8 specific inhibitor on the development of embryos is also examined. As a result, embryos treated with inhibitor at various developmental stages were arrested at each developmental stage when SETD8 was inhibited. The treatment of lower concentration of inhibitor resulted in the delay of the progression of the cell cycle not in the developmental arrest, suggesting that it is essential for H4K20 to be fully methylated by SETD8 in preimplantation development. As a result of western blotting analysis, it was confirmed that the monomethylation level of H4K20 was decreased obviously in the embryos arrested at various stages. Moreover, the shape of blastomeres distorted drastically in embryos treated with UNC0379. In particular, morula embryos showed decompaction. These results show that monomethylation of H4K20 is involved in the progression of cell cycle and maintains the morphology of blastomeres in mouse preimplantation embryos.

**P424 - CRISPR Cas9 Editing of Bovine IVF Embryos via Electroporation of Zygotes.**

Dennis Miskel, Mikhael Poirier, Luisa Beunink, Franca Rings, Dawit Tesfaye, Karl Schellander, Michael Hölker

CRISPR technologies are used to reliably edit genomes in various mammalian embryos using microinjection or electroporation. Here we assessed the feasibility of editing bovine embryos via the electroporation of IVF zygotes. Two targets were designed for the coat color related genes COPA and Tyr. Oocytes were harvested from slaughterhouse ovaries and matured and fertilized in vitro. In total, 667 zygotes were electroporated in groups of approximately 50 in 20 μl of electroporation media with 3 nM Cas9 ribonucleoprotein. Trials were performed with the verified COPA target using ATTO red fluorescence to determine the efficiency of electroporation. 30 V*mm⁻¹ was observed to be the requisite electrical potential for penetration into the cytoplasm. Direct comparisons were then performed between the COPA and Tyr targets after 10 h of fertilization using 2 ms and 3 ms pulses. Pulse number and interval were set at 5 and 100 ms respectively. Editing was determined by T7 assay. The highest cleavage rates were seen using 2 ms pulses with 32.99% and 58.16% for COPA and Tyr respectively (Control: 62.04%). Day 8 blastocyst rates were highest in the Tyr 2 ms group at 26.83%, followed by 18.21% in the 3 ms group. COPA groups were notably lower at 8.92% and 7.88% for 2 and 3 ms respectively (Control: 28.77%). Similarly, percentages of zygotes that developed to day 8 edited blastocysts were higher in the Tyr groups at 22.45% and 15.17% for 2 and 3 ms respectively. Although editing rates of blastocysts were similarly high (COPA 2 ms 73.40%; Tyr 2 ms 83.75%), low development led to COPA editing rates of 6.14% and 3.38%. While further optimization is needed to improve development rates, these initial results suggest that the electroporation of zygotes can be used for high throughput gene editing within bovine IVF workflows.

**P425 - Heat stress negatively affects embryo development, oxidative stress and IFNT production in vitro.** Carolina Amaral, Julia Koch, Eduardo Corrêa Junior, Gabrielle Corrêa, Fábio Comim, Paulo Gonçalves, Alfredo Antoniazzi
Heat stress (HS) is one of the most important causes of bovine productive and reproductive losses, and negatively influences several physiological conditions. We hypothesized that hyperthermia before embryonic genome activation alters the expression of interferon tau (IFNT) and increases oxidative stress in bovine embryos produced in vitro. The aim of this study was to evaluate HS effect on developmental rates, IFNT expression and reactive oxygen species (ROS) production during embryonic development. In vitro produced embryos were used in the present study. The study was completed in seven replicates. Results are significant at P≤ 0.05. Embryos were allocated in five groups: Control; Oocytes matured under HS conditions; Oocytes fertilized under HS conditions; Zygotes on the first culture day under HS conditions; and Embryos submitted to HS during the first 3 days of embryo production. HS conditions were established when temperature was gradually elevated from 38.5°C to 40.5°C, sustained for 6h and slowly diminished to 38.5°C during distinct periods of in vitro embryo production. The temperature of 40.5°C for 6h was determined based on body temperature in lactating dairy cows during the summer. Cleavage and blastocyst rates were higher (P<0.0001) in control group when compared to all HS groups. ROS were quantified by spectrophotometry staining, and the production increased (P=0.03) in all HS groups when compared to control. Interferon tau mRNA and protein expression were determined by RT-qPCR and immunofluorescence staining. Expression of IFNT (mRNA and protein) decreased (P=0.02) in all HS groups when compared to control. Although HS was studied as a limiting factor in reproduction due to the decrease in hormones, gametes quality and embryonic viability, it is showed IFNT production is reduced on embryos under HS. In conclusion, HS induces ROS and impairs IFNT production, suggesting ROS-mediated stress may be associated to IFNT production in HS embryos.

P426 - Duration of gonadotropin support influences follicular growth and oocyte competence in prepubertal calves. Ana Rita Krause, Fernanda Dias, Gregg Adams, Reuben Mapletoft, Jaswant Singh

We tested the hypothesis that ovarian follicular response and oocyte developmental competence in prepubertal calves will be increased by 7 days of follicular growth under FSH support compared to 4 or 6 days of support. Follicular wave emergence was synchronized in 21 crossbred Hereford calves (144±0.7 days; 205±4.6 Kg) by transvaginal ultrason-guided ablation of follicles ≥5mm, followed by 600mg of progesterone in 2 ml im of a sustained-release formulation (BioRelease Technologies, Lexington, KY, USA). Calves were assigned randomly to one of three treatment groups and given pFSH for 4 days, 6 days, or 7 days (25mg im at 12 hours intervals starting 36h after ablation; n=7 calves per group). Six hours after the last pFSH treatment, oocytes were collected by ultrasound-guided follicular aspiration and submitted to standardized in vitro maturation, fertilization and culture using commercial media (IVF Bioscience). One-way ANOVA (numerical data) and GLIMMIX (proportion data) were used to compare differences among groups. The number of follicles ≥6mm at the time of oocyte collection was greater (P=0.03) in the 7- and 6-days groups than in the 4-days group (37.3±5.5, 30.1±7.8, 14.7±2.5, respectively). The number of oocytes collected (overall 9.5±1.6 oocytes per animal; P=0.1), and the recovery rate (34.5±2.8%; P=0.7) did not differ among groups. The cleavage rate was higher (P=0.01) in the 6-days group than in the 4- and 7-days groups (73.2±5.3, 51.3±8.0, 47.2±5.3%, respectively). The proportion of oocytes that developed to morula and blastocyst stages by Day 7 was similar (P=0.5) among groups (overall 20.9±4.6%),
but the proportion that developed into blastocysts on Day 9 was higher (P=0.02) in the 6-days group than in the 4- or 7-days groups (40.9±5.8%, 20.5±6.5, and 20.2±4.3, respectively). In summary, 6 days of follicular growth under exogenous FSH in 5-month-old calves resulted in greater oocyte developmental competence than either 4 or 7 days.

**P427 - SETD8/PR-SET7-Mediated Histone H4K20 Monomethylation Is Required for Mouse Preimplantation Development.** Daiki Shikata, Takuto Yamamoto, Shinnosuke Honda, Naojiro Minami

The early mouse embryo undergoes chromatin remodeling, which allows a new developmental program to start. Histone post-translational modifications which can be associated with either gene activation or repression are required for early embryonic development. The previous works demonstrate that histone H4 lysine 20 (H4K20) is involved in heterochromatin formation, regulation of gene expression, and cell cycle progression through its mono-, di- and tri-methylation. It is reported that histone H4K20 monomethyltransferase, Setd8-null embryos are arrested by the 8-cell stage, however, little is known about the function of H4K20 methylation itself in preimplantation development. It is reported that gene mutation of lysine (K) to methionine (M) in histone coding region is often detected in cancer cells and this mutation not only inhibits methylation in the histone transcribed from mutated genome, but also inhibits non-mutated lysine methylation at the same site. In this study, we investigated the effect of inhibiting H4K20 methylation on preimplantation development. Zygotes were injected with mRNAs coding histone H4K20 wild-type (WT) and H4K20M (lysine is substituted with methionine) into cytoplasm and cultured in potassium simplex optimized medium (KSOM) until the blastocyst stage. We found that embryos overexpressing H4 K20M were arrested at the 2-cell stage. To reveal which methylation state (mono-, di-, or tri-) is critical for preimplantation development, embryos were cultured in KSOM containing UNC0379, an inhibitor of monomethyltransferase (SETD8), or A196, an inhibitor of di- and tri-methyltransferases (SUV4-20H1/2). As a result, while embryos treated with A196 at a concentration of 50 μM developed normally, embryos treated with UNC0379 at a concentration of 20 μM were arrested at the stage when the inhibitor was added. These results suggest that H4K20 monomethylation is essential for preimplantation development.

**P428 - Biochemical changes in uterine fluid composition at the initiation of conceptus elongation in cattle.** Constantine Simintiras, José Sánchez, Michael McDonald, Pat Lonergan

Conceptus elongation is a prerequisite for successful bovine pregnancy establishment and coincides with a period of significant pregnancy loss. The process, which has yet to be recapitulated in vitro, is positively correlated with maternal circulating progesterone (P4) concentrations in vivo, and directly driven by uterine gland secretions. To better understand the environment facilitating this central developmental event, uterine luminal fluid was recovered on Days 12-14 of the estrous cycle – the window of conceptus elongation initiation – from cycling heifers supplemented, or not, with P4. Subsequent advanced high-throughput biochemical profiling for >5000 metabolites by UPLC-MS/MS revealed the consistent presence of 233 biochemicals spanning 8 super-pathways [amino acid (33.9%), lipid (32.2%), carbohydrate
(8.6%), nucleotide (8.2%), xenobiotic (6.4%), cofactor and vitamin (5.2%), energy (4.7%), and peptide (0.9%) metabolism] and 66 sub-metabolic pathways. Regarding metabolic activity, 54 compounds exhibited a Day by P4 interaction (p<0.05) — i.e. comprised metabolites whose concentrations differed between groups (normal vs. high P4) at different times (Days 12 vs. 13 vs. 14). Corresponding biochemical pathways enriched by Day and P4, and therefore of potential importance to conceptus elongation-initiation, include (i) methionine, cysteine, and s-adenosylmethionine, (ii) phospholipid, and (iii) (hypo)xanthine and inosine purine metabolism. Lastly, P4 supplementation elevated total biochemical abundance at a linear rate of 0.41-fold per day, leading to a difference (p<0.0001) by Day 14. In summary, the bovine uterine luminal biochemical landscape is dominated by amino acids and lipids, and is altered by a high P4 environment consistent with advanced conceptus elongation, in addition to time. This work was supported by Science Foundation Ireland [13/IA/1983 (PL)], an Irish Research Council Government of Ireland Postdoctoral Fellowship [GIOPD/2017/942 (CAS)], and a University College Dublin Career Development Award [CDA54580 (CAS)].

**P429 - Abundance of transcript for bovine interferon tau is decreased in blastocysts produced from ovaries with increased numbers of antral follicles.** Robert Cushman, Emmalee Northrop, Jerica Rich, George Perry, Jeremy Miles, Chadwick Chase Jr.

Conflicting reports exist regarding the influence of increased numbers of follicles in bovine ovaries on the competence of oocytes to develop to blastocysts in vitro. Interferon tau (IFNT) is considered a marker for blastocyst developmental competence because IFNT production was negatively correlated with blastocyst development in vitro. Therefore, we hypothesized that IFNT transcript abundance on day 8 would be greater in blastocysts produced in vitro from ovaries with decreased numbers of follicles compared to blastocysts produced in vitro from ovaries with increased numbers of follicles. Bovine ovaries were collected at a local abattoir (n = 12 reps) and classified as Low (<11) or High (>19) based on numbers of visible antral follicles. Cumulus oocyte complexes (20-40/group for each rep) were matured and fertilized in vitro using semen from a single bull. On day 8, blastocysts were collected in pools of 10 (n = 9 pools/group). Total cellular RNA was extracted and relative IFNT transcript abundance was quantified using real-time RT-PCR. Percentage developing to the blastocyst stage was analyzed using GLIMMIX and relative transcript abundance of IFNT was analyzed using GLM. There was no difference in the percentage developing to the blastocyst stage by day 8 in vitro due to follicle number (Low: 18.6 ± 7.6% vs High: 27.6 ± 8.8%; P = 0.45). Relative transcript abundance of IFNT was decreased in blastocysts from ovaries with increased numbers of follicles compared to blastocysts from ovaries with diminished numbers of follicles (P < 0.01). While there was no difference in ability to develop to the blastocyst stage, the decreased abundance of IFNT transcript in blastocysts suggests that embryonic survival after day 8 might be improved in blastocysts produced from ovaries with increased numbers of antral follicles. USDA is an equal opportunity provider and employer.

**P430 - Embryo Development and Survival in Pubertal Ewe Lambs.** Jennifer Juengel, Laurel Quirke, Jacqui Peers-Adams, Peter Johnstone, Peter Smith
The pubertal ewe, with reproductive loss to day 35 of pregnancy approximately double that of adult ewes, represents a model to better understand why ova fail to develop into viable offspring. Therefore, our aim was to better define early embryo development, and key times of loss, in the pubertal ewe. To do this, health and development of embryos from naturally cycling ewes approximately 8 months of age were recorded on day 3.5 (n=80), 14.5 (n=83) and 35 (n=87) following mating. By day 3.5 of gestation, 22.9% of the ova released were not present as a healthy embryo (P<0.05). Survival scores at day 14.5 tended (P < 0.10) to further decrease by 11% to 66.0 ± 4.5%, but did not decrease between day 14.5 and 35 of gestation. Structures collected on day 3.5 after mating ranged from 1 to 12 cells, with 11% being 1 cell (unfertilized or cleavage failure), 49% being 2-4 cells, with the remainder > 6 cells. Embryos collected on day 14.5 ranged in length from 5.3 to 200.0 mm with high variation observed between and within animals, the within animal variation was 67 % of the between animal variation. When examining developmental stage of the embryo at day 3.5 and 14.5, neither the concentration of progesterone at time of collection, nor whether the ewe had attained puberty prior to mating with the fertile ram, was associated with developmental stage. In conclusion, reproductive loss in the pubertal ewe lamb primarily occurred prior to day 14.5 of gestation. The majority of this loss occurred prior to day 3.5, highlighting the important role of the quality of the oocyte and oviduct in supporting normal fertilization and early embryo development in pubertal ewe lambs. Funding provided by AgResearch’s Strategic Science Investment Fund from NZ Ministry of Business, Innovation & Employment.

**P432 - Conceptus Prostaglandin Synthase 2 is Not Essential for Early Development and the Establishment of Pregnancy in the Pig.** Caroline Pfeiffer, Ashley Meyer, Lee Spate, Josh Benne, Raissa Cecil, Kelsey Brooks, Thomas Spencer, Randall Prather, Rodney Geisert

Pig conceptuses secrete estrogens (E2), interleukin 1 beta 2 (IL1B2), and prostaglandins (PGs) during the period of rapid trophoblast elongation for establishment of pregnancy. Previous studies established that IL1B2 is essential for rapid conceptus elongation, whereas E2 is not essential for conceptus elongation or early maintenance of the corpora lutea (CL). The objective here was to determine if conceptus expression of prostaglandin-endoperoxidase synthase 2 (PTGS2) and release of PGs are important for early development and establishment of pregnancy in pigs. To evaluate the role of conceptus PTGS2-derived PGs in early pregnancy, PTGS2 null embryos were generated by deleting exon 1 of the PTGS2 gene in fibroblast cells using CRISPR/Cas9 genome editing followed by somatic cell nuclear transfer. Content of PG in day 7 culture media of PTGS2+/+ blastocysts was undetectable compared to 4,000 pg containing PTGS2+/+ blastocysts (P= 0.006). PTGS2 was detectable by immunolocalization in PTGS2+/+ but absent in PTGS2−/− blastocysts. PTGS2+/+ and PTGS2−/− blastocysts were transferred into surrogate gilts, and the reproductive tracts were collected on either day 14 or 17 of pregnancy. After flushing from the uterus, filamentous conceptuses were cultured for 3 h to determine PG production. Conceptus release of total PG, PGF2a, and PGE2 in culture media was lower (P = 0.016) from PTGS2−/− conceptuses compared to PTGS2+/+ conceptuses on day 14 and 17. However, total PG, PGF2a, and PGE2 content of the uterine flushings was not different. Of note, pregnancy was maintained beyond day 30 in gilts gestating PTGS2−/− embryos. Thus, conceptus-derived PGs do not have a biological role in early pregnancy in terms of conceptus
elongation and pregnancy establishment. Research supported by USDA NIFA grant 2017-12211054.

**P433 - Dysregulated expression of XIST and imprinted genes of the KCNQ1 locus in bovine haploid androgenetic embryos.** Luis Aguila, Jacinthe Therrien, Monica Garcia, Karine Mattos, Lawrence Smith

Although haploid development can be achieved experimentally in mammals, haploid androgenetic embryos (hAE) show poor preimplantatory development compared to haploid parthenogenetic embryos (hPE), suggesting that the chromosomes contributed by the sperm are developmentally restricted compared to those contributed by the oocyte. Therefore, to further characterize the parental contribution to preimplantatory development using the bovine model, in vitro matured oocytes were fertilized using X-sorted sperm and the maternal chromosomes were removed to obtain hAE, which were compared to hPE, diploid parthenotes and ICSI fertilized male and female counterparts. Although the initial cleavage divisions were similar among all groups, further preimplantatory development to the blastocyst stage was severely affected in haploid embryos (17% and 2% for hPE and hAE, respectively) compared to diploid counterparts (>25%, p=0.001). However, cytological assessment of arrested zygotes at 48 h of culture showed mitotic and cytokinetic errors at similar levels between ICSI and haploid embryos, suggesting micromanipulation artefacts as the main cause of cell division anomalies during early development. On the other hand, although the X-related transcripts HPRT and PGK1 did not differ among haploid and diploid morula-stage embryos, XIST transcripts were 2-fold higher in hAE than hPE, indicating either incomplete inactivation of the paternal X-chromosome during early development. Moreover, although transcripts levels of maternally expressed IGF2R did not differ among groups, paternally expressed KCNQ1OT1 and the maternally expressed CDKN1C, PHLDA2, and TSSC4 genes were upregulated in hAE compared to hPE and diploid embryos, suggesting a dysregulation of the KCNQ1 imprinted locus. In conclusion, we demonstrate for the first time that bovine hAE show altered expression of X-related and imprinted genes, suggesting that the preimplantatory developmental arrest of haploid androgenetic embryos is regulated at an epigenetic level. This research was supported by grant from NSERC Canada with L'Alliance Boviteq (LS), Conicyt-Chile (LA), Conacyt-Mexico (MG), and UFRGS-Brazil (KM).

**P434 - Effect of downregulating AGO1 transcripts by RNA interference on early development of porcine embryos.** Yuriko Saito, Ayako Sasaki, Natsuko Emura, Ruri Miura, Ken Sawai

MicroRNAs (miRNA) are small noncoding RNAs that play important roles in post-transcriptional regulation of gene expression. This miRNA-mediated RNA interference regulates various biological functions including embryonic development. Argonaute family proteins (AGO1, AGO2, AGO3 and AGO4) bind to miRNA, and they constitute RNA induced silencing complex (RISC) that is essential for mRNA silencing. In this study, we evaluated the expression status of AGO genes in porcine oocytes and embryos, and investigated the role of AGO1 during the early development of porcine embryos using AGO1 siRNA injection. AGO1, AGO2, AGO3 and AGO4 transcript levels in porcine IVM oocytes and IVF embryos at various developmental
AGO1 siRNA or Control siRNA were injected with 1-cell stage embryos, developmental competences of siRNA injected or uninjected embryos were evaluated. Total number of cells and rate of cells expressed OCT-4 in blastocyst stage embryos obtained from siRNA injection or uninjection were investigated. AGO1 expression levels in 1-cell to 8-cell stage embryos were significantly (P<0.05) higher than that in 16-cell to blastocyst stage embryos. AGO2 transcript levels in IVM oocytes and 1-cell to 16-cell embryos were significantly (P<0.05) higher than that in morula and blastocyst stage embryos. AGO3 level at 16-cell stage was significantly (P<0.05) higher than that in IVM oocytes, 4-cell, 8-cell and blastocyst stage embryos. No differences in AGO4 transcript levels were observed among different cell stages. Developmental rates for 8-cell≤, 16-cell≤ and blastocyst were significantly (P<0.05) decreased in AGO1 siRNA and Control siRNA injected embryos compared with uninjected embryos. Total number of cells and rate of cells expressed OCT-4 in the AGO1 siRNA injected embryos were significantly (P<0.05) lower than that in uninjected or Control siRNA injected embryos. Our results suggested that AGO1 is an important factor for the preimplantation development of porcine embryos.

**P435 - Newly synthesized lipid droplets are essential for preimplantation embryonic development in mouse.** Ryutaro Aizawa

Lipid droplets (LDs), which are a ubiquitous organelle consisting of a neutral lipid core coated with a phospholipid monolayer, play a key role in regulating cellular lipid metabolism. The content of LDs varies significantly among animal species. In mammalian oocytes and embryos, LDs are contained in the cytoplasm. Recent studies have revealed that the morphology of LDs changes dynamically throughout embryonic development and in response to culture conditions, indicating the critical role of LDs during embryonic development. In this study, we developed a method to remove LDs from mouse MII-oocytes based on two-step centrifugation combined with piezo-micromanipulation. Immunofluorescence and electron microscopy analysis confirmed that almost all LDs were efficiently isolated from MII-oocytes, which were collected from C57BL/6 females, without extra-cytoplasmic contamination. We found that MII-oocytes were able to be fertilized in vitro even after delipidation and developed normally to the blastocyst stage even when these embryos were cultured in the absence of extracellular fatty acids. We noticed that LDs were newly synthesized soon after delipidation, probably due to the endogenous fatty acid pool. In support of this observation, the fatty acid content in MII-oocytes was largely unchanged before and after delipidation. Furthermore, inhibition of fatty acid acyl-CoA synthetases completely blocked subsequent synthesis of LDs, resulting in the impairment of early embryonic development. Our results suggest that LDs are necessary for proper embryonic development, and that LDs are not only accumulated but also newly formed endogenously during preimplantation embryonic development.

**P436 - Beneficial effects of N-acetylcysteine supplementation on the development of toluene-exposed mouse preimplantation embryos in splitted monozygotic twin embryos model.** Jin Hyun Jun, Jung Eun Park, Yoon Ji Choi, Jihyun Kim, Wontae Kim, Jung Won Choi, Jaewang Kim
Toluene, one of representative volatile organic compounds, could produce free radicals and impair preimplantation embryonic development. N-acetylcysteine (NAC) is known as an effective antioxidant, and able to reduce free radicals. It has been used for in vitro culture of cells and preimplantation embryos as a beneficial supplement. This study was performed to evaluate the effects of toluene and NAC on development of mouse preimplantation embryos. We want to minimize the variations of inter-embryos using mouse preimplantation embryos. Mouse two-cell embryos were removed ZP and mechanically divided and produced paired splitted monozygotic twin embryos (SMTEs). Firstly, paired SMTEs were separately cultured under either basal media with 5% SPS (control; CON) or control with 5 μM toluene (CON+T). Secondly, paired SMTEs was individually cultured under either control condition with 5 μM toluene (TOL) or control with 5 μM toluene and 10 mM NAC (TOL+N). Blastulation rate and total cell number of CON+T were significantly decreased than those of CON in paired SMTEs. Apoptotic indexes were significantly increased in toluene exposed blastocysts of SMTEs. By the way, blastulation rate and total cell number of TOL-N were significantly increased than those of TON in paired SMTEs. Apoptotic indexes were significantly decreased in NAC supplemented blastocysts of SMTEs. We found that toluene showed significantly detrimental effects on the development of SMTEs model. It was overcome by NAC supplemented culture media. Expressions of developmental toxicity related Arnt gene was higher in CON+T than CON. The expression of Survivin was increased and the expression of Bax was decreased in the CON+T. We suggested that this SMTEs model might be valuable for subtle experiments of in vitro culture systems of preimplantation embryos. In addition, NAC treatment could be applied to improve the in vitro culture condition of mammalian preimplantation embryos.

P437 - Effect of Galectin-1 on Anti-Inflammatory and Pro-Inflammatory Related Gene Expression Within Bovine Endometrium. Lindsay Grose, Heather Baldwin, Jeanna LaBarbara, Patrick Lonergan, Daniel Mathew

Early embryonic mortality, a major cause of pregnancy failure in cattle, may result from inappropriate maternal immune responses to the conceptus. Galectin-1, an immune-modulatory protein, is secreted by the bovine conceptus. Studies suggest galectin-1 increases expression of genes associated with regulatory T cells (Treg) and anti-inflammatory proteins within the bovine endometrial caruncle. The present study investigated the effect of galectin-1 on expression of genes related to Treg and anti-inflammatory (FOX3, IL2RA, CD11c, and IL10) as well as pro-inflammatory (CXCR4, LIF, IL33, IL1B, and IL6) activities in inter-caruncular endometrium. Bovine inter-caruncular endometrial explants were collected from uteri (n=4) during the mid-luteal phase of the estrous cycle and cultured in RPMI media containing 0, 10, 100, or 1000 ng/mL of recombinant bovine galectin-1 for 6 or 24 h. Explant mRNA abundance was then determined using RT-qPCR. A MIXED procedure in SAS was utilized to statistically analyze the data. A contrast statement was included comparing control to all galectin-1 treatments. Treatment did not affect expression of IL33. At 6 h, there was an effect of treatment on IL10 (P ≤ 0.01), LIF, IL1B, and IL6 (P ≤ 0.001) mRNA. At 24 h, there was an effect of treatment on CXCR4, CD11c, IL2RA, FOX3 (P ≤ 0.05), IL10, LIF (P ≤ 0.01), IL6, and IL1B (P ≤ 0.001) mRNA. Expression in both pro- and anti-inflammatory genes seemed to increase with increasing concentration of galectin-1. Thus, bovine conceptus-derived galectin-1 may have important functions in regulation of the maternal immune environment prior to implantation by inducing a
balance of pro-inflammatory and anti-inflammatory states. Funding was provided by West Virginia University and Science Foundation Ireland.

**P438 - Functional role of L-arginine in the transport of water by porcine trophectoderm (pTr2) cells.** Mohammed Elmetwally, Cassandra Herring, Erin Posey, Fuller Bazer, Gregory Johnson, Guoyao Wu

L-Arginine, a conditionally essential amino acid for gestating mammals, is a precursor for synthesis of biologically active molecules such as nitric oxide (NO), creatine, ornithine and polyamines. This study was conducted with porcine trophectoderm (pTr2) cells to determine effects of L-arginine on the expression of aquaporins (AQPs) which are integral membrane proteins that serve as channels for water transport. Cells were cultured for up to 96 h in customized arginine-free DMEM medium containing physiological concentrations of all amino acids and 0, 50, 100, 250, or 500 μM L-arginine. The proliferation of pTr2 cells increased \((P<0.05)\) in response to different doses of L-arginine at both 48 h and 96 h of culture. L-Arginine also stimulated \((P<0.05)\) migration of pTr2 cells in a dose-dependent manner with the maximum effect at 250 μM. L-Arginine (250 μM) increased \((P<0.05)\) expression of AQP 1, 3, 4, 5, 9, and 11 mRNAs, NO production, intracellular cGMP, and transport of water (measured with the use of \(^3\)H\(_2\)O) by pTr2 cells, compared to effects of other doses of arginine. DETA-NO (15 μM, an NO donor) also increased \((P<0.05)\) the transport of water by pTr2 cells. Blockage of NO synthesis by 0.4 mM NG-monomethylarginine inhibited \((P<0.05)\) the L-arginine-induced increase in NO synthesis and the stimulatory effect of L-arginine on the transport of water by pTr2 cells. Results of this study indicate that L-arginine effects changes in the placental transport of water from the dam to the conceptus (embryo and placental membranes) is essential for its survival, growth and development. This research was supported by Agriculture and Food Research Initiative Competitive Grants No. 2016-67015-24958 and 2015-67015-23276 from the USDA National Institute of Food and Agriculture.

**P439 - The Degradation of CITED2 Is Important for Early Embryonic Development in Mouse.** Juan Lin, Yuan Fang, Shenming Zeng

Yet little is known about the role of CBP/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2 (CITED2) in mammalian embryonic development, we designed experiments to gain insight into the functions of CITED2 during preimplantation embryonic development in mouse. The results from quantitative real time polymerase chain reaction showed that there was no difference of the \textit{Cited2} mRNA expression among metaphase II (MII) oocyte, zygote and 2-cell embryo, but the level of this gene was sharply decreased at the 4-cell stage, and reached the lowest level from morula to hatched blastocyst (oocytes \((n=100)\) and embryos \((n=50)\)). Western blot for the expression CITED2 protein demonstrated a similar trend during embryo development (oocytes \((n=100)\) and embryos \((n=50)\)): the levels of CITED2 were higher in MII oocyte and zygote than other stages, then degraded from the 2-cell stage, and significantly decreased at the 4-cell stage and maintained the lowest levels from 8-cell to hatched blastocyst. When \textit{green fluorescent protein (GFP)}-tagged \textit{Cited2} mRNA was microinjected into the zygotes
to elevate the level of CITED2 protein, most of the embryos were blocked at the 2-cell stage with fewer reaching the 4-cell stage (46.2% for GFP-Cited2 mRNA injection (n=162) vs. 88.6% for GFP mRNA injection (n=160)). Injecting GFP-Cited2 mRNA also decreased the percentage of blastocyst (8.1% for GFP-Cited2 mRNA injection (n=13) vs. 62.5% for GFP mRNA injection (n=100)), the percentage of hatched blastocysts (3.4% for GFP-Cited2 mRNA injection (n=1) vs. 35.8% for GFP mRNA injection (n=36)) and the cell number per blastocyst (47.3 for GFP-Cited2 mRNA injection vs. 78.7 for GFP mRNA injection). In summary, our results indicate that the selective degradation of CITED2 is necessary for mouse early embryo development.

Research supported by the National Key Technology Research and Development Program of China (2017YFC1002003).

**P440 - Zona pellucida does not affect the pre-implantation embryonic development in mice.**
Jihyun Kim, Jaewang Lee, Sooseong You, Jin Hyun Jun

The zona pellucida (ZP) is a glycoprotein layer surrounding the mammalian oocytes. In vitro suboptimal environment could possible affect the structural changes of ZP such as flexibility and elasticity. Failure to hatch has been largely attributed to ZP hardening, and it might fail to implantation in vivo upon transfer. We have observed the preimplantation embryonic development to evaluate and compare the development competence with presence or absence of ZP using time-lapse monitoring system. Mice 2-cell embryos were collected on 1.5 dpc, and their ZP were removed by treatment with acid Tyrode’s solution. Embryos with presence or absence of ZP were in vitro cultured from 2-cell to the outgrowth stage (on 1.5 to 7.5 dpc). There was no difference in blastocyst formation and cumulative time for each developmental stage with or without the ZP. Proportion of embryos showing abnormal compaction process was similar in two groups. The presence or absence of ZP did not affect the localization of cell-cell contact molecules (beta-catenin and E-cadherin) in compacting embryos, revealed by immunocytochemistry. Our results have substantiated that the ZP is not essential for pre-implantation embryonic development in vitro. We suggest that the modification of the ZP could be an useful challenge for various purposes without decreasing the developmental competence of mice embryos in conventional human ART program and advanced animal reproductive technologies.

**P441 - Extracellular Vesicles from the Uterine Microenvironment and Polyamines in Embryo Dormancy.**
Ranran Cheng, Weimin Liu, William S.B. Yeung

Embryonic diapause is a physiological reproductive strategy widely employed in animals to ensure that the young are born in a favorable environment. However, how does the embryo entry into diapause is still unknown. Here, we hypothesize extracellular vesicles (EVs) and polyamines affect embryo dormancy and reactivation, respectively. EVs isolated from uterine luminal fluid (ULF) can be internalized by embryos and affect their transcriptome and proteome. ULF-EVs isolated from the delayed implantation mice (Dor-EVs) extended mouse blastocysts survival and induced pausing of embryo development ex vivo. MiRNA deep sequencing showed that members of let-7 family had a higher expression in Dor-EVs than in D4-EVs and Act-EVs. Polyamines nullified the effect of Dor-EVs on EdU incorporation. Estrogen supplementation,
which activates dormant embryos, decreased expression of let-7 and upregulated polyamines in vivo. Taken together, these studies show that the expression of EVs miRNAs and Polyamines determines the embryo dormancy or implantation. The Project was supported by GRF 17119117, GRF 17107915.

**P442 - Oxygen concentration alters mitochondrial function in in vitro fertilized (IVF) preimplantation mouse embryos.** Ling Zhang, Elena Ruggeri, Annemarie Donjacour, Xiaowei Liu, Paolo Rinaudo

IVF is widely used and has resulted in the birth of more than 8 million children. While a variety of media and oxygen concentrations are used, embryos cultured under physiological O2 tension (5%) reach the blastocyst stage faster and have fewer alterations in gene expression when compared with embryos cultured under atmospheric oxygen (20%). The mechanisms by which oxygen tension affects preimplantation development remain unclear, but mitochondria are believed to play an important role. The aim of this study was to evaluate how mitochondrial function in IVF embryos was affected by culture under physiologic (5%) or atmospheric(20%) oxygen concentrations. Zygotes, 2 cell, 4-cell, morula and blastocyst were flushed out of the uterus after natural fertilization (CF1X B6D2F1) as controls. IVF was performed with CF1 x B6D2F1 mice and embryos were cultured in KSOM medium with amino acids under 5% and 20% O2 until the blastocyst stage. Mitochondrial function was assessed by measuring mtDNA copy number, mitochondrial membrane potential, reactive oxygen species (ROS) production, ATP levels, and the expression of selected genes. At the blastocyst stage the mitochondria of IVF embryos cultured in 20% O2 had a lower mtDNA copy number than controls. IVF blastocysts, regardless of O2 level had statistically lower mitochondrial membrane potential. IVF in the 20% O2 group had significantly higher ROS levels, while those in 5%O2 were intermediate. There were also changes in the expression of selected mitochondrial genes. ATP levels were significantly lower than controls only under 5% O2, with the 20% O2 IVF group having intermediate levels. Unexpectedly, adding antioxidant to the culture medium did not improve development. This study suggests that changes in mitochondria may explain why lower oxygen concentration leads to better embryo development and further suggest mitochondria as a locus of reprogramming.

**P443 - Phosphorylation of Mechanistic Target of Rapamycin (MTOR) in Porcine Blastocysts is Dependent on the Concentration of Glutamine in the Medium.** Paula Chen, Lee Spate, Raissa Cecil, Joshua Benne, Taylor Hord, Randall Prather

Supplementation of glutamine to porcine embryo culture medium improves blastocyst development, increases leucine consumption, and enhances mitochondrial activity. In cancer cells, glutamine has been implicated in the phosphorylation and activation of MTOR to support rapid cellular proliferation. The objective of this study was to determine if phosphorylation of MTOR in porcine blastocysts was dependent upon the concentration of glutamine in the medium and presence of leucine, another activator of MTOR. Presumptive zygotes (n=732 per treatment across 6 replicates) were split into four groups and cultured in 0, 1, 3.75 or 10mM GlutaMAX, an L-glutamine alternative, with or without leucine. On day 6, percentages of blastocysts were
recorded for culture with leucine. Blastocysts (n=40 per treatment) were collected for immunoblotting to detect MTOR and phosphorylated(Ser2448) MTOR (p-MTOR) with 3 replicates per target. Data were analyzed by using the GLM procedure in SAS 9.4, and differences between means were detected by using one-way ANOVA followed by a least significant difference test with $P<0.05$ declared significant. Compared to the other treatments, 0mM GlutaMAX resulted in decreased blastocyst development (29.1±3.1%). Embryos cultured in 3.75mM GlutaMAX had increased blastocyst development (51.3±1.8%) compared to culture in 1mM (44.5±1.6%). With leucine, culture in 0mM resulted in decreased MTOR expression compared to all other groups, and p-MTOR expression was decreased in 0mM compared to 3.75 or 10mM GlutaMAX. Without leucine, embryos cultured in 0mM GlutaMAX had decreased expression of MTOR compared to all other groups, and p-MTOR expression was decreased in embryos cultured in 0 or 1mM compared to 3.75 or 10mM GlutaMAX. Therefore, glutamine supplementation is sufficient to prompt MTOR phosphorylation in porcine embryos regardless of leucine. Further analyses are examining the phosphorylation statuses of MTOR downstream targets and effects of inhibiting enzymes involved in glutamine catabolism. Funding from Food for the 21st Century and R01HD08636.

**P444 - Sexually dimorphic transcriptional changes in murine inner cell mass (ICM) generated in vivo or by in vitro fertilization (IVF).** Elena Ruggeri, Xiaowei Liu, Royce Harner, Saul Albarran, Annemarie Donjacour, Paolo Rinaudo

There are indications that even during the preimplantation period XX and XY embryos differ in their physiology. Artificial reproductive technologies, most notably IVF, expose the actively developing embryo to sub-physiologic conditions, the effects of which are just beginning to be uncovered. It is currently unknown what transcriptomic changes exist in the ICM of male and female embryos following natural mating, and how the IVF process might alter these underlying differences. The objective of this study was to determine changes in the transcriptomic ICM of male and female mouse blastocysts generated in vivo or in vitro (n=3/group, 12 samples total). Immunosurgery was performed in mouse blastocysts (CF-1 x B6D2F1) generated by IVF (cultured with KSOM + amino acids under 5% O2) or flushed out of the uterus (FB group, control). DNA isolated from trophectodermal cells was used for sexing. Library preparation was performed from individual ICM. After sequencing, reads were aligned to the mouse mm9 reference. Differential expression was calculated using edgeR (FDR £0.05); DAVID was used to identify biological pathways. Mapping efficiency was 70%. Interestingly, while only 13 (in vivo) and 4 (IVF) genes were differentially expressed between male and female embryos, genes involved in translational initiation pathway were different between sexes. The IVF process resulted in 91 differentially expressed genes (78 upregulated) compared to control. Pathways involved in leukocyte and lymphocytes homeostasis were the most dysregulated. In summary, we found only few significant genes differentially expressed in ICM of male and female embryos. Importantly, the IVF process resulted in transcriptomic changes with particular alteration in pathways involved in immune response. These findings provide insight into early differences between the sexes including the differential response of male and female embryos to culture conditions/environmental stress. Funded by 1R01HD082039-01A1.
**P445 - The influence of preovulatory estradiol on trophectoderm and uterine gene transcript abundance around maternal recognition of pregnancy in beef cattle.** Emmalee Northrop, Jerica Rich, Robert Cushman, Jeremy Miles, Amanda Lindholm-Perry, Brittney Keel, Runan Yao, Xijin Ge, George Perry

In cattle, most embryonic losses occur during the first month of pregnancy. Embryo survival and pregnancy success is increased among animals that exhibit estrus prior to fixed time AI, but there are no differences in conceptus survival to d16. The objective of this study was to examine differences in uterine and conceptus transcriptomes on d16 of pregnancy based on preovulatory estradiol (E2) exposure. We hypothesized that differences in uterine environment have a greater impact on pregnancy success than differences in conceptus development on d16. Beef cows/heifers were synchronized, artificially inseminated (d0), and grouped into high (highE2: n=11) or low (lowE2: n=3) preovulatory E2. Uteri were flushed to collect d16 conceptuses, and endometrial samples were collected from the ipsilateral uterine horn. Total cellular RNA was extracted from endometrium for RNA sequencing. Real-Time PCR (RTPCR) was performed on trophectoderm (TE) RNA for relative abundance of *IFNT, PTGS2, TM4SF1, C3, PPARG, FGFR2, and GAPDH*. Transcript abundances in the endometrium were quantified using kallisto, differentially expressed genes (DEGs) were determined using DESeq2 (FDR <0.05, FC>2), and IPA was used for pathway analysis. RTPCR data were analyzed using the MIXED procedure in SAS. There were no differences in mRNA abundances in TE, but there were 432 DEGs among the highE2/conceptus versus lowE2/conceptus groups, 253 were downregulated (*PRKCG, PRND, MRAP2*) and 179 were upregulated (*PRSS2, CRYGS, C1QL2*) in the highE2/conceptus group. Previously, we reported 48 differentially expressed proteins in uterine luminal fluid between highE2 and lowE2 animals on d16, 4 of these were differentially expressed (FDR <0.10) at the mRNA level. Similar pathways for mRNA and proteins included: calcium signaling, protein kinase A signaling, and connective tissue development. These results demonstrate greater differences in uterine function than in conceptus developmental competence between highE2 and lowE2 animals on d16. USDA is an equal opportunity provider and employer.

**P446 - Mimicking hypoxia in donor cells improves SCNT embryo development.** Raissa Cecil, Joshua Benne, Taylor Hord, Paula Chen, Lee Spate, Randall Prather

Somatic cell nuclear transfer (SCNT) is a tool useful for creating genetically modified animal models for biomedical, agricultural and xenotransplantation purposes. However, the efficiency of SCNT is low (<5%) likely due to incomplete remodeling and reprogramming of the somatic nucleus to allow for proper embryonic and fetal development. One way to promote remodeling is by altering the donor cells to become more blastomere-like prior to SCNT. Within blastomeres, the primary energy pathway utilized is glycolysis. The cellular survival response to hypoxic conditions includes a switch from oxidative phosphorylation to glycolysis. Cobalt chloride (CoCl2) is a known hypoxia mimic that triggers this hypoxic survival response in cells. Therefore, the objective of this study was to determine the effect of CoCl2 treatment on donor cell viability and subsequent blastocyst development following SCNT. Donor cells were treated with varying concentrations of CoCl2 (50, 100, or 150 µM) and durations (24, 36, or 72 hours) to
determine the appropriate treatment conditions. Since CoCl₂ treatment became cytotoxic at concentrations above 100 µM or for durations longer than 24 hours, a 24-hour 100µM CoCl₂ treatment was chosen. Micromanipulation drops were also treated with 100 µM CoCl₂ during the cell injection phase of the SCNT process to ensure that the treatment effect was sustained. Data were analyzed by using a Student’s t-test (P<0.05 was considered significant). Blastocyst development (50.3±2.6% vs 32.6±1.9%) and total number of nuclei (52.0±3.3 vs 39.0±3.0) were increased in the CoCl₂ treatment group compared to the control. No differences were detected in the number of apoptotic nuclei between groups after TUNEL staining. Future studies will determine changes in gene expression in donor cells and SCNT embryos following donor cell CoCl₂ treatment. Funding from R01 HD080636 and Food for the 21st Century.

**P447** - H2A variants are involved in reprogramming of gene expression in preimplantation embryos and primordial germ cells. Dai Tsukioka, Fugaku Aoki

A dramatic change in gene expression pattern, or reprogramming, occurs after fertilization and during development of primordial germ cells (PGCs). To elucidate the mechanisms underlying reprogramming in these cells, we examined the nuclear localization of histone variants TH2A, H2A.X, H3.1/H3.2, and H3.3, which are involved in regulating the looseness of the chromatin structure, as the chromatin structure is loosened in preimplantation embryos and PGCs in the 1-cell stage and E11.5 stages, respectively, when reprogramming occurs. The global changes in the nuclear localization of histone variants were examined by immunofluorescence, and the intensity of the fluorescent signal was quantified. TH2A and H2A.X were abundantly localized in the nucleus of 1-cell-stage embryos, and signal intensity was highest in this stage during preimplantation development. In contrast, the H3.1/H3.2 signal was barely detected in the 1-cell stage, became readily detectable in the 2-cell stage, and signal intensity increased further in the 4-cell stage. H3.3 was always detected, and its level was almost constant during preimplantation development. Nuclear localization of TH2A increased in the E11.5 stage in PGCs. However, H2A.X, H3.1/H3.2, and H3.3 remained unchanged between E10.5 and E11.5. These results suggest that histone variants regulate the global chromatin structure and are involved in reprogramming both preimplantation embryos and PGCs, but that the species of those histone variants are different between them. TH2A, H2A.X, and H3.1/H3.2 in preimplantation embryos, but only TH2A in PGCs, may be important in the regulation of reprogramming.

**P448** - Cytokine supplementation improves the in vitro culture of bovine embryos. Katy Stoecklein, M. Sofia Ortega, Lee Spate, Thomas Spencer, Randall Prather

Preimplantation development of the bovine embryo relies on factors from the maternal environment that are not easily mimicked in an in vitro system. We have identified three cytokines (FGF2, LIF, and IGF1 termed FLI) that play a role in oocyte maturation, fertilization, and embryo development in vivo. Our hypothesis is that supplementation of culture medium with FLI improves preimplantation embryonic development in vitro. Embryos were produced in vitro using abattoir-derived bovine oocytes and fertilized with sperm from a single Holstein bull known to have high fertility. After an 18-20 h fertilization period, putative zygotes were cultured
in synthetic oviductal fluid with or without FLI for 8 days. The number of embryos that underwent at least one cellular division (cleavage), and the number of embryos that developed to the blastocyst stage was recorded on days 3 and 8 after insemination, respectively. There were 443 embryos supplemented with FLI, and 449 embryos in the control group across 5 replicates. There was no difference (p=0.09) in the cleavage rate between the two treatments. The FLI-treated embryos had a higher (p<0.05) blastocyst rate (35.2% ± 1.3) than the control group (25.1% ± 1.3). Trophectoderm and inner cell mass cell number was determined after immunolocalization of the trophectoderm marker CDX2 and a nuclear stain using control (n=72) and FLI-treated (n=89) blastocysts. There was no difference in trophectoderm (p=0.09), inner cell mass (p=0.93), or total cell number (p=0.26) between the two groups. These results support the idea that FLI supplementation to a bovine in vitro embryo production system improves the number of embryos that develop to the blastocyst stage without affecting embryo cell number. Further research is required to elucidate the mechanisms by which FLI supplementation improves development in vitro. Supported by Food for the 21st Century and the Clifton Murphy scholarship fund.

P449 - Proteomic assessment of the porcine intra-uterine secretome during the peri-implantation period in response to heat stress. Malavika Adur, Matthew Romoser, Katie Bidne, R Schultz, Aileen Keating, Lance Baumgard, Jason Ross

Heat stress (HS), resulting from environment-induced hyperthermia, compromises fertility through multiple mechanisms including communication disruption at the conceptus-endometrial interface. Study objectives were to assess HS effects on conceptus-endometrial communication by evaluating the uterine fluid composition during the peri-implantation period. Cyclic or bred gilts exposed to HS or thermal neutral (TN) conditions were euthanized during the peri-implantation period and uterine flush fluid (UFF) was collected for evaluation by relative quantification of protein content, using LC-MS/MS. For the first comparison, cyclic and bred gilts were exposed to diurnal HS (31 to 35°C) or TN (21 ± 1°C) conditions from d 3 to 12 post-estrus (n = 6 - 7 gilts/treatment). A total of 400 proteins were identified, of which 199 differed (P ≤ 0.05) due to pregnancy status and/or thermal treatment. Pathway analysis revealed that a majority of differentially abundant proteins were chaperone proteins (HSPB-1, HSPA8, 1433B) or endopeptidases (TIMP1, MMP2) involved in growth and metabolic pathways. For the second comparison, bred animals from each thermal treatment group were supplemented with altrenogest (ALT; 15 mg/d; a progesterone receptor agonist) and were compared to bred animals not given ALT (n = 6 - 7 gilts/treatment). A total of 332 proteins were identified, of which 196 differed (P ≤ 0.05) due to the effect of ALT and/or thermal treatment. Pathway analysis revealed proteins differentially regulated were metalloproteases (MMP2, ADAMTS1) and protease inhibitors (UFAP2, SERPINA11) involved in binding and catalytic activity. Protein expression was influenced (up- or down-regulated) more by pregnancy status or ALT than thermal treatments in the respective comparison groups. These results support the involvement of dynamic processes governing conceptus and endometrial remodeling during pregnancy establishment and demonstrate an intra-uterine profile associated with the HS response. This study was supported by the Iowa Pork Producers Association.
**P450 - NRF2 is required for normal development of porcine embryos.** Werner Glanzner, Leticia Sousa, Karina Gutierrez, Vitor Rissi, Mariana de Macedo, Luke Currin, Naomi Dicks, Felipe Perecin, Vilceu Bordignon

The nuclear factor erythroid 2–related factor 2 (NRF2) is a transcription factor that binds antioxidant response elements and acts as a central regulator of oxidative stress. The role of NRF2 in the regulation of early development of embryos exposed to stressful environments have not been thoroughly investigated. Using a well-established in vitro embryo production system, we first demonstrated that NRF2 mRNA is expressed in porcine embryos between D2 and D7 of development. The relative mRNA abundance decreased from D2 to D3, and then increased on D5 and D7 of development. Attenuation of NRF2 mRNA abundance by microinjecting dicer-substrate short interference RNA (si-NRF2) into oocytes (10pl of 25mM) significantly decreased embryo development to the blastocyst stage (34.8%) compared to oocytes injected with control (si-CT) sequences (49.6%). The knockdown efficiency in the NRF2 mRNA levels at 72 h after si-NRF2 injection was ~80% compared to si-CT. When microinjected oocytes were cultured in the presence of 3.5mM or 7mM glucose until D3 of development, and then transferred to standard culture conditions until D7, embryo development to blastocyst stage was dramatically decreased in oocytes injected with si-NRF2 compared to si-CT (15.9% vs. 27.8% for 3.5mM glucose, and 5.4% vs. 25.3% for 7mM glucose). Findings in this study revealed that: i) NRF2 mRNA expression is regulated during porcine embryo development from cleavage to the blastocyst stage; ii) attenuation of NRF2 mRNA precludes develop to the blastocyst stage in a significant proportion of embryos; and iii) the negative effect of NRF2 attenuation on embryo development was more pronounced when embryos were exposed to high glucose concentrations during the first 72 h of culture. These findings indicate that NRF2 is an important oxidative stress regulator during early development of porcine embryos.

**P451 - Embryo Mortality: A transcriptome perspective in Holstein cows.** Carolina Gonzalez-Berrios, Leticia D.P. Sinedino, Hanah Georges, Jeanette Bishop, Hana Van Campen, Milton Thomas, Thomas Hansen

Exactly how failure in conceptus-endometrial signaling causes embryo mortality (EM) is unknown. The hypothesis tested that early EM is associated with adaptive immune responses in the conceptus, and impaired IFNT production and anti-luteolytic action on the endometrium to signal pregnancy recognition. Holstein dairy cows were sorted on day 16 into nonpregnant (not exposed to semen; n=7) or pregnant groups (based on conceptus morphology): Normal (N) = translucent and elongated (n=9) and EM = pink, red, opaque and/or non-elongated (n=6). Conceptus and endometrial RNA were submitted to RNA-Seq. Raw data were analyzed using DESeq2 package and Benjamini-Hochberg procedure to control for false discovery rate in RStudio and submitted to Ingenuity Pathway Analysis® (IPA; P≤ 0.05, fold change>1.5). N conceptuses were: longer (P<0.006) and had greater (P < 0.0001) IFNT mRNA raw counts (from RNA-Seq data) than EM conceptuses. N uterine flushings had greater (P<0.004), but EM only tended (P<0.07) to have greater IFNT protein concentrations than NP uterine flushings. Likewise, N endometrium had greater (P<0.003), but EM tended (P<0.09) to have greater ISG15 mRNA raw counts than NP endometrium. IPA revealed 1,275 up- and 3,751 downregulated genes in N vs EM conceptuses. The top 2 canonical conceptus pathways (P≤10^-13) were: hepatic fibrosis/hepatic
stellate cell activation and Th1/2 activation. Top upstream regulators (P≤10^{-49}) mediated apoptosis, adaptive immune and estradiol responses. In N vs NP endometrium, 159 genes were differentially regulated, with the top canonical pathway representing IFN signaling (P≤10^{-4}), as expected. In N vs EM endometrium there were only 15 upregulated genes, most of which (14/15) were associated with estradiol signaling. It is concluded that EM was associated with apoptotic and adaptive immune responses in the conceptus and impaired IFNT production and action on the endometrium, causing upregulation of estradiol-mediated luteolytic responses. USDA-AFRI-NNF 2016-38420-25289

**P452 - Effects of embryo aggregation on preimplantation development and mitochondrial function of porcine embryos.** Sanghoon Lee, Pil-Soo Jeong, Mun-Hyeong Lee, Hyo-Gu Kang, Hae-Jun Yang, Jae-Jin Cha, Seon-A Choi, Seung Hwan Lee, Young-Ho Park, Jong-Hee Lee, Bong-Seok Song, Bo-Woong Sim, Sun-Uk Kim

Embryo aggregation is a useful method for production of blastocysts with high developmental competence in various mammals, but its relationship with mitochondrial function remains unclear. Here, in the present study, we investigated the effects of embryo aggregation using 4-cell stage embryos on the in vitro developmental competence and mitochondrial function of porcine preimplantation embryos. We conducted embryo aggregation using well of the well system and the possibility of this method was examined by fluorescent carbocyanine dye staining (DiI and DiO). Successful aggregation of porcine embryos using well of the well system was confirmed by DiI and DiO staining. In terms of embryo development, the 3X-aggregated blastocysts showed significantly improved developmental competence including blastocyst formation rates, total cell numbers, ICM ratio and cellular survival rates compared to non-aggregated embryos. In addition, the 3X-aggregated blastocysts showed significantly increased expression of pluripotency-, implantation- and antiapoptosis-related genes and decreased expression of proapoptosis-related genes. In investigation of the relationship between embryo aggregation and mitochondrial function, mitochondria DNA copy number and mitochondrial membrane potential was significantly increased in the 3X-aggregated blastocysts. Furthermore, expression of mitochondria-related genes was significantly higher in 3X-aggregated blastocysts. Therefore, these results indicate that embryo aggregation improves the developmental competence and mitochondrial function of porcine embryos. Collectively, embryo aggregation could be a useful method for successful production of transgenic pigs.

**P453 - Glycine receptor α4 subunit supports the early development of mouse embryos.** Hirofumi Nishizono, Mohamed Darwish, Takaho Endo, Hiroyuki Abe

Unraveling the interaction between fertilized oocytes and oviduct fluid components during early mammalian development is important for understanding preimplantation development and developing in vitro culture techniques. Oviduct fluid contains many free amino acids, including glycine; however, the mechanisms by which embryos utilize glycine are not fully understood. In particular, the relationship between glycine receptors (GlyRs), directly coupled with glycine, and embryos is unknown. Therefore, we aimed to investigate whether GlyRs are involved in the development of mouse fertilized oocytes. The addition of GlyR inhibitors to embryo culture
medium dose-dependently inhibited embryonic development at the 4-cell, morula, and blastocyst stages. Transcriptomic analysis using the public database and immunohistochemical experiments showed that among GlyRs, the α4 and β subunits were expressed in mouse oocytes and early embryos. Mice lacking the α4 (Glrα4) subunit were generated using the CRISPR/Cas9 system. Phenotypic analysis of fertilized oocytes produced from ovaries of Glrα4 knockout mice revealed decreases in the early developmental rate and the number of cells in blastocysts. Our study shows that Glrα4s involved in the embryonic preimplantation development and provides new insights into the interactions between oviduct fluid components and oocytes/fertilized oocytes.

**P454 - Effect of saponin treated spermatozoa on efficiency of EGFP-expressing transgenic mice produced by ICSI-SMGT.** Sanghoon Lee, Mun-Hyeong Lee, Hyo-Gu Kang, Ju-Hyun An, Hae-Jun Yang, Jae-Jin Cha, Seung Hwan Lee, Young-Ho Park, Jong-Hee Lee, Bong-Seok Song, Bo-Woong Sim, Sun-Uk Kim

Transgenic (Tg) mice are widely used in many fields, such as gene function, transgenesis and biomedical researches. Intracytoplasmic sperm injection-sperm mediated gene transfer (ICSI-SMGT) has benn a useful tool for generating Tg mice. However, ICSI-SMGT is still not optimal condition for efficiently production of Tg mice. Here, we assessed whether saponin can efficiently permeabilize spermatozoa membrane to introduce the exogeneous DNA in mice and produce transgenic embryo and fetus with high efficiency using ICSI-SMGT and embryo transfer. First, In order to confirm the permeabilization ability of saponin, we have treated saponin to spermatozoa and incubated with Cy-3-labeled DNA. The fluorescence of Cy-3 was prominently detected in the head region of saponin-treated spermatozoa. Next, we confirmed that the optimal conditions of saponin treatment for production of transgenic blastocyst with high efficiency were pretreatment with 0.01% saponin for 1min and co-incubation with 1ng/ul of EGFP expressing vector for 1min through ICSI using saponin-treated spermatozoa. Interestingly, saponin-treatment has more efficient than freeze-thawed and NaOH treatment methods to increase EGFP positive blastocyst rate, blastocyst development rate, blastomere number, and cellular survival. Consistent with this, after transferring those embryo to recipient mice, saponin treatment was enhanced to get a high proportion of transgenic mice offspring. Thus, we have established a high production method for transgenic mice using saponin, which can be used as useful tool for transgenic mice production for biomedical research.

**P455 - Imaging oocytes: Optical clearing and mathematical modeling.** Alexandre Bastien, Claude Robert

Imaging oocytes is challenging as they are giant spherical cells with high turbidity. The motto “Seeing is believing” should not be applied here, particularly in confocal laser scanning microscopy (CLSM), since it could lead to important misinterpretation of the cellular physical organisation. In CLSM, photons coming from the focal plane needs to converge at the pinhole in order to get detected. Organelles, lipid droplets and cytoplasm turbidity create refractive index (RI) mismatches that scatter the photons preventing them from being detected. Because of the large spherical shape of the oocyte, photons coming from cortical areas will be less scattered since they will cross less RI mismatches. This leads a common cortical artifact. In this study, we
describe a method to evaluate the local scattering coefficients of denuded porcine oocytes compartments and we establish a mathematical model used to correct cortical artifacts. We also evaluate several optical clearing methods to reduce scattering and enable deep imaging within specimen using direct stains and immunofluorescence. Among the tested methods are Scale, BABB, passive CLARITY and simple Glycerol/H₂O for dyes such as MitoTracker Orange, Hoechst 33342, SiR-Actin and Bodipy FL, and AF488 and AF555 secondary antibodies bind to primary against FMRP, TOMM20 and TUBA4A. Finally, we evaluate objective magnification and numerical aperture (NA) effect in both real-life and mathematical models. While high NA improves resolution and light collection, it also augments scattering which prevents deep imaging. Depending on the user needs, lower NA might be preferable. No single clearing agent works in all cases since they interact with particular dyes and antibodies. In addition, the clearing processes alter cellular composition such as lipids that can modify the natural distribution and localisation of targets.

**P456 - Effect of seminal plasma on uterine capacity to support conceptus elongation in cattle.** Sandra Recuero, Jose Sanchez, Sandra Bages-Arnal, Michael McDonald, Alan Kelly, Marc Yeste, Beatriz Fernandez-Fuertes

Seminal plasma (SP) has been reported to improve early embryo development and implantation in mice by regulating the maternal environment. However, there is little and conflicting evidence for a role of SP in fertility in cattle, where dilution or removal of SP before artificial insemination is routine. In cattle, a significant proportion of pregnancy losses occurs before embryo implantation during which time the trophoderm undergoes elongation associated with interferon-tau secretion for maternal pregnancy recognition. The aim of this study was to determine the effect of exposing the female reproductive tract to SP on bovine conceptus elongation. Crossbred heifers were estrous synchronised and either mated to a vasectomised bull (n=12 heifers, 3 bulls), or left unmated (control, n=13). Seven days later, the corpus luteum (CL) was measured by ultrasonic scanning, and in vitro-produced blastocysts were transferred (n=12-15 blastocysts/heifer). All heifers were slaughtered on Day 14. No differences (P>0.05) between groups were observed in CL volume at Days 7 or 14, nor in CL weight at Day 14. Conceptus recovery rate did not differ between control or mated heifers either (51%). Large variation in conceptus length within heifer was observed in both groups (CV 44%- 79%). Distributions of ovoid (<5 mm), tubular (5-19 mm) and filamentous (>19 mm) conceptuses were 28%, 46%, 26% in control heifers and 27%, 40%, 33% in mated heifers. Heifers exposed to SP, tended to have longer conceptuses compared to unmated controls (15.9±1.3 vs. 11.6±1.2, respectively; P=0.07). In conclusion, SP exposure at mating may be beneficial to conceptus elongation in cattle. However, further investigation is required into the biological processes underlying these effects to determine their fertility benefit in cattle. Supported by EU Horizon 2020 Marie Sklodowska-Curie (No 792212).

**P457 - Elongating Pig Conceptuses Utilize Glucose via Glycolytic Branching Pathway, and Compensate for the Decrease in Available Pyruvate by Utilizing Glutaminolysis to Provide**
Carbon for the TCA Cycle Anaplerotic Pathway. Heewon Seo, Avery Kramer, Bryan Mclendon, Guoyao Wu, Robert Burghardt, Fuller Bazer, Greg Johnson

Conceptus (embryo/placental membranes) elongation in pigs involves rapid growth and remodeling that involves extensive cell proliferation and migration requiring significant amounts of energy. Therefore, conceptuses likely employ multiple biosynthetic pathways to assure optimal utilization of energy sources within the uterine lumen. Our preliminary studies suggest that the endometrium of pigs transports glucose into the uterine lumen where the conceptus trophectoderm directs the majority of the carbon away from the TCA cycle and into aerobic glycolysis for use in the pentose phosphate pathway, one-carbon metabolism, and hexosamine biosynthetic pathway. This limits available pyruvate for maintaining the TCA cycle; therefore, the trophectoderm may compensate via anaplerosis to convert glutamine into a TCA cycle intermediate (α-ketoglutarate) through glutaminolysis. Indeed, glutamine increases in the uterine lumen during the peri-implantation period of pregnancy and increases proliferation of porcine trophectoderm cells in vitro. We hypothesized that glutamine is used by porcine conceptuses as an alternate carbon source to maintain TCA cycle flux through glutaminolysis, and determined: 1) if glutamine synthetase (GLUL, converts glutamate to glutamine) is expressed in conceptuses; and 2) if enzymes of glutaminolysis that convert glutamine to glutamate and then to α-KG (glutaminase (GLS), glutamate dehydrogenase (GLUD), and aminotransferases) are expressed in conceptuses. Results demonstrated that: 1) expression of GLS increased in trophectoderm and GLUL increased in endoderm of conceptuses, suggesting that endoderm synthesizes glutamine, and trophectoderm converts glutamine into glutamate; and 2) expression of GLUD decreased while expression of aminotransferases (glutamate–oxaloacetate transaminase (GOT), glutamate–pyruvate transaminase (GPT), phosphoserine aminotransferase (PSAT1)) increased in trophectoderm, suggesting generation of α-KG, serine, alanine, and aspartate which contribute to the biosynthesis of nucleotides. We conclude that the trophectoderm of porcine conceptuses utilize: (1) glucose for branching pathways of glycolysis, and (2) glutamine as an alternate carbon source to maintain TCA cycle flux.


The high lipid content in porcine oocytes and embryos is a well-established fact. Recent studies have demonstrated that fatty acid metabolism is active in porcine pre-implantation embryos. Fatty acid binding proteins (FABPs) form a family of small and abundant cytoplasmic proteins that are known to bind fatty acids and other small hydrophobic molecules. FABP3 have been detected in mammalian early embryos but it is not known if this protein plays a role in early embryo development. To investigate the presence and changes in FABP3 mRNA abundance during porcine embryo development, RNA was extracted from in vitro fertilized embryos at Days 2, 4 and 7 of development, which represent pre- during and post-stages when the embryo genome is activated, and analyzed by quantitative RT-PCR. To evaluate the role of FABP3 during embryo development, two dicer-substrate short interference RNAs (DsiRNA) specifically targeting FABP3 mRNA (si-FABP3) or a scramble sequence (si-CT) were microinjected into in vitro matured oocytes, which were then parthenogenetic activated and cultured until blastocyst
We observed that FABP3 mRNA is present in porcine embryos and increased in blastocysts compared to 2-cell stage embryos. Injection of si-FABP3 aattenuated FABP3 mRNA abundance, altered the expression of genes involved in fatty acid metabolism and mitochondrial network (CPT2 and MFN2, respectively) on Day 5 embryos, and dramatically reduced embryo development to the blastocyst stage (42.28% ± 3.9 vs. 17.52% ± 1.14) and embryo quality (43.20 cells vs. 30.21 cells) when compared to control embryos injected with si-CT. These results suggest that FABP3 is important for normal embryo development in the porcine species.

**P459 - Porcine Conceptuses Differentially Metabolize Glucose and Fructose to Produce Pyruvate and Lactate.** Avery Kramer, Heewon Seo, Bryan McLendon, Guoyao Wu, Robert Burghardt, Fuller Bazer, Gregory Johnson

The abundance of glucose and fructose increases in uterine flushings of pigs during the peri-implantation period, and the temporal and cell-specific expression of facilitated diffusion symporters of the solute carrier family 2A can potentially transport glucose and fructose from the maternal blood, through the endometrium and into the placenta during the peri-implantation period. However, it was not known if glucose and fructose are actively metabolized by porcine conceptuses. We utilized isotope-labeled glucose or fructose to determine if elongating conceptuses metabolize glucose and fructose. We collected conceptus tissues from Day 16 of pregnancy and incubated the tissues with either $^{14}\text{C}\text{-glucose}$ or $^{14}\text{C}\text{-fructose}$ in medium containing unlabeled glucose and/or fructose as follows: 1) $^{14}\text{C}\text{-glucose} + 4\text{ mM glucose}$; 2) $^{14}\text{C}\text{-fructose} + 4\text{ mM fructose}$; 3) $^{14}\text{C}\text{-glucose} + 4\text{ mM glucose}$ and fructose; and 4) $^{14}\text{C}\text{-fructose} + 4\text{ mM glucose}$ and fructose. The radioactivity of CO$_2$ released from conceptus tissues were then measured to compare the oxidation rates between glucose and fructose. Results demonstrated that: 1) either glucose or fructose alone are actively metabolized to produce pyruvate and lactate; 2) glucose is preferentially oxidized in the presence of fructose; and 3) glucose and fructose have no synergistic effects. We conclude that: 1) both glucose and fructose can be directly transported into elongating conceptuses, and 2) glucose is preferentially metabolized in the presence or absence of fructose, while fructose can be metabolized by the conceptus in the absence of glucose, suggesting an important mechanistic role(s) for both glucose and fructose in support of elongating/implanting conceptuses. Our results are the first to directly demonstrate utilization of glucose and fructose by elongating porcine conceptuses, and provide the basis for ongoing research to identify metabolic pathways activated within conceptuses to effectively utilize these hexose sugars to support conceptus development.

**P460 - Glyceraldehyde-3 phosphate dehydrogenase (GAPDH) levels as a possible marker of embryonic metabolism and health.** Xiaowei Liu, Rhodel Simbulan, Sky Feuer, Annemarie Donjacour, Paolo Rinaudo

GAPDH is not only a glycolytic enzyme, but also a multifunctional protein with diverse activities. Importantly, GAPDH has been found to play a critical role in modulating intracellular pathway that lead cells to abnormal glucose homeostasis. For example, GAPDH is changed in tissues and cells of animal models of type 2 diabetes. Hyperglycemia-induced GAPDH inhibition is a consequence of poly ADP-riboosylation of GAPDH by poly-ADP-ribose-polymerease
(PARP). Production of reactive oxygen species (ROS) due to physiological stress conditions, such as diabetes, has been shown to increase PARP levels, followed by the decrease of GAPDH, causing a downstream altered glucose homeostasis and disease condition. Given that increase in ROS has been shown in preimplantation embryos generated by in vitro fertilization (IVF), and given that offspring generated by IVF manifest glucose intolerance, we hypothesize that mouse blastocysts and tissues of offspring generated by might show alterations in GAPDH levels. GAPDH and PARP levels were measured at the mRNA level (by PCR) and at the protein level (by western blot) in embryos and by Western blot in adult tissues. We found that mouse blastocysts generated by IVF had a decrease in GAPDH protein and increase in PARP protein levels compared to control. Adult IVF offspring showed a tissue and sexual dimorphic change in GAPDH protein levels compared to control. IVF offspring had an increase of GAPDH in liver tissue (P<0.05 for both sexes) and muscle (female only p<0.05), while there was a decrease in fat tissue (female only, p <0.01). Our findings demonstrate that GAPDH is decreased in embryos and altered in adult tissue of mouse offspring generated by IVF. We speculate that oxidative stress derived from embryo culture could be the source of variations in GAPDH levels and that measurement of GAPDH level could provide a snapshot of metabolic activity in different tissues.

**P461 - Developmental programming of bovine preimplantation embryos by choline chloride.** Eliab Estrada-Cortes, William Ortiz, Elizabeth Jannaman, Charles Staples, Jeremy Block, Peter Hansen

Supplementation with rumen-protected choline has been reported to improve reproductive performance in dairy cows. Such an effect could represent actions on the embryo because choline is a precursor of betaine, a methyl donor for DNA methylation, and there is extensive DNA demethylation and re-methylation during the preimplantation period. The objective was to determine whether addition of choline chloride to culture medium improves the competence of in vitro-produced embryos to become blastocysts, to establish pregnancy after embryo transfer (ET) and alter characteristics of resultant offspring. Oocytes were obtained by ovum pick-up from Brahman cows (n=11). Oocytes from each donor were fertilized using conventional (n=4) or sexed semen (n=1) from five Brahman sires. Half of the presumptive embryos from each donor were incubated in BBH7 culture medium (control; n=387 oocytes) and half with culture medium containing 1.8 mM choline chloride (n=332 oocytes). Treatments were isotonic. At day 7, blastocysts were transferred into Angus and Brangus recipients. Pregnancy diagnosis was performed at day 28 after ET and resultant offspring were evaluated at birth. There was no effect of treatment (P>0.1) on the percent of cleaved embryos becoming a blastocyst (15.9±3.4% control vs 15.8±3.3% choline) or pregnancy per ET [50.0% (31/62) vs 40.0% (20/50) for control and choline, respectively]. Birth weight was affected by the treatment x sex of calf interaction (P=0.0022). For females, choline calves were heavier than control calves [49.4±2.5 (n=4) vs 34.3±2.1kg (n=7)] whereas the opposite was observed for male calves [27.5±3.2 (n=3) vs 36.2±3.2kg (n=4)]. In conclusion, addition of 1.8 mM choline chloride to culture medium did not affect competence of embryos to become blastocysts but programmed development to alter fetal growth in a sex-dependent manner (Support: Larson Endowment).
**P462 - Utilization of glucose and fructose by the ovine conceptus during the peri-implantation period of pregnancy.** Robyn Moses, Katherine Halloran, Claire Stenhouse, Heewon Seo, Gregory Johnson, Guoyao Wu, Fuller Bazer

Glucose is a key substrate for ATP production via glycolysis and is important for the synthesis of other biomolecules, including fructose by the placentae of ungulates and cetaceans. The role of glucose at the ovine feto-maternal interface is well defined, but how fructose is utilized by the conceptus (embryo and extra-embryonic membranes) is unknown. This study tested the hypothesis that ovine conceptus trophectoderm utilizes glucose and fructose during the peri-implantation period of pregnancy to support conceptus development. Rambouillet (n=2) and Suffolk (n=2) ewes were bred to fertile rams during estrus (Day 0), then euthanized and hysterectomized on Day 17 and uterine horns were flushed with 20mL phosphate buffered saline to collect conceptuses. Conceptus tissue was cultured in Krebs-Henseleit Bicarbonate buffer with either radiolabeled U-14C-glucose and U-14C-fructose, U-14C-glucose, or U-14C-fructose and unlabeled glucose and fructose (4mM each), glucose (4mM), or fructose (4mM). Cultures were analyzed by quantification of radiolabeled carbon dioxide produced from the metabolism of the hexose sugars via the Krebs cycle by the conceptus. Concentrations of pyruvate and lactate in the culture medium were determined via fluorometric assays after homogenization of conceptus tissue. Conceptus tissue cultured with radiolabeled glucose and 4mM unlabeled glucose produced greater amounts of carbon dioxide than that cultured with radiolabeled fructose and 4mM unlabeled glucose or fructose alone (P<0.05). There was no treatment effect on concentrations of lactate and pyruvate in culture media (P>0.05). These results indicate glucose is metabolized through oxidative metabolism via the Krebs cycle and that fructose, through aerobic glycolysis, can produce lactate and pyruvate. Pyruvate may be used by the trophectoderm for gluconeogenesis via the Cori cycle. This research was supported by Agriculture and Food Research Initiative Competitive Grant no. 2018-67015-28093 from the USDA National Institute of Food and Agriculture.

**P463 - Warburg metabolism is utilized by developmentally competent in vivo produced mouse embryos, but is disrupted by in vitro culture.** Ben Goheen, Sandeep Rajput, Courtney Grimm, John Becker, William Schoolcraft, Rebecca Krisher

Metabolic processes fueling rapid cell proliferation, as occurs in cancer cells, require glycolytic metabolites upstream of the tri-carboxylic acid (TCA) cycle for synthesis of macromolecules for biomass production and redox regulation. This can be achieved by engaging in aerobic glycolysis, also known as the Warburg Effect. Warburg metabolism has been proposed to occur in preimplantation embryos, and although gene expression data would support that hypothesis there is to date no firm evidence. Our objective was to characterize presence and functional activity of pyruvate kinase M2 isoform (PKM2), pyruvate dehydrogenase (PDH), and lactate dehydrogenase (LDH) proteins in mouse (CF1) blastocysts produced in vivo (IVO) and in vitro (IVC). Replicate (n=3) pools of 30 blastocysts were subjected to western blot analysis for both phosphorylated (p) and total (t) protein; p:t ratio reflects protein activity. Phosphorylation of PKM2 was elevated 2.2-fold (p=0.0034), and PDH was elevated 4.2-fold (n=1) in IVO compared to IVC blastocysts. Phosphorylation inhibits PKM2 activity and redirects glycolytic metabolites away from the TCA cycle, a key component of the Warburg Effect. Phosphorylation of PDH is
also inhibitory and acts to slow entry of pyruvate into the TCA cycle. Another hallmark of the Warburg effect is lactate production from pyruvate, catalyzed by LDH. We observed an 11.1-fold increase in total LDHB protein in IVO blastocysts compared to IVC (p<0.0001). These results demonstrate that Warburg metabolism is active in mouse blastocysts produced in vivo. However, culture of mouse embryos in vitro results in metabolic alterations such that Warburg metabolism is no longer utilized. Thus, in vitro produced embryos may aberrantly rely on the TCA cycle and oxidative phosphorylation for energy generation, potentially compromising biomass production and redox control and resulting in the reduced developmental competence that is a well-known hallmark of in vitro embryo production.

**P464 - IFNT does not affect extracellular vesicle release into the uterine lumen of sheep.**
Eleanore O'Neil, Gregory Burns, Thomas Spencer

In sheep, the blastocyst develops into an ovoid conceptus by day 12 and then elongates into a filamentous conceptus by day 14. Coincident with conceptus elongation, the trophectoderm produces interferon tau (IFNT) that signals pregnancy recognition and modulates uterine receptivity. Both the uterine endometrium and conceptus trophectoderm produce and release exosomes and microvesicles, collectively extracellular vesicles (EVs), that contain select RNAs, proteins, and lipids. In sheep, progesterone increases EVs in the uterine lumen and represent a novel form of conceptus-endometrial communication. In Study One, the uterine lumen of day 14 cyclic (C) and pregnant (P) ewes (n=5 per status) was gently flushed with 20 ml of sterile PBS. The conceptus was removed if necessary, and uterine lumen flush clarified by centrifugation and stored at -80°C. EVs were then isolated by size exclusion chromatography and ultrafiltration. Nanoparticle tracking analysis revealed that total EVs in the uterine lumen was lower (P<0.05) in day 14 P than C ewes. To determine effects of IFNT on endometrial EV production (Study Two), Alzet 2ML1 osmotic pumps were secured to the mesosalpinx of one uterine horn of ewes on day 8 post-estrus and the attached catheter inserted into the uterine lumen. Osmotic pumps contained either: (a) 2 ml saline (n=4); or (b) 202 µg recombinant ovine IFNT in saline (n=5). Ewes were collected on day 14, and EVs in the uterine lumen determined as in Study One. Intrauterine infusions of IFNT did not (P>0.10) affect EV size (218 ±18 nm in saline treated) or number (1.14E+11 ± 3.8E+10 in saline treated) in the uterine lumen. These studies support the hypothesis that the pregnancy-dependent decrease in EV content of the uterine lumen is due to uptake by the elongating conceptus. Supported by AFRI 2015-67015-23678 and 2016-67015-24741 from the USDA National Institute of Food and Agriculture.

**P465 - Medium renewal on day 3 and its effect on blastocyst formation: a randomized retrospective research study.**
Dorina Dulaj, Sara Cnudde, Alanna White, Sule Dogan, Fang Li, Michael Fakih, Nicholas Shamma, Ahmad Hammoud, Iqbal Khan

**Objective:** To evaluate the development and blastocyst formation of embryos cultured in a single medium with and without day 3 medium change.

**Design:** Randomized retrospective research on sibling embryos.
**Methods:** Data were collected from randomly selected patients (n=38); a total of 724 embryos from were evaluated from November, 2017 – February, 2018 in our clinic. We excluded those patients who had less than 8 oocytes to be able to divide the sibling embryos into two groups. Oocytes were incubated in table top incubators (Miri, ESCO) following intracytoplasmic sperm injection (ICSI). All embryos were cultured in the single medium, Life Global Total ® (LGGT), and randomly divided into two groups to avoid patient related bias. In Group 1, medium renewal was performed on day 3 by simply replacing of 0.5ul LGGT with freshly equilibrated LGGT medium. Group 2 did not receive any medium renewal/ replacement on day 3. Both groups of embryos were cultured until Day 5, 6 or 7 and the blastocyst formations were assessed on day 5, 6, and 7. Statistical analysis was done to demonstrate the difference between two groups using two sample t-test and the differences were reported as significant when p < 0.05.

**Results:** The overall blastocyst rates for Group 1 and 2 were 54.3% and 57.7 % respectively. There were no significant differences in the blastocyst formation between group 1 and group 2 (p > 0.05).

**Conclusion:** Because the comparison of medium renewal/replacement was done using sibling embryos under similar conditions, no limitations or biases were reported in this study. We concluded that the medium renewal/ replacement on day 3 does not have a significant impact on embryo development or blastocyst formation because of our well stabilized culture conditions in the lab. These results may vary depending up on different lab culturing systems and patient profiles.

*P466 - Maintenance of developmental competence in feline oocytes following 24 h of meiotic arrest with physiological agents.* Shelley Sandmaier, Jason Herrick

In vitro matured (IVM) feline oocytes exhibit reduced developmental competence compared to in vivo matured oocytes, which is further compromised in oocytes collected during the non-breeding season or from pre-pubertal females. Extending the culture period for immature oocytes by arresting meiosis has been shown to improve oocyte developmental competence in other species. In this study, cumulus-oocyte complexes (COCs) from domestic cats were matured in vitro (5 COCs per 50 µl, 1.0 IU eCG and 25 ng/ml EGF, 24 h in 6.5% CO2 in air) immediately after collection or following 24 h of culture with natriuretic peptide C (100 nM), hypoxanthine (100 µM), 17β-estradiol (100 nM) and eCG (0.01 IU/ml). These factors delay the resumption of meiosis in other species by maintaining cumulus-oocyte communication and preventing the reduction in cyclic AMP (cAMP) associated with meiotic resumption. Following IVM, COCs were fertilized in vitro (22 h, 0.5 x 10⁶ motile sperm/ml, 6.5% CO2 in air) and resulting embryos were cultured in a sequential, feline-specific medium (5 embryos per 20 µl, 6 days, 6.5% CO2 and 5% O2). Embryonic cleavage (62.5% ± 2.8% vs. 60.3 ± 2.8%), development to blastocyst (41.8 ± 2.9% vs 39.4 ± 2.8% of oocytes; 67.0 ± 3.5% vs. 65.4 ± 3.6% of embryos), hatching (10.3 ± 2.2% vs. 13.9 ± 2.6% of embryos), and blastocyst cell numbers (180.7 ± 9.5 vs. 194.2 ± 12.9) were not different (P>0.05) for oocytes subjected to meiotic arrest compared to control oocytes, respectively. While meiotic arrest did not improve embryo development, our results indicate it is possible to maintain feline oocytes in culture for 24 h without compromising
developmental competence. Manipulation of the culture environment during this period of meiotic arrest could be a novel means of improving the quality of feline oocytes.

**P467 - Effect Of Physiological Heat Stress On HSP70 And CASPASE 3 Levels In Bovine Oocytes.** Luiz Camargo, Luiz Siqueira, Naiara Saraiva, Gilson Maia, Beatriz Nogueira, Carolina Quintao, Clara Oliveira

CASPASE 3 is an apoptosis downstream effector enzyme. HSP70 chaperones help to fold many proteins and are upregulated in response to stress. We wanted to evaluate if CASPASE 3 and HSP70 would be affected after physiological heat stress and could be used as markers of thermal injury in bovine oocytes. Girolando (Gir x Holstein crossbred) cows were housed for 23 days in controlled-climate chamber programmed with three climatic conditions along 24h: THI (Temperature and Humidity Index)=74.8 between 00:00 and 10:00h; THI=88.6 from 10:00 to 18:00h and THI=79.7 from 18:00 to 00:00h, with 12h light and 12h dark. Control group was cows housed in a free-stall barn located beside the climate chamber with an average THI=66.5. After the end of induced-heat stress, immature oocytes were collected by ultrasound-guided ovum pick-up (four replicates). Cumulus cells were removed and fixed in paraformaldehyde. Oocytes (n=197) were stained for immunofluorescence using primary antibodies mouse anti-HSP70 (SCBT, 1:50) and rabbit anti-active CASPASE 3 (Sigma, 1:750) and secondary antibodies Alexa 555-conjugated donkey anti-rabbit and Alexa 488-conjugated donkey anti-mouse. Images of each oocyte were recorded and analyzed for average fluorescence level in ImageJ software. Results are shown as mean± SEM. Means were compared using the T-test. Levels of CASPASE 3 (14.9 ± 1.2 vs 15.8 ± 0.8, heat-stressed and controls, respectively; P=0.54) and HSP70 (6.9 ± 0.5 vs 7.7± 0.6, heat-stressed and controls, respectively; P=0.31) did not differ between groups. Our results suggest that HSP70 levels remain unaltered in bovine oocytes after short-term physiological heat stress. This highlights the importance of non-HSP70-based responses in bovine oocytes subjected to heat stress. Similarly, the apoptosis cascade was not affected by heat stress. In conclusion, CASPASE 3 and HSP70 may not be reliable markers of thermal injury in immature oocytes derived from heat-stressed Girolando cows. Financial support: CAPES, Fapemig and Faperj.

**P468 - Correlation analysis between C natriuretic peptide and pregnancy outcome.** Lidan Guo, Weina Yang, Donghui Huang

**Aim:** CNP was proved to played a key role in female reproduction, and be related to oocyte quality. This study were to found the relationship of CNP with pregnancy outcome, to provide a new indicator to access pregnancy outcome. **Methods:** Follicular fluid(FF) were collected from 158 patients undergoing IVF/ ICSI procedure at the Center for Reproductive Medicine, Tongji Medical College, Huazhong University of Science and Technology. CNP and cGMP levels in human FF were tested by ELISA. Then Distribution Patterns of CNP and NPR-B from GV oocyte to blastocyst in mouse were tested by Confocal microscopy. Finally CNP were added in the fertility or embryo development medium respectively, to observe the development of embryo. **Results:** CNP levels in FF from non-pregnancy patients is significant higher than that from pregnancy patients. a strong positive correlation between CNP and cGMP concentrations in
human FF, both CNP and NPR-B expressed in the plasma of cells at different stages from GV to blastocyst. 2-cell rate of embryos and rate of blastocysts cultured in fertility culture medium added CNP were higher than that in the control group. CNP could also promote rate of blastocysts when added to embryo culture medium. **Discussion:** CNP in human FF could predict pregnant outcome of IVF patients, the mechanism CNP in follicular fluid have relation with the quality of oocytes or embryos competence and could promote the development of embryos.

**P469 - Histone H3 lysine 23 acetylation and methylation are drastically changing during oocyte maturation and embryos cultured in vitro in pig.** Dong Jin, Tao Lin, Reza Oqani, Jae Lee, Hyeong Shin, Joo Lee

Histone modifications have important roles in regulating the expression of developmental genes during preimplantation embryonic development. Modifications of canonical histone H3 at lysine 4, 9, 27 and 36 etc. have been intensively studied in mammalian oocyte and early embryos. Here, we analyzed temporal and spatial distribution of acetylation, mono-, di- and tri-methylation of noncanonical histone H3 lysine at 23 (H3K23ac, H3K23me1, H3K23me2 and H3K23me3) during porcine oocyte maturation and pre-implantation development, as well as porcine fetal fibroblasts. In interphase cells, H3K23ac, me1, me2 and me3 were evenly distributed throughout the nucleoplasm, but not the nucleolus. In mitotic-phase cells, H3K23ac, me1, me2 and me3 were observed over the entire genome of cells. H3K23ac, me1, me2 and me3 actively expressed in EdU-positive S phase cells when compared to EdU-negative cells. More than 91% DNA replication foci were well colocalized with H3K23 methylation sites. After DNA replication inhibitor hydroxyurea treatment, H3K23 me1, me2 and me3 expression levels were significantly reduced. H3K23ac and me3 expressions were detectable through oocyte meiotic resumption. H3K23me3 is only barely detectable in the pronuclei of zygotes and the nuclei of blastocysts after parthenogenetic activation (PA). After in vitro fertilization (IVF), no H3K23me3 signals were expressed in nuclei of IVF-derived embryos except in residual polar bodies. However, H3K23ac signals were clearly detected in the nuclei of PA and IVF-derived blastocysts. Transcription signal was seen in blastocyst after EU staining. However, RNA polymerase inhibitor actinomycin D not only inhibits transcription activity, but also reduces H3K23ac signal expression in porcine blastocysts. These findings may serve as a valuable reference for further study of the mechanistic relevance of H3K23 modifications for the regulation of oocyte maturation and early embryonic development in mammals.

**P470 - Evaluating CRISPR/Cas9 Guide RNA Formulations for Gene Editing Efficiency in Rhesus Macaque Pre-Implantation Embryos.** John Statz, Fernanda de Carvalho, Cathy Ramsey, Trevor McGill, Martha Neuringer, Carol Hanna, Jon Hennebold

Non-human primate (NHP) models of human diseases are critical for understand their etiology and developing treatments. While CRISPR/Cas9 revolutionized the possibility for engineering disease models, its use in NHP zygotes has not been fully optimized. Two guide RNA (gRNA) formulations were tested for editing efficiency of the *MYO7A* gene in rhesus macaque zygotes, including two-component CRISPR RNA (crRNA)/trans-activating crRNA (tracrRNA) hybridized gRNAs (IDT, referred to herein as Hyb-gRNAs) and chemically synthesized single
guide RNAs (sgRNAs; Synthego). The gRNAs target exon 3 of MYO7A, which when mutated in humans leads to hearing loss and balance disorders at birth, as well as progressive vision loss. Hyb-gRNAs were injected into 53 zygotes, whereas 166 zygotes were injected with sgRNAs. Of the 53 Hyb-gRNA injected zygotes, 11 developed into blastocysts (21%), while 16 of 166 zygotes (10%) injected with sgRNAs developed into blastocysts (P<0.05). For embryos that developed into blastocysts, the trophectoderm was biopsied using a laser-mounted microscope objective. Arrested embryos and biopsied samples were flash frozen and subjected to whole-genome multiple displacement amplification (Qiagen). Of the embryos that successfully underwent genome amplification, DNA sequencing revealed that the editing efficiency was significantly (P<0.001) greater in those embryos receiving sgRNAs (110 out of 149; 74%) compared to zygotes injected with Hyb-gRNAs (14 out of 36; 38%). These results demonstrate that, although embryonic development rates may be lower, high editing efficiencies can be achieved in rhesus macaque zygotes by using multiple, chemically synthesized sgRNAs along with Cas9 protein. Future studies are necessary to determine that the high level of MYO7A editing with multiple sgRNAs applies to other gene targets. Funding: Foundation Fighting Blindness and NIH P51 OD011092.

**P471 - Overlapping roles and requirement of histone deacetylase 1 and 2 in lineage development and genome-wide DNA methylation during mouse preimplantation.** Kun Zhang, Panpan Zhao, Huanan Wang, Han Wang, Yanna Dang, Lei Luo, Shuang Li, Yan Shi, Lefeng Wang, Shaohu Wang, Jesse Mager

Extensive epigenetic modifications, including DNA methylation and histone modifications, occur following fertilization during mammalian preimplantation development. Aberrant epigenetic reprogramming is a major factor limiting the developmental success of embryos generated by assisted reproductive technologies, including somatic cell nuclear transfer. To date, little is known about the precise role of histone modifications in preimplantation development. We found that co-knockdown (cKD) of histone deacetylases 1 and 2 (Hdac1 and 2), but not individually, led to lethality during the morula to blastocyst transition with reduced blastocyst rate and number of live offspring after embryo transfer. This developmental arrest was further validated with the use of FK228, a small-molecule Hdac1/2-specific inhibitor. Cell proliferation was compromised whereas incidence of apoptosis increased in cKD embryos. These two defects may be explained by increased acetylation of Trp53. RNA-seq analysis revealed 991 genes were differentially expressed (Fold changes (FC) >2 or <0.5, P adjusted<0.05), 72% of which were upregulated, in agreement with the well-known role of Hdac1/2 as transcriptional repressors. Moreover, both RNA-seq and immunostaining analysis identified a failure of lineage specification to give rise to trophoderm and pluripotent cells with a substantial reduction of Cdx2 whereas Hippo pathway was also affected. Increased genome-wide DNA methylation was found with increased DNA methyltransferases and Uhrf1. Taken together, Hdac1 and 2 are required for preimplantation development with critical roles in regulations of lineage specification, cell viability and global DNA methylation.

**P472 - Sin3a is required for mouse preimplantation development via regulating Hdac1.** Panpan Zhao, Yanna Dang, Shuang Li, Lefeng Wang, Tong Liu, Kun Zhang
Preimplantation development is dynamic in epigenetic modifications, including DNA methylations and histone modifications. Sin3a complex is a Hdac1/2-containing epigenetic complex that is well known for its capability of transcriptional repression. Previously we found that Sin3a knockdown (KD) embryos arrest at morula stage. However, the mechanism of the functional requirement remains unclear. To determine the molecular basis of the developmental failure of Sin3a KD embryos, RNA-seq was performed with embryos collected at 16 cell. Results show 717 genes were differentially expressed in Sin3a KD morulae relative to control (Up:407 and down:310; Padj<0.05, Log2 (Fold change) ≥ 0.8 or ≤ -0.8). Gene ontology analysis of downregulated genes revealed top enriched GO terms involve protein deacetylation, regulation of histone acetylation and transcriptional corepressor activity. In particular, among downregulated genes, we identified genes associated with cell cycle progression and epigenetic regulation genes, including Hdac1 and Hdac5. We observed a decrease in phosphorylation of histone H3 serine 10, a marker for late G2 or mitosis, in Sin3a KD morulae, suggesting a defect in mitosis. This defect may be explained by increased acetylation of p53 lysine 379. Microtubule bridges between cells have been shown crucial for protein transport in preimplantation embryos. Immunostaining analysis showed number of microtubule bridges was reduced in Sin3a KD morula. qPCR and immunostaining analysis verified that Hdac1 were decreased at both mRNA and protein level in Sin3a KD morulae. We further asked if we can rescue the developmental potential of Sin3a KD embryos by injection of exogenous Hdac1 mRNA. Results showed blastocyst formation rate was improved significantly when Hdac1 mRNA was co-injected with Sin3a siRNAs into zygote. Moreover, p53 acetylation level dropped to normal compared to Sin3a KD group. In summary, we propose Sin3a is functionally required for preimplantation development with critical roles in regulation of p53 acetylation and Hdac1 transcription.

**P473 - EPGN improves preimplantation embryo development of porcine oocyte.** Ga-hye Kim, Sang-Hwan Hyun

Epigen is a recently identified ligand of the epidermal growth receptor (ErbB1). In this study, we examined the effects of the epigen during *in vitro* culture (IVC) on porcine embryonic development after *in vitro* fertilization (IVF). Zygotes were treated with 0 ng/mL (Control), 3 ng/mL, 6ng/mL, and 30ng/mL of epigen from day 1 to day 7. There was no significant difference in cleavage rate and intracellular in IVC (Cleavage rate; 0ng/ml (60.10 ± .2.77), 3ng/ml (57.8 ± 3.28), 6ng/ml (58.72 ± 5.03) and 30 ng/ml (60.65 ± 1.21). The blastocyst formation rates were determined at 0ng/ml (25.39 ± 1.44), 3ng/ml (41.6 ± 4.96), 6ng/ml (35.5 ± 3.85) and 30ng/ml (23.33 ± 4.47). When the blastocysts were divided into 3 groups according to the morphology of the blastocysts, there was a significant difference in the expended blastocyst concentration of 3ng/ml (p <0.05). The expended blastocyst formation rates were determined at 0ng/ml (15.55 ± 3.20), 3ng/ml (27.47 ± 1.04), 6ng/ml (20.05 ± 5.26) and 30ng/ml (12.54 ± 41.69), and total blastocyst formation rates were determined at 0ng/ml (25.39 ± 1.44), 3ng /ml (41.6 ± 4.96), 6ng/ml (35.5 ± 3.85) and 30ng/ml (23.33 ± 4.47). Supplementation with epigen in the IVC medium significantly increased blastocyst formation in 3ng /ml and 6 ng/ml (p<0.05). Although it is necessary to analyze the mechanism of the effects of epigen on embryonic development, these results suggest that supplementing IVC medium with epigen during IVF of porcine embryos improves their developmental potential.
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**P474 - Culture of human 3PN zygotes in physiologically relevant reduced nutrients results in normal peri-implantation development after extended culture.** Deirdre Logsdon, Ye Yuan, William Schoolcraft, Rebecca Krisher

Previous studies from our laboratory have shown that mouse embryos thrive in culture medium formulated to provide only what embryos consume in vitro, based upon metabolomic analyses. Our objective was to determine if reduced nutrient (RN) medium could support human embryo development and quality. Human 3PN zygotes were cultured in two media treatment groups (Control n=22, RN n=21) to the blastocyst stage using time-lapse imaging, with patient consent. On E5 and E6, blastocysts were dezonated and placed into extended culture (Control n=5, RN n=9). Surviving embryo outgrowths (control n=4, RN n=5) were fixed at E10, and stained with DAPI and antibodies against F-actin and pluripotency marker Pou5f1 to determine the number of epiblast cells and total cells using 3D confocal microscopy. Although not reaching significance, culture in RN medium resulted in improved embryo cleavage (RN 81%, Control 68%; p=0.34) and E5/E6 blastocyst development (RN E5 24%, E6 43%; Control E5 9%, E6 23%; p=0.20, 0.16) compared to control. Embryos cultured in RN medium tended to cleave to the two-cell stage earlier (RN 10.9hr, Control 18.8hr; p=0.09) and reach the morula stage sooner (RN 78.2hr, Control 100.0h; p=0.08) than control embryos. Although embryos cultured in RN resulted in outgrowths with numerically more epiblast cells (RN 60.25 ± 41, Control 18.25 ± 1) and total cells (RN 177 ± 55, Control 97 ± 41) compared to control, this also did not reach significance likely due to the low number of embryo outgrowths assessed in this preliminary experiment. Extended embryo culture offers insight into developmental and implantation potential and has provided information to suggest that RN medium may support human embryo development to the blastocyst stage and beyond as well as, if not better than, traditional embryo culture medium.

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**P475 - Neurogenin 3 disruption by CRISPR/Cas9 electroporation of porcine zygotes.** Insung Park, Seung Kim, Pablo Ross

Genetically modified pigs are a promising model for advancing biomedical applications. Neurogenin 3 (NGN3) is responsible for generating pancreatic endocrine cells. NGN3 KO embryos would represent a useful diabetic pig model and a host for testing the functionality of human pancreatic cells derived from pluripotent stem cells. CRISPR/Cas9 microinjection into pig zygotes has been successfully used to generate gene knock-out pigs. However, microinjection is a laborious process and requires individual manipulation of each embryo, resulting in a time consuming, low throughput process. Delivery of CRISPR/Cas9 by
electroporation has been used to circumvent the drawbacks of microinjection. In this study, we first used fluorescent dextran electroporation to establish conditions that allowed for high permeabilization efficiency maintaining embryo viability, as assessed by development to blastocyst stage. Next, we further determined the efficiency of NGN3 disruption when Cas9 protein and single guide RNA (sgRNA) targeting NGN3 were electroporated into zygotes. Different concentrations of sgRNA were evaluated. Three different total concentrations of NGN3-targeted gRNA, 100ng, 200ng, and 500ng, were tested maintaining 1:2 ratio of sgRNA and Cas9 protein to determine which concentration has the most effective disruption rate retaining cell viability. Among the groups, a 500ng:1μg group had the highest insertion/deletion rate (80%) in parthenogenetically activated porcine embryos with comparable blastocyst development rate (25%) to the control group.

P476 - Supplement of GDF8 during in vitro maturation of porcine oocyte enhanced subsequent embryonic development after somatic cell nuclear transfer. Junchul Yoon, Sang-Hwan Hyun

Growth differentiation factor 8 (GDF8) is a member of the transforming growth factor-β that has been identified as a strong physiological regulator. The purpose of this study is to investigate the effects of GDF8 on porcine oocytes during in vitro maturation (IVM). The 0 or 2 ng/mL of GDF8 were added during IVM followed experiment design as control and GDF8 supplemented groups. After IVM, we performed somatic cell nuclear transfer (SCNT) and investigated intracellular reactive oxygen species (ROS) levels on cleaved SCNT embryo and observed subsequent embryonic development. Data were analyzed by independent-sample t-tests. After 42 h of IVM, SCNT was performed and then the SCNT embryos were cultured in vitro (IVC). After 48hr of IVC, the GDF8 supplement was shown significantly (p<0.05) higher four to six cell formation rate (10.2% VS 16.5%) than control. After additional 120hr of embryo culture, the GDF8 supplement showed significantly (p<0.05) enhanced blastocyst formation rate and total cell number (22.3% and 54.2±7.3 VS 30.3% and 74.4±8.7). To determine the effect of GDF8 supplementation on cellular apoptotic regulation during IVM and subsequent influence during early embryonic development, each group of four to six cell stage embryos were collected at 48hr of IVC and immediately assayed. The GDF8 supplemented group showed a significant (p<0.05) decrease in intracellular ROS level compared with control. In conclusion, the supplement of GDF8 during IVM improved the developmental potential of porcine SCNT embryos by decreased the intracellular ROS level during preimplantation embryonic development.

Keywords: in vitro maturation, GDF8, SCNT, porcine, embryo

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*Corresponding author: shhyun@cbu.ac.kr.

**P477 - Folate deficiency induces to impaired embryo implantation and decidualization via estrogen and progesterone signaling during pregnancy in mice.** Junya Ito, Yui Nambu, Takafumi Namiki, Yui Kawata, Atsuko Kageyama, Naomi Kashiwazaki

During pregnancy in mammals, it is thought that folate has an essential role in the fetal growth. In addition, some previous studies showed that folate also affected uterine function, for example decidualization and placental development. However, it still remains unclear the role of folate in molecular mechanism. In this study, it was confirmed expression and localization of folate receptor (Folr1) in the mouse uterus during pregnancy. We also examined whether folate deficiency affect uterine function and pregnancy-associated gene expression during pregnancy in mice.

Matured C57BL6/J female mice were mated with fertile male mice. The appearance of the vaginal plug was considered as Day 1 (D1) of pregnancy. From D1 to D10, uterine tissues were collected at the different time point and then used for further studies. A folate-deficient pregnant mouse model was prepared as previously reported (Gao et al., 2012). At D1, Folr1 was only expressed at the epithelium and gradually spread to the stroma cells beyond D5. In folate-deficient (FD) mice, implantation rate at D5 was slightly lower than that of wild type (WT) mice but there was no significant difference (55.6% vs 75.0%, \( p > 0.05 \)). There was also no significant difference at the number of implantation (FD 6.3 vs WT 8.0, \( p > 0.05 \)). However, expression of ERa, PR and LIF at D4 of FD group was lower than that of WT group. At D10 in FD group, pregnancy rate was significantly lower than that of WT group (22.6% vs 66.7%, \( p < 0.05 \)). In addition, the weight of implantation site in FD group was also decreased compared to that of WT group (54.7 mg vs 66.7 mg, \( p < 0.05 \)). Taken together, our present results show that folate deficiency negatively affects the maintenance of pregnancy via estrogen and progesterone signal during pregnancy in the mouse.

**P478 - Prediction of implantation competence of pre-implantation mouse embryos by time lapse-monitoring and outgrowth assay.** Jaewang Lee, Jihyun Kim, Wontae Kim, Jung Won Choi, Jin Hyun Jun

Time-lapse monitoring system has been applied for prediction of developmental competence and selection of transferable embryos. We evaluated the integration of time lapse-monitoring system and outgrowth assay to estimate implantation competence of pre-implantation embryos. Mouse 2-cell embryos were collected on 1.5 dpc and individually cultured with Quinn’s Advantage blastocyst medium with SPS in a well-off-well dish. For outgrowth assay, the culture medium was changed to Dulbecco’s modified Eagle media with 10% FBS on 4.5 dpc. Embryo development were collected by time-lapse monitoring with the Primo system from 1.5 to 7.5 dpc. Developmental stages were classified as 3-cell, 4-cell, 8-cell, morula, early blastocyst, late blastocyst, hatching blastocyst, hatched blastocyst, and outgrowth. The prediction power of
Implantation competence was statistically analyzed by the cumulative time of early, medium and late group, and the interval time of short, middle and long group in each developmental stage. The implantation competence of each embryo was confirmed by successful development to outgrowth stage. In the early cleavage stage of 4-cell stage, the medium development group (62.5%) showed the highest outgrowth rate rather than the early development group (45.0%). The highest outgrowth rate was showed in early development to 8-cell stage group as 68.2% among cumulative time groups, and in the short time from 4-cell to 8-cell stage group as 69.6% among the interval groups, respectively. The outgrowth rate was significantly increased to 75.0% of 8-cell stage (early and short interval group) and 72.7% of blastocyst stage (early, medium or long interval group) by the combination of the cumulative time and the interval time of development stage. Analysing the morphokinetic parameters for implantation competence could provide the better selection value of transferable embryo. We suggest that implantation outcome could be increased by the optimized selection of 4-cell, 8-cell and blastocyst stage embryos in human IVF-ET program.

**P479 - Conceptus-derived proteins, CAPG & P4HB, alter the transcriptome of bovine endometrial cells cultured in vitro to enhance the pregnancy recognition process.** Haidee Tinning, Anna Pullinger, Ruth Sutton, Georgios Oikonomou, Miguel Velazquez, Achim Treumann, Niamh Forde

Successful early pregnancy in cattle is depended in part, on the production of sufficient quantities of Interferon Tau (IFNT: the pregnancy recognition signal) by the conceptus to induce successful maternal recognition of pregnancy (MRP). However, the presence of the conceptus alters additional transcripts in the endometrium in addition to those that change in response to IFNT alone. We have shown previously that the bovine conceptus secretes additional proteins coordinate with IFNT production including macrophage capping protein (CAPG) and protein disulphide isomerase (P4HB). We tested the hypothesis that these proteins may act alone or in synergy with IFNT to alter the endometrial transcriptome to facilitate MRP and successful early pregnancy in cattle. Recombinant bovine CAPG and P4HB were produced in E.coli and purified using immobilised metal affinity chromatography. Primary endometrial cells were obtained from abattoir-derived mid-to-late luteal phase bovine uteri (n=3) by enzymatic digestion and purification. Stromal and epithelial-enriched cells were cultured for 24 hours with the following treatments: 1) Control, 2) Vehicle control, 3) IFNT 4) P4HB, 5) CAPG, 6) IFNT+CAPG, and 7) IFNT+P4HB. RNA sequencing was performed to determine transcriptional changes induced by CAPG, P4HB +/- IFNT. Treatment of epithelial cells with IFNT, CAPG or P4HB alone altered 2941, 1020 and 1016 transcripts respectively. Interestingly, 728 transcripts were altered in all three treatment groups, while 32 and 34 differentially expressed genes (DEGs) were detected in CAPG and P4HB treated cells respectively. CAPG & P4HB altered the expression of 131 DEGs that were not altered by IFNT. Comparison of IFNT treatment alone with IFNT plus CAPG or P4HB resulted in 131 and 88 DEGs respectively. Collectively these data indicate that the conceptus-derived proteins P4HB & CAPG have a functional role in enhancing MRP alone and in combination with IFNT.
**P480 - Seminal plasma promotes decidualization of endometrial stromal fibroblasts and induces a potent transcriptional response featuring IL-11 induction.** Ashley George, Karen Jang, Mette Nyegaard, Jason Neidleman, Trimble Spitzer, Guorui Xie, Joseph Chen, Eytan Herzig, Warner Greene, Linda Giudice, Nadia Roan

Seminal plasma (SP) is the liquid fraction of semen that serves as a vehicle for sperm, but numerous clinical studies have suggested that SP can also promote implantation, indicating a role for SP beyond sperm transport. A prior *in vitro* study demonstrated that SP can promote decidualization, a differentiation program that endometrial stromal fibroblasts (eSF) undergo to render the endometrium receptive for implantation, suggesting that SP may promote implantation by increasing the efficiency of decidualization. The objective of this study was to determine whether SP can promote decidualization of eSF from women with endometrial diseases associated with decreased decidualization efficiency (PCOS/endometriosis), which SP components promote decidualization, and the signaling pathways underlying this activity. We found that SP promoted decidualization in eSF from women with as well as without PCOS or endometriosis (n=9). Proteins associated with SP microvesicles >3500 Da were responsible for this activity. To assess the signaling pathways associated with SP-enhanced decidualization, we assessed by RNAseq the global transcriptional response of eSF to various SP constituents, and found that SP fractions with decidualization-promoting activity induced cellular pathways associated with cell survival, cell movement, and cellular development. The cell survival and cell movement responses were associated with a marked upregulation of IL-11, a cytokine with a key role in promoting implantation. We confirmed by Luminex and ELISA that IL-11, as well as a number of other growth factors and cytokines (e.g., EGF, FGFβ, HGF, IFNy, IL-8, MCP-1, MIP-1β, VEGF), were secreted by eSF in response to SP exposure vs control. The role of IL-11 signaling in SP-enhanced decidualization is currently being examined using CRISPR/Cas9-based gene knockout approaches. Our results suggest that proteinaceous SP constituents associated with microvesicles actively signal to cells of the endometrium to promote decidualization of eSF in preparation for implantation, possibly through induction of IL-11.

**P481 - Amazingly active peristaltic movements and fluid production of the mouse oviduct: their roles in fluid and sperm transport and fertilization.** Toshiaki Hino, Ryuzo Yanagimachi

In mammals, fertilization normally takes place in the ampullary region of the oviduct. How do spermatozoa deposited in the lower segment of the female tract during coitus reach the ampulla to effect fertilization? Although much important information on sperm–oviduct interactions and fertilization can be obtained after isolation of oviducts from the animal's body, one must be aware that what happens in isolated reproductive organs does not necessarily occur *in situ*. Here, we adopted the technique of Battalia and Yanagimachi (1979), which allowed us to examine the behavior of spermatozoa and fluid within oviducts still attached to the animal's body without disturbing the blood circulation or parasympathetic innervation. Injection of boluses of India ink and/or transgenic mouse spermatozoa into the lower isthmus of the mouse oviduct allowed us to examine the contractile movements of the oviduct, sperm and fluid ascent and fertilization of oocytes within the ampulla. We found that: (1) the isthmus actively secretes fluid along its entire length; (2) there is undisturbed upward fluid flow; and (3) adovarian peristalsis of the isthmus...
wall is most active during the periovulatory period. These factors combined with the spermatozoon's own movements all contribute to efficient sperm ascent and fertilization in the oviduct.

**P482 - Screening and characterization of molecules modulating embryo implantation using a high throughput in-vitro co-culture model.** Xian Chen, Nga Leung, Raymond Li, William Yeung, Fai Lee

Embryo attachment is critical to the establishment of pregnancy. Whether small molecules could be used to enhance pregnancy outcome remains largely unexplored. The use of in-vitro trophoblastic spheroids (blastocyst surrogate) and Ishikawa cell (endometrial surrogate) co-culture model could enhance the screening of drugs in a laboratory setting. Here, we report the use of a high-throughput in-vitro spheroid-endometrial cells co-culture model to screen a library of pharmacologically active compound (LOPAC) containing 1280 small molecules in order to identify molecules that either enhance or suppress spheroid attachment. In this study, we used AggreWell to produce spheroids with homogenous size from trophoblast cells (BeWo) and co-cultured with the Ishikawa monolayer. The spheroids were labeled with the fluorescent dye Calcein-AM. After 1 h co-culture, a linear florescent signal could be produced when 0 to 100 spheroids were seeded on Ishikawa monolayer in 96-well plates. Then we screened the LOPAC by treating the Ishikawa monolayer in duplicates at 10µM for 24h, and the attachment rates of spheroids were determined in repeated experiments. Each screening of assay contained negative control and vehicle control (0.1% DMSO). A significant different at p<0.05, one-way ANOVA was used. Until now, 320 LOPAC compounds have been screened and 6 primary hits have been found can suppress the attachment rate. In summary, this result indicates that some constituents of the LOPAC panel can modulate the implantation process. Our study will shed light on identifying potential small molecules that can enhance or suppress embryo implantation which may have great potential in the enhancement of IVF treatment or development of emergency contraception.

**P483 - Effect of Exogenous Estradiol on Long-term Culture of Equine Endometrial Organoids In Vitro.** Riley Thompson, Aime Johnson, Margherita Turco, Tulio Prado, Christopher Premanandan, Graham Burton, Brian Whitlock, Budhan Pukazhenthi

Equine endometrial organoids provide a novel three-dimensional culture method to investigate cell signaling and inflammatory mechanisms in the uterus in vitro. Our objectives were to determine the effect of estradiol supplementation on 1) proliferation (number) of endometrial organoids and 2) gene expression. Endometrial biopsies (3 mares) previously cryopreserved in 20% (v/v) dimethyl sulfoxide using a slow cooling method were thawed and dissociated to isolate endometrial glands. Glandular fragments were embedded in Matrigel and overlaid with a basal organoid culture medium (DMEM/F12 phenol red-free, penicillin/streptomycin, B27, N2, insulin/transferrin/selenium, nicotinamide, epidermal growth factor, fibroblast growth factor 10, Noggin, A83-01, N-acetyl-l-cysteine, SB202190) in the absence (Control) and presence (2 nM) of estradiol. Organoids were dissociated and re-plated (passaged) in their corresponding medium every 6-8 days for 20 days. The rate of proliferation was determined by the difference in organoid count at the start of a passage versus the end, expressed as a fold change. Then,
organoid RNA was extracted and gene expression (estrogen receptor-α, ER-α; progesterone receptor, PR; e-cadherin; ki67) analyzed using quantitative PCR. Data was analyzed using ANOVA with Tukey’s post-hoc test, and significance was set at P<0.05. Organoid numbers increased faster (2.4±0.2; mean±SEM fold; P<0.01) in Control compared with estradiol-treated counterparts (1.8±0.1). When cultured under Control conditions, endometrial organoids increased at a higher (P<0.001) rate during Passage 2 (3.6±0.3) compared to Passage 0 (1.7±0.3) or 1 (1.4±0.1). ER-α and PR gene expression decreased (0.36- and 0.32-fold, respectively; P<0.001) in the presence of estradiol compared to Control. No difference was observed in expression of e-cadherin (cell adhesion) or ki67 (proliferation) between treatment groups. Results demonstrate that equine endometrial organoids proliferate long-term in vitro in basal culture medium, but continuous supplementation of estradiol (2 nM) exerts a suppressive effect on proliferation and ER-α and PR gene expression. Research supported by Mr. Mike Baudhuin.

**P484 - PRNP genome engineering in cattle using all-in-one CRISPR/Cas9 piggybac vector.**

Kyeong-Min Kim

Genome engineering application in cattle can provide a powerful model to understand a basic genetic function or disease-resistance in livestock. Several genome engineered technologies such as viral gene delivery has been introduced to them but its progress is very slow. Here, we reported that the gene PRNP, which are well known for a causative agent of BSE, was efficiently mutated by all-in-one CRISPR/Cas9 piggybac (PB) transposon. To construct the all-in-one CRISPR/Cas9 vector, Cas9, GFP and sgRNA for PRNP were cloned into PB backbone vector in our previous study (Yum et al., 2016). PB-all-in-one vector was microinjected into in vitro fertilized embryos. The embryos were cultured for seven days in two-step chemically defined media. Because the DNAs contained GFP, only GFP expressing blastocysts were selected for mutation assay or frozen for embryo transfer to recipient cow in future. As results, every blastocyst showed mutated pattern and various deletion pattern in sequencing. Based on mutation assay, frozen or fresh blastocysts were transferred into ten recipients. After embryo transfer, at around 60 days, pregnancy was diagnosed with ultrasonography. In five cow, normal fetus was confirmed and is going on to the term. In conclusion, the data demonstrated that all-in-one CRISPR/Cas9 PB system for genome engineering system could be chosen for various applications in genome editing of livestock.

**P485 - Bipotent stem cells support the cyclical regeneration of murine uterine endometrial epithelium.**

Shiying Jin

Disorders of uterine endometrial epithelial regeneration result in severe pathological conditions, including uterine cancer, endometriosis, and infertility. The lack of understanding of cellular mechanisms underlying uterine endometrial epithelial regeneration during the mouse estrous cycle or human menstrual cycle hinders the effective treatment of these diseases. Here, a CreER$^{T2}$-LoxP based single-cell lineage tracing in the whole mouse uterus reveals that
endometrial epithelium is maintained by adult tissue stem cells. These uterine epithelial stem cells are bipotent and persistent, able to survive the cyclical uterine tissue loss and generate two functionally distinct luminal and glandular epithelia, by which uterine endometrial epithelial regeneration is fueled. 5-ethynyl-2’-deoxyuridine (EdU) pulse-chase experiments further reveal that this stem cell population may reside in the intersection zone between luminal and glandular epithelial compartments. Such tissue distribution endows these uterine epithelial stem cells with a bidirectional differentiation ability to maintain homeostasis and regeneration of mouse endometrial epithelium under physiological conditions. This uterine epithelial stem cell population also supports the regeneration of uterine endometrial epithelium post parturition. Thus, uterine function over a mouse reproductive lifespan relies on stem-cell-maintained rhythmic endometrial regeneration. Further work will be conducted to address the niche environment of these uterine epithelial stem cells and their roles in uterine diseases progress.

**P486 - Reliability of ISGs Expression in Peripheral Blood Leukocytes for Prediction of Gestational Conditions in Embryo Transferred Cows.** Keiichiro Kizaki, Hitomi Yoshino, Toh-ichi Hirata, Kosuke Iga, Hideo Matsuda, Tadayuki Yamanouchi, Yutaka Hashiyada, Kei Imai, Toshina Ishiguro-Oonuma, Toru Takahashi, Kazuyoshi Hashizume

Many studies have been used the expression of interferon-stimulated genes (ISGs) in peripheral blood leukocytes (PBLs) for prediction of bovine gestational statuses, but there are contradictory results. In the present study, we tried to validate the prediction method for early gestational statuses in embryo transferred (ET) cows. Peripheral blood from ET and artificial inseminated (AI) cows were collected from day 17 to 24 after estrus. Gestation statuses were determined by ultrasonographic examination (UE). The ISG15, MX1, MX2 and OAS1 mRNA in PBLs were determined by RT-qPCR. Cutoff values of ISGs to predict the accuracy of pregnancy diagnosis were estimated by the average gene expression during estrous cycle. Results are indicated as follows; true positive (TP): both prediction values and UE were positive, false positive (FP): prediction values were positive but UE were negative, true negative (TN): prediction values and UE were negative, false negative (FN): prediction values were negative but UE were positive: In ET cows, positive predictive value (PPV; TP/TP+FP) from day 17 to 21 of gestation were about 0.5 and increased on day 23, on the contrary these values increased with the progress of gestation days until day 21 in AI cows. Negative predictive value (NPV; TN/TN+FN) were rather higher even on 17 of gestation in ET cows and higher statuses had kept until day 24 of gestation. These results suggest that NPV in ET is reliable but PPV are lower, and ISGs are detectable in PBLs at least 10 days after ET with rather large intensities. Although the results are quite reasonable because the expressions of ISGs may reflect embryonic signals, PPV is not suitable for prediction of early gestational statuses in ET cattle. The study was granted by the Research Program on Innovative Technologies for Animal Breeding, Reproduction, and Vaccine Development from the MAFF of Japan.

**P487 - Osteopontin modulates gene expression in peri-implantation trophoblast.** John Aplin, Stephane Berneau, Susan Kimber, Melissa Westwood, Daniel Brison, Peter Ruane
Osteopontin (OPN) is expressed under hormonal regulation in endometrial epithelium and is suggested to play attachment and signalling roles during embryo implantation. OPN in the human endometrial epithelial Ishikawa cell line was characterised using three different monoclonal antibodies, revealing at least nine distinct molecular weight forms and a novel secretory pathway localisation that was modulated by cell organisation within the epithelial layer. Mouse blastocysts co-cultured with Ishikawa cell layers served to model embryo-epithelial interaction at implantation. Embryos are activated during the first 24h in coculture (E4.5-5.5), after which they attach weakly, then progress to stable attachment, breaching of the cell layer and invasion, with appearance of trophoblast giant cells and displacement of epithelial cells. Exogenous OPN mildly delayed attachment. More strikingly, the presence of exogenous OPN from E4.5 in coculture inhibited subsequent embryonic invasion. This corresponded with altered transcription factor expression in trophoblast: Hand1, which is associated with giant cell formation and invasion, was inhibited, while genes (Cdx2, Gata2 and Gata3) associated with the transition from trophectoderm to TS and other mononuclear trophoblast lineages, were retained or upregulated. These data suggest that a major function of OPN at implantation involves modulating trophoblast lineage allocation.

P488 - The Concentration of Some Inflammatory Cytokines, Prostaglandin E2, MUC-1 and Cortisol in Uterine Washing of Repeat Breeder Dairy Cows with and without Subclinical Endometritis. Gamal El-Amrawi

Subclinical endometritis as a problem in dairy farms may contributes to severe economic losses due to increased open days, calving interval and number of inseminations to achieve conception; moreover, it is considered as one of the main causes of repeat breeding in cows. This study was performed to evaluate the uterine immunological and biochemical changes related to subclinical endometritis as one of the most common causes of repeat breeder in dairy cows. This study was carried on total number of 60 Holstein dairy cows, the experimental groups consisted of 20 repeat breeder cows with subclinical endometritis (Group-I), 20 repeat breeder cows without subclinical endometritis (relying on cytobrush samples PMNs% threshold of 10%) (Group-II) and a control group of 20 healthy cows (Group-III). The level of tumor necrosis factor alpha (TNF-alpha), interleukin-10 (IL-10), prostaglandin-E2 (PGE-2), mucin-1 (MUC-1) and cortisol were detected in uterine lavage of all the tested cows. Uterine washings had a significant higher level of TNF-alpha, IL-10, PGE-2 and cortisol in subclinical endometritis cows (group-I) when compared to animals without subclinical endometritis (group-II) and control animals (group-III) (P<0.05), while uterine level of MUC-1 was significantly elevated (P<0.05) in both of group I and II as compared to control cows. In conclusion, diagnosis of subclinical endometritis in repeat breeder dairy cow might be achieved depending on assessment of such immune-biochemical substance in uterine washing of suspected cows; the elevated level of these tested factors may share fundamentally in fertility disturbance associated with subclinical endometritis.
**P489 - Diversity of the Endometrial Microbiota is Enhanced in Women with Endometriosis.** Jocelyn Wessels, Miguel Dominguez, Nicholas Leyland, Sanjay Agarwal, Warren Foster

Endometriosis is a chronic, estrogen-dependent gynecological condition for which there is no cure. It is characterized by growth of endometrial glands and stroma outside the uterus, and women with endometriosis often suffer debilitating pelvic pain and infertility. Endometriosis occurs in up to 10% of women and 50% of women with infertility, yet the cause of endometriosis-associated infertility remains poorly understood. Recent reports challenge the notion of a sterile uterine cavity, and the endometrial microbiota of women with endometriosis remains poorly defined. Therefore in our prospective cohort study we collected endometrial biopsies and profiled the endometrial microbiota using 16S rRNA gene sequencing, in women with endometriosis (N=12) and controls (N=9), undergoing gynecological laparoscopy for pelvic pain. Diversity metrics were calculated and linear discriminant analysis (LDA) effect size (LEfSe) was used to determine if there were significant taxonomic differences between the endometrial microbiota of cases and controls. We found women with endometriosis had an endometrial flora that verged on being significantly different from the endometrial microbiota of surgically confirmed, symptomatic controls (β-diversity, P=0.09, PERMANOVA). Additionally, the endometrial microbiota of women with endometriosis had a significantly greater bacterial diversity (α-diversity, P<0.05, Shannon Diversity Index) at all levels of rarefaction compared to controls. LEfSe analysis revealed enrichment of taxa including bacteria in the Actinobacteria phylum, Oxalobacteraceae and Streptococcaceae families, and Tepidimonas genus in women with endometriosis, while symptomatic controls were found to have enrichment of the Burkholderiaceae family, and Ralstonia genus. Finally, some clustering of endometrial microbiota by disease status was evident during principal coordinates analysis, and three clusters were present in the data. Our results suggest the endometrial microbiota is altered in women with endometriosis, compared to symptomatic control women; who have pelvic pain but do not have endometriosis. Furthermore, an altered endometrial microbiota in women with endometriosis may contribute to endometriosis-associated infertility.

**P490 - Uterine extracellular vesicles- meaningful component of developing conceptuses’ milieu.** Joanna Najmula, Yael Heifetz, Monika Kaczmarek

Extracellular vesicles (EVs) gained great attention in the last decade as an important element of cell-to-cell communication. EVs can transport numerous biomolecules (e.g., lipids, proteins, mRNAs and miRNAs), which maintain the ability to fulfil specific role at the place of delivery. Thus far, EVs have been detected in several biological fluids and shown to be released by many cell types in vitro. There are also reports of their presence in the uterus of pregnant animals, but precise impact of EVs on the developing embryos remains elusive. To understand the role of EVs in conceptus development and pregnancy maintenance, we decided to: (1) characterize EVs present in the uterine lumen of pregnant pigs using variety of methods (transmission electron microscopy, Western blot, nanoparticle tracking analysis), (2) profile EVs’ miRNA cargo and (3) assess the effect of EVs delivery on trophoblast in vitro. After characterization, EVs collected on Days 12, 14, and 16 of pregnancy were subjected to miRNA profiling with custom made TaqMan Low Density Array Cards, covering 166 miRNAs of either endometrial or embryonic
origin. Detected miRNAs (79) enriched processes important during early pregnancy, e.g., signal transduction, cell communication and growth. To determine the effect of uterine EVs on trophoblast, appropriate dose and time of incubation of porcine primary trophoblast cells with EVs was determined by evaluating cell migration and proliferation. The strongest inhibition (P=0.03) of migration was observed after 6 hours of treatment with medium supplemented with 2% of EVs. The same treatment significantly increased trophoblast cells proliferation (P=0.02). Collectively, uterine lumen is enriched with EVs transporting miRNAs, which may have an impact on pregnancy maintenance. Uterine EVs affect trophoblast cells physiology, i.e. migration and proliferation. Altogether, these results show importance of EVs in a proper embryo-maternal communication.

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**P491 - Uterine epithelial deletion of Gp130 causes implantation failure due to the downregulation of progesterone receptor and Snail family transcriptional repressor 1 in mice.** Takafumi Namiki, Jumpei Terakawa, Takiko Daikoku, Junya Ito, Naomi Kashiwazaki

In mice, embryo implantation is orchestrated by estrogen (E2) and progesterone (P4). The Leukemia inhibitory factor (LIF) produced by uterine glands, the downstream target of E2, are essential for embryo implantation, because LIF null female mice show implantation and decidualization failure (Stewart et al., 1992 *Nature*). LIF induces activation of the signal transducer and activator of transcription 3 (STAT3) via LIF receptors and GP130 in the uterus. Previous study demonstrated the mice with uterine deletion of GP130 or STAT3 (*PgrCre/+Gp130floxflox* and *PgrCre/+Stat3floxflox*) were both infertile due to implantation failure, suggesting the LIF-GP130-STAT3 pathway is indispensable for embryo implantation in mice. However, the role of the pathway in the uterine epithelium is still unclear. Lactoferrin-iCre (*LtfCre/+*) mice have been produced and it is useful for studying the function of uterine epithelial genes in adult female mice (Daikoku et al., 2014 *Endocrinology*).

In this study, we generated conditional KO (cKO) mice with the deletion in uterine epithelial of *Gp130* (*LtfCre/+Gp130floxflox*: *Gp130 cKO*) and analyzed molecular mechanism of Gp130 during embryo implantation and decidualization in mice. *Gp130* cKO mice were completely infertile due to incomplete embryo attachment to the uterine luminal epithelium and consequent implantation failure. Expression of P4 receptors (PR), phosphorylated STAT3 (pSTAT3) and Snail family transcriptional repressor 1 (SNAI1) which are important for stromal cell proliferation, differentiation and epithelial-mesenchymal transition, were downregulated in the uterine epithelial cells of *Gp130* cKO. In addition, artificial stimulation did not induce decidualization in *Gp130* cKO mice. Taken together, our results revealed that Gp130 in the uterine epithelium is indispensable for embryo attachment and implantation via pSTAT3-PR/SNAI1, which is critical pathway for successful implantation and decidualization in mice.

**P492 - Involvement of specific Akt isoforms in decidualization processes in the mouse uterus.** Pascal Adam, François Fabi, Laurence Tardif, Sophie Parent, Eric Asselin
Infertility is a rising problem currently touching 16% of North American couples trying to conceive. To achieve conception, successful implantation is primordial and necessitates a prepared and receptive endometrium which is dependent of the decidualization process. Under the effect of progesterone and AMPc, the endometrial stromal cells undergo phenotypical changes, transitioning from fibroblasts to epithelial, secretory, glycogen-filled cells. During this process, many signaling pathways involved in proliferation and apoptosis are modulated such as PI3K/Akt. Three isoforms of Akt have been identified and are well recognized to have distinct physiological and pathological roles. It has been previously demonstrated in vitro that the expression and activity of specific Akt isoforms are downregulated during decidualization, but little is known about its implication in cell survival, apoptosis and glycogen synthesis in the endometrium. We hypothesize that each Akt isoform have specific roles during decidualization and the successful pregnancy onset.

Therefore, we developed a novel endometrial-targeted mouse model with simple and combined KO of each Akt isoforms using the PR-Cre mouse model. Using artificial decidualization activation, specific cellular localization, expression and activation of each Akt isoforms and their downstream targets was investigated during this process in order to evaluate the role of PI3K/Akt pathway. Preliminary results suggest that Akt downstream targets activity of p70S6K and IκBα is regulated in an isoform specific manner during a non-gestational, progesterone driven context in the endometrium that could reveal distinctives isoforms role during decidualization. Moreover, a variation of the average mouse litter number and capacity to decidualize has been observed depending of the KO genotype. Thus, it is clear that the PI3K/Akt pathway has an important role in fertility. Further studies will allow us to understand the precise signaling mechanisms by which this pathway is regulated leading to a better understanding of the cellular and molecular aspects of infertility.

**P493 - Global Transcriptomics of the Mouse Uterus and Oviduct.** Elle Roberson, John Wallingford

The uterus and oviduct – like other female reproductive organs – respond to the steroid hormones produced during the estrous cycle. This cyclical responsiveness is critical to female fertility, however, the global transcriptional response of these tissues to steroid hormones is unknown. For this reason, we performed RNAseq of the uterus and oviduct at each stage of the estrous cycle. We further investigated the oviduct, because while the oviduct is a critical site for female fertility, how oviduct physiology is regulated at the genetic, molecular, and cellular level remains mysterious. Further, the oviduct displays intriguing, yet understudied, anteroposterior cellular patterning. To identify the underlying transcriptional mechanisms underlying this cellular patterning, we performed additional RNAseq on anterior and posterior portions of the oviduct. We have identified >1,800 differentially expressed genes so far, and have confirmed a fraction of these using qPCR on at least five independent uterus and oviduct samples. These differentially expressed genes participate in various signaling pathways that are potentially
important for the cyclical maintenance of the uterus and oviduct during the estrous cycle, and may be required for implantation and pregnancy maintenance. In addition to transcriptomic analysis, we are using quantitative high-resolution imaging and mouse genetics to provide a more comprehensive understanding of how the uterus and oviduct epithelium are maintained during the estrous cycle and in early pregnancy.

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**P494 - Effect of the restriction of essential amino acid on lipid metabolism and milk protein synthesis in vitro.** Ricardo Córdoba, Camilo Calle, Mark Hanigan, Tatiana Ruiz-Cortés

The objective of this study was to identify the metabolic pathways and proteins related to lipid metabolism and milk protein synthesis in lactocytes (MEC) under essential amino acid (EAA) restriction in vitro. MEC were exposed to seven treatments: a control group (T1-100%EAA), two depletion groups, (T2: 2%EAA-8h, T3: 2%EAA-24h), four depletion+repletion groups (T4: 2%EAA8h+100%EAA8h, T5: 2%EAA8h+100%EAA24h, T6: 2%EAA24h+100%EAA8h; T7: 2%EAA24h+100%EAA24h). Casein expression was calculated with Western Blot; proteins were analyzed by label-free quantitative proteomics. The pathways related to lipid metabolism were identified through KEGG software. ANOVA was performed to compare protein synthesis between treatments; a multiple correspondence analysis detected significant metabolic pathways and their implicated proteins. Intracellular casein levels were unaffected by treatment with a mean of 0.086±0.02ug/mL for the control MEC and of 0.081±0.002ug/mL and 0.069±0.004ug/mL with 2% EAA D and D + R treatments respectively. Five significant metabolic pathways and twenty-one proteins were found as important for lipid metabolism in MEC restricted. Three of those pathways with six proteins had significant difference (p<0.05) compared to control. MEC depleted (T3) resulted in reduction of very-long-chain 3-oxoacyl-CoA reductase protein, it is assumed that biosynthesis of unsaturated acid and mitochondrial fatty acid elongation were “blocked” pathways. However, after repletion (T4, T6, T7) the same decreased of this enzyme was found. Conversely, the quantity of medium-chain specific acyl-CoA dehydrogenase was significantly higher in depleted (T3) and repleted MEC (T6) compared to control group (T1), suggesting that the degradation of fatty acid pathway is activated, and consequently the production of Acetyl-CoA increases; this allows the production of β-Alanine and probably regulates the protein synthesis as alanine participates in 3% of casein synthesis, in 1% of α-lactoalbumin and in 7% of β-lactoglobulin. It is concluded that the EAA restriction can activate alternative metabolic pathways (homeorhesis) to maintain cellular homeostatic processes as casein expression.

**P495 - Genome-wide siRNA Screen Reveals the Human Decidualization Genetic Network.** Liang Ma, Meade Haller, Yan Yin
Failure of embryo implantation accounts for a significant percentage of pregnancy failure during both natural pregnancy and in vitro fertilization procedures. Exquisitely coordinated interactions between the competent blastocysts and the receptive uterus require the precise interplay between hormonal, growth factor, and intercellular signaling. Decidualization, the rapid proliferation and differentiation of fibroblast-like endometrial stromal cells into epithelioid-like decidual cells, is an integrated part of the implantation process. Decidualization defects can directly lead to implantation failure. In addition, early decidualization defects commonly result in unhealthy pregnancies due to defects in placentation, decreased intrauterine fetal growth, and early parturition leading to pre-term birth. Here we developed a novel immortalized human endometrial stromal cell line that contains a YFP-fluorescent readout of the decidualization response under the control of the prolactin promoter, hESC-PRLY cells. Upon stimulation with hormone cocktail, high throughput imaging and quantification reveals that the measurement of (YFP intensity x cellular area) is reliably induced 10 fold. Using this cell line, we performed a genome-wide siRNA library screen to identify novel genes required for decidualization (promoters), as well as those actively inhibiting this process (inhibitors). The screen identified around 1000 promoters and 2000 inhibitors of decidualization. In addition to about 280 transcription factors and many components of various signal transduction pathway components, around 90 olfactory and several taste receptors were identified. This is the first time sensory GPCRs are implicated in the decidualization process. We are currently examining the expression profile of gene of interest and generating knockout cell lines to corroborate our siRNA screening results. These results will help to build a thorough genetic map of the decidualization network and will aid in the development of patient-specific treatments for recurrent pregnancy loss, subfertility and infertility due to decidualization defects. 

**P496 - Effect of interferon tau on bovine endometrial epithelial and fibroblast cell transcriptomes in 3D cell culture.** Heather Baldwin, Lindsay Grose, Gilles Charpigny, Susanta Behura, I Sheldon, James Cronin, Patrick Lonergan, Thomas Spencer, Daniel Mathew

Interferon tau (IFNT), secreted by the elongating bovine conceptus, is the maternal recognition of pregnancy signal that suppresses the endometrial luteolytic mechanism, thus maintaining progesterone secretion from the corpus luteum to support development of the conceptus. To achieve this, IFNT modifies gene expression in specific cell types within the endometrium. This study examined the effect of IFNT on bovine endometrial epithelial and fibroblast cell transcriptomes when the cells were cultured in a three-dimensional (3D) environment. Endometrial cells were isolated from mid-luteal phase endometrium (n=3-4 cows). Epithelial cells, cultured in Transwell inserts above the fibroblast cells, were treated with RPMI media alone (control) or RPMI containing 100 ng/mL recombinant ovine IFNT for 6 h. Total RNA was extracted from both cell types and the transcriptomes determined by RNA-Seq. Interferon tau regulated 673 and 83 genes (differentially expressed genes or DEGs) in epithelial and fibroblast cells, respectively (FDR P ≤ 0.05). Many of the DEGs identified in the epithelial (205) and fibroblast cells (64) were previously reported as IFNT and bovine conceptus-induced DEGs in intact bovine endometrium, including well known IFN stimulated genes (ISGs) such as *ISG15, MX1, MX2, OAS1* and *OAS2*. Surprisingly, the *ZC3HAV1* gene, which was recently found to be up regulated in intact bovine endometrium in response to the conceptus, was only up regulated in fibroblast cells despite applying IFNT to the apical surface of the epithelial cells in 3D culture.
This study supports the idea that 3D culture of bovine endometrial cells may be utilized to study early conceptus-maternal crosstalk and identify endometrial cell type-specific responses to the conceptus. Funding was provided by West Virginia University and Science Foundation Ireland.


In a previous study, preovulatory estradiol (E2) on d0 and postovulatory progesterone (P4) on d7 were the most important factors affecting establishment of pregnancy. We hypothesized that a decrease in preovulatory estradiol and/or postovulatory progesterone would decrease pregnancy rates following embryo transfer. The objective was to differentiate between independent effects of Low or High preovulatory estradiol and Low or Normal postovulatory progesterone on pregnancy. Ovulation was synchronized in postpartum beef cows (n=679) as follows: d-9 GnRH (GnRH1) administered and an intravaginal progesterone implant (CIDR) inserted, on d-2 the CIDR was removed and PGF administered, and on d0 GnRH (GnRH2). Cows detected in estrus between d-2 and d0 were removed from the study (n=278). Remaining cows were classified based on d0 concentrations of estradiol as either Low (mean±SEM; 2.60±0.07 pg/ml; n=140) or High (5.76±0.08 pg/ml; n=147; P<0.0001) and subsequently assigned to a Low (PGF injections on d3, 3.5, and 4) or Normal progesterone group. Cows (n=114) with intermediate d0 estradiol were removed from the study. Final treatment groups were as follows: Low E2-Low P4 (LL; n=71), Low E2-Normal P4 (LN; n=69), High E2-Low P4 (HL; n=74), and High E2-Normal P4 (HN; n=73). Each cow received an in vivo produced embryo on d7 and pregnancy diagnosis occurred on d34. Concentrations of progesterone on d7 were decreased (P<0.05) in HL compared to HN, and in LL compared to LN. Estradiol on d-2, 0, and change in estradiol (d-2 to d0) positively influenced pregnancy rates (P<0.008), and pregnancy rates for LL, LN, HL, and HN were different (22%a, 30%a, 47%b, and 58%b; P<0.001). In summary, estradiol had a significant positive effect on the establishment of pregnancy; whereas, a reduction in progesterone did not reduce pregnancy rate in postpartum beef cows. Supported by AFRI Grant 2013-67015-21076.

**P499 - New transcriptomic insights into processes associated with formation of egg-white in the magnum of laying hens.** Nirvay Sah, Donna Kuehu, Rajesh Jha, Birendra Mishra

The magnum is the site in the hen’s oviduct where egg-white, composing more than 60% of an egg, is formed. The epithelial cell-lining of the magnum synthesizes, stores, and secretes the constituent proteins of the egg-white. The functionality of the magnum determines the size and quality of the egg. However, selection and breeding based on quantitative traits in the hen have yet to be employed due to the paucity of information on genes/proteins governing egg formation. In this study, we analyzed transcripts in the magnum to detect genes regulating the formation of egg-white. Magnum tissues of Hy-line laying (n=3, of 35 weeks) and non-laying (n=3, of 35-60 weeks) hens were subjected to RNA-Sequencing. At q-value <0.05 and fold-change >3, a total of
540 genes were differentially expressed. Among them, 152 genes were up-regulated in laying hens. Temporal effects on gene expression in the magnum of laying hens at 3 h post-ovulation (p.o., n=5, egg in the magnum), 15-20 h p.o. (n=5, egg in the shell gland), non-laying (n=4), and molting hens (n=5, 60 weeks of age) were determined using qPCR. Genes expressed include proteases [CAPN2, TMPRSS9, MMP1, and MMP9 (protein maturation, ECM degradation, and angiogenesis)], enzymes [PHGDH, PSPH, and PSAT1 (amino-acid biosynthesis)], antimicrobials (AVD, AvBD11), antioxidant (GPX3), solute carrier proteins (SLC26A4, SLC22A3, SLC51B, SLC7A17, SLC1A4, SLC7A11) relaxin (RLN3), renin (REN), and angiotensin converting enzyme (ACE) for albumen synthesis and secretion, and egg transport. Each of those genes encodes for essential molecules linked to egg-white formation in the magnum. In conclusion, the findings of this study revealed some novel genes that participate in the signaling pathways for synthesis and secretion of egg-white. These genes can be used as markers for formulating strategies to improve the size and quality of eggs. (Supported by USDA Multistate Fund, CTAHR, UH Manoa to BM).

P500 - Lysosomes and early pregnancy. Xiaoxin Ye, Zidao Wang, Christian Andersen, Yuehuan Li

Lysosomes are the most acidic intracellular organelles in the eukaryotic cells. They have been implicated in the female reproductive system, such as atresia of granulosa cells and regression of corpus luteum (CL) in the ovary. Our novel finding of uterine epithelial lysosomal acidification upon embryo implantation led us to the investigation of lysosomes in the establishment of uterine receptivity for embryo implantation. Lysosomal acidity regulates lysosomal activity and function. It is mainly maintained by V-ATPase to pump H+ into lysosomal lumen and counter ion channels to dissipate the transmembrane voltage built up by V-ATPase. Our microarray analysis reveals that *Mcoln1* (encoding transient receptor potential cation channel, mucolipin subfamily, member 1 (TRPML1)) has the highest mRNA expression level among counter ion channel genes in the peri-implantation mouse uterine luminal epithelium. Mutations in *MCOLN1/Mcoln1* can cause mucolipidosis type IV (a progressive and severe lysosomal storage disorder with a slow onset) in both humans and mice, indicating a critical role of TRPML1 in the lysosome. TRPML1 is highly expressed in wild type (WT) mouse uterine epithelium and luteal cells during early pregnancy. We demonstrate that despite normal mating activities, *Mcoln1−/−* female mice have reduced fertility at 2 months old and quickly become infertile at 5 months old, with embryo implantation failure, uterine luminal fluid retention, and P4 deficiency. Histology of CLs from 5-6 months old *Mcoln1−/−* mice shows comparable number of CLs to their age-matched controls but extensive luteal cell degeneration, which inevitably leads to P4 deficiency. Exogenous P4 fails to rescue embryo implantation or uterine luminal fluid resorption in 5-6 months old *Mcoln1−/−* mice. These data demonstrate novel functions of TRPML1 and lysosomes in CL maintenance and uterine luminal fluid resorption during early pregnancy.

P501 - Comparable developmental competence of mouse blastocysts in outgrowth in vitro and implantation in utero. Jin Hyun Jun, Jihyun Kim, Wontae Kim, Jung Won Choi, Jaewang Lee
Developmental competences for peri-implantation and post-implantation of mouse blastocysts from various in vitro culture conditions were usually evaluated by animal experimentations of the embryo transfer in utero. By the way, the mouse blastocysts have ability to attach and to outgrowth in vitro on the extracellular matrix coated dish without serum supplementation for several days. This study was performed to compare the developmental competence of mouse blastocysts in outgrowth in vitro and implantation in utero. Two-cell embryos were collected from superovulated ICR mice (1.5 dpc), and cultured in basal medium, Quinn's advantage blastocyst medium, up to blastocyst (4.5 dpc). In vitro cultured blastocysts were transferred to be outgrowth on fibronectin-coated dish without serum supplementation for 3 days (7.5 dpc). The in vitro cultured embryos were transferred into the uterine horns pseudopregnant recipients mated with vasectomized male. To assessment of implantation potential in vitro, on 7.5 dpc, outgrowth embryos were fixed in 4% paraformaldehyde and assessed by outgrowth rates, spreading area and morphological scoring of inner cell mass and trophectoderm. To assessment of implantation potential in utero, the cultured embryos on 3.5 dpc were transferred into the uterine horns of pseudopregnant recipients. Two days later (6.5 dpc), the implantation sites in uterine horns were detected by intravenous injection of Chicago blue dye. Clear blue bands in utero were considered as implantation sites. We found that the significant correlation of developmental competence between the blastocysts outgrowth in vitro and implantation in utero by embryo transfer. Also, the outgrowth rate was significantly associated with blastocyst formation and hatching rate. This study suggested that the in vitro outgrowth assay should be an alternative method for assessment of peri-implantation and post-implantation potential in utero. It could be successfully applied to reduce the number of scarified animals in certain experiments of embryo transfer.

P502 - Picturing the dynamics of trophoblast differentiation in peri-implantation stage human embryos by single cell RNA sequencing. Ye Yuan, Hao Ming, Rachel West, Deirdre Logsdon, Rebecca Kile, Courtney Grimm, Sandeep Rajput, Jiangwen Sun, William Schoolcraft, Rebecca Krisher, Zongliang (Carl) Jiang

Trophoblast differentiation plays a critical role in implantation and formation of the maternal-fetal interface. To understand the dynamics of trophoblast differentiation during human implantation, we cultured human blastocysts in vitro until embryonic day 8, 10 and 12, then collected cells for single-cell RNA-sequencing. Cells were categorized as “small” (cytotrophoblast), “large” (syncytiotrophoblast), or “migratory” (extravillous trophoblast; EVT) based on morphology. A total of 139 single cell samples from 12 embryos were collected and analyzed. After cell lineage analysis and data filtration, 122 cells were identified as trophoblast lineage for further analysis. By performing unsupervised clustering analysis, we identified groups of intermediate state cells transiting from cytotrophoblast to EVT (17 cells) or syncytiotrophoblast (15 cells). The remaining cells were used to identify the unique transcriptomic signature of cytotrophoblast, syncytiotrophoblast, and EVT. Gene ontology and pathway analysis revealed that cytotrophoblast-specific genes are highly enriched in cell proliferation, transcription, and energy metabolism, suggesting their role in supporting placental formation with a sufficient number of founding cells. Syncytiotrophoblast-specific genes are enriched in protein folding, transport, and hormone processing, suggesting their role as a powerhouse of hormone production and transportation in the placenta. EVT-specific genes are related to exocytosis, cell migration, blood vessel remodeling, immune modulation, response to
hypoxia, and response to cytokines and hormones. As EVT are responsible for invasion and remodeling of the maternal spiral arteries, these upregulated pathways delineated some well-understood roles of EVT. In addition, our data have shed light on some new immunomodulatory mechanisms of how EVT are responsible for the formation of the maternal-fetal interface. Further analysis of these cell types on different embryonic developmental days will provide a more comprehensive picture to assist in understanding the dynamics of trophoblast differentiation during human implantation. This research was funded by CCRM and approved by WIRB (Study no: 1179872).

**P503 - Identification of putative factors associated with pelvic organ prolapse in sows during late gestation.** Zoe Kiefer, Amanda Chipman, Jamie Studer, Lucas Koester, Lucas Showman, Aileen Keating, Stephan Schmitz-Esser, Jason Ross

Sow mortality, as the result of pelvic organ prolapse (POP), has increased in the past five years in the U.S. swine industry and continues to worsen. A perineal scoring (PS) system to assess potential for prolapse during late gestation was developed and 2906 individual sows were scored, (PS1 - a presumed low risk of prolapse; PS2 - a presumed moderate risk; and PS3 - a presumed high risk) while laying. Subsequently, 1.1, 0.8, and 7.2% of sows scored as PS1, PS2, or PS3, respectively, experienced a uterine and/or vaginal prolapse. The objective of this study is to identify putative biological factors associated with risk of POP in sows. We hypothesized that sows differing in PS would have differences in serum factors and vaginal microflora. During perineal scoring in late gestation, (days 105-115) serum samples and vaginal swabs were collected from sows of two different farms. Serum was analyzed by gas chromatography mass spectroscopy (GC-MS) while microbiome DNA was extracted from vaginal swabs and 16S rRNA sequencing was performed. Non-targeted metabolite analysis following GC-MS revealed candidate small molecules for POP risk. There were 61 and 16 different compounds between PS1 and PS3 for each farm (> 4-fold change; \(P< 0.05\)). 16S rRNA gene sequences were clustered into operational taxonomic units (OTUs) based on a sequence similarity threshold of 97% using Mothur software. Overall, 76 and 58 OTUs differed between PS1 and PS3 for each farm (\(P< 0.05\)) while whole-community comparisons revealed differences between PS1 and PS3 samples using AMOVA and ANOSIM. Collectively, these data indicate differences in serum metabolites and vaginal microflora exist in sows differing in POP risk level and provide an initial and novel characterization of the sow vaginal microbiome. This project was supported by the National Pork Board and the Foundation for Food and Agriculture Research.

**P504 - Decreased Expression of MicroRNA-210 in Ectopic Lesion May Promote Endometriotic Lesion Development in Baboons and Women with Endometriosis.** Kentaro Kai, Niraj Joshi, Gregory Burns, Samantha Bond, Erin Vegter, Ariandna Ochoa-Bernal, Yong Song, Genna Wilber, Agerally Fazleabas

**Introduction:** The objectives of this study were to explore the expression of miR-210 in the context of ectopic endometriotic lesions and the potential role of its target proteins in promoting lesion development.
Methods: RNA-Seq analysis for differentially expressed miRNAs was performed on disease-free (Ctrl) and matched eutopic (EuE) and ectopic (EcE) endometrium obtained from baboons with endometriosis. This study focused on miR-210 expression and function in ectopic lesions. qRT-PCR analysis was performed to validate expression of miR-210 and its predicted targets IGFBP3 and COL8A1 in baboon disease free and matched EuE and EcE tissues as well as during the menstrual cycle in controls. IHC was performed to detect the localization of IGFBP3 and COL8A1. Additionally, the translational relevance was validated in Ctrl, EuE and EcE tissues from women with and without disease.

Results: RNA-Seq analysis revealed a 5-fold (p=0.0000183) decrease in miR-210 expression in baboon EcE tissues compared to matched EuE obtained at 15 months. In baboon and human EcE, miR-210 expression was significantly decreased compared to EuE. The miR-210 target proteins IGFBP3 and COL8A1, were significantly increased in the EcE compared to control endometrium and EuE obtained from animals with disease. In disease free baboons, miR-210 expression was significantly decreased in proliferative phase compared to mid-secretory phase, however IGFBP3 and COL8A1 were not significantly altered. IHC analysis revealed that IGFBP3 is predominantly expressed in stromal cells while COL8A1 was primarily localized to the glandular epithelium.

Conclusions: Our data indicate that miR-210 expression in our baboon model and women with endometriosis is decreased in ectopic endometrium while its targets, IGFBP3 and COL8A1, are increased, suggesting a role in cell survival and promoting vessel growth, a postulated function of both these proteins. We suggest that alterations in miRNA expression and their target genes contribute to the development of endometriosis (HD083273).

P505 - Menopause drives malignant transformation of endometrial hyperplasia. Jumpei Terakawa, Vanida Serna, Makoto Taketo, Adrian Suarez, Takeshi Kurita

Endometrioid endometrial carcinomas (EECs) carry multiple driver-mutations even when they are low grade. However, the biological significance of these concurrent mutations is unknown. We explored the interactions between three signature EEC mutations: loss-of-function (LOF) mutations in PTEN, gain-of-function (GOF) mutations of phosphoinositide 3-kinase (PI3K), and CTNNB1 exon 3 mutations, utilizing in vivo mutagenesis of the mouse uterine epithelium. While epithelial cells with a monoallelic mutation in any one of 3 genes failed to propagate in the endometrium, any combination of two or more mutant alleles promoted the growth of epithelium, causing simple hyperplasia, in a dose-dependent manner. Notably, Ctnnb1 exon 3 deletion significantly increased the size of hyperplastic lesions by promoting the growth of PTEN LOF and/or PI3K GOF mutant cells through the activation of neo-adenogenesis pathways. Although these three mutations were insufficient to cause EEC in intact female mice, castration triggered malignant transformation, leading to myometrial invasion and serosal metastasis. Treatment of castrated mice with progesterone or estradiol attenuated the neoplastic transformation. This study demonstrates that multiple driver mutations are required for premalignant cells to break the growth-repressing field effect of normal endometrium maintained by ovarian steroids, and that CTNNB1 exon 3 mutations play critical roles in the growth of pre-
neoplastic cells within the endometrium of premenopausal women and in the myometrial invasion of EECs in menopausal women.

**P506 - The Role of COUP-TFII in the Uterus During the Pre-implantation Period.** Yeong Seok Oh, San-Pin Wu, Francesco DeMayo

Chicken ovalbumin upstream promoter transcription factor II (COUP-TFII) is an orphan nuclear receptor transcription factor. Conditional ablation of COUP-TFII in the mouse uterus results in female infertility due to implantation failure, suggesting a role of COUP-TFII in the function of uterus. To date, it has been known that in the mouse uterus COUP-TFII is expressed in the stroma of the endometrium and in the myometrium and is involved in epithelial proliferation and decidualization, but its regulatory mechanisms remain to be elucidated. In an effort to elucidate the regulatory mechanism of COUP-TFII in the uterine function, RNA-seq, RT-qPCR, and immunohistochemistry were performed using the uteri of PR\textsuperscript{Cre}, COUP-TFII\textsuperscript{flox/flox} (COUP-TFII\textsuperscript{d/d}) female mice at gestational day 3.5 (GD3.5), and ChIP-seq was performed using the uteri of randomly cycling female mice. Gene ontology and canonical pathway analyses following RNA-seq showed that COUP-TFII regulates the gene set of inflammatory response. In addition, upstream analysis showed that loss of uterine COUP-TFII results in a gene signature of increased inflammatory responses, suggesting a role of COUP-TFII in uterine inflammation which is important for embryo implantation. Correlation analysis showed that transcriptomic change in COUP-TFII\textsuperscript{d/d} uterus is highly correlated with transcriptomic profiles of Foxa\textsuperscript{2d/d}, Sox1\textsuperscript{7d/d}, and Arid1\textsuperscript{a/d} uterus, which previously showed implantation failure, suggesting that COUP-TFII regulates the transcriptome for uterine receptivity. In COUP-TFII\textsuperscript{d/d} uteri at GD3.5, stromal PR and Hand2 expression were decreased together with an increase of Fgf1 and Fgf18 expression, indicating a defect of inhibition of epithelial proliferation during pre-implantation period. ChIP-seq analysis revealed that both COUP-TFII and PR occupy the Hand2 locus in the uterus, suggesting that both COUP-TFII and PR regulate Hand2 expression directly. Taken together, our data suggest that COUP-TFII is an important regulator in establishing uterine receptivity for embryo implantation.

**P507 - Stromal cell-derived factor-1 Stimulates Invasion of Human Extravillous Trophoblast Cells.** Seoung O Jung, Wooyoung Jeong, Seunghye Peak, Jinyoung Kim

The extravillous trophoblast (EVT) invasion into the maternal uterine decidua is critical for successful placental formation. Stromal cell-derived factor-1, also known as C-X-C motif chemokine 12, acts as a chemoattractant regulating various cellular functions. It has been shown that SDF-1 and its receptor are expressed in the placenta during pregnancy and up-regulated in women with pre-eclampsia. However, it is unclear the molecular mechanism underlying the effect of SDF-1 on trophoblast function. In here, we demonstrated that treatment of human trophoblast cells (HTR) with SDF-1 stimulated cell invasion and activated phosphatidylinositol 3-kinase (PI3K)/AKT and mitogen-activated protein kinase (MAPK) pathway proteins. In the presence of PI3K inhibitor (Wortmannin), ERK1/2 MAPK inhibitor (U0126) or P38 MAPK inhibitor (SB203580), SDF-1-mediated trophoblast invasion was reduced. Expression of matrix
metalloproteinase (MMP)-2 and MMP-9 was increased at transcriptional level in SDF-1-treated HTR cells, while the SDF-1-induced MMPs expression was blocked in the presence of AMD3100, an inhibitor of the SDF-1 receptor (CXCR4). In addition, the SDF-1-induced increase of MMP-2 and MMP-9 were inhibited by blockage of PI3K and/or MAPK pathways. These findings provide evidence that SDF-1/CXCR4 system activates PI3K and MAPK signaling pathways and up-regulates expression of MMPs, which subsequently contributes to increased invasiveness of trophoblasts.

P508 - Effects of IL-1beta in human trophoblast cells. Seung Hye Paek, Seoungo Jung, Jinyoung Kim

Trophoblast invasion and migration through the decidua and maternal spiral arteries are crucial for establishment of implantation during pregnancy. Interleukin-1 beta (IL-1β) and IL-1β receptor are present at the feto-maternal interphase and maternal decidua. These data suggest that IL-1β is an important regulator of that communication regulating development of the fetus and opening the window of implantation during early pregnancy. However, little is known about IL-1β mediated intracellular signaling cascades and functional effects in trophoblasts during implantation period of pregnancy in human. Therefore, this study determined the intracellular signaling cascade responsible for activities of IL-1β in human trophoblast (HTR) cells and the changes in cellular activities induced by IL-1β. IL-1β stimulated phosphorylation of RPS6 proteins in HTR cells in a dose-dependent manner. Ten ng/ml IL-1β increased levels of phosphorylation of ERK1/2, RPS6K and RPS6 proteins in HTR cells within 15min, and this IL-1β induced phosphorylated status was inhibited by increasing doses of rapamycin (MTOR inhibitor). Moreover, immunofluorescence analyses revealed that IL-1β increased abundance of p-RPS6K protein in nuclei and p-RPS6 in cytoplasm of HTR cells. Expression of matrix metalloproteinase (MMP)-1 was increased at transcriptional level in IL-1β treated HTR cells, while the IL-1β induced MMPs expression was blocked in the presence of IL-IRA, an antagonist of the IL-1β receptor. In addition, the IL-1β induced increase of MMP-1 were inhibited by blockage of RPS6K and/or ERK1/2 pathways. Collectively, the results of this study indicate that IL-1β / IL-1β receptor system activates RPS6K, RPS6 and ERK1/2 signaling pathways and up-regulates expression of MMPs, leading to enhance invasion of trophoblasts.

P509 - Taurine Protects Pregnant Rats and Their Fetuses Against Lead Toxicity. Hoda Aglan, Marwa Safar, Afaf Ain-Shoka, Asmaa Kandil, Samuel Gebremedhn, Karl Schellander, Dawit Tesfaye

Lead (Pb²⁺) is a prevalent environmental heavy metal proven to be embryotoxic in both human and animals. It is evident that there is no safe level of lead exposure and even small doses are believed to induce health hazards, where the soundest approach is to minimize Pb²⁺ exposure rather than treatment. Taurine (TA), an antioxidant that is abundant in almost all mammalian tissues, is essential for the normal functioning of different organs. The beneficial role of TA in development is well documented. Here we aimed to evaluate the role of TA against lead-induced toxicity in pregnant albino rats and their fetuses. For this, pregnant rats at day 1 of the gestation were divided into four groups (10 rats of each), where group 1 was given distilled water and served as control.
Group 2 received lead acetate (233.25 mg/kg) orally from day 7 to 16 of the gestation. Groups 3 and 4 received taurine (50 mg/kg) orally all over the gestation period, while group 4 received also lead acetate from day 7 to 16. Dams were sacrificed on day 20 and fetuses were removed by cesarean section. Fetal mortality, morphological examination, body weight and length were recorded. Blood samples were collected for hematological and biochemical parameters assessment. Hepatic malondialdehyde, reduced glutathione and catalase were also analyzed. The results indicated that Pb2+ caused a reduction in the maternal body weight gain, increase in the rate of abortion, as well as fetal growth retardation and malformations in their skeleton. Additionally, Pb2+ induced hematological and biochemical alterations in both dams and fetuses. The toxicity of Pb2+ was emphasized by histopathological examination of the placenta and hepatic DNA fragmentation. However, these events have been mitigated by TA pretreatment, which could be a promising prophylaxis against environmental heavy metals toxicity.

**P510 - Let-7 derived from endometrial extracellular vesicles is a main factor inducing embryo diapause in mice.** Weimin Liu, Ziru Niu, Ranran Cheng, WSB Yeung

MicroRNA Let-7 is up-regulated in the dormant mouse blastocysts. The onset of embryonic diapause is under maternal control. We hypothesized that maternal let-7 regulates the process. In this study, we demonstrated that force-expression of let-7a by electroporation or by incubation with let-7-enriched extracellular vesicles from endometrial epithelial cells prolonged blastocyst survival in vitro, and the treated blastocysts developed to term when transferred back to foster mothers. Let-7-induced dormant blastocysts exhibited reduction in proliferation, apoptosis and nutrient metabolism similar to in vivo dormant blastocysts. Transcriptomic comparison between let-7-induced and in vivo dormant blastocysts suggested that inducer(s) other than let-7 were involved. Let-7 also blocked trophoblast differentiation and implantation potential of human embryo surrogates, and prolonged the survival of human blastocysts in vitro, consistent with the suggestion that embryonic diapause is an evolutionary conserved phenomenon. The project was supported by GRF 17119117, GRF 17107915 and NSFC 31471398.

**P511 - Histological changes in oviducts and uterine horns of adult domestic cats during the estrous cycle.** Julie Lamy, Pierre Comizzoli, Adrienne Crosier

Cellular and molecular changes associated with estrous cycles remain unclear in domestic cats; thus, negative outcomes in assisted reproductive techniques such as artificial insemination in felids are difficult to interpret. The aim of this study was to determine structural changes within oviductal and uterine tissues at different stages of the estrous cycle. After collection during routine ovariohysterectomies, reproductive tracts from adult queens (1-3 years old) were classified as: inactive (n=4), early follicular (n=5), or luteal phase (n=3). After fixation in 4% paraformaldehyde and cross sections from infundibulum (OV1), ampulla (OV2), isthmus (OV3), uterotubal junction (OV4), cranial uterine horn (UT1) and caudal uterine horn (UT2) were stained with eosine/hematoxylin. Complexity of the epithelium structure was determined by its full length, and potential glandular activity was assessed by the mucosa:total area ratio. Ciliated and non-ciliated cell heights were determined for each area to assess the epithelium differentiation. Secretory
activity was measured by counting the number of glands per mm² in uterine sections. Cycle stage had no effect on any of the metrics measured in OV2, OV3, OV4 and UT1. Moreover, there were no effect of the stage on epithelium length for OV1 and UT2. In OV1, ciliated cell height was significantly increased from inactive (6.7±0.2 μm) to both early follicular (9.4 ±0.5 μm, p<0.01) and luteal phase tissues (10.82 ±0.09 μm, p<0.01). The number of glands in UT2 was higher in luteal phase (136.5 ±35.3 glands/mm²) compared to both inactive and early follicular phase (69.3 ±13.5; 88.0 ±8.3 glands/mm², p<0.05). For UT2, the ratio mucosa:total area was higher in inactive compared to luteal phase (0.37 ±0.05 and 0.32 ± 0.02, respectively; p<0.05). Collective results suggest an effect of the stage of the estrous cycle on the oviductal epithelium differentiation in the infundibulum and on uterine glandular activity.

**P512 - Induced uterine disease reduces oocyte quality and embryo development in the cow.**
Mackenzie Dickson, Jeremy Block, Jose Santos, I. Martin Sheldon, John Bromfield

Uterine disease is common in postpartum cows and is associated with subsequent subfertility even after resolution of disease. We used an experimental infection model of uterine disease to characterize the impact of bacterial endometritis on the oocyte and its ability to develop into an embryo in culture. Estrous cycles were synchronized in 23 non-lactating, primiparous Holstein cows, and cows were blocked by days postpartum then assigned randomly to receive an intrauterine infusion of vehicle (Luria-Bertani broth; Control, n=11), or endometrial pathogenic bacteria (*Escherichia coli* and *Trueperella pyogenes*; Bacteria, n=12) on day 2 of the estrous cycle. Data were analyzed by ANOVA for continuous data and logistic regression for binomial data. Increased endometrial expression of *CXCL8*, *IL1B*, *IL6*, *PTGS2* and *TNF* on day 6 after infusion, and the presence of vaginal pus confirmed acute endometrial inflammation in bacteria infused cows compared with controls. Oocytes were collected 2, 24, 45, and 66 days after intrauterine infusion by transvaginal ultrasound guided oocyte pick-up and used for in vitro fertilization. The number of oocytes collected did not differ between treatments, but there was a tendency (P = 0.08) for a greater proportion of oocytes to undergo cleavage when collected from bacteria infused cows compared with controls. Interestingly, the proportion of cleaved zygotes to reach the morula stage of development was reduced (P = 0.04) when oocytes were collected from bacteria infused cows (33.9%) compared with controls (42.2%). Morulae derived from bacteria infused cows also had increased (P = 0.02) expression of heat shock 70 kDa protein 1A (*HSPA1A*) compared with controls. These data provide evidence that uterine disease reduces the capacity of oocytes to progress to the morula stage of development following in vitro fertilization. This work is supported by NICHD R01HD084316

**P513 - Lysophosphatidic acid as a regulator of prostaglandin secretion in equine endometrium during the estrous cycle: different effects in the course of endometrosis.**
Dariusz Skarzynski, Natalia Leciejewska, Beata Zelmańska, Joanna Staszkiewicz-Chodor, Graca Ferreira-Dias, Anna Szóstek-Mioduchowska

We have investigated whether lysophosphatidic acid (LPA) could play a role in the regulation of endometrial function under physiological (during the estrous cycle) and pathological (endometrosis) conditions in mares. LPA levels and *LPARI-4* mRNA transcription and protein
expression were studied in equine endometrium in the mid luteal phase (n=6 for each Kenney and Doig’s endometrium category: I, IIA, IIB, III) and in follicular phase of the estrous cycle (n=5 for each category). In the follicular phase, the level of LPA and LPAR1-4 protein expression were down-regulated in categories IIA, IIB and III endometria compared to category I (P<0.05). The effect of LPA (10^-9M) on the secretion of PGE2 and PGF2α and mRNA transcription of PG synthases in equine endometrium in different stages of endometrosis were determined in vitro. In mid luteal phase, LPA stimulated PGE2 secretion from all endometrium categories (P<0.05), while in the follicular phase LPA only stimulated PGE2 secretion in category I endometrium, when compared to the respective control groups (P<0.05). In the follicular phase, LPA down-regulated PGF2α secretion by category IIB endometrium, but in category III it stimulated PGF2α secretion (P<0.05). LPA concentration was up-regulated in mid luteal phase compared to follicular phase, and LPA increased PGE2 (P<0.05) but did not affect PGF2α secretion in both investigated phase of the estrous cycle by the healthy endometrium (Category-I; P>0.05). In the course of endometrosis endometrial LPA concentration and expression of LPAR1-4 in the follicular phase were disturbed. We suggest that the effect of LPA on the secretion of PGF2α and PGE2 from endometrial tissue was altered and PGE2:PGF2α ratio is changed in the course of endometrosis, which may contribute to disturb endometrial homeostasis.

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_P514 - C/EBPbeta, a transcription factor, genome-widely regulates gene expression through H3K27ac modifications during decidualization of human endometrial stromal cells (ESCs)._ Haruka Takagi, Yuichiro Shirafuta, Ryo Maekawa, Hiroshi Tamura, Norihiro Sugino

We previously reported that H3K27ac (active marker of histone modification) is genome-wide increased with the up-regulation of many gene expressions during decidualization of ESCs. We also reported that C/EBPb up-regulates IGFBP-1 and PRL expressions through the increase of H3K27ac of their promoters. In this study, we investigated the involvement of C/EBPb in the genome-wide changes of gene expressions and H3K27ac levels during decidualization by using RNA-sequence and ChIP-sequence. cAMP was used to induce decidualization. C/EBPb was knockeddown by siRNA. Three types of cells (control cells, cAMP-treated cells and cAMP-treated + C/EBPb-knockdowned cells) were generated. 4190 genes were up-regulated by cAMP, and 2239 genes (53.4%) were under the regulation of C/EBPb. Among 2239 genes, 1272 genes had H3K27ac-increased regions by cAMP. C/EBPb knockdown abolished these increases in almost all genes (1263 genes, 99.3 %), suggesting that C/EBPb is closely associated with the genome-wide increases of H3K27ac levels during decidualization. To investigate how C/EBPb genome-widely regulates H3K27ac levels, we first examined the expression levels of genes with histone acetyltransferase (HAT) activities. But, they were not under the regulation of C/EBPb. Therefore, we hypothesized that C/EBPb genome-widely binds to its binding regions and recruits cofactors with HAT activities. To identify these cofactors, we collated our ChIP-sequence data with public ChIP-sequence database of transcription factors and found that p300 is the most candidate cofactor that binds to the H3K27ac-increased regions with C/EBPb. ChIP-qPCR confirmed that C/EBPb binds to the promoter regions of several genes, which was crucial for the p300 recruitment and the increase of H3K27ac by cAMP. These data suggest that C/EBPb binds to the promoter regions and recruits p300, which is one of the mechanisms that
C/EBPb genome-widely increases H3K27ac levels. Our work revealed that C/EBPb, a transcription factor, genome-widely regulates gene expression through H3K27ac modifications during decidualization of ESCs.

**P515 - The role and regulation of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1A)α in decidualization of human endometrial stromal cells.**
Haruka Takagi, Isao Tamura, Yuichiro Shirafuta, Ryo Maekawa, Hiroshi Tamura, Norihiro Sugino

Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α) is a transcriptional coactivator that regulates the expression of genes involved in mitochondrial energy metabolism, glucose metabolism and fatty acid oxidation. PGC1α is also expressed in human ESCs and its expression increases with decidualization. However, the function of PGC1α in endometrium is still unclear. This study was undertaken to investigate the involvement of PGC1α in decidualization. PGC1α protein was expressed in stromal cells in the late proliferative phase endometrium, and its expression increased in those in the late secretory phase. When ESCs were incubated with cAMP to induce decidualization, cAMP increased PGC1α expression with the induction of IGF-binding protein-1 (IGFBP-1) and prolactin (PRL). Knockdown of PGC1α inhibited cAMP-induced expressions of IGFBP-1 and PRL. Next, we focused on C/EBPb, a transcription factor, that regulates many gene expressions, including IGFBP-1 and PRL during decidualization, and hypothesized that C/EBPb regulates PGC1α expression induced by cAMP. Knockdown of C/EBPb decreased cAMP-induced PGC1α expression. Furthermore, because public ChIP-sequence data of other cell lines shows the existence of C/EBPb binding sites in the enhancer region of PGC1α gene, the C/EBPb binding to these enhancer regions and the endogenous function of these regions were examined. cAMP increased the C/EBPb binding to these regions and PGC1α mRNA levels were inhibited by the deletion of those regions by CRISPR/Cas9 system. To investigate how PGC1α is involved in the regulation of IGFBP-1 and PRL, PGC1α was knockdowned and C/EBPb binding to the promoter regions of IGFBP-1 and PRL were examined. Knockdown of PGC1α decreased cAMP-induced C/EBPb binding to the promoter regions of IGFBP-1 and PRL. Taken together, C/EBPb regulates PGC1α expression by binding to the novel enhancer regions. PGC1α is involved in the induction of IGFBP-1 and PRL by promoting C/EBPb binding to their promoter regions.

**P517 - E-Cigarette Exposure Delays Pregnancy Onset and Impairs Future Offspring Health.**
Margeaux Wetendorf, Lewis Randall, Mahlet Lemma, Sophia Hurr, Claire Doerschuk, Kathleen Caron

E-cigarette usage is prevalent, perceived among the public as a harmless alternative to cigarette smoking. Many reproductive-aged women use E-cigarettes as a seemingly safe substitute, as smoking during a pregnancy can result in the increased risk for fetal developmental abnormalities and impaired fertility for adult male offspring. However, little is known how E-cigarette exposure can affect pregnancy. A successful pregnancy is dependent on proper
functioning of the receptive uterus at the time of embryo implantation, occurring at day 4.5 within the mouse. The uterus elicits dynamic changes of the epithelial and stromal compartments, resulting in molecular alterations to support the growing embryo. Because early pregnancy is highly regulated, we hypothesized that E-cigarette exposure impairs embryo implantation and fetal health. To test this hypothesis, pregnant wildtype C57BL/6J mice were exposed daily for 5 weeks to E-cigarette vapor or sham via a Sci-Req inExpose chamber. After 4 months, females exposed to E-cigarette exhibited a significant delay in the onset of the first litter (n=5). Based on this result, new mice were mated and exposed daily to E-cigarette or sham until euthanasia on day 4.5 or 5.5 of pregnancy. Sham treated day 5.5 mice demonstrated normal implant sites, while implant sites were absent from E-cigarette exposed animals, despite exhibiting high levels of progesterone, indicative of pregnancy (n=4). RNA microarray from day 4.5 pseudopregnant mice resulted in significant changes in the integrin, chemokine, and PI3K/AKT signaling pathways (n=4). Moreover, females exposed in utero to E-cigarette exhibited a significant weight reduction at 8.5 months (n=5), while males experienced only a minor, insignificant impairment in fertility (n=5). Thus, E-cigarette exposure delays embryo implantation and harms female offspring health, but not male offspring fertility. Further investigation is needed to understand how E-cigarette exposure delays the initiation of pregnancy. Supported by UNC TCORS pilot project: 5-P50-HL120100-04-05.

**P518 - Function of TRPML1 in uterine fluid resorption prior to embryo implantation.**
Zidao Wang, Ahmed Zowalaty, Yuehuan Li, Christian Anderson, Xiaoqin Ye

TRPML1 (encoded by *Mcoln1*) is a six transmembrane protein predominantly localized on lysosomes or late endosomes and lysosomes in different cell types. Mutations in *Mcoln1* can cause mucolipidosis type IV (MLIV) in both humans and mice, indicating a critical role of TRPML1 in the lysosome. TRPML1 serves as a lysosomal counter ion channel to regulate pH in lysosomal lumen. Our microarray analysis revealed *Mcoln1* being the most highly expressed counter ion channel gene in mouse peri-implantation uterine luminal epithelium. Although *Mcoln1*<sup>+/−</sup> mouse providers indicate fertile *Mcoln1*<sup>−/−</sup> mice, the fertility of *Mcoln1*<sup>−/−</sup> female mice has not been systemically investigated. Our study demonstrates that despite normal mating activities, *Mcoln1*<sup>−/−</sup> female mice have reduced fertility at 2 months old and quickly become infertile at 5 months old. *Mcoln1*<sup>−/−</sup> female mice at 5-6 months old have embryo implantation failure accompanied with progesterone (P4) deficiency and uterine luminal fluid retention. However, exogenous P4 treatment, which rescued embryo implantation in our *RhoA<sup>fl/fl</sup>-PR-Cre* mice that had embryo implantation failure and P4 deficiency but no uterine luminal fluid retention, fails to rescue embryo implantation, or alleviate uterine luminal fluid retention, or facilitate the absorption of Dextran from the uterine lumen to the uterine endometrium in the 5-6 months old *Mcoln1*<sup>−/−</sup> mice, despite its effect on shortening the height of uterine epithelium in these mice. These data demonstrate a local uterine defect in the 5-6 months old *Mcoln1*<sup>−/−</sup> mice. Since a prerequisite for establishing uterine receptivity is the resorption of uterine luminal fluid that will facilitate uterine lumen closure and subsequent embryo implantation, our data reveal a novel function of TRPML1 in the uterus for uterine luminal fluid resorption. The mechanisms of TRPML1 in uterine luminal fluid resorption and establishment of uterine receptivity are being investigated.
Embryo is considered as a semi-allograft and several immunologic mechanisms orchestrated by trophoblastic cells are involved to maintain integrity of embryonic and uterine/placental tissues during pregnancy. Secretion of gestational, pro-inflammatory factors such as IFNγ (interferon gamma) or GM-CSF (granulacyte macrophage-colony stimulating factor) are involved in different stages of pregnancy. However, they are highly produced during uterine bacterial infection, thereby affecting trophoblast function and compromising survival of the developing embryo. Therefore, in response to pathogen-induced inflammation, several trophoblastic and placental factors are produced, such as oncostatin M (OSM) and amphiregulin (AREG), but their role as immune regulators are yet elusive. The aim of this study was to determine whether and how OSM and AREG regulate the inflammatory response of human trophoblastic cells to IFNγ and GM-CSF. In a first step, we sought to define the optimal treatment with OSM and AREG to induce specific signaling molecules (STAT3, ERK1/2) in trophoblast cells. Next, we determined the minimal concentration to activate pro-inflammatory IFNγ/STAT1 and GM-CSF/STAT5 signaling pathways. Once these different conditions were defined, we found that pretreatment with either OSM or AREG efficiently inhibited the activation of STAT1 by IFNγ and STAT5 by GM-CSF. In mechanistic investigation for the regulatory role of OSM, we determined that OSM stimulated the expression of suppressor of cytokine signaling (SOCS) 1 and 3, which could play a common role in the inhibition of STAT1 and STAT5. This work was supported by grants from the Natural Sciences and Engineering Research Council of Canada (NSERC), the Fonds Québécois de la Recherche sur la Nature et les Technologies (FQRNT), and the Réseau Québécois en Reproduction (RQR).

The ability of the uterine epithelium to support embryo implantation requires loss of the progesterone receptor expression. Previous studies have demonstrated that forced expression of PGRA in the uterine epithelium results in impaired embryo implantation and reduced LIF and nuclear FOXO1 expression, both of which are uterine receptivity regulators. Here we investigate the phenotypes of forced expression of the PGRB isoform in the uterine epithelium and the involved signalings.

The Wnt7aCre mouse model was bred with a Cre inducible PGRB, PGRB<sup>LaL/+</sup> transgenic mouse to generate the Wnt7aPRB mice, with forced PGRB expression in the uterine epithelium. Similar to Wnt7aPRA mice, these mice showed impaired female fertility and disrupted embryo implantation. Histological analysis and 3D visualization showed enlarged and cystic uterine glands in Wnt7aPRA and Wnt7aPRB mice suggesting abnormal uterine gland function. These mice also showed reduced LIF production and altered nuclear localization of FOXO1. Administration of rLIF to these mice at pregnancy Day 3.5 restored the embryo implantation and the nuclear localization of FOXO1 at Day 4.5.
We next investigated the mechanism by which LIF restores the nuclear localization of FOXO1. Phosphoinositide 3-kinase (PI3K) - Serum/glucocorticoid regulated kinase 1 (SGK1) is the common signaling pathway that results in phosphorylation of FOXO1 and export from the nucleus. Increased SGK1 expression was found at the apical side of uterine epithelium in Wnt7aPRA and Wnt7aPRB mice. To test the involvement of PI3K-SGK1 in the nuclear localization of FOXO1, human endometrial cancer cell line HEC1A was treated with SGK1 inhibitors. This treatment reduced pFOXO1 resulting in the nuclear localization of FOXO1.

In conclusion, we demonstrate that loss of PGR expression in the uterine epithelium is critical for the induction of LIF expression and LIF regulates the window of receptivity in part by regulating the nuclear localization of FOXO1 through SGK1.

**P521** - Short and long term effects of uterine disease on oocyte transcriptome in dairy cows. Rachel Piersanti, Jeremy Block, Jose Santos, I. Martin Sheldon, John Bromfield

Postpartum uterine disease is a common cause of reduced fertility in dairy cows. The effect of uterine disease on subsequent fertility may be related to the accumulation of pathogen-associated molecules and inflammatory mediators in follicular fluid, and their impact on oocyte development. In order to better understand the influence of uterine disease on the oocyte, we used an experimental model of induced uterine disease in virgin heifers. Animals received an intrauterine infusion of sterile medium (control, n=5) or bacteria (pathogenic *Escherichia coli* and *Trueperella pyogenes*, n=4). Uterine disease was confirmed in bacteria infused animals by the presence of clinical endometritis symptoms. Ovum pick-up was performed on day 4 (during disease) and day 60 (after resolution of clinical disease) relative to infusion, and zona-free oocytes were subjected to RNAseq analysis. Using a *P*-value < 0.05 cut-off, 474 genes were differentially expressed in oocytes from bacteria infused animals on day 4, and 929 genes on day 60. Only 56 genes were differentially expressed in oocytes of bacteria infused animals on both day 4 and day 60. Further analysis of the differentially expressed genes identified canonical pathways impacted at day 4 including, interferon signaling, TGFβ signaling, TNFR2 signaling, IL-6 signaling and BMP signaling; while at day 60 glycolysis, bile acid biosynthesis neutral pathway, gluconeogenesis, ILK signaling, interferon signaling and chondroitin sulfate degradation pathways were affected. Predicted upstream regulators of differentially expressed genes in oocytes of bacteria infused animals on day 4 included SOX2, LPS and NFκB; while upstream regulators at day 60 included IL-1β, IL-6, and LH. These data provide evidence that uterine disease alters the oocyte transcriptome, and that unique effects are also apparent in the oocyte after the resolution of disease. This work is supported by NICHD R01HD084316.

**P522** - The autophagy protein FIP200 (RB1CC1) mediates progesterone responses governing uterine receptivity and decidualization. Arin Oestreich, Sangappa Chadchan, Alexandra Medvedeva, John Lydon, Emily Jungheim, Kelle Moley, Ramakrishna Kommagani

Between 40% and 70% of miscarriages are unexplained and may occur because the uterus fails to become receptive to embryo implantation. Receptivity requires endometrial stromal cells to transdifferentiate into decidual cells that secrete factors necessary for embryo survival and
trophoblast invasion. Decidualization is accompanied by activation of the cellular recycling pathway autophagy. The objective of this study was to determine the extent to which autophagy is required for endometrial function. We generated mice in which the autophagy protein FIP200 was conditionally knocked out in the female reproductive tract by crossing FIP200flox/flox mice with mice expressing cre recombinase under control of the progesterone receptor promoter (PRcre/+). Compared to control (FIP200flox/flox) mice, cKO (FIP200flox/flox PRcre/+) mice had reduced fecundity due to implantation failure (P≤0.05; n=6-9; Student’s t-test). Staining for the proliferation marker KI-67 revealed prolonged epithelial proliferation in the cKO uteri at 4 days post-coital (dpc). Using a controlled steroid hormone regimen to induce receptivity, we found that progesterone signaling failed to inhibit estrogen-dependent epithelial proliferation in the cKO uteri. Furthermore, histological analysis of the cKO uteri at 6 dpc revealed a failure of stromal cells to decidualize. In artificial decidualization experiments, stimulated uteri from cKO mice were 84% smaller than those from control mice and had lower expression of the decidualization markers Wnt4 and Bmp2 (P≤0.05; n=5-8; 2-way ANOVA with Sidak’s correction). Finally, siRNA knockdown of FIP200 impaired expression of the decidualization markers PRL and IGFBP1 (P≤0.05; n=3; 2-way ANOVA with Sidak’s correction) in human endometrial stromal cells. We conclude that the autophagy protein FIP200 is important for uterine receptivity and decidualization. Thus, autophagy-stimulating strategies may provide a means to improve outcomes for women experiencing recurrent miscarriages. This work was supported by grants R01HD065435, R00HD080742, and T32DK007120.

P523 - Female fertility in Atp6v0d2/-/Mcoln1/- double knockout mice. Yuehuan Li, Ahmed Zowalaty, Zidao Wang, Christian Anderson, Xiaqin Ye

Our previous microarray analysis of peri-implantation uterine luminal epithelium (LE) revealed a dramatic upregulation of Atp6v0d2 in the LE upon embryo implantation initiation. It led us to the novel finding of uterine epithelial lysosomal acidification upon embryo implantation. Lysosomal acidity is mainly maintained by V-ATPase to pump H+ into lysosomal lumen and counter ion channels to release positively charged ions from lysosomal lumen to dissipate the transmembrane voltage. Mcoln1 (encoding transient receptor potential cation channel, mucolipin subfamily, member 1 (TRPML1)) is the most highly-expressed gene encoding a counter ion channel in the peri-implantation LE. Mutations in MCOLN1/Mcoln1 can cause mucolipidosis type IV in both humans and mice, indicating the importance of TRPML1 in lysosomes. We have been studying in vivo functions of Atp6v0d2 and Mcoln1 in embryo implantation using Atp6v0d2-/- and Mcoln1-/- mouse models. Although Atp6v0d2-/- female mice have overall normal fertility, they have decreased implantation rate from 1st mating. On the other hand, Mcoln1-/- female mice have reduced fertility at 2 months old (2M) and quickly become infertile at 5M despite normal mating activities. Infertility in Mcoln1-/- female mice is associated with embryo implantation failure, progesterone deficiency, and uterine luminal fluid retention that couldn’t be alleviated by exogenous progesterone treatment. Since ATP6V0D2 and TRPML1 regulate lysosomal lumen pH in opposite directions, we hypothesize that deletion of both Atp6v0d2 and Mcoln1 would offset their effects in cells with their co-expression. To test this hypothesis, we generated Atp6v0d2-/- Mcoln1-/- double knockout mouse model. Fertility test in the limited Atp6v0d2-/-Mcoln1-/- females generated so far shows decreased pregnancy rate only after 6M compared to age-matched wild
type control. This ongoing study will fill in the knowledge gaps about the electro-chemical balance for lysosomal functions in female reproduction.

*P524 - PSAT1, present in the uterine luminal fluid in cattle, alters the endometrial cell transcriptome and blastocyst quality in vitro.* Tiago Henrique De Bem, Haidee Tinning, Niamh Forde

Phosphoserine aminotransferase 1 (PSAT1), is a component of extracellular vesicles from confirmed pregnant heifers on Day 16 as well detected in conceptus-conditioned medium. We tested the hypothesis that PSAT1 may act on the embryo to support development or on the endometrium during pregnancy recognition. In vitro produced embryos were cultured in serum-free media in the presence or absence of recombinant bovine PSAT1 from day 4-9 of culture. Primary endometrial cells were obtained from abattoir-derived mid-to-late luteal phase bovine uteri (n=3) and epithelial-enriched cells were treated with 1) control, 2) vehicle, 3) recombinant IFNT, 4) PSAT1 or 5) IFNT+PSAT1 for 24 hours. Extracted RNA from epithelial cells was produced and single end 75bp RNA sequencing was performed. For gene expression Tophat Cufflink Cuffdiff analysis pipeline was used. Differences in blastocyst development was analysed using chi-square both an adjusted p value of < 0.05. In total, cleavage rate in serum-free culture was 72.4% (n=346). Following addition of PSAT1 to culture media on day 4, no effect on the number of embryos developing to the blastocyst stage on days 7 (15.6% n=34 and 19.5% n= 25), or 8 (15.2% n=33 and 20.3% n=26) were observed for control and PSAT1 treated embryos respectively (P>0.05). However, significantly more blastocysts hatched on Day 9 in PSAT1 treated embryos (46.2% n=12) compared to controls (17.7% n=6). Exposure of cells to IFNT resulted in 2941 differentially expressed genes (DEGs) compared to vehicle controls. Treatment of epithelial cells with PSAT1 alone altered the expression of 125 DEGs. Comparison of these revealed 106 DEGs in common, of which 94 are significantly upregulated including MX1, MX2 ISG15 and RSAD2. Collectively these data demonstrated a functional role for PSAT1 in modifying the endometrial epithelium during the pregnancy recognition period and may also enhance post-hatching embryo development in cattle.

*P525 - Ovarian superstimulation down-regulates expression of follicular fluid-derived exossomal microRNAs in Nelore cows.* Fernanda Franchi, Priscila dos Santos, Patricia Fontes, Ana Clara de Ávila, Juliano Da Silveira, Edson Mareco, Anthony Castilho

To gain insight about impacts of ovarian superstimulation (OVS) on profile of exossomal miRNAs derived from follicular fluid, we submitted Nelore cows to superstimulatory protocols using only FSH (FSH group; n=4) or FSH plus eCG (FSH/eCG group;n=4) or only submitted to estrous synchronization (NS group; n=4). The exossomes were recovered from follicular fluid of follicles > 10 mm by successive centrifugations and submitted to analysis of 384 precursors and mature miRNAs by RT-qPCR. The miRNA was considered present when detected at least in two of four samples. The data were normalized by endogenous miRNA. The effect of OVS on detected miRNAs (at least three of four different samples) was tested by ANOVA and the mean values compared by Tukey test. Differences was considered significant when $P \leq 0.05$. To predict miRNA targets using TargetScan tool, we found 239 miRNAs present in exossomos, among then, 110
miRNAs were exclusively detected in NS group. A total of 1738 target genes are predicted. Moreover, using a gene ontology (GO) analyses, we infer alterations on regulation of biological process, nucleus, multicellular organismal development, cellular differentiation, protein binding and intracellular pathways. On the other hand, only four miRNAs were exclusively detected in FSH group and six in FSH/eCG group which 273 e 258 target genes are predicted respectively. Furthermore, when we analyzed miRNAs detected at least in three samples from each group, 13 were differently expressed and 12 were up-regulated in NS group and a total of 138 target genes are predicted and GO analysis infers alterations on protein binding, intracellular e cytoplasm pathways. Taken together we enforce there is a miRNAs regulation by exossomes on follicle microenvironment and specifically, that superstimulatory treatments down-regulate follicular fluid-derived exosomal miRNAs in Nelore cattle.

P526 - Antiphospholipid Autoantibodies Accelerate Human Endometrial Stromal Cell Decidualization and Induce Senescence and Senescence-Associated Inflammation, Mancy Tong, Teimur Kayani, Lawrence Chamley, Vikki Abrahams

Spontaneous miscarriage is the most common early pregnancy complication affecting 1 in 7 women. Women with antiphospholipid autoantibodies (aPL) are at increased risk. aPL specific for B2-glycoprotein I (B2GPI) readily interact with placental trophoblasts and the maternal decidua/endometrium. While the effects of aPL on trophoblast function are well-established, how aPL affect human endometrial stromal cell (HESC) function remains unclear. The objective of this study was to determine the effects of aPL on HESC decidualization, senescence and inflammation; all of which, if altered, could impact the window of receptivity. HESCs were exposed to decidualizing conditions (10nM estradiol, 1µM MPA and 0.5mM cAMP), in the presence or absence of anti-b2GPI aPL or control IgG (20mg/ml). In some experiments, SB203580, a p38 MAPK inhibitor, was also added (10mM). After 48hrs, insulin growth factor binding protein-1 (IGFBP-1) and prolactin (PRL), key markers of decidualization, were quantified by ELISA. Senescence was evaluated by cellular staining for senescence-associate β-galactosidase (SA-β-gal) activity and Western blotting for pS6 ribosomal protein. HESC cytokine secretion was profiled by multiplex analysis and validated by ELISA. Treatment with control IgG did not affect HESC function compared to the untreated control. aPL significantly increased HESC secretion of IGFBP-1 (7.4±3.1-fold) and PRL (2.1±0.3-fold) compared to control IgG (p<0.05, n=6). aPL, but not control IgG, induced HESC senescence, as evidenced by SA-β-gal staining and augmented pS6 expression (n=3). Compared to control IgG, aPL significantly increased HESC secretion of the senescence-associated inflammatory cytokines, IL-6 (534±376-fold) and IL-8 (2.2±0.2-fold, p<0.05, n=6). SB203580 inhibited aPL-induced IGFBP-1 by 55.4±22.1%; PRL by 48.6±14.3%; and IL-8 by 35.5±1.8%. In summary, aPL accelerated HESC decidualization, and induced senescence and senescence-associated inflammation. aPL augmented decidualization and inflammation via p38 MAPK activation. Thus, in women with aPL, these autoantibodies may promote a hostile uterine environment that may negatively impact implantation and placentation.
P527 - Hypoxia-induced vesicular trafficking between uterine cells is critical for embryo implantation and establishment of pregnancy. Arpita Bhurke, Athilakshmi Kannan, Qiuyan Ma, Alison Hantak, Milan Bagchi, Indrani Bagchi

Embryo implantation is initiated when the blastocyst trophectoderm attaches to the uterine luminal epithelium and subsequently penetrates into the underlying stroma to firmly embed into the endometrium. These events are followed by the formation of an extensive vascular network in the decidua that supports the growing embryo prior to placentation. If any of these processes fail to proceed normally, it leads to pregnancy complications, such as recurrent miscarriage, preeclampsia, and intrauterine growth restriction. The current challenge is to understand the complex processes by which intercellular communications occur between endometrial epithelial-stromal and stromal-endothelial cells to ensure successful implantation and establishment of pregnancy. Interestingly, in many mammalian species, these complex processes of early pregnancy occur in hypoxic environment. However, the mechanisms of maternal adaptation to hypoxia during early pregnancy remain unclear. In this study, we show that the transcription factor Hypoxia-inducible factor 2 alpha (Hif2α) is selectively induced in sub-epithelial stroma surrounding the embryo, and plays a crucial role in regulating maternal adaptation to hypoxia during early pregnancy. Using a conditional knockout mouse model we demonstrate that in the absence of HIF2α, the embryo attaches to the uterine epithelium but fails to breach through it, resulting in implantation failure. Using genetic, molecular and cell biological approaches, we demonstrate that stromal HIF2α regulates the production of extracellular vesicles, which mediates stromal-epithelial crosstalk to promote epithelial remodeling during implantation. As pregnancy progresses, HIF2α-directed vesicular communication between stromal and endothelial cells promotes the development of the vascular network critical for the establishment of pregnancy. Collectively, our study provides novel insights into the molecular basis of hypoxia adaption critical for embryo implantation and establishment of pregnancy.

P528 - VEGF suppresses scar formation during endometrial and cutaneous wound healing. Muruganandan Shanmugam, Sanket Nayak, Subhendu Das, Margaret Bruce, Sabita Dhal, Pravansu Mohanty, Nihar Nayak

Background: The postmenstrual endometrial repair provides a unique model for studying non-scarring adult tissue repair. Earlier we found that Vascular Endothelial Growth Factor (VEGF) regulates angiogenesis and reepithelialization during postmenstrual endometrial repair. The present study examined the role of VEGF during scar formation in non-scarring postmenstrual endometrium and in the naturally scarring mouse cutaneous wounds.

Methods: Effect of VEGF blockade by VEGF-trap on postmenstrual healing in artificially cycling ovariectomized rhesus macaques was examined. Scar forming genes such as TNFa, COL1A1, COL3A1, TGFb, IL-6, and DSTN were assessed by quantitative real-time PCR (qPCR). Surgically created mouse cutaneous wound was examined for the effect of VEGF blockade by adeno viral delivered Flk1 (AdFlk). Reepithelialization, CD31, and markers for scar formation at the wound site were analyzed by histology and qPCR. Keratinocytes and fibroblasts isolated from mouse skin were used to assess migration and scar formation, respectively. The VEGF activities were tested on vitrified VEGF after exposure to high ambient temperatures.
Results: Significant and marked (>10-fold) increase in the expression of scar formation marker genes was observed in endometrium during postmenstrual healing in VEGF-trap treated macaques. VEGF blockade by AdFlk resulted in significant (P<0.05) and marked loss (~75% loss) of reepithelialization and CD31 immunoreactivity in mouse cutaneous wounds. Consistently, recombinant VEGF stimulated (>2-fold) the migration of keratinocytes in a KDR-dependent manner. AdFlk treated mice exhibited a robust (>10-100 fold) increase in scar forming gene expression at the wound site. Consistent to this, VEGF significantly (P<0.05) abrogated the LPS induction of scar-forming genes in cultured dermal fibroblasts in a KDR-dependent manner. Vitrified VEGF replicated both endothelial and non-endothelial VEGF effects for 15 days after exposure at 55 °C.

Conclusions: VEGF/KDR signaling regulates scar formation, reepithelization that promotes postmenstrual endometrial and cutaneous wound healing suggesting clinical implications when enhanced stability was conferred by vitrification.

P529 - Expression and regulation of metallopeptidases in equine endometrium in the course of endometrosis. Anna Szóstek-Mioduchowska, Mariola Słowińska, Joanna Pacewicz, Dariusz Skarzynski, Kiyoshi Okuda

Equine endometrosis is characterized by excessive deposition of extracellular matrix (ECM) components around the endometrial glands and stroma that leads to the destruction of tissue architecture and impairment of endometrial function. Matrix metallopeptidases (MMP) are a group of zinc-dependent endopeptidases which mediate ECM turnover and are important factors in the process of fibrosis. In other species, MMPs production is regulated by profibrotic cytokines including transforming growth factor (TGF)-β1. However, the knowledge about MMP expression and regulation in equine endometrosis is limited. Thus, the goal of the study was to establish (i) the level of MMPs in equine endometrium in the course of endometrosis; (ii) the effect of TGF-β1 on secretion of MMPs in equine endometrial cells.

In Experiment 1., endometrial tissue from mid-luteal and follicular phase of the estrous cycle (n=5 for each category I, IIA, IIB, III according to Kenney and Doig and each examined phase) were used. The level of MMP-1, -2, -3, -9, -13 was performed using ELISA. The level of MMP-1, -2, -9 was up-regulated in the course of endometrosis compared to category I endometrium in follicular phase of estrous cycle (P<0.05). In turn, the level of MMP-3 was down-regulated in category III endometrium compared to category 1 endometrium in mid luteal phase of estrous cycle (P<0.05).

In Experiment 2., endometrial epithelial cells (n=5) and fibroblast (n=6) were stimulated with TGF-β1 (5 ng/ml) for 24 and 48 h. TGF-β1 up-regulated MMP-1, -9, -13 but down-regulated MMP-3 secretion from fibroblasts in time-dependent manner (P<0.05). In epithelial cells, TGF-β1 up-regulated MMP-1, -9, -13 in time-dependent manner (P<0.05). The level of endometrial MMPs is disrupted in course of endometrosis. Transforming growth factor-β1 seems to participate in the pathogenesis of endometrosis modulating MMP secretion in equine endometrium.

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**P530 - Morphometric evaluation of the ovaries, preantral follicles and oocytes of six-banded armadillos (Euphractus sexcinctus).** Andreia Silva, Andreza Brasil, Luana Bezerra, Erica Camila Praxedes, Samara Sandy Moreira, Lívia Campos, Alexandre Silva

To aggregate information about the reproductive morphology and physiology of the six-banded armadillo, we characterized the morphometry of their ovaries, preantral follicles and oocytes. After euthanasia, ovaries from four adult females were recovered and washed. Right and left ovaries were separately measured for length, width and thickness by a caliper and weighted at using a precision balance. Ovaries were fixed in Carnoy and then processed for histology. Preantral follicles were classified as primordial, primary and secondary, and measured by using an ocular micrometer. The average of two perpendicular measurements from the outer layer of granulosa cells and of the oocyte was used as a measurement of follicle and oocyte diameter, respectively. The measurements were made in 30 follicles of each category, with a visible oocyte nucleus. Microphotographs were obtained to evaluate preantral follicles using Image J software. Data were expressed as mean and standard error of means (SEM). The armadillo ovaries were characterized as ovoid bodies, relatively symmetric, with a smooth surface where the growing follicles were observed as translucent bodies. Right and left ovaries presented 0.90 ± 0.12 and 0.89 ± 0.10 for length, 0.41 ± 0.03 and 0.41 ± 0.06 for width, 0.25 ± 0.06 and 0.25 ± 0.06 for thickness, respectively. Both ovaries weighted 0.06 ± 0.2 g. The diameters for primordial, primary and secondary follicles were 14.59 ± 0.18 µm, 23.43 ± 0.34 µm, and 45.01 ± 1.45 µm, for their oocytes were 11.21 ± 0.16 µm, 16.19 ± 0.24 µm and 28.44 ± 0.95 µm, and for their oocyte nuclei were 6.94 ± 0.10 µm, 8.53 ± 0.12 µm and 11.93 ± 0.30 µm, respectively. We provide data regarding ovarian characteristics of *Euphractus sexcinctus* and this valuable information will contribute to the comprehension of the reproductive physiology of the species. Research supported by CAPES (Financial Code 01), Brazil.

**P531 - Beta2-adrenergic receptor dependent and independent mechanisms of pre-implantation embryo movement.** Ripla Arora, Diana Diaz

How a mammalian embryo approaches its site of attachment is a mystery that has puzzled researchers for decades. While in monocotous mammals it is essential to select a ‘good’ site of attachment, in polycotous species such as mice, it is also essential to ensure that the embryo is spaced far enough from neighboring embryos to avoid competition for maternal resources. We use our enhanced confocal imaging and 3D image reconstruction technology to evaluate murine embryo location in the uterus, at detailed time intervals along the longitudinal oviductal-cervical axis and the mesometrial-anti mesometrial axis. We show that embryos enter the uterus around midnight at the beginning of day 3 (day of plug is day 0.5) of pregnancy. Embryos move through the oviductal-cervical axis unidirectionally, until they have traveled about half the length of the uterine horn and then move bidirectionally to space themselves out equally. Facilitated by 3D uterine folds that are perpendicular to the oviductal-cervical axis, the embryos move towards the anti-mesometrial end to be in close proximity to the uterine glands. Our data support the
hypothesis that uterine implantation sites are not predetermined but instead are determined by the number of eggs ovulated and/or the number of embryos entering the uterine lumen. This allows for observed smaller distances between implantation sites when more embryos are present as compared to when fewer embryos are present in the uterine horn. Unidirectional movement of the embryos is facilitated by smooth muscle contractions that lie downstream of beta-adrenergic receptor signaling where as bi-directional movement of the embryos is independent of this signaling pathway. We are currently investigating the mechanisms of bi-directional embryo movement as determined by embryo-uterine interactions to allow for equal spacing and embryo attachment at the implantation site.

**P533 - Effects of progesterone and interferon tau on polyamine synthesis and secretion in the ovine uterus.** Katherine Halloran, Robyn Moses, Claire Stenhouse, Guoyao Wu, Fuller Bazer

Agmatine and polyamines are essential for cell survival and function and play important roles in the survival and growth of conceptuses in all species. This study tested the hypothesis that progesterone (P4) and interferon-tau (IFNT) stimulate expression of genes involved in the metabolism of arginine to agmatine and polyamines, and their transport, in the ovine uterus. Mature Rambouillet ewes (n=24) were observed for estrus using vasectomized rams and were surgically fitted with intrauterine catheters on Day 7 of the estrous cycle. They received daily intramuscular injections of 50mg P4 in corn oil and/or 75mg progesterone receptor (PGR) antagonist (mifepristone, RU486) (Days 8-15) and twice daily intrauterine infusions of either control serum proteins (CX) or IFNT (Days 11-15; 50µg/horn/day): 1) P4 and CX; 2) RU486, P4, and CX; 3) P4 and IFNT; or 4) RU486, P4, and IFNT. On Day 16, maternal blood was collected before all ewes were euthanized and hysterectomized. The uteri were flushed with 10mL phosphate buffered saline, and sections of the uterus and endometrium were processed to assess expression of mRNA and proteins. RT-qPCR revealed that expression of *ODC1, ADC, AGMAT, ARG2, SLC2A3, SLC12A8*, and SLC22A3 mRNAs were not affected by treatment (*P*>0.05), but there was a trend (*P*<0.1) for RU486 to decrease expression of those genes. This suggests that P4 production alone is sufficient to regulate expression of those genes or that they are constitutively expressed to produce polyamines to meet the requirements for survival of uterine epithelial and stromal cells. Future analyses of uterine flushings will include quantification of amino acids, with particular interest in the abundance of agmatine, polyamines and catecholamines. This research was supported by Agriculture and Food Research Initiative Competitive Grant no. 2016-67015-24958 from the USDA National Institute of Food and Agriculture.

**P534 - Cell-Type-Specific Gene Expression Signature in the Oviductal Epithelium During Different Stages of Early Pregnancy in Mice.** Emily Harris, Nathan Law, Sierra Olsen, Wipawee Vinuthayanon

Fertilization and pre-implantation development take place in the oviduct (or the Fallopian tube in humans). In vitro fertilization (IVF) makes the bypass of Fallopian tubes possible. However, more evidence has shown that babies born from IVF techniques had a higher risk of diseases
later in life compared to naturally-conceived babies. This finding emphasizes the importance of the oviduct function during fertilization and embryo development. It is established that steroid hormones (both estrogen and progesterone) regulate epithelial cell morphology and function in the female reproductive tract. Yet, how the oviduct works in the context of pregnancy establishment is virtually unknown. As such, our study aims to characterize the gene expression signature in each epithelial cell population during fertilization (at 0.5 days post coitus; dpc) and embryo development (1.5 and 2.5 dpc) in comparison to non-pregnant controls using single-cell RNA sequencing analysis (scRNA-seq) in a mouse model. We found the heterogeneity of the epithelial cell population in the oviduct using transcriptome-based analysis. These epithelial cell populations comprise secretory, ciliated, decorin-expressing, and the other two previously unknown cell populations. More importantly, gene expression signatures of each cell population vary between 0.5, 1.5, and 2.5 dpc in comparison to non-pregnant controls. Our data suggest the dynamic transcriptional profile of each epithelial cell type in response to different stages of early pregnancy. These findings may provide a better understanding of the function of each epithelial cell type at fertilization and pre-implantation embryo development in mammals that can be applicable to IVF settings.

\textit{P535 - Decreased Live Birth Rate in Mouse Surrogate Pregnancy Receiving Embryos Generated by In-Vitro Fertilization (IVF) Compared to Natural Conception.} Royce Harner, Zhuoni Xiao, Rhodel Simbulayan, Priyanka Manadhar, Elena Ruggeri, Xiaowei Luo, Annemarie Donjacour, Emin Maltepe, Adrian Erlebacher, Paolo Rinaudo

IVF is commonly used in both animals and humans; however an increased incidence of obstetrical complications including placentation abnormalities, preeclampsia, and pre-term birth has been described; the incidence of miscarriage is controversial. The aim of this investigation was to assess if IVF conceived embryos, as opposed to naturally conceived embryos, displayed disparity in live birth rate, and to understand if in vitro culturing affects decidualization, a key process in preparing the endometrium for gestation. IVF embryos were generated and cultured in either Whitten’s medium (WM, suboptimal conditions) or KSOM medium with amino acids (KAA, optimal conditions). Control blastocysts from superovulated mice were flushed out of the uterus 3.5 days after mating. Resulting blastocysts were transferred to non-superovulated CF1 recipients mated to vasectomized males. Interestingly, live birth rate was statistically lower in IVF generated embryos compared to control embryos, both in inbred (C57Bl6) (FB: 36.29%, IVFKSOM: 12.12% and IVFWM: 6.39%) or outbred (CF1xB6D2F1) (FB: 58.24%, IVFKSOM: 26.04% and IVFWM: 19.30%) mice (p<0.05). IVF conceptuses had a decreased fetal placenta ratio at day 18.5. The reduction in live birth rate led us to examine if abnormalities in decidualization or increase in senescence related pathways were present at two different time points (E7.5 and E18.5). Expression of selected genes (n=15) involved in decidualization was analyzed by RT-PCR and cellular senescence was assessed by immunohistochemistry using p-S6 (mTOR pathway) Cox2 and B-galactosidase staining. No differences in gene expression or decidua staining were noted between the groups. In summary, IVF conceived embryos, independently of the culture media used, displayed a lower live birth rate compared to in vivo conceived embryos. Obvious abnormalities in the decidua were not detected, suggesting that abnormalities in the IVF concepti rather than abnormalities in the decidua may be responsible for these findings.
**P536 - Peri-implantation stage human embryos cease cell proliferation and increase metabolism to prioritize important cellular events for implantation.** Sandeep Rajput, Rachel West, Hao Ming, Deirdre Logsdon, Rebecca Kile, Courtney Grimm, Jiangwen Sun, William Schoolcraft, Rebecca Krisher, Zongliang (Carl) Jiang, Ye Yuan

Human blastocysts have the ability to self-organize in vitro and recapitulate some key events of in vivo development during the implantation window. We performed single cell RNA-seq on trophoblast cells collected on embryonic days 8, 10 and 12 during extended embryo culture and found that the cytotrophoblast underwent dynamic changes in development, from concentrating on cell proliferation on d8, to primitive syncytium formation and hormone production on d10, and finally, focusing on extravillous trophoblast formation and invasion on d12. The objective of this study was to elucidate the relationship between major metabolic signaling pathways and the rapid progression of trophoblast differentiation during the peri-implantation period. Using western blot analysis of single extended culture embryo from these same time points, the ratio of phosphorylated to total protein of AKT, mTOR, ERK1/2, and STAT1 was used to determine the activity of each signaling pathway (n=3). Total cell number of embryos from each time point was determined by DAPI counterstaining followed by 3D confocal imaging. Embryo cell number rapidly increased from d8 to d10, and then remained constant from d10 to d12. All of the proteins examined, except mTOR, steadily increased their phosphorylation state during extended culture, peaking at d12. This demonstrates continued upregulation of PI3K-AKT, ERK, and JAK-STAT signaling pathways during the implantation window in human embryos. Therefore, the observed hiatus in cell proliferation at the later time point of extended culture may be not caused by any detrimental effect of the in vitro environment. Instead, embryos appear to pause cellular proliferation around the time of implantation and concentrate on other cellular events, such as hormone production, cell invasion, and immunomodulation, all of which require active cellular metabolism and are critical for the success of implantation. This research was funded by CCRM and approved by WIRB (Study no: 1179872).

**P537 - Evaluating the endometrial transcriptome following controlled ovarian stimulation in the St. Kitts African green monkey (Chlorocebus sabeus).** Kimicia Isaac, Shervin Liddie, Matthew Lawrence, John Callanan, Pompei Bolfà, Ronan Whiston, Aspinas Chapwanya

Women 15-45 years commonly suffer from infertility due to ovarian dysfunction, damaged fallopian tubes or dysregulated hormones. *In vitro* fertilization (IVF) is often used to treat infertility, however, success rates remain below 50%. We hypothesize that ovarian hyperstimulation treatment induces changes in endometrial innate immune gene expression that perturbs fertility. Since the uterus is a privileged immune organ, the type of proinflammatory response induced by IVF is not well-known. To better understand the molecular mechanisms of these changes, we assessed differential gene expression in the endometria of African green monkeys (AGM) following ovarian hyperstimulation. Twelve adult AGM (n=6 hyperstimulated, n=6 non-stimulated) were enrolled in the study. On study days 1-15 subjects received 25 µl leuprolide acetate depot (Lupron), followed by combined doses of 25 µl leuprolide and 25 IU follicle-stimulating hormone (Follistim) on days 16-30. Approximately 10-12 hours before tissue
collection subjects were given a single dose (250 µg) of choriogonadotropin (Ovidrel) to trigger final oocyte maturation. Following ovariohysterectomy, endometrial RNA transcripts were sequenced using the Illumina TruSeq protocol. Preliminary RNA-sequencing identified 699 transcripts. Four showed significant differential gene expression (3 down-regulated and 1 up-regulated with +/- 1.5 log fold change or higher and p-values and false discovery rate (FDR) less than 0.05 between the hyperstimulated and non-stimulated subjects. TGFβ-2 gene encoding the transforming growth factor beta-2 (TGFβ-2) proprotein showed a 2.3-fold downregulation in the hyperstimulated versus non-stimulated cohorts. TGFβ-2 is involved in numerous molecular and biological processes including regulation of cell growth and immune responses. The gene dopamine D2 receptor was significantly up-regulated in hyperstimulated subjects with a log fold-change of 3.8. These results demonstrate that the hyperstimulation protocol adopted in this study perturbs gene expression profile of the AGM uterus and have identified possible gene targets that warrant further investigation of their roles in pregnancy outcomes.

**P538 - Prostaglandin E2 Signaling Gene Signatures in Human Endometriotic Epithelial and Stromal Cells by RNA-Seq.** Joe Arosh, Jone Stanley, Esther Davidraj, Kaylon Bruner-Tran, Kevin Osteen, Sakhila Banu

Prostaglandin E2 (PGE2), a proinflammatory mediator, plays several important roles in the pathogenesis of endometriosis. Studies from our laboratory have indicated that inhibition of PGE2 receptors EP2 and EP4 regulate growth, survival, adhesion, and invasion of human endometriotic cells in vitro and in vivo in the xenograft mouse model of endometriosis of human origin. In the present study, we determined the gene or transcriptome signatures regulated by inhibition of EP2 and EP4 in human endometriotic epithelial cells 12Z and stromal cells 22B by RNA-Seq analysis. In both cell types, among the 14,203 transcripts, 3551 transcripts are highly expressed, 7101 are moderately expressed, and 3551 transcripts are lowly expressed. Differential expression analysis indicated that: (i) inhibition of EP2 and EP4 decreased the expression of 1506 transcripts in stromal cells, 229 transcripts in epithelial cells, and among them, 97 transcripts are commonly decreased in both stromal and epithelial cells; and (ii) inhibition of EP2 and EP4 increased expression of 745 transcripts in stromal cells, 43 transcripts in epithelial cells, and among them, 28 transcripts are commonly increased in both cell types. IPA analyses indicated that pathways associated with cell cycle, survival, apoptosis, adhesion, invasion, metabolism, inflammation and epigenetics are regulated in both stromal and epithelial cell types. These results together suggest that PGE2 signaling is an important pathogenic mediator of endometriosis that could be targeted for the treatment of endometriosis.

**P539 - Expressions and functions of microRNA Lethal-7b in mouse pre- and peri-implantation embryo.** Jihyun Kim, Jaewang Lee, Sooseong You, Wontae Kim, Jung Won Choi, Jin Hyun Jun

Alteration of various microRNAs expression may be involved and regulated in pre- and peri-implantation embryo development. Lethal-7b (let-7b) is a member of let-7 family, and it was suggested that a functional regulator of physiological events. This study aimed to evaluate the expression pattern of let-7b in mouse pre- and peri-implantation embryos. Also, we want to
clarify the role of let-7b in implantation process between trophoblasts and endometrial cells. We found that the let-7b expression was maintained and similar from 1-cell to blastocyst stage in mouse pre-implantation embryos. However, it was significantly increased in mouse peri-implantation embryos of in vitro outgrowth stage. Biological pathway of target genes regulated by let-7b miRNAs was analyzed by an in-silico analysis. The in-silico analysis showed let-7b of miRNA have regulated the expression of 35 mRNAs with high conservation and functions of targets have been involved in the insulin/IGF, gonadotropin releasing hormone receptor, and integrin signaling pathway. Among the target genes, we hypothesized that integrin/let-7b signaling pathway may play a crucial role in embryo attachment to uterine endometrium. To demonstrate the roles of let-7b in implantation process, human uterine endometrium cell line (Ishikawa and ECC-1) and/or human trophoblastic JAr cell line were transfected with either let-7b mimic or inhibitor. We found that let-7b inhibitor transfected JAr cells significantly decreased the attachment on the endometrial cells by an inhibition of mRNA translation in integrin beta 3 which is strongly related to mediating adhesion, migration, invasion, and cellular signaling. These results suggested that Let-7b might be highly expressed in peri-implantation embryos, and it involved in the regulation of embryo attachment in embryo implantation. However, the exact physiological functions of let-7b would be required to be further elucidated the implantation process, and it may even contribute to treatment implantation failure and other reproductive diseases.

**P540 - Involvement of Estrogen Receptor β with Cell Proliferation during Postpartum Uterine Regeneration Period.** Tomomi Sato, Marina Miyamori, Yukari Yamashita

The mouse uterus consists of the luminal epithelium (LE), the glandular epithelium (GE), the stroma, and the myometrium. The uterus is damaged after parturition but it is immediately regenerated. At postpartum day (PPD) 0 (the day of delivery), 1 and 3, cell proliferation is detected in the LE, GE and the stroma. Cell proliferation of LE is mediated by estrogen receptor α (ERα) in the stroma, whereas ERβ functions to suppress the cell proliferation in the epithelium. However, the major part of the underlying mechanism of uterine cell proliferation during postpartum regeneration period remains unclear. This study was aimed to investigate the role of ERβ in the uterine cell proliferation after parturition.

The role of ERβ in the uterine cell proliferation after parturition was examined by BrdU immunohistochemistry in wild-type (WT) and ERβ knockout (KO) mice at postpartum day 0-3. The percentage of BrdU-labelled uterine cells in ERβKO mice was higher than that in WT mice. The percentage of BrdU-labelled GE cells in postpartum ovariectomized (OVX) mice was significantly decreased compared with that in sham OVX mice, indicating that cell proliferation in the GE of the postpartum mouse uterus could be dependent on ovarian hormones. Finally, mRNA expression of growth factors and receptors involved in cell proliferation of adult uterus was investigated by real-time RT-PCR. Igf1 mRNA was low before birth and immediately increased after parturition, however, the expression of Igfr, Egfr, Met, Vegfa, Flt1 and Kdr mRNAs remained high during peripartum period. The mRNA expression of growth factors and receptors was not changed in WT and ERβKO mice. Thus, IGF, EGF, HGF and VEGF signaling were involved with the postpartum uterine regeneration, however, an increase of uterine cell
proliferation in ERβKO mice is not due to changes in the expression of growth factors and receptors.

**P541 - Contribution of early growth response-1 (EGR1) to mouse implantation through decidual reaction regulation.** Min Young Lee, Minji Kang, Chongsuk Ryou, Yong-Pil Cheon

For a successful pregnancy process, the embryo has to achieve the competence and the microenvironmental condition of the uterus, either. For the embryo to be conceived, a receptive uterus is needed. Endometrium thickness, and the number of glands and blood vessels are key factors in diagnostic predictors and decidual response is a main process in those. Early growth response-1 (EGR1) is a zinc finger transcription factor belonging to the Egr family and under the control of estrogen in uterus. It well evaluated that the EGR1 is involved in cell proliferation and differentiation but not fully evaluated in the decidualization. In this study, using the in vitro decidualization model of Egr-1 null mice, the role of Egr1 in decidualization was studied.

Tecidual markers such as progesterone receptor, decidual prolactin-like protein, and alkaline phosphatase were decreased in Egr1 null mice. Besides, the amount of lipid and glycogen in decidualizing cells decreased. The cell stability affected in Egr-1 null stroma cells. Those suggest that EGR1 contributes to mouse implantation through decidualization regulation

Key word: EGR1, implantation, decidualization

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**P542 - Insulin signaling is crucial for successful decidualization and implantation in mice.** Nikola Sekulovski, Allison Whorton, Mingxin Shi, Kanako Hayashi, James MacLean II

INSR and IGF1R, have been correlated with a multitude of physiological processes. Being interested in the function of these receptors in the female reproductive tract we utilized a Pgr-CRE to drive the knockout of both genes. We have previously reported that these mice have impaired ovulation, mainly due to reduced Pgr expression and reduced P4 production. To assess the function of INSR/IGF1R in the uterus we first performed artificial decidualization on ovariectomized mice. Both single receptor-knockout mice had significantly reduced uterine size with partial decidualization of the horns, the double-knockout on the other hand had no morphological or histological signs of decidualization compared to controls (n=5). This was confirmed by qPCR of Akp2 and Dtprp, which expression was not detectable in the uterus of double-knockout mice; the single-knockouts had variable expression levels. Histologically the uterus of a 2mo double-knockout mouse is similar to the wildtype (n=3). However, the thickness of the uterine endometrium is significantly reduced by 50% in the double-knockouts (p<0.05). This suggests that proliferation of stromal and epithelial cells is compromised, confirming previously published data of the role of IGF1R in the uterus. ESR1 is the main regulator of proliferation in the uterus, thus we analyzed its relative mRNA expression and observed a 2-fold increase (p<0.01) in the double-knockout compared to the wildtype, suggesting that INSR and
IGF1R act downstream of ESR1. We also analyzed the expression of PGR; the relative mRNA levels of Pgr were more than 2-fold reduced (p<0.01) compared to the wildtype in the uterus; this is supported by immunohistochemistry where there was no detectable PGR in either decidualized uterus or uterus from a 2mo female. This data suggests that ESR1 acting through INSR/IGF1R not only controls proliferation of the endometrium, but also regulates the expression of Pgr, thus regulating decidualization.

**P543 - Scanning Electron Microscopy of the Surface Epithelium of the Bovine Endometrium.** Fayth Kumro, Lauren Ciernia, Joao Moraes, Martin Schauflinger, Scott Poock, Matthew Lucy

The surface epithelium of the bovine endometrium is composed of at least 2 cell types (ciliated cells and secretory cells with microvilli) but their distribution and morphological changes over time are poorly understood. The objective was to quantify the number of ciliated cells across the uterus and assess morphological changes in secretory cells on the surface epithelium during the estrous cycle. Three heifers on day 0 (estrus) and four heifers on day 14 (mid-luteal phase) of the estrous cycle were humanely slaughtered and caruncular tissue was collected in three locations; the body (B), the horn ipsilateral to the corpus luteum (HCL) and the contralateral horn (NCL). Biopsies were prepared for scanning electron microscopy at 1000X magnification. A minimum of 4 fields (256X225µm) for each sample were examined (n=186 images). The number of ciliated cells was counted and the surface scored for the morphology of the secretory cells (0= no microvilli, 1= >0 to 50% of surface contained cells with microvilli, 2= >50% to <100% of the surface contained cells with microvilli, 3= 100% microvilliated). The morphological score was affected by location (B: 1.6±.3; HCL: 1.9±.3; NCL: 2.0±.3; P<.0002) and the location by day interaction (P<.0007; greater score on day 0 in HCL). The number of ciliated cells per field (11.2±1.7) was similar and was not affected by day. In conclusion, relatively few ciliated cells were detected on the surface epithelium. The presence of microvilli on the surface epithelium was dependent on the location. In general, the uterine surface was composed of patches of villiated and nonvilliated secretory cells. The loss of microvilli on secretory cells appeared to be explained by localized regions of apocrine secretion. Supported by NIH R01HD092254.

**P544 - Impact of Cage Type and Caloric Availability on Estrous Cyclicity in Mice.** Moniece Lowe, Simranjit Kalotia, Ryan Scott, Joseph Tash, Lane Christenson, Joshua Alwood, April Ronca

The impact of spaceflight on female reproductive-health has had limited investigation. These impacts may affect short-term endocrine signaling and future long-term reproductive and multigenerational studies in space. In a previous ground-based study using mice, we used the hindlimb-unloading (HU) model to analyze effects of simulated weightlessness on estrous cycling. Surprisingly, estrous cycling was disrupted in control mice housed in HU cages (but not experiencing unloading) and pair-fed to match intake of unloaded subjects. Variables of housing perturbation and food availability each modulate stress and inflammation. The aim of this study was to determine whether exposure to HU cages, pair feeding, or the combination disrupted
estrous cyclicity in mice - namely, experiencing a prolonged diestrus phase of at least one typical cycle (=5 days). We hypothesize that the combination of cage perturbation and pair-feeding exacerbates cage-induced disruption of the estrous cycle. Sixteen-week old, C57BL/6J mice were divided (n=10/group) into the following: HU-cage + pair-fed (using consumption from previous study), HU-cage + fed ad libitum, and vivarium controls fed ad libitum. We performed vaginal lavages for 4 days to acclimate and then performed and manually scored smears for cycle stage over 28 days during treatment. Control mice cycle normally throughout the experiment. For HU-cage + fed ad libitum, prolonged diestrus presents in 3/10 mice (p=0.06 vs. control), with 1/3 lacking evidence of recovery. For HU-cage + pair-fed, prolonged diestrus presents in 5/10 mice (p<0.01 vs. control), with 2/5 lacking evidence of recovery. These preliminary data demonstrate deleterious effects on estrous cyclicity when combining cage permutation with caloric availability. Pathways implicated in dysfunction of the reproductive organs are important for understanding the interwoven organ systems and risk factors of all spacefaring endeavors. This research will help improve ground-based models of spaceflight and understanding of environmental effects on the reproductive system.

**P545 - Relationship between anogenital distance and fertility in beef cows submitted to timed artificial insemination.** Sarah Battista, Shaun Wellert, Justin Kieffer, Kaitlin Brown, Luis Moraes, Alvaro Garcia-Guerra

Anogenital distance (AGD) is a sexually dimorphic characteristic defined, in females, as distance between the anus and base of the clitoris. Relatively lesser androgen effects during gestation leads to differentiation of the genital tubercle and urogenital sinus, into structures of the reproductive tract. There is great variation in AGD among individuals and increased AGD is associated with adverse reproductive outcomes in multiple species. The objective of this study was to test the hypotheses that: 1) increased AGD is associated with reduced pregnancies per artificial insemination (P/AI) in suckled cows, 2) body size and postpartum interval (PPI) affect AGD in beef cows. Suckled beef cows (n=563) were submitted to a 5-day CO-Synch protocol for timed AI (TAI). Briefly, cows were treated with a progesterone intravaginal device (CIDR) and 100 μg of GnRH on D-8. Five days later (D-3), there was CIDR removal and prostaglandin F2α (PGF) administration (1000 μg cloprostenol). Ovulation was induced 72 h after CIDR removal (D0) with 100 μg GnRH and cows were inseminated. Pregnancy diagnosis was performed by ultrasonography 35 days after AI. Anogenital distance was measured on D-8 using a caliper. Weight, rump fat thickness, age, estrus and PPI were determined on D0, while hip height was determined on D35. Data were analyzed using the generalized linear mixed models. Mean AGD was 100.8 ± 0.7 mm (ranged 60 to 160 mm) and overall P/AI was 56.1%. Pregnancies per AI weren’t associated with AGD (P=0.10). Cows expressing estrus before AI, however, were more likely (OR=3.09; P<0.01) to become pregnant and increased PPI was associated with increased P/AI (OR=1.02; P<0.01). Anogenital distance was affected (P<0.01) by age and postpartum interval (AGD = 102.4 + 1.93*age – 0.109*PPI). In conclusion, even though AGD varies greatly among cows, fertility is not associated with AGD.
The evidence of heterochromatic of endometrial glandular epithelium in a case of equine endometrosis. Malgorzata Domino, Anna Krajewska, Lukasz Zdrojkowski, Maria Sady, Zdzislaw Gajewski

Degenerative lesions of endometrium became one of the most important causes of equine infertility in mares over 12-years old. In reference to Kenney & Doig’s categorization of endometrosis, the quantification of the histological features became the crucial point of clinical examination of the mare with lower probability of fertility. In contrast to the method pursued so far, we proposed the new possibility of mare uterine biopsy examination - semiautomatic quantitative histometry. Biopsies of uterine horn were taken from mares with unaffected (n=10, category I of endometrosis) and with significantly affected endometrium (n=10, category III). Samples were stained with HE and trichrome Masson protocols independently and examined histometrically using semiautomatic quantitative analysis of slides on brightfield system for the scanning and analyzing TissueFaxes Plus (TissueGnostics, Austria). In all slides, affected (ROIsA) and unaffected regions of interests (ROIsU) were demonstrated.

The quantitative measurements (mean±SD) show significantly higher (p<0.0001) area of ROIsA in category III compared to category I (24.10%±18.52 vs 3.18%±2.33). In category III the clear evidence of heterochromatic of endometrial glandular epithelium were confirmed. The percentage of the area of light-pink cytoplasm and the size of cells with light-pink cytoplasm was higher in ROIsA than in ROIsU in both categories (p<0.05). The percentage of the area of dark-pink cytoplasm and the size of cells with dark-pink cytoplasm were comparable (p>0.05) in ROIsA and ROIsU also in both categories. The percentage of the area of collagen was always higher (p=0.0122) in category III (49.97%±12.18) vs category I (24.61%±12.68), when ROIsA and ROIsU were taken together under account.

We demonstrated the new protocol of biopsy samples of mare's endometrium is effective in endometrosis lesions quantification. Our findings demonstrate the high usage of semiautomatic quantitative examination under tissue cytometer, which may be applied as a diagnostic tool in the case of mare's infertility.

Potential Dysregulation of the Phosphatidylinositol 3-Kinase Signaling Pathway in Adult Female Mice Exposed to Di(2-ethylhexyl) Phthalate and Diisononyl Phthalate. Karen Chiu, Daryl Meling, Catheryne Chiang, Jodi Flaws

Di(2-ethylhexyl) phthalate (DEHP) is a common phthalate that is used to make polyvinyl chloride flexible. It can be used in many end products such as toys, blood bags, and building materials. DEHP is a reproductive toxicant and endocrine disruptor that can adversely affect female fertility and interfere with folliculogenesis. Manufacturers have begun to replace DEHP with diisononyl phthalate (DiNP); however, little is known about DiNP and its effects in the ovary. Thus, this study tested the hypothesis that subchronic exposure to DEHP or DiNP alters gene expression related to the phosphatidylinositol 3-kinase (PI3K) signaling pathway. To test this hypothesis, 39-40 day old female CD-1 mice were orally dosed with corn oil (control), DEHP (20 or 200 mg/kg/day), or DiNP (20 or 100 mg/kg/day) for 10 days and euthanized during
the diestrous stage of the cycle immediately after dosing. From the ovaries, total RNA was collected, extracted, and subjected to qPCR for measurement of the following PI3K signaling factors: Tsc1, Pdpk1, Akt, Foxo3a, Mtor, Rps6, Kit, Foxl2, and Pten. DEHP and DiNP exposure did not affect ovarian expression of Pdpk1, Akt, Foxo3a, Mtor, Rps6, Kit, Foxl2, and Pten compared to control. However, exposure to 20 μg DEHP and 20 μg DiNP downregulated the expression of Tsc1 compared to controls (p<0.05). Downregulation of Tsc1 has been indicated to play a role in cell growth and proliferation. Thus, these data suggest that DEHP and DiNP exposure may interfere with cell growth or proliferation in the ovary. Supported by NIH T32 ES 007326 and NIH R01 ES028661.

P548 - Comparison of Circulating and Uterine microRNA in Pregnant and Non-pregnant Beef Heifers. Savannah Speckhart, Andi Lear, Rhianna Wallace, Jon Green, Michael Smith, S. Gunewardena, Lane Christenson, Ky Pohler

MicroRNA (miRNA) have successfully been used as noninvasive biomarkers of different physiological conditions in many species. In cattle, identifying pregnancy-associated miRNA to diagnosis pregnancy status early in gestation would be beneficial, since current methods lack accuracy until the third to fourth week post-insemination. The objective of this study was to identify pregnancy-specific extracellular vesicle miRNA on day 18 of gestation in cattle. We hypothesized that extracellular vesicle miRNA from pregnant and non-pregnant heifers at day 18 post-insemination would differ in both circulation and uterine flush samples. Angus crossbred heifers were randomly assigned to be inseminated at day 0 using live semen (n = 21) or dead semen (n = 7) regardless of estrus expression. At day 18 post-insemination, all heifers were sacrificed, reproductive tracts excised, and samples obtained. Interferon-stimulated gene expression in endometrium was measured at day 0 and 18 post-insemination, and the presence or absence of a conceptus was recorded at sacrifice to classify heifers as pregnant or non-pregnant. All fluids were ultracentrifuged to obtain an extracellular vesicle enriched pellet, and RNA isolated via Trizol extraction. Small RNA sequencing was performed on 3 pregnant and 3 non-pregnant heifers to produce 12 total samples; 2 from each heifer for a comparative analysis between serum and uterine flush miRNA. Sequencing identified 334 known miRNA. Differential expression analysis identified 5 known miRNA (let-7c, miR-125b, -146b, -328, and -1246) as differentially expressed between pregnant and non-pregnant heifers in both serum and uterine flush. In summary, pregnancy-associated miRNA candidates located in both serum and uterine flush samples were associated with pregnancy status at day 18 post-insemination in beef heifers. This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2017-67015-26457 from the USDA National Institute of Food and Agriculture.

P549 - Deep Learning Classification of Estrous Stages in Mice. Andrew Jong, Ryan Scott, Lane Christenson, April Ronca, Tony Lindsey, Joshua Alwood

Spaceflight may cause deleterious changes to ovarian health and potentially disrupt normal estrous cycling in mice, a concern for reproduction in space. More data and analysis tools are needed to determine whether animals are cycling normally and ascertain the root cause. Current lab standards involve manual data labeling, a time consuming and arduous task. To optimize data
production and analysis rates, we propose an automated deep learning method to classify estrous phases in microscopic images of vaginal smears from mice. Our dataset of microscopic images consists of 1362 human-scored images of crystal-violet stained smears. We present two approaches to classify this dataset: first, a two-class classifier to identify the visually distinct diestrus phase, dominated by small leukocytes, from the other three phases (proestrus, estrus, metestrus); second, a four-class classifier to recognize each of the four phases in the estrous cycle. These two approaches provide a tradeoff between interpretation and accuracy; while the two-class classifier only identifies the presence of diestrus, the easier task yields significantly higher accuracy; in addition, the identification of one class is sufficient to detect healthy estrous cycling. Importantly, both deep learning approaches require no programming of explicit classification rules, which allows future model improvements without domain expertise. We successfully train, evaluate, and compare algorithm performance with ten leading deep learning architectures. By utilizing transfer learning for training, as well as Bayesian optimization for hyperparameter tuning, we achieve 94% and 85% test accuracy on the two-class and four-class classifier respectively. We support these accuracy results with several performance evaluation metrics. Most significantly, we achieve a speed increase over manual labeling by a factor of 200 to 600. This tool will aid reproductive studies using the rodent model and improve our understanding of female reproductive health and fertility on Earth and in Space.

**P550 - The Development of a Uterine Gland 3D Culture Model To Understand Pregnancy Establishment In Women.** Harriet Fitzgerald, Pramod Dhakal, Susanta Behura, Daniel Schust, Thomas Spencer

In humans, an unreceptive uterus and disrupted maternal-conceptus interactions can cause infertility due to pregnancy loss or later pregnancy complications. Recent studies in mice revealed that uterine glands and, by inference, their products and secretions impact uterine receptivity, blastocyst implantation, stromal cell decidualization, and placental development. In order to investigate the roles of uterine glands in first trimester human pregnancy, we used an established protocol to generate three-dimensional human uterine gland organoid (HUGO) cultures from endometrial biopsies of two different patients. The HUGO expand long-term, are genetically stable, differentiate following treatment with reproductive hormones, and can be cryopreserved. After passaging, HUGO were grown for 4 days in expansion medium and then treated with estradiol-17b (E2) for 2 days and then either nothing (Control), E2 and medroxyprogesterone acetate (MPA) or E2+MPA+cAMP for a further 6 days (n=3 replicates per patient and treatment). Immunofluorescence analyses found that HUGO cells were positive for the gland marker, FOXA2, as well as steroid receptors (ESR1 and PGR). Real-time qPCR analysis found that E2 increased PGR, IHH and OLFM4 expression, whereas MPA increased HSD17B2, LIF, PAEP and SPP1, which recapitulates changes in proliferative and secretory phase endometrium in vivo. EdgeR robust analysis of RNA-sequencing data revealed that E2 increased 3,077 and decreased 1,394 genes. Relative to the Control, E2+MPA increased 2,638 genes, such as PAEP, SPP1, LIF and STC1, and decreased 1,722 genes, whereas E2+MPA+cAMP treatment increased 3,149 and decreased 1,877 genes and E2+MPA+cAMP increased 860 genes relative to E2+MPA treatment. Of note, differential gene expression signatures were detected in each patient. The HUGO model will serve as an important platform for studying gland-decidualizing stromal cell-trophoblast interactions and discovering essential
genes involved in human pregnancy establishment. Supported by NIH Grants R01 HD096266 and R21 HD087589.

**P551 - Sperm Capacitation-induced Zinc Efflux is Necessary for Increased Proteasomal Activity and Release from Oviduct Glycans of the Sperm Reservoir.** Karl Kerns, Momal Sharif, Michal Zigo, David Miller, Peter Sutovsky

For decades, the role of calcium (Ca\(^{2+}\)) was the focus of understanding mechanisms leading to sperm fertilization competency. There has been little study of other divalent cations, including zinc ions (Zn\(^{2+}\)). Building on two of our recent discoveries: 1) the zinc signature phenomenon present in boar, bull, and human spermatozoa (four distinct zinc localization patterns), and 2) sperm oviductal glycan release being mediated by the 26S proteasome, we have further characterized the role of Zn\(^{2+}\) in the spermatozoon’s pathway to fertilization in domestic boar and bull. We have identified the sperm head Zn-efflux, reflected by transition from a zinc signature 2-to-3 state (Kerns et al., Nat Comm, 2018) and necessary for sperm acrosomal remodeling, is associated with a posterior-to-anterior plasma membrane and nuclear envelope permeability wave in bull spermatozoa. On a subcellular level, the capacitation induced Zn\(^{2+}\) efflux appears to be necessary for boar sperm release from oviductal epithelial cell glycans. Sperm release from oviductal glycans in response to progesterone was reduced in the presence of 0.5 mM ZnCl\(_2\) (\(P<0.05\)) and completely inhibited by 2.5 mM ZnCl\(_2\) (\(P<0.05\)), compared to equivalent concentrations of CaCl\(_2\). To understand this pathway further, we performed a fluorometric proteasomal activity assay. Decreased trypsin-like activity of the boar sperm-borne 26 proteasomes was detected in the presence of 0.5 mM ZnCl\(_2\) (\(P<0.05\)). Altogether, our findings support a model in which the Zn\(^{2+}\) efflux during sperm capacitation facilitates sperm release from oviductal epithelial cell glycans. Sperm knowledge may allow more accurate male fertility assessment in livestock and humans. Supported by NIH 1R01HD084353 (PS), USDA National Institute of Food and Agriculture (NIFA) 2015-67015-23231 (PS, DM), USDA NIFA Graduate Fellowship 2017-67011-26023 (KK), and MU F21C Program (PS). *Equal contribution.

**P552 - NOTCH1 activation enhances invasiveness of endometriotic epithelial cells through epithelial-to-mesenchymal (EMT) and contributes to the development of endometriosis.** Yong Song, Ren-Wei Su, Niraj Joshi, Tae Hoon Kim, Jae-Wook Jeong, Asgerally Fazleabas

The etiology of endometriosis is still undefined, but it has the characteristic of being an invasive disease with cells having an enhanced capacity to migrate, invade and survive. In our previous study we demonstrated that NOTCH1 expression was up-regulated by IL-6 mediated E-proteins in the ectopic epithelial cells of women as well as baboon and mouse models with endometriosis. Aberrant NOTCH1 expression has been reported in various malignancies and it has been linked to tumor invasion and metastasis by inducing EMT transition through repression of E-cadherin. Therefore, in this study we investigated whether NOTCH1 promotes EMT and invasiveness of endometriotic epithelial cells and contributes to the development of endometriosis. Our results showed that the overexpression of the NOTCH1 intracellular domain (N1ICD) in endometriotic epithelial cells (12Z) cells significantly lead to a decrease of E-cadherin and beta-catenin, and an
increase of their transcriptional repressor Snail and Slug. Additionally, the wound healing and matrigel invasion assay showed that overexpressing N1ICD in 12Z cells greatly increased their migration and invasion ability. To determine whether ligand-triggered NOTCH1 activation could suppress E-cadherin expression, 12Z cells were treated with 18nM recombinant JAG2-Fc for 48h with or without pretreatment with g-secretase inhibitor (GSI-0.5uM), a NOTCH cleavage inhibitor, for 1h. Our data showed that E-cadherin was significantly down-regulated while Slug was up-regulated after JAG2-Fc treatment. However, this effect was neutralized by GSI treatment. Finally, we induced endometriosis using Ltfcre/+N1ICDf/+ mouse model, in which N1ICD is specifically overexpressed in the epithelial cells. We found that the development of ectopic sites was significantly increased in Ltfcre/+N1ICDf/+ mice compared to the wild-type mice. In conclusion, our study suggests that NOTCH1 activation in ectopic epithelial cells could enhance lesion development by inducing EMT and cell invasion. These may contribute to the development of endometriotic lesions. (HD042280)

P553 - Spatially mapping matrisome protein abundance across ovarian compartments identification of glycoproteins that may affect folliculogenesis. Nathaniel Henning, Kelly Even, Sofia Petukhova, Monica Laronda

Patients with a disease or treatment regimen that affect their gonads, are at risk for premature gonadal insufficiency (PGI). Pediatric patients at risk of PGI may have diagnoses that can render preserved tissue unsafe to transplant. Our goal is to understand the ovarian microenvironment and its role in maintaining the follicle pool and folliculogenesis to restore fertility and ovarian hormones. The extracellular matrix (ECM) is a network of proteins providing structural and biochemical support. We developed a method for mapping the distribution of the ECM and associated proteins, or matrisome, in the porcine ovary. The ovary is divided into two compartments: the cortex, containing the ovarian reserve, while the medulla contains the majority of activated and growing follicles.

To spatially map the ovarian matrisome we deconstructed porcine ovaries, axially and sagittally, into 0.5mm slices, enriching for matrisome proteins through decellularization, and performed unbiased proteomics analyses. The cortex is the first 0.5 mm slice and the medulla makes up the ovary’s center. We identified 317 proteins that were significantly different (FDR <0.01) across depths of the ovary, of which 96 proteins belonged to the matrisome. We chose to focus on two differentially expressed mechanosensing proteins: AGRN (Cortex 23.58±0.12 v Medulla 22.87±0.077, P-value 0.002) and EMILIN1 (cortex 30.47±0.009 v medulla 29.77±0.054, P-value<0.001). The greater abundance of these proteins in the cortex were validated using immuno-PCR. These proteins are part of the Hippo and PI3K-Akt pathways respectively, which have both been implicated in ovarian follicle activation. We aim to investigate how these proteins influence folliculogenesis. To this end, we have established an in situ culture method for ovarian tissue and have observed ECM remodeling overtime.

This foundational work will further define how the ovarian microenvironment supports or is remodeled by folliculogenesis and inform the design of a scaffold for a bioprosthetic ovary transplant.
**P554 - Pig conceptuses secrete interferon gamma to recruit T-helper and T-regulatory cells to the endometrium to contribute to a controlled inflammatory environment that supports implantation.** Bryan McLendon, Heewon Seo, Avery Kramer, Guoyao Wu, Robert Burghardt, Fuller Bazer, Gregory Johnson

The emerging paradigm in the immunology of pregnancy is that conceptus (embryo and placental membranes) implantation does not progress in an immunologically suppressed environment. Rather, the uterine endometrium undergoes a controlled inflammatory response that supports stromal remodeling and vascularization required for successful placental development and pregnancy. T cells play critical roles in the induction of tissue inflammation and maintenance of tissue homeostasis. Our preliminary studies showed that trophectoderm cells of pig conceptuses secrete interferon gamma (IFNG) to recruit activated T cells, expressing PDCD1, ICOS, and CTLA4, to the endometrium to contribute to an inflammatory environment during the peri-implantation period. However, the T cell subtypes that populate the uterine endometrium during conceptus implantation are unknown. Therefore, we performed flow cytometry to determine the percentages of the subpopulations of T cells within the endometrium during the peri-implantation period. We collected endometria from gilts on Day 16 of pregnancy, digested the tissue using Accutase, and filtered the cells through a 70 mm mesh. We then immunofluorescence stained the cells using monoclonal antibodies generated against CD4, CD8, and CD25, sorted the cells using a Beckman Coulter Moflo Astrios Cell Sorter, and analyzed the data using FlowJo software. The major T cell subpopulation was CD4+ (22.59%), with minor subpopulations of CD8+ (1.38%), CD4+CD25+ (1.08%), and CD4+CD8+ (0.61%) T cells. Results suggest that CD4+ T-helper cells support endometrial inflammation, and that this inflammatory response is limited by CD4+CD25+ T-regulatory cells to prevent hyperinflammation that would compromise the pregnancy. We conclude that trophectoderm cells of pig conceptuses secrete IFNG to recruit various subpopulations of T cells to the endometrium to contribute to a carefully controlled inflammatory environment that supports the active breakdown and restructuring of the endometrium in response to implantation of the conceptus.

**P555 - Progesterone decreases diameter at follicle selection during either low or high circulating FSH or LH in bovine.** Victor Gomez-León, Oliver Ginther, Rafael Domingues, Milo Wiltbank

Follicle selection has been related to diameter deviation between the future dominant follicle (F1) and the future largest subordinate follicle (F2). F2-Undersized deviations have been reported as a complexity of this process based on the F2 smaller diameter (<7mm) compared to conventional deviations at expected diameter deviation (F1 ~8.5mm). Experiment 1 manipulated Progesterone (P4) to test whether elevated circulating P4 increases the likelihood of F2-undersized, decreases LH, and increases FSH concentrations. Experiment 2 tested whether increased LH activity, in presence of elevated P4, stimulates subordinate follicle growth and thereby increases the likelihood of conventional deviation. Holstein dairy heifers had ovaries evaluated by ultrasound and blood samples were collected every 12h after a follicular wave was synchronized by follicle aspiration on D6 after ovulation. Data were normalized to F1≥7.5mm and compared using SAS software. All heifers (n=20) in experiment 1 had their corpus luteum regressed with PGF2α analog and were randomized (D6) into: Control (minimum P4);
P4@3mm; and P4@6mm (P4=75 mg of P4 i.m. every 12h/5 days when F1 ≈3 or 6 mm, respectively). Treatment with P4 increased undersized deviation frequency from 0% (controls) to 54%, decreased LH, and increased FSH concentrations (0.20±0.01 vs. 0.14±0.01 ng/mL).

Heifers (n=27) in experiment 2 were randomized (D6) into: Control (saline), pLH (1.25 mg of porcine luteinizing hormone/12h), and hCG (160 IU of human chorionic gonadotropin and subsequently 96 IU/24h). Despite differences (P=0.04) in P4 (hCG: 8.6±0.8 ng/mL, pLH: 6.4±0.3 ng/mL, and Control: 4.6±0.3 ng/mL), and FSH (control: 0.46±0.03 vs Combined-pLH/hCG: 0.34±0.02 ng/mL), the F1 and F2 dynamics as well as the frequency of conventional (37%) and undersized (48%) deviations, were similar between the control and the combined pLH/hCG group. In conclusion, elevated P4 was linked to undersized deviation and occurred either during increased or decreased LH activity/FSH concentration.

P556 - Seminal Plasma or TGFβ Increases Expression of IL6 and TNF in Bovine Endometrial Cells. Jason Rizo, John Bromfield

Seminal plasma is the cell-free fraction of semen that induces endometrial inflammation following coitus in rodents and swine. Seminal plasma-derived TGFβ is the active molecule that modulates inflammation in female reproductive tissues. In rodents and swine, endometrial responses to seminal plasma promote embryo development and improve pregnancy outcomes. However, the endometrial response to seminal plasma is not well-characterized in cattle, where diluted semen is routinely used for artificial insemination. We hypothesize that seminal plasma or TGFβ increases expression of proinflammatory cytokines in bovine endometrial cells. Bovine endometrial (BEND) cells were treated for 24 hours with increasing concentrations of pooled seminal plasma (0.001% to 20%), rhTGFβ-1 (1 to 100 ng/mL) or rhTGFβ-2 (0.1 to 10 ng/mL). Cell viability was measured by MTT assay and gene expression was quantified by qPCR. Viability of BEND cells was reduced following exposure to 1% seminal plasma and higher concentrations. Heat-treatment of seminal plasma or TGFβ did not affect CSF2 or IL1B expression. Exposure to TGFβ did not affect CSF2 or IL1B expression. These data suggest that seminal plasma is cytotoxic to BEND cells and alters expression of proinflammatory cytokines. Further investigation is required to determine the extent by which semen components elicit endometrial inflammation in the bovine, which may be useful to optimize reproductive efficiency. This research was supported by Select Sires and the Southeast Milk checkoff.

P557 - Müllerian inhibiting substance and calcitriol induce growth inhibition and apoptosis of human endometrial stromal cells in endometriosis. Seung Ju Oh, Jieun Kang, In-Bai Chung, Hyuck Dong Han, Yeon Soo Jung
Purpose This study aimed to investigate whether Müllerian inhibiting substance (MIS) in combination with calcitriol modulates proliferation and apoptosis of human endometrial stromal cells in endometriosis and to identify the signaling pathway by which MIS mediates apoptosis.

Materials and Methods Endometrial stromal cells in endometriosis were treated with MIS in the absence or presence of calcitriol. Cell viability and proliferation were evaluated using the Cell Counting Kit-8 assay and apoptosis was evaluated by DNA fragmentation assay. Western blot and enzyme-linked immunosorbent assay were used to determine the signaling pathway.

Results The cells showed specific staining for the MIS type II receptor. Treatment of endometrial stromal cells with MIS and calcitriol led to dose- and time- dependent inhibition of cell growth and survival. The combination treatment significantly suppressed cell growth, down-regulating the expression of B-cell lymphoma 2 (Bcl-2); and up-regulating the expression of Bcl-2 associated X protein (BAX), caspase-3, and caspase-9.

Conclusion These results suggest a rationale for testing the therapeutic potential of MIS, alone or in combination with calcitriol, in the treatment of endometriosis.

P558 - Comparison of the clinical outcome of FET with and without pretreatment with the GnRH agonist. Seung Ju Oh, Jieun Kang, Yingmei Wang, In-Bai Chung, Hyuck Dong Han, Yeon Soo Jung

Objectives: To describe the clinical outcomes of frozen-thawed embryo transferred (FET) performing artificial preparation of the endometrium using a combination of estrogen (E2) and progesterone (P4), with or without the gonadotropin releasing hormone agonist (GnRHa), and modified natural cycle (MNC) with the use of human chorionic gonadotropin (hCG) trigger.

Materials & Methods: This was a retrospective study, evaluated 187 patients during a three-year period (February 2012–April 2015). The patients were allocated to treatment groups, comprising group A, in which 113 patients (181 cycles) received GnRHa+E2+P4, group B, in which 49 patients (88 cycles) received E2+P4 and group C, in which 25 patients (42 cycles) received hCG+P4. The inclusion criteria were: regular menstrual cycles (length 24–35 days), 21–45 years of age.

Results: The results of the present study revealed that the primary outcome of the study - the implantation rates (IRs) per embryo transferred - were not statistically different among the three groups. A similar results were found for the IRs with fetal heart beat per embryo transferred (68/181 (37.6%) in Group A versus 22/88 (25.0%) in Group B versus 14/42 (33.3%)), and for the live birth rates (LBRs) per embryo transferred (56/181 (30.9%) in Group A compared to 18/88 (20.5%) in group B, and 11/42 (26.2%) in group C).
**Conclusions:** Although the pregnancy outcomes were better in the hormone therapy (HT) with GnRHa group, HT FET with GnRHa for pituitary suppression does not result in significantly improved IRs and LBRs when compared with HT FET without GnRHa or in the MNC FET.

**P559 - Insights on the establishment of the uterine microbiome in virgin holsteins heifers.**
Joao Gabriel Nascimento Moraes, Lauren Ciernia, Fayth Kumro, Tamara Gull, Scott Poock, Matthew Lucy.

The interest in understanding the relationship between the microbiome and physiological processes has increased tremendously over the past decade but little is known about the influence of the uterine microbiome on reproductive processes. Historically, a sterile uterus was thought to be a prerequisite for a healthy uterus and successful pregnancy, but recent data in humans and domestic animals have challenged this theory. Here, we tested the hypothesis that the uterus is sterile at puberty and that the microbiome is introduced into the uterus when a heifer is first inseminated. For that, post-pubertal virgin Holstein heifers were subjected to artificial insemination (AI, n=10) or not (CON, n=10), and were slaughtered on days 13-14 post-breeding. Samples for microbiological analysis were collected using aseptic techniques from the outside of the tract (perimetrium) to control for contamination during tissue collection, and from three locations in the endometrium [uterine body, and uterine horns ipsilateral (IPSI) and contralateral (CONTRA) to the corpus luteum]. Additionally, the semen used for AI was also cultured to access microbial growth. Bacteria was cultured from the perimetrium in all heifers (20/20). No differences were observed in the proportion of AI and CON heifers with bacterial growth in the uterine body (AI=0/10; CON=3/10; P=0.17), IPSI horn (AI=2/10; CON=2/10; P=1.00) or CONTRA horn (AI=1/10; CON=1/10; P=1.00). With the exception of three bacteria found exclusively in the endometrium (Enterobacter cancerogenus, Bacillus infantis, Paenibacillus spp.) the remaining bacteria cultured from endometrium were also present in the perimetrium, perhaps indicating contamination. A yeast, Lodderomyces elongisporus, was cultured from semen but not detected in the uterus of AI heifers. Collectively, these results indicate that AI does not cause major changes in abundance of culturable microorganisms in the endometrium of virgin heifers, suggesting that their uteri are either sterile or colonized by nonculturable microbes. Supported by NIH 1R01HD092254.

**P560 - Understanding The Role Of Hemoglobin During Oocyte Maturation.** Megan Lim, Hannah Brown, Jeremy Thompson, Kylie Dunning

Hemoglobin is most associated with its gas transport function in erythrocytes. However, recent work has identified hemoglobin in a diverse number of non-erythroid cells, ranging from retinal epithelial cells to cancer cells, with several potential roles including gas binding and transport.

Cumulus oocyte-complexes (COCs) of mouse and human origin express hemoglobin in high amounts in vivo, which is drastically lower in vitro. COCs are found in the avascular ovarian follicle, and we hypothesize that hemoglobin is strategically placed to regulate oxygen levels, initiating hypoxia-induced gene expression. The molecule 2,3-bisphosphoglycerate (2,3-BPG),
synthesized by 2,3-BPG mutase (Bpgm), binds oxygenated-hemoglobin in the erythrocyte, releasing oxygen to the surroundings. During in vitro oocyte maturation (IVM) at 2% oxygen, exogenous hemoglobin addition significantly decreased Bpgm expression compared to control. Correspondingly, in vivo COC levels of Bpgm were 15-fold lower than hemoglobin. This inverse relationship between hemoglobin and Bpgm suggests an inherent mechanism for oxygen binding and release in COCs, potentially contributing to changes in oxygen-regulated gene expression.

To investigate the interaction of hemoglobin and 2,3-BPG and its effect on oxygen-regulated gene expression during IVM, we conducted a CRISPR-Cas9 knock-down of Bpgm, the 2,3-BPG synthesizing enzyme, in murine COCs using lentiviral delivery. Murine COCs were collected 44 h post-eCG and transduced with lentivirus containing Bpgm-specific guide RNA and Cas9 protein. COCs were maintained in meiotic arrest in maintenance culture media containing 1 x 10^{-8} M estradiol and 1 uM milrinone. Thirty hours post-transduction, GFP fluorescence was observed to confirm viral transduction. Successful gene knockdown was confirmed by qPCR and immunohistochemistry for Bpgm mRNA and protein, respectively. We hypothesize that the addition of hemoglobin to COCs depleted of Bpgm during IVM will increase oxygen-regulated gene expression as a result of hemoglobin binding more oxygen in the absence of 2,3-BPG.

**P561 - Comparison Of The Pregnancy Rates According To The Stage Of Development Embryos Produced In Vitro And Transferred Into Brahman Recipients.** Erly Carrascal-Triana, Juan García-Jimenez

Embryo transfer (ET) is one option that can increase a cow’s reproductive efficiency, allowing her to have numerous calves per year. However, pregnancy rates in ET can vary according to the stage of development and quality of the embryos. The aim of this study was to compare the pregnancy rates according to the stage of development Girolando (Gyr x Holstein) embryos produced in vitro and transferred in Brahman recipients. We synchronized 350 potential Brahman recipients for estrus using synchronization protocol: (1) insertion of a progesterone releasing device and 2 mg, i.m., oestradiol benzoate (EB) on Day 0; (2) 400 IU equine chorionic gonadotrophin (eCG) and 75 mg, i.m., d-cloprostenol on Day 5; (3) device removal and 0,5 mg, i.m., oestradiol cypionate (EC) on Day 8; and (4) embryo transfer on Day 17. The data collected were provided over 5 years (2014-2019), where the recipients selected participated on more than one occasion. A total of 644 embryo transfers are performed: 100 initial blastocyst, 211 blastocyst, 318 expanded blastocyst and 15 hatching blastocysts. Pregnancy rates, at 30 days, for initial blastocyst, blastocyst, expanded blastocyst and hatching blastocysts were, respectively, 27.72%, 34.28%, 37.96% and 6.32%. Pregnancy rates were greater (P < 0.05) with expanded blastocyst than the other treatments. We concluded that the stages of development of the embryos interfere in the rate of pregnancy of cattle, and, in Brahman race expanded blastocysts improve the pregnancy rates.

Keywords: Brahman, Girolando, embryo transfer, implantation

**P562 - Expression and activation of STATs in porcine endometrium during peri-implantation period.** Beenu Moza Jalali, Karolina Lukasik, Dariusz Skarzynski
Early pregnancy is associated with the uterine endometrial remodeling under the influence of ovarian steroid hormones and during this time various cytokines are also secreted in uterine lumen. This hormonally induced remodeling is an essential prerequisite for embryo implantation and role of signal transducers and activators of transcription (STAT) proteins in these processes is under investigation.

The aim of study was to evaluate expression and activation of STAT1, STAT2 and STAT3 genes/proteins in porcine endometrium on Days 10-16 of estrous cycle and pregnancy and evaluate effect of cytokines IL-6, LIF and IFNG on STAT expression and activation. We used real time PCR, and western blot, to evaluate the expression of STAT genes and proteins in porcine endometrium (n = 5). The activation of STATs was evaluated by determining their phosphorylation status on different days of cycle and pregnancy.

An increase in expression of STAT1 and STAT2 was observed on Day 13 of pregnancy as compared to corresponding day of cycle. STAT2 was additionally expressed at higher levels on Day 16 of pregnancy. Immunofluorescence of STAT proteins was primarily detected in endometrial luminal and glandular epithelium with a higher intensity in pregnant endometrium on Days 13 and 16. The phosphorylated proteins were detected only in endometria of pregnant animals on Days 13 and 16. Although there was no significant difference in the expression of STAT3 across the group of animals investigated, phosphorylated STAT3 was detected only in pregnant endometrium on Days 13 and 16. Whereas, IL-6 and LIF induced STAT3 expression and its phosphorylation in endometrial explants collected on Day 13 of cycle, IFNG induced an increase in expression of STAT1.

In conclusion, STAT genes/proteins are significantly expressed and activated during early pregnancy in pigs that might play a role in endometrial remodeling.

Study was supported by NCN grant no. UMO-2016/21/B/NZ9/03616

**P564 - Differential response of bovine endometrium ipsilateral and contralateral to the corpus luteum to a Day 14 conceptus.** Sandra Bagés-Arnal, José Sánchez, Beatriz Fernández-Fuertes, Michael McDonald, Susanta Behura, Tom Spencer, Trudee Fair, Pat Lonergan

In cattle, embryo transfer into the uterine horn contralateral to the CL results in a higher incidence of pregnancy loss compared to transfer into the ipsilateral horn. We have previously reported differing temporal changes in the endometrial transcriptome during the estrous cycle between uterine horns. The objective of this study was to compare the transcriptomic response of endometrium from the ipsilateral and contralateral horns to a Day 14 conceptus. Cross-bred beef heifers were synchronized and either used to generate Day 14 conceptuses following the transfer of in vitro-produced blastocysts into the ipsilateral horn on Day 7 (n= 9; 15 embryos/recipient; Day 0 = expected ovulation) or were used to obtain Day 14 endometrial explants (n= 5). Conceptuses were recovered on Day 14 by post-mortem uterine flushing and placed individually on top of explants collected from the ipsilateral (IPSI-D14) or the contralateral (CONTRA-D14) uterine horn, and co-cultured for six hours. As a negative control, explants from each horn were also cultured without a conceptus (IPSI-CTRL, CONTRA-CTRL). All explants were snap frozen
for subsequent RNA-seq. The response to a Day 14 conceptus was markedly different between horns with 61 differentially expressed genes (DEG) between IPSI-D14 and IPSI-CTRL compared to 239 DEG between CONTRA-D14 and CONTRA-CTRL (FDR < 0.05). Direct comparison between IPSI-D14 and CONTRA-D14 revealed 32 DEG, including CXCL11, CXCL10, IFIT2, RSAD2, and SAMD9. Gene Ontology analysis of these 32 genes revealed ten enriched biological processes, mainly related to immune response and response to an external stimulus. These data indicate that the endometrial response to the presence of a conceptus varies between uterine horns in the same uterus and may contribute to the higher incidence of pregnancy loss following embryo transfer to the contralateral horn. Supported by EU Horizon 2020 Marie Sklodowska-Curie REPBIOTECH 675526 and Science Foundation Ireland 13/IA/1983.

**P565 - Critical role of High-Mobility Group Protein Box-1 (HMGB1) in embryo implantation and pregnancy outcomes.** Shizui Aikawa, Wenbo Deng, Xiaohuan Liang, Jia Yuan, Amanda Bartos, Xiaofei Sun, Sudhansu Dey

A reciprocal communication between the implantation-competent blastocyst and the receptive uterus is essential to successful implantation and pregnancy outcomes. As progesterone (P4) is known as a hormone of pregnancy for most eutherian mammals, an optimal P4 signaling is absolutely critical to uterine receptivity for implantation. Here, we show that High-mobility group protein Box-1 (HMGB1), an evolutionally highly conserved nuclear protein, plays a critical role in implantation in mice. HMGB1 is expressed in the uterus in a spatiotemporal manner during early pregnancy. First, it is expressed in epithelial cells on days 1-3 of pregnancy followed by stromal cell expression prior to (day 4) and during blastocyst attachment (day 5). Its role in periimplantation uterine biology was explored by uterine-specific deletion using a Pgr-Cre driver (Pgrcre/+ Hmgb1f/+). We observed that these mice have either implantation failure or inferior implantation as detected by reduced intensity of blue bands and histological appearance of smaller implantation chambers on day 5. We discovered that implantation defects arise from dysregulated activation of stromal progesterone receptor (PR) as assessed by luciferase reporter activity. This finding corroborates with diminished PR-responsive Hoxa10 expression and poor cell proliferation in the stroma, despite of comparable serum P4 levels and uterine PR expression in the deleted mice. These results are consistent with the failure of exogenous P4 administration to rescue implantation deficiency in Pgrcre/+ Hmgb1f/+ females. These defects result in subsequent adverse ripple effects, giving rise to severe subfertility. Our findings provide evidence that nuclear HMGB1 contributes to successful blastocyst implantation by preserving P4-PR signaling.

**P566 - Designing in vitro Gelatin Hydrogel Platforms for Monitoring Endometrial Function and Trophoblast Invasion.** Samantha Zambuto, Kathryn Clancy, Brendan Harley

Each menstrual cycle, the endometrium cycles through rapid phases of growth, remodeling, and breakdown. Trophoblast cells also drive significant vascular and matrix remodeling during pregnancy. Despite the importance of early events in successful establishment of pregnancy, initial blastocyst adhesion to the endometrial epithelium has never been observed in humans and other integral processes have been observed only from single specimens. Understanding cellular
processes associated with pregnancy has significant value in improving our understanding of human reproduction. The objective for this study was to develop a three-dimensional tissue engineering endometrial platform to investigate dynamic processes associated with endometrial function and trophoblast invasion. We report adaptation of a methacrylamide-functionalized gelatin hydrogel system that integrated: (i) a hydrogel matrix stiffness relevant to the in vivo environment; (ii) the ability to monitor endometrial angiogenesis via culture of an artificial endometrial perivascular niche (endothelial-stromal cell co-culture); (iii) spatial structure similar to the endometrium luminal epithelium; (iv) capacity to present hormonal cues via decidualization of endometrial stromal cells; and (v) capability to monitor the kinetics of trophoblast invasion in three dimensions. We validated this model by quantifying endometrial angiogenesis metrics (n=3 hydrogels, n=6 images/hydrogel), stromal cell decidualization status (n=3 hydrogels) and trophoblast invasion (n=6 spheroids) in gelatin hydrogels as well as qualitatively analyzing various phenotypic markers pertaining to endometrial epithelial cell function. With these studies, we provide a series of techniques that will instruct researchers in the development of endometrial models of increasing complexity. Ongoing efforts include moving toward a tissue-specific model with human endometrial microvascular endothelial cells as the endothelial cell source and investigating the effects of decidualization status on endothelial network complexity.

P567 - Uterine Expression of Basigin is Required for Proper Decidualization in Female Mice. Kailiang Li, Romana Nowak

Basigin (BSG) is a transmembrane glycoprotein involved in cell proliferation, angiogenesis and tissue remodeling. BSG has been shown to be essential for male and female reproduction. We hypothesized that uterine expression of BSG is required for proper decidualization in mice. To investigate our hypothesis, we generated a uterine tissue-specific knockout using PR/CRE-Lox method. Fertility study data showed that Bsgd/d females had significantly reduced fertility compared to Bsgf/f females (n=6-7) when bred to wild type males. Ovarian function appeared normal with no difference in the number of superovulated oocytes collected or in serum progesterone levels between Bsgd/d and Bsgf/f females. Uterine tissues collected at various times of gestation showed increased abnormalities in implantation, placentation and parturition in Bsgd/d females. We then tested the artificially-induced decidualization response. Ovariectomized mice of each genotype were given 100 ng estradiol for three days, rested for two days, then given 10 ng estradiol and 1 mg progesterone for three days. We then injected one uterine horn with 15 µl corn oil as a stimulus while the other horn was an un-injected internal control. Mice were supplied with 1 mg progesterone for four days before tissue collection and analysis. The injected uterine horn weight increased 2.2 fold in Bsgd/d mice compared to the un-injected horn, which was significantly lower than the 9 fold increase observed in Bsgf/f mice. Histology analysis determined that the decidua cross-section was much smaller in the Bsgd/d mice. Immunohistochemistry showed significantly lower expression of the decidualization marker CEBP/b in the Bsgd/d decidua. qPCR analysis demonstrated significantly lower expression of the decidualization gene BMP2. Immunofluorescent staining for the angiogenic marker CD31 showed markedly lower expression in day 6 samples, indicating impaired angiogenesis. Our results support that uterine expression of BSG is required for normal decidualization in female mice. Supported by NIH HD40093 (RAN).
**P568 - Hyaluronan-NK cell Interaction Controls the Primary Vascular Barrier during Early Pregnancy.** Ron Hadas, Eran Gershon, Aviad Cohen, Sima Stroganov, Ofir Atrakchi, Shlomi Lazar, Ofra Golani, Bareket Dassa, Michal Elbaz, Gadi Cohen, Elena Kartvelishvily, Raya Eilam, Nava Dekel, Michal Neeman

Successful embryo implantation is associated with a unique spatial pattern of vascular remodeling, characterized by profound peripheral neo-vascularization surrounding a peri-embryo avascular niche. We hypothesized that hyaluronan controls the formation of the unique vascular pattern encompassing the embryo. This hypothesis was evaluated by inhibition of maternal hyaluronan synthesis and by genetic modification of hyaluronan metabolism specifically targeted to the trophectoderm. The outcome of altered hyaluronan deposition on uterine vascular remodeling and post-implantation development were analyzed by MRI, detailed histological examinations and RNA-sequencing of uterine NK cells. Our experiments revealed that elimination of hyaluronan, led to elevated expression of MMP-9, VEGF-A and its receptor VEGFR-2 and a decrease in VEGFR-3 expression. These molecular modifications were accompanied by impaired formation of mesometrial vascular sinuous folds, ectopic decidual angiogenesis and impaired differentiation of uterine NK cells. Conversely, enhanced deposition of hyaluronan resulted in the expansion of the maternal-embryo barrier, leading to an increased diffusion distance and aborted implantation. These results demonstrate a pivotal role for hyaluronan in successful pregnancy by fine-tuning the peri-embryo avascular niche and maternal vascular morphogenesis.

**P569 - Integrated analysis of mRNA and microRNA expression in the bovine oviduct: can we pinpoint embryo receptivity?** Angela Gonella-Diaza, Ricardo Perecin Nociti, Gabriella Mamede Andrade, Everton Lopes, Juliano Coelho Da Silveira, Mario Binelli

*In silico* predictions of miRNA-mRNA interactions not consider the specific transcriptomic status and the occurrence of false positives could be high. The integration of real miRNA and mRNA expression data into *in silico* target predictions could be a more reliable method of prediction. A model for receptivity based on size of the pre-ovulatory follicle (POF) was used to study miRNAs and mRNAs abundance on oviductal samples (Ampulla and isthmus) collected on day 4 of the estrous cycle. Growth of the POF of Nelore (*Bos indicus*) cows was manipulated to produce two groups: large POF-large corpus luteum (CL) group (LF-LCL; greater receptivity; n=8) and small POF-small CL group (SF-SCL; n=8). Using RNAseq we identify 698 and 591 differential expressed mRNAs in ampulla and isthmus, respectively. Using RT-qPCR of 348 miRNAs, we identify 14 and 34 differentially expressed miRNAs in ampulla and isthmus, respectively. Bioinformatic analyses was carried out with a custom R script. First, using variance stabilizing transformation we combined miRNA data (CT values) with RNAseq data and using CEMItool we identify 12 specific cluster of mRNAs and 8 of miRNAs. Then, using a Kendall correlation we verify the relationship between miRNA and their predicted targets. Next, we performed a gene ontology (GO) and enrichment analyses using clusterProfiler and we build
a network analysis with igraph to summarize the relationships between miRNA, mRNA and GO terms. For all analyzes, results were significant when \( p \text{ adjusted (Benjamini-Hochberg)} < 0.05 \). We identify molecular pathways that were exclusively presented in the high receptivity group: ribosome, secretion and extracellular space. This pathways are exclusively present in the LF animals and are under the control of the miRNAs. We conclude that mRNA-miRNA interactions are present in the bovine oviduct and could change in regard of the fertility status of the cow.

**P570 - Uterine luminal microenvironment during the bovine estrous cycle as defined by proteins secreted by the endometrium.** Kasey Schalich, Prasanthi Koganti, Juan Castillo, Soon Hon Cheong, Vimal Selvaraj

Secretions mark the sole functional basis by which the intercaruncular endometrium supports vital events in the uterine lumen, from the transit of sperm through the preimplantation stages of embryo development. Despite knowledge of these engendering actions, study of endometrial secretions has been focused exclusively on proteomic evaluations of uterine luminal fluid in the context of the presence or absence of a conceptus. To gain fundamental understanding of temporal changes to the uterine luminal microenvironment and interpret uterine physiology, we analyzed the endometrial transcriptome of Holstein heifers at days 0, 5, 10, 14 and 18 of the estrous cycle using a bioinformatic algorithm to identify secreted proteins. Our results identified 2293 transcripts that coded for secreted proteins in the uterine endometrium across all timepoints. Of these, 777 transcripts (34%) were significantly up- or down-regulated during different stages of the estrous cycle underscoring the dynamics of luminal endometrial secretions. Analysis of temporal patterns revealed 16 distinct clusters indicative of cyclical changes to secreted protein transcripts that also defined stage-specific transformations relevant to events supported by the luminal microenvironment. Based on absolute abundance, we also delineated quantitative changes within the different temporal patterns to refine this dataset. Proteins classes within the different clusters included extracellular matrix proteins, growth factors, cytokines, enzymes and other elements that can mediate/influence paracrine communication. Prominent in our results were numerous factors that have not been previously described to align with uterine physiology. Our findings establish the physiological ‘secretory cycle’ of the bovine uterine endometrium. Functional shifts to secretory patterns established provide a core framework for further discovery through synchronizing maternal-embryo signaling essential for early developmental events in the uterine lumen.

**P571 - Effects of the estrous cycle and early pregnancy on circulating immune cells in dairy heifers.** Neha Oli, Frankie Gambonini, Joy Pate, Troy Ott

Pregnancy affects immune cell populations in the uterus and peripheral blood of placental mammals. Work from our lab showed that macrophages increased in the uterus during early pregnancy and that there was greater expression of immunomodulatory molecules, signal regulatory protein alpha (SIRPA) and indoleamine 2-3 dioxygenase (IDO). We hypothesized that circulating monocytes contribute to the increase in uterine macrophages and that their function is affected by the developing conceptus. Blood was collected from Holstein dairy heifers (n=4
heifers/status/Day) on Days 14, 17 and 20 of the estrous cycle and, subsequently, on Days 14, 17, 20 and 23 of pregnancy. Immune cells were isolated for flow cytometry and labeled with antibodies against CD11c, CD14 to quantify monocytes, CD172a to quantify SIRPA and against aryl hydrocarbon receptor (AhR), IDO and peroxisome proliferator-activated receptor gamma (PPARG) to assess the functional status of the cells. A greater proportion of monocytes was detected in pregnant compared to cyclic heifers (status*day, P= 0.02), particularly on days 14 and 17. SIRPA expression per cell was greater in pregnancy compared to the estrous cycle (P=0.01) and the proportion of CD14+ cells tended to increase during pregnancy (P =0.07). Day of Pregnancy affected the proportion of CD11c+ cells (cubic effect of day, P =0.03) with a transient reduction at day 20 and a tendency for the same in SIRPA expression (P =0.06). Proportion of cells expressing IDO declined at day 23 of pregnancy (quadratic effect of day, P =0.05). There was no change in AHR or PPARG expression. In summary, proportions of circulating immune cells changed during early pregnancy consistent with the hypothesis that these cells contribute to an increase in myeloid lineage cells in the uterus. Furthermore, pregnancy resulted in an increase in expression of molecules associated with tolerance.

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**P572 - Significance of Adrenomedullin and Pinopodes during Endometrial Receptivity.**
Kelsey Quinn, Brooke Matson, Kathleen Caron

Successful embryo implantation is vital for pregnancy establishment and is a major concern in the management of infertility. Implantation is a complex and highly regulated process involving precise synchronization between the embryo and endometrium. In preparation for implantation, the uterus must become receptive to embryo attachment. Uterine protrusions, termed pinopodes appear at the time of implantation and are hypothesized to be associated with fertility in women. However, how pinopodes form, what they produce, and why they exist during endometrial receptivity remains unclear. Our laboratory previously identified adrenomedullin (AM=protein Adm=gene) as an important contributor to endometrial receptivity and implantation. Maternal reduction of Adm in mice leads to defective endometrial receptivity, including a decrease in pinopode numbers and subfertility. Additionally, intrauterine delivery of AM increases pinopode coverage and improves implantation rate. Based on the previous known roles of AM in implantation, in this study, we hypothesized that a genetic increase in Adm is beneficial to endometrial receptivity by contributing to pinopode formation and increasing genes essential for implantation. Uterine tissue was collected from pseudopregnant wildtype mice and mice with a genetic overexpression of Adm (Admhi/hi). Admhi/hi females exhibited a pronounced increase in pinopodes and uterine glands. The increase in pinopode numbers in Admhi/hi mice also led to investigations related to understanding the biological function of pinopodes. Using scanning electron microscopy, we identified morphological differences among pinopodes at different stages of pseudopregnancy in mice including spherical shaped pinopodes at pp2.5 and an increase in pinopodes regressing or bursting at the time in which an embryo would implant (pp4.5). Our current results signify the importance of AM in supporting pinopode and glandular development and preparing the uterus for implantation. Our findings also raise new questions
related to the morphology and biological function of pinopodes during the peri-implantation period.

**P573 - Circulating CD31+ Exosomes are Significantly Elevated with a Proliferative and Angiogenic but Anti-apoptotic mRNA Signature in Pregnant Women with Placenta Accreta Spectrum.** QR Qi, J Makhoul, YH Yang, QY Zhang, TL Lechuga, A Al-Khan, NP Illsley, L Liu, J Song, WB Tan, S Zamudio, DB Chen

Placenta accreta spectrum (PAS) is an obstetric condition of deep myometrial invasion of trophoblast villi, which is histologically categorized as: 1) accreta when villous trophoblast is firmly attached to myometrium with no intervening decidua; 2) increta when trophoblast invasion penetrates the myometrium; and 3) percreta when the myometrium is completely penetrated. PAS is the most dangerous obstetric disease that threatens pregnant women with unknown pathogenesis. This study was to determine the mRNA signature of CD31+ exosomes isolated from sera of PAS vs. normal pregnant women. Total serum exosomes from normal pregnancy (n = 8), accreta (n = 7), increta (n = 4), and percreta (n = 15) were extracted using the ExoQuick kit. The exosomes were labeled by a fluorescence dye and used to quantify EC and trophoblast specific exosomes respectively by CD31 or placental alkaline phosphatase (PLAP) antibody-conjugated magnetic beads and flow cytometry. CD31+ exosomal mRNAs were profiled by RNA-seq and their function were analyzed by bioinformatics. Circulating CD31+ (but not PLAP+) exosomes were significantly higher (P<0.001) in sera of PAS vs. normal pregnant women and correlated (P<0.05) with PAS severity, increased by 8.93±4.75, 9.34±6.02, and 15.37±17.83-fold in accreta, increta, and percreta, respectively. There were 1360 differentially expressed mRNAs in circulating CD31+ exosomes in PAS vs. normal pregnant women, with 841 upregulated and 519 downregulated. Clusters of apoptotic and anti-proliferative genes were downregulated, whereas clusters of pro-proliferative and pro-angiogenic genes were upregulated in PAS CD31+ exosomes. Thus, significantly greater quantity of circulating CD31+ exosomes and its positive correlation with disease severity suggest that circulating CD31+ exosomes is a potential biomarker for PAS; the anti-apoptotic, proliferative, and angiogenic mRNA signature suggest that circulating CD31+ exosomes participate into the pathogenesis of PAS. (Supported by NIH RO1 HL70562, AR073172 & U01 HD087209).

**P574 - Loss of REST in uterine fibroids leads to aberrant CaV2.1 expression.** Fatimah Aljubran, Michelle McWilliams, Faezeh Koohestani, Sornakala Ganeshkumar, Vargheese Chennathukuzhi

Uterine fibroids are benign tumors of the myometrium. They are characterized by smooth muscle proliferation and overproduction of extracellular matrix proteins. Pregnant women with uterine fibroids are at higher risk for pregnancy complications such as preterm labor and cesarean section. The etiology of such complications is not clear. Irregular uterine contractions have been reported in uterine fibroids, which may play a role in preterm labor. REST (RE1 Silencing Transcription factor) is a transcriptional repressor of neuronal genes in non-neuronal tissues. We had earlier shown that REST expression is lost in uterine fibroids leading to upregulation of target genes and manifestation of uterine fibroid features. RNA sequencing data from our Rest
cKO mice (Rest f/f Amhr2+/Cre) showed a significant upregulation of CACNA1A, which encodes the pore forming subunit of calcium voltage gated channel 2.1 (CaV2.1). According to ChIP-seq data, CACNA1A is a target gene for REST mediated repression. CaV2.1 is predominantly expressed in the brain and mediates the entry of calcium ions into excitable cells. We evaluated mRNA and protein levels of CaV2.1 in human tissue samples using qPCR, western blot, and immunofluorescence. We found CaV2.1 overexpressed in uterine fibroid tissue samples compared to matched myometrium. Ca++ ions provide one of the major regulatory mechanisms of smooth muscle contraction. We hypothesize that elevated intracellular Ca++ concentration can disrupt the normal peristalsis of the myometrium. Loss of REST and the ensuing expression of calcium voltage-gated channel CaV2.1 may play a role in abnormal uterine contractions in women with uterine fibroids, resulting in pregnancy complications.

This research was supported by NIH Grants 1R01HD076450 and R01 HD094373.

P575 - The impact of oxygen on early trophoblast column establishment. Alexander Beristain, Jenna Treissman, Hoa Le

In early pregnancy, a shift from relatively low (i.e. 3-5%) to relatively higher oxygen levels (6-8%) occurs. However, the role that differing levels of oxygen play in the establishment of the placenta, and particularly in trophoblast differentiation into invasive extravillous trophoblast (EVT) is poorly understood. This study aims to define how differing levels of oxygen control trophoblast column formation and outgrowth. Matrigel-imbedded placental explants cultured in low (1%), physiological (5%), and high (20%) oxygen levels were used to assess the affect of oxygen on column outgrowth. Column outgrowth was examined microscopically, where morphometric measurements were correlated with global gene expression signatures and pathways generated by gene microarray analysis (N=5 explants per oxygen condition). Grossly, 1% oxygen promoted greater column outgrowth than either 5% or 20% oxygen; outgrowth length or area was not different between 5% or 20% oxygen levels. Gene array analyses (FDR < 0.05) identified 978 differentially expressed genes between 1% vs. 20%, and 769 genes between 5% vs. 20% conditions. Comparison of global gene signatures between 1% and 5% oxygen did not identify any differentially expressed genes. Anchoring columns cultured in 1% or 5% oxygen showed enrichment of genes involved in hypoxia, hormone, and growth factor signaling and extracellular matrix attachment. By comparison, anchoring columns established at 20% oxygen showed enrichment in genes involved in the promotion of the cell cycle. The gene lysyl oxidase (LOX) was consistently upregulated in trophoblast columns established at low oxygen, where inhibition of LOX activity led to impaired column outgrowth. Our findings suggest that low oxygen promotes column outgrowth by stimulating EVT migration and matrix interaction, while higher levels of oxygen promote anchoring column expansion through pro-proliferative mechanisms. Together, these findings provide novel insight into how specific levels of oxygen in early pregnancy control placenta development by regulating trophoblast biology.

P576 - Apelin in the human trophoblastic cells: expression, signalling pathway, proliferation, cell cycle and hormone secretion. Ewa Mlyczyńska, Monika Dawid, Patrycja Kurowska, Eliza Drwal, Malgorzata Opydo-Chanek, Waclaw Tworzydlo, Agnieszka Rak
Placentation requires the production of many growth factors, hormones and transcription factors. Many of them, like the adipose tissue-derived leptin or adiponectin, have been identified in the placenta, and their role has been established in proliferation and subsequent development of placenta. Apelin is another adipocyte-derived signalling molecule known to exert proliferative effects in various cell types. In the present study, using real-time PCR, immunoblotting and immunocytochemistry, we showed expression of apelin and its receptor (APJ) in syncytiotrophoblast (BeWo) and cytotrophoblast (JEG-3) cells. Apelin was higher in JEG-3 than BeWo (n=3, p<0.05), while APJ was the same in both placenta cells. Immunocytochemistry revealed high cytoplasmic and/or membrane apelin localisation in JEG-3, while BeWo cells exhibited markedly weaker apelin signal in the cytoplasm. Additionally, the effect of different doses of apelin (0.02 to 200 ng/ml) on cell proliferation, cell cycle, protein expression of cyclins, phosphorylation level of ERK1/2, Akt, Stat3, AMPKα kinases and hormones (progesterone – P4, estradiol – E2 and human chorionic gonadotropin – hCG) concentration were studied in BeWo cells. Statistical analysis were performed using GraphPad Prism 5 and a one-way ANOVA test. We observed that apelin significantly increased cell proliferation as well as the percentage of cells in G2/M phase of cell cycle, cyclin proteins and all kinases phosphorylation (n=3, p<0.05). Moreover, we showed that ERK1/2, Stat3 and AMPKα were involved in the mitogenic action of apelin. Additionally, this effect was abolished when cultured in the presence of ML221, an APJ antagonist. Our results clearly demonstrated that apelin have inhibitory action on hormones secretion (n=3, p<0.05). In conclusion, apelin by promotion placenta cell proliferation via APJ activation, and different signalling pathways can be considered as newly identified regulator of early placental development.

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**P577 - Characterization of natural and synthetic molecules to reduce inflammation associated with gestational complications.** Jovane Hamelin Morrissette, Julie Girouard, Gervais Bérubé, Carlos Reyes-Moreno

The population of the uterine macrophages (Mφs) is exceptional in its ability to protect the placenta and the developing embryo from a variety of pathogens. However, aberrant activation of inflammatory pathways in uterine/placental Mφs can affect trophoblast survival and function and potentially induce pregnancy complications, such as preterm birth (PTB) in human. To this day, treatment options for PTB remains scarce. This study aims to characterize the anti-inflammatory action of a natural molecule, leukemia inhibitory factor (LIF), and a novel class of synthetic molecules derived from aminobenzoic acid (DABs), which could be used to reduce macrophage-mediated inflammation in the case of PTB. In a first attempt to provide evidence of our premise, we studied the ability of LIF and DABs to regulate inflammatory signal transduction in human monocyte-derived Mφs (hMDMφs) and mouse peritoneal Mφs (mPMφs), which were activated with the pro-inflammatory cytokines gamma-interferon (IFNγ) and granulocyte-macrophage colony-stimulating-factor (GM-CSF), or bacterial lipopolysaccharide (LPS). We found that LIF and DABs reduced the activation levels of pro-inflammatory signaling molecules (STAT1, STAT5, NFkB, p38), and the expression levels of surface markers (CD40 and MHC-II) in cytokine-activated Mφs. In mice, intraperitoneal injections with either LIF or DABs reduce the expression of inducible nitric oxide synthase (iNOS) expression and NO production by mPMφs.
Subchronic toxicity studies on mice show that DAB molecules have no effect on normal development and viability in mice. These results indicated that the inflammatory signaling pathways associated with pregnancy complications or gynecological diseases may be targeted effectively with a combination of LIF and DABs. This work was supported by grants from the Natural Sciences and Engineering Research Council of Canada (NSERC), the Fonds Québécois de la Recherche sur la Nature et les Technologies (FQRNT), and the Réseau Québécois en Reproduction (RQR).

**P578 - Progesterone receptor membrane component-1 (PGRMC1) is upregulated by progesterone in the highly dynamic uterine junctional zone of the macaque myometrium.**

Christopher Keator, Ov Slayden

Rhesus macaques display menstrual cycles with an extended luteal phase similar to women. While the cyclic change in nuclear progesterone receptors (PGR) has been well characterized, much less is known about membrane receptors. We reported that PGRMC1 and PGRMC2 are expressed in the primate endometrium; levels of PGRMC1 transcript were highest during the proliferative phase whereas PGRMC2 mRNA was elevated under the influence of progesterone and highest during the secretory phase of the menstrual cycle. Recent studies indicate relatively high levels of both PGRMC1 and PGRMC2 in the myometrium of women during pregnancy and both proteins decline markedly during the onset of labor, which is likely due to the withdrawal of progesterone at the end of gestation. In this study we determined the temporospatial changes in PGRMC1 and PGRMC2 in the rhesus macaque myometrium during the menstrual cycle. Samples were collected during menses, the proliferative phase, the mid-secretory (equivalent with the window of implantation) and the late-secretory phase of the cycle. Quantitative real-time PCR (qPCR) revealed a significant (P<0.05) regulation in the myometrial expression of PGRMC1, with a notable increase detected during the late-secretory phase of the cycle. In contrast, qPCR revealed no change (P>0.05) in PGRMC2 during the cycle. Localization with in situ hybridization and immunocytochemistry for PGRMC1 revealed relatively steady levels in the outer two-thirds of the myometrium, whereas the inner junctional zone, the layer of the myometrium closest to the endometrium, exhibited dynamic regulation during the cycle, with the strongest expression level and staining during the late secretory phase. Coupled with the other studies in the gravid uterus, we hypothesize that PGRMC1 could mediate steroid trafficking in the myometrium. Further studies are required to determine if PGRMC1 in the uterine junctional zone is essential for progestational quiescence required for a successful pregnancy. Funded by HD055744 and P51-OD011092.

**P579 - Sire influences gene expression in trophoblast cell markers in vitro.**

M.Sofia Ortega, Thomas Spencer

Pregnancy establishment in cattle is complex and encompasses ovulation, fertilization, blastocyst formation and growth into an elongated conceptus, pregnancy recognition signaling, and development of the embryo and placenta. Previously, Holstein bulls were classified as high or low fertility based on their sire conception rate (SCR) value (Biol Reprod 2018;99:1244).
Several low fertility sires (SCR<-5) exhibited a two-fold increase in pregnancy loss between days 19 and 32 post-breeding compared to high fertility sires (SCR>+5). Our hypothesis is that low fertility sires have a defect in placental development that compromises pregnancy establishment. Here, trophoblast cell lines were derived from in vitro produced blastocysts generated using high or low fertility Holstein bulls. Groups of cumulus-oocyte complexes were split and fertilized with semen from a high fertility or a low fertility sire. After 8 days of culture in synthetic oviduct fluid, hatched blastocysts were collected and placed individually in a 48-well plate coated with type-IV collagen with bovine fetal fibroblast-conditioned medium containing 10% fetal bovine serum for at least 7 days. Ten cell lines per bull were produced from two in vitro embryo production sets. Trophoblast cell outgrowths contained both mononucleate (MNC) and binucleate cells (BNC). At second passage, expression of MNC marker (CDX2, FGF4, GATA2, GATA3, HAND1, IFNT) and BNC marker (TKDP1, CSH1, PAG1) genes was assessed by qPCR. Expression of HAND1, CDX2, FGF4, CSH1, and PAG1 was lower (P < 0.001) in cells produced with low fertility bulls compared to high fertility bulls, whereas GATA2 and GATA3 were not different. This study and others highlight the importance of understanding the genetic contributions of the sire to pregnancy establishment that is crucial to increase reproductive efficiency in dairy cattle. Supported by AFRI 2019-67015-28998 from the USDA-NIFA.

**P580 - Fetal T cells secrete cytokines elevated during labor in response to antigen at the fetal-maternal interface.** Jessica Toothaker, Collin McCourt, Stephanie Stras, Oluwabunmi Olaloye, Liza Konnikova

During pregnancy, the maternal immune system maintains must tolerate paternal antigens to prevent rejection of the developing semi-allogenic fetus. In healthy pregnancies, labor is marked by the well-defined transition to an inflammatory state in reproductive tissues. It has been postulated that cytokine release is the resultant of maternal immune cells’ loss of tolerance towards the fetus. However, recent data show the presence of memory T cells in cord blood that secrete cytokines in response to maternal antigens suggesting that the fetal immune system might also play a role in initiating labor. To address directly if fetal T cells are found at the fetal-maternal interface we performed deep immunophenotyping and functional analysis of cells present at the feto-maternal interface we characterized immune cells using mass cytometry (CyTOF) that allows for simultaneous evaluation of ~40 antigens on a single cell basis. We identified diverse T cell populations in the fetal placental tissues (placental villi and fetal membranes), including effector, central and tissue resident memory T cells in the second trimester and at full term. These T cells secrete cytokines under baseline conditions. Additionally, our work agrees with previous work detecting an abundance of CD8+ effector memory T cells in the maternal decidua. Furthermore, after stimulation with maternal (decidual) components from the same pregnancy, 30% of fetal T cells from the second trimester placental villi secrete TNFa to an even greater extent than when stimulated with PMA/Ionomycin (27%) but fail to secrete TNFa when stimulated with unrelated PBMCs (1.62%). This result suggests fetal T cells within placental villi respond to antigens on the maternal side of the interface, but not when exposed to blood antigens from unrelated donors. Collectively these findings illustrate a dynamic, functional arsenal of fetal T cells in the second trimester, that could potentially contribute to the onset of labor.
**P581 - Wnt-/Cadherin- signaling relating genes cause madibular dyplasia in cloned dogs.**
Sun-A Ock, Dajeong Lim, Seunghoon Lee, Yong-Ho Choe, Hyeon-Jeong Lee, Sung-Lim Lee

Brachygnathia inferior, abnormal shortness of the lower jaw is mostly hereditary malformation in the animals, but it’s very rarely found in dogs. We produced four lived cloned puppies (NT-1, -2, -3 and -4) from a single normal original dog (OD), and their microsatellites sequence was analyzed and confirmed as identical. However, Two (NT-1 and -3) of them show serious shortness of mandibular and were diagnosed as brachygnathia inferior. Karyotyping and whole genome sequencing (WGS) were performed to investigate the genetic cause of brachygnathia inferior. While chromosomal abnormality was not detected in karyotype, 214 unique single nucleotide variants (SNVs), only found in NT-1 and NT-3, were filtered from WGS data. These brachygnathia inferior dogs-specific SNVs were distributed in certain 50 different genes, we discovered that these genes were specifically associated with Wnt-/Cadherin- signaling pathway and were also key components of them. Especially arrestin beta 2 (ARRB2), frizzled class receptor 4 (FZD4), and disheveled binding antagonist of beta-catenin 1 (DACT1) have an important role in Wnt-/Cadherin signaling and are closely interacting with other components. Besides each variant in these 3 genes are located in protein coding region, so are predicted as a high and moderate disease-damaging effect. Although other 211 SNVs in 47 genes are located in intergenic and non-coding region, they also interfere with the regulation of transcription and its binding process. Furthermore, because the number of variants in these single genes has been accumulated, that interfered process can be worsen.

Our results suggest that multi-layer variants of Wnt-/Cadherin- signaling-related genes are occurring in the reprogramming or development of cloned dogs, which may affect mandibular development. This is the first report of cloned dog with serious brachygnathia inferior and reveals that the multiple mutations in Wnt-/Cadherin- signaling pathway cause brachygnathia inferior in dogs.

**P582 - FGL2-Associated Transcriptional and Histopathological Features of Immunological Preeclampsia.** Pascale Robineau-Charette, Shannon Bainbridge, Barbara Vanderhyden

Preeclampsia (PE) is a hypertensive disorder affecting 5-8% of pregnancies, caused by faulty placentation. Significant heterogeneity exists in clinical presentation of the disease, fetal health outcomes and placental histopathology, such that no universal treatment or predictive marker is known. Our laboratory has shown the existence of molecular subtypes of preeclampsia, with distinct histopathological phenotypes and transcriptional patterns. Fibrinogen-like protein 2 (FGL2) is a secreted immunomodulator and procoagulant, two functions related to the pathology of preeclampsia. Gene expression analysis of a large cohort of healthy and preeclamptic placentas revealed differential expression of FGL2 between predetermined transcriptional clusters. Expression was low in a cluster that includes preeclamptic patients exhibiting features consistent with the traditional paradigm for disease pathology. Expression was high in an intriguing cluster that includes preeclamptic patients with transcriptional and histological evidence of profound inflammation. Gene Ontology analysis revealed that genes whose
expression best correlates with that of FGL2 are predominantly involved in immune response. Placentas with higher FGL2 expression were more likely to be affected by inflammatory lesions such as villitis of unknown etiology or massive perivillous fibrin deposition, but less likely to be affected by maternal vascular malperfusion lesions, commonly associated with PE diagnosis. Placentas affected by inflammatory lesions showed upregulation of FGL2 and genes involved in macrophage signaling and interestingly, downregulation of commonly accepted PE markers such as FLT1 and ENG. In the villous space, FGL2 expression localized to cytotrophoblast, syncytiotrophoblast and leukocytes, including macrophages. Knockout or overexpression of FGL2 in HTR-8/SVneo and BeWo cells led to only minor functional impairments. Several inflammatory cytokines upregulate FGL2 expression, suggesting FGL2 is a downstream or feed-forward factor in trophoblast-immune cell crosstalk. Overall, this work suggests an important contribution of FGL2 to features specific to immunological PE and highlights the potential of this secreted factor as a subtype-specific biomarker.

**P583 - Temporal Expression Pattern of Fabp3 during Mouse placenta Development.** Hui Gao, Yichen Wang, Liu Tian, Changjun Zhang, Honglu Diao

Placenta is unique and temporary organs during pregnancy. It plays an important role in the matter of transportation between maternal and fetal, and also protects a fetal from maternal immune attack and external toxic substances. The placenta can produce a variety of peptide and steroid hormone to maintain pregnancy and fetal growth. Fatty acids in maternal blood, transfer to the fetus via the placenta. Fatty Acid Binding proteins (FABPs) is coding of 15 KDa protein gene family members, it has the ability of combining thin biological molecules, such as fatty acid, to ensure the normal metabolism and transport. Fabp5 is a member of, family of highly homologous cytosolic proteins and can bind a variety of hydrophobic compounds, including fatty acids, various cyclooxygenase and lipoxygenase metabolites, and retinoic acid. The functions of Fabp3 are still unclear in the uterus during the process of placenta development. The aim of this project is to examine the uterine expression and regulation of Fabp3 during pregnancy in mice and its regulation under steroid hormone treatment conditions and in vitro culture of HTR-8/Snevo cells line by Realtime PCR, In Situ Hybridization, and Immunofluorescence. Our Pre-limited data showed the Fabp3 mRNA and protein mainly expressed in the labyrinthica in the mouse placenta and the expression of FABP3 mRNA was up-regulated in the steroid hormone treated mouse uterus and HTR-8/Snevo cell line treated by E2 + P4 and E2 respectively. Our Pre-limited data showed that Fabp3 should have the important function during placenta development. Next step, we will identify molecules and proteins that can directly interact with Fabp3 and downstream signaling pathways affected by Fabp3 deletion in mouse in vitro HTR-8/Snevo cells line and we will also learn the effect on transport of fatty acids.

**P584 - Uterine Gland Influence on Development of the Placenta and Fetus.** Pramod Dhakal, Susanta Behura, Harriet Fitzgerald, Andrew Kelleher, Thomas Spencer

Forkhead box a2 (FOXA2) is a gland-specific transcription factor critical for postnatal uterine gland development and adult uterine gland function. In the mouse uterus, Foxa2 regulates a large number of hormone-regulated, gland-specific genes, including the critical implantation factor
leukemia inhibitory factor (Lif). Foxa2 conditional knockout (cKO) mice, generated using lactotransferrin (Ltf)-iCre and floxed Foxa2 mice, are infertile due to defects in blastocyst implantation, however injections of Lif on gestational day (GD) 4 restored fertility with the birth of live pups at term (PNAS 2017; 114:E1018). Although fertility was restored, subtle differences in decidual marker gene expression were observed on GD 6 and 10 in Lif-replaced uterine Foxa2 cKO mice. The goal here was to begin testing the hypothesis that Foxa2-deficient uterine glands will impact placental and fetal growth. First, placentas from Lif-repleted wildtype control and Foxa2 cKO mice were assessed on GD 15. Placentas were not histologically different. RNA-Seq analysis found 385 genes were decreased (FDR P<0.05) in placentas from uterine Foxa2 cKO mice, including prolactin (Prl) family members and cyclins, but no genes were increased in Foxa2 cKO mice. Next, a littermate study was conducted using bred Control and Foxa2 cKO female mice. On GD 12.5, implantation site number was lower (P<0.05) in the Foxa2 cKO as compared to Control dams (7.3±1.8 vs 9.0±1.1). Placentae of female pups, but not male pups, weighed 15.2% less (P<0.001) in Foxa2 cKO dams. Male and female fetuses from Foxa2 cKO dams weighed 13.8% and 22.2%, respectively, less (P<0.001) than from Control dams. These results support the idea that Foxa2-regulated factors produced by the uterine glands directly or indirectly, via the decidua, impact placental and fetal growth in a sex-dependent manner. Supported in part by NICHD Grants R21 HD076347 and R01 HD096266.

P585 - Normalizing the immune system of the rat for analysis of immune cell function at the maternal-fetal interface. Regan Scott, Michael Soares

Rats are a useful model to study pregnancy disorders because they possess a hemochorial placenta with deep trophoblast invasion and spiral artery remodeling. We know that immune cells play an essential role in placental development, and disruption of immune cell interactions within the uterus can compromise the establishment of pregnancy. However, recent studies have shed light on the limitations of experiments performed in specific pathogen free (SPF) laboratory rodents because these animals exhibit limited immune system development that does not resemble the complexity of the adult human immune system. Accurate recapitulation of a complex immune system in an animal model is critical for drawing biologically relevant conclusions of the role of immune cells in pregnancy. To address this limitation, we have developed a co-housing strategy to generate laboratory rats with mature immune systems. Laboratory rats are co-housed with rats previously maintained in an environment of robust pathogen exposure (“dirty rats”). Pathogen transmission to exposed laboratory rats was validated through PCR of rodent infectious agent panels, and after thirty days of co-housing, profiles of peripheral blood mononuclear cells (PBMCs) exhibited an up-regulation of transcripts associated with several pathways reflecting an activated immune response. Spleen weights increased at thirty days of co-housing but returned to normal weight at sixty days of co-housing, suggesting laboratory rats’ immune systems had been activated by exposure to pathogens, but had “normalized” by day sixty. Following 60 days of pathogen exposure, laboratory rats were mated. In contrast to SPF laboratory rats, pregnancy outcomes of pathogen-exposed laboratory rats were highly variable and included significant fetal loss. Thus, the immune adaptations to pathogen exposures were not favorable for pregnancy success. We hypothesize that this immune adapted rat model will provide a more accurate representation of the complex interplay of immune cells at the maternal fetal interface.
P586 - Embryonic Deletion of Atypical Chemokine Receptor 3 (Ackr3) Stimulates Immune Cell Recruitment and Spiral Artery Remodeling in the Decidua. Kelsey Quinn, Kathleen Caron

Preeclampsia (PE) is one of the leading causes of fetal and maternal mortality. Although the cause of PE is unknown, it is strongly associated with the placenta, as the only cure is pre-term delivery. A critical characteristic of PE is an imbalance of immune and trophoblast cells, leading to the persistence of narrow, hypertensive spiral arteries during mid-gestation. Chemokines are regulators of immune cell recruitment and angiogenesis during placental development. Atypical chemokine receptor 3 (ACKR3) is a unique chemokine receptor that can sequester ligands and thus control their availability to bind to other receptors. However, the role of this receptor during placentation is still unknown. Recently, through single cell analysis, ACKR3 was shown to localize to endothelial cells of the human placenta during early gestation. Therefore, we hypothesized that ACKR3 may contribute to placental development by governing immune cell recruitment and trophoblast migration.

Placental tissue from embryos lacking Ackr3 (Ackr3−/−) at e12.5 exhibited an increase in cell density, signifying potential changes in immune cell populations. Indeed, fluorescent staining and flow cytometry of immune cells showed significantly higher numbers of uNK and dendritic cells in placentas of Ackr3−/− mice compared to littermate controls. Interestingly, fetal sinus area within the labyrinth of Ackr3−/− also increased with very little trophoblast invasion. Consistently, knockdown of Ackr3 in trophoblast cells resulted in decreased migration. In Ackr3−/− e14.5 placenta, lumen size of spiral arteries significantly increased compared to their wildtype counterparts, suggesting that the influx of immune cells and decrease in trophoblast migration may augment remodeling. Thus, the ACKR3 receptor serves as a direct mediator of spiral artery remodeling by fine-tuning precise levels of immune cells and promoting trophoblast migration within the placenta. This work was supported by The Lalor Foundation Fellowship (to KEQ) and NIH Grant 5R01HD060860-07 (to KMC).


Besides serving as a conduit for solutes, the placenta possesses a tightly regulated endocrine system that is, of itself, vulnerable to endocrine-disrupting chemicals (EDC) that interfere with the production of or simulate the actions of hormones. Yet, there is limited information on how EDCs affect the placenta. In rodents and humans with a hemochorial placenta, fetal placenta cells are susceptible to such compounds as they are bathed directly in maternal blood. To understand how EDCs affect the placenta, C57BL6J mouse dams were fed 200 ug/kg body weight bisphenol A (BPA) or bisphenol S (BPS) daily for two weeks and then bred to C57BL6J males. Dams received these chemicals through 12.5 days post-coitus, whereupon placental and fetal tissues were collected, and the sex of each conceptus determined. Phenotypic profiles of male and female placenta exposed to BPA and BPS were compared to unexposed controls. BPA
and BPS altered placental transcriptome profiles, including reductions in placental enriched genes, e.g., Gm9513 and Sfrp4. Functional enrichment analysis revealed the Wnt signaling pathway was likely disrupted in the placenta of both BPA and BPS exposed individuals. Treatments caused changes to the fetal placental architecture, including maternal blood vessel dilation within the labyrinth region of BPA exposed females and reductions in the spongiosotrophoblast zone to multinucleated giant cell area. Both chemicals increased placental dopamine but dramatically reduced serotonin concentrations. BPS exposed female placenta also had significant reductions in fatty acids, including docosahexanoic acid (DHA), cholesterol, and amino acids. Placental concentrations of estradiol were decreased with BPA exposure. Current results show that while some BPA/BPS induced effects in the placenta were similar, others diverged. Imbalances in neurotransmitters, fatty acids, amino acids, and estradiol caused by BPA and BPS would likely impair fetal brain development, and thus, could be means whereby these chemicals affect the placental-brain axis.

**P588 - Cell Morphology and Differentiation in a 3D Human Uterine Myometrial Tissue Model.** Anutr Sivakoses, Craig Ulrich, Jiavanna Wong-Fortunato, Janet Lambert, Iain Buxton, Heather Burkin

Our goal is to develop a 3D model of human uterine myometrial tissue for use in experiments to understand molecular pathways that lead to the onset of uterine contraction, parturition, and preterm birth. Regulation of human parturition is not fully understood and often differs from regulation in animal models, and two-dimensional cell culture models do not always mimic the characteristics of three-dimensional tissue. The objective of this work is to develop and validate a new 3D human myometrial tissue model for use in contractile and other functional studies. We performed experiments to test the hypothesis that synthetic human myometrial tissue contains cells that are morphologically similar to native uterine smooth muscle cells and appropriately express differentiation markers. Term human uterine myometrial tissue was obtained with informed consent and IRB approval. Myometrial cells were mechanically dissociated, isolated, and allowed to proliferate in 2D culture for 5-9 passages. Trypsinized cells were rinsed with calcium-free PBS and resuspended to 50 million cells/mL in alginate solution (Aspect Biosystems) with 5 mg/mL purified human myometrial extracellular matrix protein. A microfluidic device was used to deposit myometrial cells in 15 mm rings. Cells within synthesized rings were allowed to proliferate for 24 h and then transferred to differentiation media containing insulin, transferrin, and selenium (ITS) with estrogen and progesterone and allowed to differentiate and form interconnected networks for at least 7 days. Rings were incubated with Calcein AM, Hoescht, and propidium iodide to assess cell viability. Confocal microscopy was used to assess cell morphology and to detect uterine smooth muscle proteins. Cells within the synthetic tissue rings displayed >90% viability and appropriately expressed cell smooth muscle actin and oxytocin receptor. These results indicate cultured uterine myometrial cells retain some ability to re-differentiate when printed into synthesized tissue rings.

**P589 - CITED2 Disruption in the Mouse and Rat Exhibit Distinct Developmental Phenotypes.** Marija Kuna, Pramod Dhakal, Lindsey Kent, Khursheed Iqbal, Michael Soares
CBP/p300-interacting transactivator with glutamic acid/aspartic acid-rich carboxyl terminal domain 2 (CITED2) is a member of the CITED protein family and has been shown to possess critical roles in cellular development and differentiation. CITED2 regulates the recruitment of CBP/p300, histone 3, lysine 27 acetyltransferases, to transcription factors such as HIF1 and TFAP2C. The activity of some transcription factors is enhanced by CITED2, whereas the activity of others is repressed. Evidence indicates that CITED2 is a conserved regulator of both rodent and human placentation. Our laboratory recently generated a rat model possessing a deletion of the complete coding sequence of CITED2 using Crispr/Cas9 genome editing. As we began characterizing the mutant CITED2 rat strain it became evident that differences exist between CITED2 null rat and mouse phenotypes. In this study, we compared phenotypes of our mutant CITED2 rat strain with a CITED2 mutant mouse model (PNAS 99:10488-10493, 2002). CITED2 disruptions were investigated on outbred genetic backgrounds (rat: Holtzman Sprague-Dawley; mouse: CD1). Heterozygous males and females of each species were mated, and placentation sites and fetal development examined at various gestational stages. CITED2 deficiencies in the mouse and rat shared deficits in placentation, intrauterine fetal growth restriction, and dysmorphic lung development. However, prominent species differences were also observed. CITED2 disruption in the mouse resulted in prenatal lethality, exencephaly, and adrenal gland agenesis. In contrast, in the rat CITED2 null fetuses progressed through pregnancy and died immediately after birth with intact adrenal glands and without signs of exencephaly. Comparison of mouse and rat models of CITED2 deficiency demonstrated some conserved roles for CITED2 in placental and fetal development but also fundamental species differences. CITED2 is probably not unique. Thus caution may be warranted in generalizing gene regulatory actions from observations of gene disruption in a single species. (Supported by HD020676; HD079363; Sosland Foundation)

P590 - Estrogen Stimulates Human Uterine Artery Endothelial Cell H2S Biosynthesis via Estrogen Receptor- and Pregnancy-Dependent Cystathionine b-Synthase Upregulation in vitro. Thomas Lechuga, Qian-rong Qi, Dong-bao Chen

Pregnancy is a physiological state with dramatically elevated endogenous estrogens that stimulate uterine blood flow important for pregnancy health. Hydrogen sulfide (H2S) is biosynthesized from L-cysteine by cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE). Pregnancy and estrogens augment H2S biosynthesis in intact uterine arteries (UA) in vivo. However, it is yet to be determined if pregnancy and estrogens play a synergistic role in UA H2S production. This study was to determine if estrogens stimulate UA H2S biosynthesis via estrogen receptor (ER)- and pregnancy-dependent mechanism. Primary human uterine artery endothelial cells (hUAEC) were isolated from primary UAs from hysterectomies of nonpregnant (NP) and late pregnant (P) women. hUAEC were serum-starved overnight and treated with or without 17b-estradiol (E2b, 10 nM) in the absence or presence of ICI 182, 780 (1 µM) for 48 h. Levels of CBS and CSE mRNA and protein were determined by qRT-PCR and immunoblotting, respectively. H2S production was determined by the methylene blue assay with or without specific inhibitors of CBS (CHH, 2 mM) or CSE (BCA, 2 mM). Baseline CBS, but not CSE, protein was significantly lower in NP- vs. P-hUAEC (P < 0.05). Baseline H2S production was also greater in P- vs. NP-hUAEC (P < 0.05). Treatment with E2b significantly
stimulated CBS, but not CSE, mRNA and protein expression and H₂S production in P-hUAEC. ICI 182,780 blocked E₂b-stimulated CBS mRNA/protein and H₂S production in P-hUAEC (P < 0.05). Moreover, incubation with CHH but not BCA blocked pregnancy-enhanced baseline and E₂b-stimulated H₂S production in P-hUAEC. Thus, pregnancy potentiates estrogen-simulated H₂S biosynthesis by estrogen receptor-dependent upregulation of CBS expression in hUAEC in vitro (AHA POST34380384 and NIH RO1 HL70562).

**P591 - Circadian Rhythms of Clock Genes and Angiogenic Factors in Bovine Placental Explants.** Zully Contreras-Correa, Racheal Lemire, Derris Burnett, Caleb Lemley

Clock genes regulate biological functions and target multiple physiological pathways leading to the entrainment of circadian rhythms. Currently, a paucity of information exists for clock gene expression and circadian rhythms in the bovine placenta. For this study, pregnant heifers (n = 12) were allotted 100% (adequate fed) or 60% (restricted fed) of nutrient requirements from day 50 to 180 of pregnancy. Placentomes were collected via Cesarean section at day 180 of gestation. Cotyledon fragments were cultured for 24 hours and sampled every 4 hours for RNA extraction. Subsequently, circadian rhythms were determined for clock genes (ARNTL, CRY1, and PER2), angiogenic factors (HIF1A and VEGFA), and nutrient sensing genes (NAMPT and NR3C1). The most stable reference genes (GAPDH, SF3A1, and RPL19) were used to normalize transcript abundance of target genes. Data were analyzed using repeated measures ANOVA. Early gestational nutrient restriction did not affect transcript abundance; therefore, time main effect is reported. Clock genes were expressed in the cotyledon explants with ARNTL decreasing and CRY1 increasing over time (P < 0.005). The transcript abundance of PER2 was not different over time (P > 0.05). Angiogenic factors and nutrient sensing genes displayed a circadian rhythm (P < 0.001). During the scotophase, HIF1A increased (P < 0.0001) while VEGFA decreased (P < 0.001) showing a negative relationship; although, both increased during the photophase (P < 0.005). Additionally, NAMPT increased during the scotophase (P < 0.001) and exhibited stability during the photophase (P > 0.05). Whereas, NR3C1 decreased during scotophase and increased during photophase (P ≤ 0.0001). In conclusion, clock genes are expressed in the cotyledon of the bovine placenta. Interestingly, circadian oscillations were observed for angiogenic factors and nutrient sensing genes suggesting that time of sample collection could influence observed results. This project was supported by AFRI Competitive Grant no.2018-67016-27580 from the USDA-NIFA.

**P592 - ASCL2 is Essential for Invasive Trophoblast Lineage Development.** Kaela Varberg, Regan Scott, Masanaga Muto, Khursheed Iqbal, Keisuke Kozai, Elin Grundberg, Michael Soares

The invasive trophoblast cell lineage is critical to spiral artery remodeling and placentation. Insufficient trophoblast invasion, and subsequent vascular remodeling, can lead to pregnancy disorders including preeclampsia, preterm birth, and intrauterine growth restriction. The objective of this study was to identify critical and conserved gene regulatory networks of trophoblast lineage development. Previous studies in the mouse identified Achaete-scute family bHLH transcription factor 2 (ASCL2) as essential to extraembryonic development, including
junctional zone and invasive trophoblast progenitor cell formation. The mouse exhibits shallow trophoblast invasion and is not an optimal animal model for investigating events at the trophoblast-uterine interface. In contrast, the rat possesses deep intrauterine trophoblast invasion, similar to human placentation. We hypothesized that ASCL2 is essential for invasive trophoblast lineage development. To test our hypothesis, we established an Ascl2 mutant rat model and investigated invasive/extravillous trophoblast (EVT) cell differentiation using a human trophoblast stem cell model. Rat Ascl2 was successfully targeted using CRISPR/Cas9 genome editing to generate a germline mutation. Similar to murine in vivo studies, Ascl2 mutant rats exhibit embryonic lethality. The temporal onset of prenatal Ascl2 mutant death and impact on placental organization, including invasive trophoblast lineage development, are under investigation. Differentiation of human trophoblast stem cells into EVT cells resulted in significant upregulation of ASCL2 (n=6; p<0.0001) and a number of transcripts indicative of EVT differentiation, including major histocompatibility complex, class I, G. shRNA-mediated knock down of ASCL2 impaired EVT cell differentiation as indicated by altered cell morphology and gene expression profiles. RNA sequencing analysis of ASCL2-depleted trophoblast cells identified both downregulation of EVT-associated transcripts and upregulation of syncytiotrophoblast-associated transcripts. Thus, ASCL2 possesses a key regulatory function in trophoblast development through promoting EVT cell differentiation, while concurrently suppressing syncytiotrophoblast differentiation. Future investigations will seek to determine the position of ASCL2 in the regulatory hierarchy controlling placentation.

P593 - Transcriptome Analysis Reveals the Key Regulators and Molecular Mechanisms Underlying Myometrial Activation during Equine Placentitis. Shavahn Loux, Yatta Boakari, Pouya Dini, Kirsten Scoggin, Ted Kalbfleisch, Alejandro Esteller-Vico, Barry Ball

Placentitis is the single most common cause of abortions, stillbirths, and perinatal losses in the mare. The key event in placentitis-induced preterm labor is the loss of myometrial quiescence with the subsequent initiation of labor. However, the molecular mechanisms underlying myometrial activation have not been studied so far in the mare. Therefore, we aimed to characterize the transcriptome of the equine myometrium during placentitis in comparison to gestationally matched controls. Myometrial samples were collected after euthanasia at 290 days of gestation in mares with experimentally induced placentitis (n=5) and un-inoculated control mares (n=4). Next-generation RNA-Sequencing was performed by using Illumina NextSeq500 and reads mapped to EquCab3.0 (STAR-2.5.2b). Differentially expressed genes (DEGs) identified with Cuffdiff-2.2.1 (FDR<0.05). Our study identified 596 DEG during placentitis as compared to the controls, including eight genes (MMP1, MMP8, S100A9, S100A8, PI3, APOBEC3Z1B, RETN, and CXCL2) that are expressed only in the inflamed myometrium. Overall, DEGs included several uterine contraction-associated genes that have been well-defined in human preterm labor, including PTGS2, PTGER3, MMP1, MMP8, and RLN. Pathway analysis elucidated that myometrial activation during placentitis is dominated mainly by inflammatory signals, Toll-like receptor signaling, and apoptosis pathways. Gene ontology enrichment analysis identified several chemoattractant factors in the inflamed myometrium, such as CCL2 and CXCL6. Upstream regulators analysis revealed 18 upstream regulators identified as DEGs, including transcription
regulators (E2F1, FOXM1, HIF1A, JUNB, NFKB1A, and STAT1), transmembrane receptors (FAS, ICAM1, SELP, TLR2, and TYROBP), growth factors (HGF and TGFB3), enzymes (PTGS2 and PRKCP), and others (S100A9, CD44 and C5AR1). Weighted gene co-expression analysis revealed the hub genes in equine placentitis including TLR2, PTGS2, and CXCL6. These findings revealed the key regulators and mechanisms underlying myometrial activation during equine placentitis for the first time, which might lead to the development of efficacious therapies by targeting the key molecules and pathways.

P594 - The inhibitory T cell co-receptor molecule B7-H4 (VTCN1) inhibits invasion and promotes E-cadherin expression in a stem cell-derived model of primitive human trophoblast. Jie Zhou, Yuchen Tian, Toshihiko Ezashi, Danny Schust

Introduction: Both tumors and placental trophoblast cells invade deeply into host tissues. Unlike tumors, trophoblast invasion is tightly controlled and both overly aggressive invasion (placenta accreta) and deficient invasion (preeclampsia, IUGR) are problematic. E-cadherins are involved in cell-cell adhesion and are known to be differentially expressed during malignant transformation and during trophoblast invasion and placental differentiation. We previously reported that trophoblast expression of the immune cell co-receptor, B7-H4 (VTCN1) is limited to early gestation. Since decreases in B7-H4 expression are linked to poor prognosis and metastatic potential in tumors, we hypothesized that low expression of VTCN1 in placental cells may associate with increased invasion that may mechanistically involve E-cadherin.

Objective: To study invasion and E-cadherin dynamics in human embryonic stem cell (hESC)--derived trophoblast cells with and without VTCN1 knockdown.

Methods: H1 HESCs were treated with transcriptional regulators (BAP treatment) to model for peri-implantation trophoblast development. Cells were transfected with silencing VTCN1 siRNA or control siRNA on differentiation day 3. RNA and proteins were collected and expression of B7-H4 and E-cadherin assessed using quantitative RT-PCR and western immunoblotting. Transwell cell invasion assays were used to determine trophoblast cell invasion with and without VTCN1 knockdown.

Results: Downregulation of VTCN1 expression in cells representing the earliest stages of placental development dramatically decreased the expression of E-cadherin protein and RNA and increased trophoblast invasion.

Conclusion: Human trophoblast cells express VTCN1 primarily in early gestation, when cell invasion is particularly critical. E-cadherin protein and RNA decrease and trophoblast invasion increases with VTCN1 knockdown in an in vitro model of peri-implantation trophoblast. In vivo, cytotrophoblast invasion is frequently and E-cadherin expression increased in preeclamptic compared to normotensive pregnancies. We hypothesize that physiologic downregulation of VTCN1 in normal early pregnancy supports appropriate placental development and describe transcriptional regulatory mechanisms that could be leveraged for therapy.
**P596 - Placental Extracellular Vesicles in Murine Pregnancy.** Sean Nguyen, Soo Hyun Ahn, Benjamin Collaer, Magaret Petroff

Extracellular vesicles (EVs) are nanosized, membrane-enclosed structures containing proteins, lipids, and RNAs that can travel and act on other cells in the body. The hemochorial arrangement of mouse and human placenta allows for placenta-derived materials such as EVs to be shed into the maternal blood space. We have demonstrated that maternal plasma EV concentrations increase across murine pregnancy and peak at gestational day (GD) 14.5. Studies have identified placental EV trafficking to the liver and lung; however, the cellular targets of these organs are not characterized. To identify the placental EVs in vivo, we generated a transgenic mouse model in which a male sire expresses GFP in all tissues. WT females bred to GFP sires give rise to pups that expresses GFP in all tissues including the placenta. Western blot analysis of EVs obtained by size exclusion chromatography from placental explant culture and EVs from maternal plasma both contained GFP, thus providing evidence of fetal EV contribution to total circulating maternal plasma EVs. To identify cellular targets of EVs in vivo, placental EVs (5 or 50ug) were labeled with a lipophilic red fluorescent dye (PKH26) and administered intravenously into non-pregnant and pregnant (GD14.5) mice. After 30 minutes, liver, spleen, thymus, and lung were harvested and analyzed by immunofluorescence microscopy and flow cytometry. Immunofluorescence revealed colocalization of EVs with CD31+ endothelial cells as well as F4/80+ macrophages in the lung, liver, and spleen. Flow cytometry revealed colocalization of EVs with 0.3% of liver NK cells (CD45+ MHCII- CD64- CD11bmid) and of 2% of liver monocytes (MHCII+ CD11b+ CD64+), and 17% of lung interstitial macrophages (MHCII+ CD11c+ CD11b+ CD64+). These results demonstrate that placental EVs may preferentially target myeloid cells within vascularized tissues in vivo.

**P597 - Matrix Metalloproteinases 2 and 9 Enhance the Contractile Response to Oxytocin in Human Uterine Tissue.** Craig Ulrich, Veronica Arinze, Heather Burkin

Matrix metalloproteinases 2 and 9 (MMP2/9) have previously been shown to be elevated in serum and amniotic fluid from women undergoing preterm labor. To investigate the effect of MMP2/9 on uterine contraction, we added purified MMP2/9 or the specific inhibitor SB-3CT to human uterine tissue strips. Uterine myometrial tissues were obtained from term non-laboring patients undergoing elective Cesarean section. Myometrial strips were treated with oxytocin to induce uterine contraction and baseline response was recorded for 10 minutes. Purified MMP2/9 or SB-3CT was added and changes in the contractile response were analyzed over 10-15 minutes. We observed a 36% increase in the contractile response as measured by area under the curve over time in MMP2/9-treated samples compared to vehicle controls (n=6 per group, p<0.05). In contrast, we observed a 34% reduction in the contractile response as measured by area under the curve over time in tissue treated with 1 μM SB-3CT compared to a 13% reduction in the vehicle treated controls (n=18 per group, p<0.05). The MMP2/9 inhibitor SB-3CT decreased the contractile response to oxytocin in a dose-dependent and reversible manner. These results indicate MMP2/9 rapidly affect the contractile response in uterine myometrium and suggest elevated MMP2/9 may contribute to the onset of preterm labor, while MMP2/9 inhibition can attenuate this effect.
P598 - *Preeclampsia differentially dysregulates female and male fetal endothelial function: roles of miR29a/c*. Chi Zhou, Qin Yan, Xin-wen Chang, Ronald Magness, Ian Bird, Jing Zheng

Preeclampsia (PE) impairs fetoplacental vascular function and increases risks of adult-onset cardiovascular disorders in children born to PE, implicating that PE programs fetal vasculature *in utero*. However, the underlying mechanisms remain elusive. Recently, we reported that miR29a/c regulated growth factors-induced endothelial function and were down-regulated in unpassaged (P0) human umbilical vein endothelial cells (HUVECs) from PE. We hypothesize that PE alters miR29a/c and their target genes in fetal endothelial cells and disturbs endothelial function.

RNAseq (Illumina HiSeq2000; n=6–8 individuals/sex/group) was performed with male (M)/female (F) P0-HUVECs from normotensive pregnancies (NT: 39.1±0.6weeks) and PE (37.5±0.6weeks). After RT-qPCR validation, functional genomics analysis (Ingenuity Pathway Analysis) was performed. Multiple testing was corrected using False Discovery Rate (FDR)-adjustment. Cell proliferation and monolayer integrity assays were performed with passage 1 (P1, ~5 days culture)-HUVECs with or without miR29a/c overexpression (n=5 individuals/sex/group/analysis).

PE dysregulated 926 and 172 genes in F- and M-P0-HUVECs, respectively. PE dysregulated TGFβ1 target genes in F- but not M-P0-HUVECs. PE downregulated miR29a/c in P0-HUVECs, with F-cells exhibiting a more dramatic downregulation than M-cells. PE correspondingly dysregulated more miR29a/c-target genes in F- (55) than M- (11) P0-HUVECs, and only 3 miR29a/c-targets were commonly dysregulated. Many of these fetal-sex specifically dysregulated miR29a/c-targets are associated with cardiovascular diseases (i.e. heart failure and atherosclerosis). 27% of the PE-dysregulated miR29a/c-targets in PE-F are TGFβ1 target genes. In P1-HUVECs, TGFβ1 stimulated cell proliferation in PE-F, but not in NT-F/NT-M/PE-M cells. MiR29a/c overexpression inhibited the TGFβ1-stimulated cell proliferation in PE-F, but enhanced this effect in PE-M cells.

In conclusion, PE differentially regulates miR29a/c and its targets genes in female and male fetal endothelial cells in association with differential cellular responses to TGFβ1. These fetal sex-specific PE-induced fetal endothelial responses may provide potential therapeutic targets and risk predictors for adult-onset cardiovascular diseases in children born to PE.

P599 - *Gene Expression Profiles of Immune Cells under the Influence of Bovine Trophoblast Cell Derived Extracellular Vesicles*. Ana Silva, Kira Morgado, Christopher Davies, Irina Polejaeva, Heloisa Rutigliano

Embryonic loss is an important problem in the cattle industry. Therefore, an understanding of mechanisms that regulate placental and embryonic development, such as the immune regulatory
interactions that exist between placental cells and immune cells during pregnancy, is relevant for this industry. Our hypothesis is that trophoblast cells from functional placentas secrete extracellular vesicles (EV) that can alter the gene expression profile of immune cells as a mechanism of immune regulation. To test this conjecture, bovine placental cells were isolated and cultured for 48h, conditioned media was harvested, and 50-1000 nm EVs were isolated by a differential ultracentrifugation method. Cells from ten animals were used to prepare the mix of EVs. Different immune cell populations (CD4+ CD25+ T lymphocytes, CD4+ CD25- T lymphocytes, CD8+ T lymphocytes, γ/δ−T cells, and monocytes) were sorted by flow cytometry. These cells were either cultured alone (control group) or supplemented with trophoblast-derived EVs for 48 hours. Total cell RNA was isolated for gene expression analysis by microfluidic chip, real-time RT-PCR (96.96 Dynamic Array; Fluidigm). Gene expression analysis was conducted for the following cytokines, transcription factors and CD markers: IL1, IL2, IL4, IL5, IL6, IL8, IL10, IL12B, IL13, IL15, IL17, IL18, IL23, IFNG, TNF, TGFB1, CSF2, FoxP3, Tbx21, Gata3, CD25, IL2ra, and CTLA4. A strong effect of trophoblast cell derived EV on the gene expression profile of immune cells was detected. The different cell populations analyzed responded differently to trophoblast derived EVs. Transcription factors Gata3 and FoxP3 were downregulated in both CD4+ populations, while they were upregulated in CD8+ cells. IL2 and IL17 were downregulated in CD4+CD25+ cells and upregulated in CD8+ cells treated with trophoblast-derived EVs. Both CD4+ cell populations downregulated TGFB1 expression when treated with EVs. The overall gene expression profile indicates that trophoblast-derived EVs regulate immune cell activity.

P600 - LIN28-let-7 axis Regulates Gene Expression in Ovine Placenta In Vivo. Asghar Ali, Russell Anthony, Gerrit Bouma, Quinton Winger

Abnormal placental development is one of the main etiological factors for intrauterine growth restriction (IUGR). The pluripotency factors LIN28A and LIN28B have both been detected in human and sheep placenta, and potentially regulate trophoblast cell proliferation. The LIN28 proteins are known to inhibit the maturation of let-7 miRNAs, which regulate expression of many genes post-transcriptionally. We have previously shown that knockdown of LIN28A or LIN28B results in increasing let-7 miRNA levels in trophoblast cells in vitro. Here we show significant reduction (P<0.05) in LIN28A and LIN28B and significant increase (P<0.05) in let-7 miRNAs in term human IUGR vs normal placentas. We hypothesize that the LIN28-let-7 axis regulates genes with known importance for placental development including Hmga1, Hmga2, Arid3b, cMyc, Vegf-A, IGF2bp1, IGF2bp2 and IGF2bp3. Using sheep as an experimental model, day 9 hatched blastocysts were collected by flushing the uteri of donors. Blastocysts were incubated for 4 h with lentiviral particles expressing LIN28A, LIN28B or scramble control (SC) shRNA and were surgically transferred to synchronized recipients to generate placenta specific gene knockdown. Conceptuses were collected at day 16 and showed significantly reduced (P<0.05) elongation of trophectoderm in LIN28A and LIN28B KD conceptuses compared to SC. Let-7 miRNA levels were significantly increased (P<0.05) in trophectoderm from LIN28A and LIN28B KD compared to SC conceptuses. Hmga1, Hmga2, Arid3b, cMyc, Vegf-A, IGF2bp1, IGF2bp2 and IGF2bp3 mRNAs were significantly reduced (P<0.05) in trophectoderm from LIN28A and LIN28B KD conceptuses suggesting increased targeting by let-7 miRNAs. These results suggest that the LIN28-let7 axis plays an important role in ovine...
conceptus development by regulating the expression of let-7 target genes in trophectoderm and is necessary for proper placental growth.

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**P601 - Identification of positive and negative regulatory pathways controlling rat hemochorial placental development.** Khursheed Iqbal, Jovana Rajovic, Ayesha Hasan, Jackson Nteeba, Masanaga Muto, Stephen Pierce, Keisuke Kozai, Michael Soares

The hemochorial placenta possesses a diverse set of functions ensuring survival and development of the fetus within the female reproductive tract. Execution of these functions requires synchronized temporal and spatial differentiation of progenitor cells into specialized trophoblast cell types and their organization into a hemochorial placenta. Placental development requires the activation of specific genetic regulatory programs. Insulin-like growth factor 2 (Igf2), an imprinted gene, and placenta specific 1 (Plac1), an X-linked gene have been implicated in the regulation of placental development. In vivo mouse mutagenesis demonstrated that Igf2 promotes placental growth, whereas Plac1 restrains placental growth. The mouse is a powerful model for biomedical research but has some limitations in placenta research. Alternatively, the rat has proven to be an effective model for investigating hemochorial placentation associated with deep intrauterine trophoblast invasion, as is also observed in the human. In this study, we sought to establish Igf2 and Plac1 mutant rat models using genome editing and to investigate the impact of each mutation on placentation. Rats possessing germline global null mutations at Igf2 or Plac1 loci were produced using Crispr/Cas9 genome editing. Placentation sites and fetal development were assessed during various stages of gestation in wild type, Igf2 null, and Plac1 null pregnancies. Disruption of Igf2 resulted in growth restriction, whereas Plac1 deficiency led to placental overgrowth. These findings implicate Igf2 and Plac1 as key regulators of hemochorial placenta development and provide new tools for evaluating the involvement of Igf2 and Plac1 signaling in regulating the invasive trophoblast lineage in an important experimental model of deep placentation. (Supported by an ADA fellowship to JN, AHA fellowships to MM and KK, NIH grants HD020676; HD079363, and the Sosland Foundation)

**P602 - Development of Immunotolerance in Bovine Fetuses Infected with BVDV.** Hanah Georges, Katie Knapek, Helle Bielefeldt-Ohmann, Hana Van Campen, Thomas Hansen

Bovine Viral Diarrhea Virus (BVDV) costs the cattle industry billions of dollars in performance loss annually, despite available vaccines. Known as “trojan horses”, persistently infected (PI) animals are generated through transplacental infection prior to day 125 of gestation and asymptotically shed the virus postnatally. It was hypothesized that PI fetuses have upregulated innate immune responses along with attenuated adaptive immune responses throughout gestation. This is thought to manifest through exposure to BVDV antigens during fetal lymphocytic development of central tolerance, resulting in immunotolerance to the virus. Naïve heifers were sham inoculated (controls) or infected with a non-cytopathic strain of BVDV on day 75 of gestation. Fetal spleens (n = 4-6) were collected on days 82, 97, 190, and 245 of gestation.
RNA, protein, and tissues were assayed by RT-qPCR (.r), western blot (.w), and immunohistochemistry (.i). In PI fetal spleens, during peak viremia on day 97 of gestation, a strong innate immune response (RIGI.r, IRF7.r, ISG15.r.w.i) and an attenuated adaptive response occurred; represented by an unresponsive T-cell response (CD4.r.w, CD8.r) and a decreased B-cell population (CD79.r.w.i). By day 190, 115 days post infection, the innate immune system returned to control levels, while the adaptive response became significantly down regulated (IFI30.r, LMP2.r, LMP7.r, STAT4.r, CD4.r, CD8.r, CD79b.r). On day 245 of gestation, 170 days post infection, gene expression levels became non-significant between control and PI animals, possibly due to the suppression of fetal immune responses in preparation for parturition. Collectively, the impaired adaptive response and diminished innate immune response on day 190 of gestation may reflect exhaustion of the immune responses heralding the development of immunotolerance to BVDV; revealing a potential target for intervention and prevention of persistent infection. USDA AFRI NIFA NNF 2016-38420-25289 and USDA NIFA W3112.

**P603 - New Insights into Gestational Endocrinology of the Mare.** Shavahn Loux, Barry Ball

Despite the importance of the placental unit in pregnancy, there has yet to be a large-scale study characterizing RNA expression in either chorioallantois or endometrium through gestation in any species, representing a major hole in the knowledge of gestational physiology. Therefore, we used next generation sequencing to characterize the transcriptome of the chorioallantois and endometrium in the mare (45d, 4m, 6m, 10m, 11m, PP; n=4/stage). Next-generation sequencing data were mapped to EquCab3.0 (STAR-2.5.2b) and quantified using ENSEMBL v.95 annotation (Cufflinks-2.2.1). Differentially expressed genes (DEG) were identified using one-way ANOVA with a false-discovery rate P < 0.05 (JMP 14). In total, 8,942 and 4,679 DEG were identified in chorioallantois and endometrium, respectively. Insights into steroidogenesis included the identification of high levels of CYP11A1 and CYP17A1 transcripts in the 45d placenta; suggesting that, at this stage, the placenta is able to convert cholesterol to DHEA via pregnenolone. This process has not been previously identified in placenta, instead attributed to the maternal ovary. The endometrium expressed many of the same endocrine-related transcripts as the chorioallantois; however, expression was uniformly lower with the exception of transcripts required for the final modifications to steroid hormones, such as 5α-reduction of progestogens and sulfonation of estrogens, which were substantially higher in the endometrium. At parturition, a precipitous decline in oxytocin receptor, 5α reductase 1, alpha keto reductase 1C1, aromatase (CYP19A1) and prostaglandin synthases (PGFS, PTGDS) was observed, while prostaglandin E synthase (PTGES3) and prostaglandin reductase (PTGR1) rose dramatically. Other changes of note include an increase in inhibin subunit B combined with a decrease in inhibin subunit A, suggesting a reversal of the ratio of activin A to activin B. Although further work is needed, these changes provide novel insight into endocrine control of equine pregnancy and parturition.

**P604 - Placental Nuclear and Membrane Progesterone Receptors (PGR) Expression from Early to Late Pregnancy in Sheep: Effects of Restricted Nutrition and Realimentation.** Thanya Bunma, Kimberly Vonnahme, Manuel Vasquez-Hidalgo, Kendall Swanson, Sheri Dorsam, Chainarong Navanukrav, Anna Grazul-Bilska
Nutrient restriction and/or realimentation may affect several placental functions, such as expression of selected regulatory factors, blood flow and other processes in sheep and other species. To determine the effects of plane of nutrition, nulliparous white face ewes (6 - 8 months) carrying singletons on day 50 of gestation were randomly assigned to two dietary treatments receiving 100% of NRC recommendations (CON) or 60% of CON (RES). CON ewes either remained on CON or RES until day 130, or CON ewes were RES from day 90 to 130, or RES ewes were realimented to CON from day 90 to 130. This resulted in 7 groups (n=5-6 ewes/group): CON (day 50, 90 and 130), RES (day 90 and 130), RES-CON (day 130) and CON-RES (day 130). At these time points, placental tissues were collected for evaluation of expression of PGR protein (whole tissue), and mRNA separately in maternal (caruncular, CAR) and fetal (cotyledon, COT) compartments. Evaluation of PGRAB protein using immunohistochemistry and image analysis demonstrated greater (P<0.05) expression on day 50 than 90 or 130, and overall it was not affected by plane of nutrition. In CAR, PGRAB mRNA expression was greater (P<0.04) on day 50 than 90 or 130, and PAQR7 (membrane PGR alpha) was lowest (P<0.05) in CON-RES on day 130. In COT, mRNA expression of PAQR8 (membrane PGR beta) was lowest (P<0.02) on day 50, and PAQR5 (membrane PGR gamma) was greater (P<0.03) in all nutrition groups on day 130 than any group on days 50 and 90. These data indicate that expression of PGR in ovine placenta depends on pregnancy stage, and may be affected by plane of nutrition. Overall, the mechanisms of diet and developmental effects on placental functions needs to be elucidated and warrant further investigation. USDA-AFRI grant 2016-67016-24884 to ATGB and KAV.

P605 - Interleukin 17F: A placental cytokine expressed at the uterine interface. Stephen Pierce, Khursheed Iqbal, Masanaga Muto, Keisuke Kozai, Michael Soares

Interleukin 17F (IL17F) is a member of the IL17 family of cytokines. It shares significant homology with IL17A. IL17F is expressed as a homodimer, or as a heterodimer with IL17A and transduces cellular signals through IL17 receptors A and C. IL17 signaling has been directly implicated in maternal immune activation. However, a role for IL17F in the biology of pregnancy is unknown. Preliminary data and genome-wide transcriptome analyses indicate that the placenta is a source of IL17F. This study describes the expression of IL17F in the rat placenta and the generation and initial characterization of an Il17f mutant rat strain using genome editing with the goals of investigating potential roles for IL17F in the modulation of pregnancy, placentation, and fetal development. Initially, we determined that IL17F is prominently expressed in the junctional zone (site of invasive trophoblast lineage development) of the rat placenta during the second half of pregnancy. Guide RNAs were designed to target two exons within the Il17f locus of functional significance (Exons 3 and 4). Out-of-frame mutations in offspring from recipient mothers were confirmed via PCR screening and DNA sequencing. These founder Il17f mutant rats were mated with wild-type rats in order to confirm germline transmission and to generate heterozygous pups. Il17f heterozygous males were mated with female heterozygotes in order to generate Il17f null offspring. Il17f null rats are viable, present with normal physical features, and are fertile. Initial findings indicate that basic pregnancy-related parameters (litter size, placental and fetal weights) are comparable in IL17F deficient and wild type rats in a specific pathogen free environment. In summary, IL17F is a placental
cytokine with potential actions regulating inflammatory events at the uterine interface. The Il17f mutant rat model will be used to investigate the biology of IL17F in pregnancies challenged by maternal immune activation.

**P606 - Uterine Glucocorticoid Signaling is Necessary for Placental Functions and Fetal Growth.** Shannon Whirledge, Andreanna Burman, Edwina Kisanga, Seth Guller

Intrauterine growth restriction (IUGR) is the second leading cause of fetal and infant morbidity and mortality, occurring in up to 10% of all pregnancies. Despite the prevalence and severe consequences of IUGR, the molecular mechanisms causing placental insufficiency and subsequent growth restriction are poorly understood. We recently developed a uterine-specific glucocorticoid receptor (Ut GR KO) knockout mouse model to evaluate the role of glucocorticoids during pregnancy and made the unexpected discovery that fetuses of the Ut GR knockout mice develop IUGR. The objective of this study was to examine the mechanism of IUGR in this new in vivo model. To accomplish this goal, timed pregnancies were evaluated in control and Ut GR KO mice. Prior to the establishment of the placenta, we found no difference in gestational sac size in the Ut GR KO mice compared to controls. However, by day 11.5 of pregnancy the gestation sac was significantly smaller in Ut GR KO mice compared to controls. On day 15.5 of pregnancy, fetuses from the Ut GR KO mice weighed 35% less than those from controls and placentas weighed 20% less. Pups from Ut GR KO dams remained smaller at birth. Histological analysis revealed altered placental morphology in the Ut GR KO mice, and the cross-sectional area of the junctional zone was reduced by over 50% compared to control mice. Transcript levels of growth factors (Igf1 and Igf2), glucose transporters (Slc2a1 and Slc2a3), and amino acid transporters (Slc38a2 and Slc38a4) were significantly decreased in the placentas of Ut GR KO mice compared to controls. These data are the first to demonstrate that glucocorticoid signaling in the uterus is critical for the development and functions of the placenta. Therefore, this new model provides a unique opportunity to investigate the pathways that lead to IUGR.

**P607 - Effects of bovine pregnancy-associated glycoproteins on gene transcription in bovine endometrial explants.** Amanda Schmelzle, Ky Pohler, Michael Smith, Jonathan Green

Pregnancy-associated glycoproteins (PAGs) are a complex gene family, whose members are expressed by trophoblasts of ruminants and related species. In cattle, the PAGs accumulate at the trophoblast-uterine interface and many can enter the maternal circulation. However, very little is known about the role they play in pregnancy although preliminary results suggest that PAGs at the placenta-uterine interface play roles involving matrix turnover and chemokine release. This study was designed to provide further insight into the biological roles of bovine PAGs by measuring changes in endometrial transcript abundance for some matrix metalloproteinases (MMPs) and chemokines/cytokines. PAGs for these experiments were purified from mid-gestation bovine placental extracts by affinity chromatography. Heifers were synchronized and bred by artificial insemination. Heifers were slaughtered at day 18 post-insemination and the reproductive tracts were obtained and flushed to determine if a conceptus was present. Endometrial explants were collected and split between 4 groups: pregnant with and without 15 µg/ml PAG (n=10) and not-pregnant with and without 15 µg/ml PAG
Endometrial explants were cultured for up to 96 hours at 37°C and 5% CO₂ and samples were harvested at 24 h time points for extraction of RNA. Transcript abundance for ten target genes was analyzed in the endometrial tissue by quantitative PCR. The normalization control transcript was PPIA. Significant increases in CXCL5 and MMP12 were measured in the PAG-treated endometrium from pregnant and non-pregnant animals (P<0.05). There was also a significant increase in message for CCL2 and MMP13 in the pregnant PAG-treated group but not in the non-pregnant groups (P<0.05). These results indicate that PAGs are capable of inducing changes in transcript abundance in bovine endometrial explants, which suggests that this model system might be useful to assess PAG function at the placenta-uterine interface. Funded by the MU Research Board.

**P608 - Deactivation of interferon-treated macrophages by gestational cytokine Leukemia inhibitory factor can promote trophoblast migration and invasion in vitro.** Laurie Fortin, Jovane Hamelin Morrissette, Céline Van Themsche, Cathy Vaillancourt, Carlos Reyes-Moreno

The immune system of the uterine endometrium is exceptional in its ability to protect the mucosa from a variety of pathogens while being supportive to a developing semi-allogeneic embryo. However, aberrant activation of inflammatory pathways in macrophages at the maternal-fetal interface can affect trophoblast survival and function and potentially induce pregnancy complications such as early embryo loss in humans. In mice, the pleiotropic cytokine LIF is essential for embryo implantation. Moreover, LIF seems to play an immune-regulating role in both mice and human. However, the ability of LIF to modulate the activation of leukocytes remains unclear. This study aimed to elucidate the molecular mechanism by which LIF modulates macrophage (Mφ) activation by the pro-inflammatory cytokine gamma-Interferon (IFNγ). This cytokine is especially important for the remodeling of uterine vasculature, but prolonged production throughout pregnancy is associated with spontaneous abortion, intra-uterine growth restriction and pre-eclampsia.

A coculture of peripheral blood monocyte-derived Mφs and immortalized trophoblastic cells was used to evaluate the effect of a treatment with LIF in the deactivation of IFNγ-treated Mφs. To elucidate the molecular mechanism by which LIF counteracts IFNγ effects in Mφs, Stat1 phosphorylation, cell motility and matrix metalloprotease-9 (MMP-9) gene expression was assessed.

Our results indicate that LIF treatment reverses the inhibitory effect of IFNγ-activated Mφs on trophoblast cells invasion. Moreover, LIF increases Mφ cell motility and reactivates cell motility when Mφs were immobilized by IFNγ. The mechanism may be in part explained by the ability of LIF to induce the expression of MMP-9. The molecular inhibition mechanism of IFNγ-triggered response in Mφs includes a decrease in phosphorylation levels of Stat1 transcription factor after LIF treatment.

In this context, we propose LIF as an important modulator of the gestational immune response, thereby supporting the survival and differentiation of the placenta.
**P609 - Comparison of developmental capacity of bovine parthenogenetic and biparental IVF embryos and its effects on placental products secretion.**  Gessica Franco, Gabriela Melo, Sydney Reese, Webb Fields, Veronica Negron-Perez, Claire Timlim, Kyungjun Uh, Kiho Lee, Vitor Mercadante, Ky Pohler

Establishment of pregnancy in cattle involves regulated interactions between maternal and paternal genetics to obtain proper conceptus development. This experiment aimed to determine differences in pregnancy establishment and placental products in parthenogenetic embryos (PA) compared with biparental embryos (CON). We hypothesized recipients receiving parthenotes will have decreased ISG expression and decreased circulating pregnancy-associated glycoprotein (PAG) concentration. Parthenote embryos were produced *in vitro* using a validated chemical activation method and control biparental embryos produced *in vitro* using industry standard techniques. Cows (n=30) were synchronized and embryos transferred 7 days after estrus onset (d0). Blood samples were collected on days 7, 15, 21 to 40 for peripheral blood leukocytes and serum. Transrectal ultrasonography was performed daily to monitor conceptus development. Trizol was used to extract RNA from buffy coats, cDNA was synthesized and RT-PCR performed to determine relative expression of ISG15, MX2 and OAS1. An in-house ELISA was used to measure serum PAG concentration. As expected, the PA group had decreased pregnancy rate at d30 (13%, 2/15) compared to CON (33%, 3/9) and pregnancy was maintained up to d40 of gestation in both groups. Fold change on d22 over the baseline was decreased on PA for both OAS1 (7.0 vs 2.4, *P*=0.02) and ISG15 (13.2 vs 4.2, *P*=0.07) compared to CON embryos. Circulating PAGs increased from d24 forward in both groups, but the PA group had reduced concentrations (0.81±0.44 vs 5.23±0.44; *P*=0.01) at d32 of gestation. These results indicate that parthenotes embryos can establish pregnancy up to d40 of gestation in cattle, with decreased but detectable amounts of conceptus-derived products in circulation, therefore seems to be a suitable model to investigate parental contribution to placental development in cattle. **This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2017-67015-26457 from USDA NIFA.**

**P610 - Bouncing back: macrophage-associated immune signaling during involution of the postpartum uterus.**  Sarah Bacon, Kristina Ramsden, Rebecca Ortiz, Banna Hussain

In early pregnancy, uterine spiral arteries are remodeled into low resistance, high volume tubes that deliver maternal blood to the placenta's intervillous space. These vessels tear at birth when the placenta shears off the uterine decidua. What prevents hemorrhage and stimulates healing of the vessels postpartum? Pilot data in our rat model showed macrophages densely aggregated around spiral arteries in the first week postpartum. In this study, we describe macrophage-associated signaling in the first week postpartum as spiral arteries heal from birth. Female Sprague-Dawley rats were paired with males on diestrus; mating was verified by sperm in a vaginal lavage. After birth, dams were sacrificed one, three, five, or seven days postpartum (n=4 dams per time point). Four virgin estrous females served as controls. At necropsy, uterine segments were (a) paraffin embedded for hematoxylin and eosin (H&E) staining, (b) frozen embedded for immunohistochemistry or (c) homogenized in Trizol for RNA extraction and quantitative real-time PCR, with relative quantification done by the delta delta Ct method. Widely dilated spiral arteries were visible on postpartum day one with marked regression by day
five. Cells immunopositive for the pan-macrophage marker CD68 densely infiltrated the mesometrial triangle. Quantitative PCR verified elevated CD68 cDNA in this period. We expected elevated pro-inflammatory cytokines early in the first week postpartum, yielding to immunomodulatory cytokines later. The pro-inflammatory cytokine *Tnf-alpha* was at its highest in uterus day 1 and 3 postpartum. But immunomodulatory cytokines *Il-10* and *Tgf-beta* were too, as was the canonical macrophage M2 marker *Mrc1*. The phosphatidyl serine receptor *Mer* was consistently low across all timepoints. We infer that while involution in the rat unfolds over ten days, the most intense cytokine activity is in the first three or four days. This intense activity is likely associated with the rapid postpartum involution of spiral arteries.

**P611 - Role Of The Human Placenta-Specific Transcription Co-Factor Vgll1 In The Specification And Maintenance Of The Trophoblast Lineage.** Francesca Soncin, Omar Farah, Mariko Horii, Donald Pizzo, Morgan Meads, Kathy Niakan, Louise Laurent, Mana Parast

The placenta is an understudied organ, whose functions not only affect pregnancy outcome but also maternal and child health long after birth. In a recent comparative study between mouse and human placentae across gestation, we identified vestigial-like protein 1 (VGLL1) as a human-specific transcription co-factor highly expressed in first trimester villous cytotrophoblast (CTB), the proliferative progenitor compartment of the placenta. We observed VGLL1 expression as early as the pre-implantation blastocyst, specifically in the trophoderm layer, the precursor compartment of the trophoblasts. We are now investigating the role of VGLL1 in trophoblast cells using multiple *in vitro* models. We differentiated human pluripotent stem cells (hPSC) into CTB-like cells using a two-step BMP4-based protocol optimized in our lab to study trophoblast lineage specification. After 24h of BMP4 addition, VGLL1 was up-regulated, temporally following GATA3 and before TP63 induction, necessary for trophoblast specification and maturation, respectively. Expression of VGLL1-targeting shRNA caused impaired expression of both TP63 and GATA3, suggesting a potential role in driving trophoblast specification into CTB. We also observed high expression levels of VGLL1 in human trophoblast stem cells (hTSC), a novel *in vitro* system isolated from first trimester placenta to investigate trophoblast progenitor maintenance and differentiation. VGLL1 interacts in a conserved manner with members of the TEA domain-containing family of transcription factors (TEADs). In all three systems (primary CTB, hPSC-derived CTB-like cells and in hTSC) VGLL1 co-localized with TEAD4 in the cell nuclei and the two factors were co-immunoprecipitated. We hypothesize that, in human placenta, VGLL1 acts in combination with TEAD4 in a trophoblast-specific gene network that drives trophoblast lineage specification and trophoblast stem cell maintenance. Future studies include identification of direct down-stream targets of the VGLL1/TEAD4 complex by ChIP-seq and the investigation of the transcription factor cascade and network necessary for trophoblast specification and maintenance.

**P612 - Administration of exogenous gonadotropins does not alter ovine endometrial estrogen receptor concentration during early diestrus.** Hayder Habeeb, Timothy Hazzard, Cecily Bishop, Fredrick Stormshak, Michelle Kutzler
PG-600® is a single dose injectable product labeled for use in swine but used off-label for out-of-season estrus induction in sheep. Our laboratory reported that PG-600® administered to cycling ewes resulted in significantly elevated serum estradiol-17β concentrations [E2] within the first day after mating and significantly reduced pregnancy rates. Elevated [E2] can increase endometrial estrogen receptor concentration [ESR], which may indirectly contribute to embryo mortality in early pregnancy. Therefore, we hypothesized that [ESR] would be increased during early diestrus in ewes treated with PG-600®. The research objective was to compare [ESR] at 4 and 7 days after treatment of cycling ewes with PG-600® or saline. Cycling Polypay ewes (n=24) were administered intravaginal progesterone (P4) for 9 days. Two days prior to exogenous P4 withdrawal, ewes were treated with intramuscular cloprostenol (Estrumate;125μg). On the day of exogenous P4 withdrawal (d0), ewes were divided randomly into two treatment and two control groups to receive a single intramuscular injection of PG-600® (400IU equine chorionic gonadotropin and 200IU human chorionic gonadotropin) or saline (5mL). On d4 and on d7, six ewes from each treatment and control group were generally anesthetized and a laparotomy was performed to obtain endometrial samples. Tissue samples were collected ipsilateral to the ovary bearing the greatest number of corpora lutea. An estradiol exchange assay was used to determine [ESR]. Two-way analysis of variance was used to determine the effect of treatment on [ESR]. Significance was defined as p<0.05. Endometrial ESR concentration did not differ by treatment but did differ between d4 and d7 (p<0.02). These data suggest that reduced pregnancy rates observed following PG-600® treatment in ewes are not related to alterations in [ESR]. This research was supported by the Oregon Sheep Commission and Ministry of Higher Education and Scientific Research, Al-Qasim Green University, Iraq.

**P613 - Role of PAX2 in the development of fallopian tube derived high grade serous ovarian cancer.** Jose Colina, Peter Varughese, Angela Russo, Joanna Burdette

Ovarian cancer is most lethal gynecological malignancy and the 5th leading cause of cancer deaths among women. The deadliest subtype of the disease is high grade serous ovarian cancer (HGSOC) with an average 5year-survival-rate of 29%. The secretory cells in the fallopian tube epithelium (FTE) can become preneoplastic lesions called secretory cell outgrowths(SCOUTS), which are hypothesized to go on to become HGSOC. PAX2 is a transcription factor that is lost in HGSOC and in SCOUTs, suggesting that loss of PAX2 is an early event in fallopian-tube-derived HGSOC tumorigenesis. In the present study, we developed PAX2shRNA knockdown and CRISPR deletion murine oviductal cell lines (MOE) to model the development of SCOUTs. We used this model to study how it potentiates the FTE for further transformation. Loss of PAX2 in MOE cells, regardless of levels of PAX2 deficiency, lead to no significant changes in adhesion, migration, or proliferation consistent with the observation that this is a benign precursor lesion. However, RNA sequencing of PAX2shRNA cells revealed a transcriptional overhaul that accurately captures the transcriptional dysregulation in key genes present in human SCOUTs as outlined by a recently published microarray. Furthermore, analysis of the RNAseq data of our newly created SCOUT model with RNAseq of estrogen stimulated MOE cells revealed remarkable overlap suggesting that loss of PAX2 regulates hormonal responses, a known pathway in gynecological tumorigenesis. Hormone responsiveness of these cells were investigated using ERE-and PRE-luciferase assays which revealed higher basal hormone activity and sensitivity to hormone treatment. Our SCOUT model also showed increased estrogen
receptor, ligand independent induction of estrogen responsive genes including progesterone receptor and decorin, and increased sensitivity to estrogen mediated genotoxicity. This presents the possibility of a known subset of HGSOC precursor lesions having a predisposition for estrogen-induced genotoxicity in the estrogen-rich environment of the fallopian tube.

**P614 - Spheroids May Mediate Metastasis from the Fallopian Tube Epithelium to the Ovary in High-Grade Serous Ovarian Cancer.** Matthew Dean, Vivian Jin, Tova Bergsten, Julia Austin, Angela Russo, Joanna Burdette

High-grade serous ovarian cancer (HGSOC) can originate in the fallopian tube epithelia (FTE), but how tumor cells from the FTE spread to the ovary are currently unclear. Our objective was to investigate the possibility that aggregates of tumor cells (called multicellular tumor spheroids, MTSs) from the FTE colonize the ovary. To determine if normal or tumorigenic cells could form a MTS, a panel of cell lines was cultured in ultra-low adhesion (ULA) conditions. Normal FTE cells from humans or mice failed to produce MTS, while cell lines representative of HGSOC (OVCAR8 and ES2) could. Mutation in p53 (R273H), activation of KRAS (G12V), or activation of AKT (myristoylated AKT) failed to induce MTS formation in murine oviductal epithelial (MOE) cells. In contrast, loss of PTEN induced MTS formation both in vitro and in vivo. To determine if normal cell could be incorporated into the MTS formed by MOE PTEN$^{shRNA}$ cells, fluorescently tagged wild type (WT) and PTEN$^{shRNA}$ cells were mixed and cultured in ULA conditions. When ≥75% of cells in each well were PTEN$^{shRNA}$, MTSs formed and wild type cells were incorporated into the MTS. However, when the PTEN$^{shRNA}$ population was ≤50%, MTSs failed to form. Investigating the functional effects of MTS formation, we found that in ULA conditions, MOE PTEN$^{shRNA}$ cells, which form spheroids, had higher viability and lower cell death than MOE SCR$^{shRNA}$ cells, which cannot form MTSs. Adhesion assays showed that MTSs preferentially attached to the ovarian stroma, which is exposed during ovulation. In an invasion assay, both activin A and norepinephrine were able to induce invasion of cells out of an MTS and into surrounding collagen. These results indicated that MTSs could play a role in metastasis of the fallopian tube-derived tumor cells to the ovary.

**P615 - A calcium-dependent phospholipase A2 (cPLA2) expression is regulated by MIG-6 during endometrial tumorigenesis.** Tae Hoon Kim, Hanna Teasley, Munseok Jeong, Jae-Wook Jeong

The ovarian steroid hormones, estrogen (E2) and progesterone (P4), are essential regulators of uterine biology. The imbalance of these ovarian steroid hormones lead to uterine diseases such as endometrial cancer. Mitogen-inducible gene 6 (MIG-6) is an adaptor protein. MIG-6 mediates P4 signaling and acts as a tumor suppressor during endometrial tumorigenesis in humans and mice. In previous studies, we developed the conditional knockout of Mig-6 in all uterine compartments ($Pgr^{flox/flo}$; Mig-6$^{f/f}$; Mig-6$^{f/f}$) and endometrial epithelial cell-specific Mig-6 knockout ($Spr2f^{flox/flo}$; Mig-6$^{f/f}$; Mig-6$^{f/f}$; Mig-6$^{Ep-KO}$) mice. Both mouse models developed endometrial hyperplasia and E2-dependent endometrial cancer. P4 treatment significantly decreases aberrant epithelial proliferation and AKT signaling in Mig-6$^{Ep-KO}$ mice but not in Mig-6$^{f/f}$ mice. In the present study, we identified cPla2 as one of the genes down-regulated by Mig-6 in the uterus. We
performed immunohistochemistry analysis to investigate the regulation of cPLA2 by MIG-6 as well as determine the expression patterns of cPLA2 in the uterus. While the expression of cPLA2 was stronger at the uterine epithelial cells of Mig-6KO and Mig-6Ep-KO mice compared to control mice, P4 suppressed the expression of cPLA2 in Mig-6Ep-KO mice but not in Mig-6KO mice. To determine the ovarian steroid hormone regulation of cPLA2, we examined the expression of cPLA2 in ovariectomized control, Mig-6KO, Mig-6Ep-KO, and PRKO mice treated with P4 or E2. After P4 treatment, cPLA2 expression was remarkably reduced in Mig-6Ep-KO mice but not in Mig-6KO mice. However, the expression of cPLA2 was not changed in PRKO mice. Our results identified cPLA2 as a novel target of MIG-6 in the murine uterus and its important role during endometrial tumorigenesis.

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Endometriosis is an inflammatory disease characterized by the presence of endometrial tissue outside of the uterine cavity. It is believed that some immune populations are dysfunctional in diseased women. Inflammation is necessary for the development of the disease, but it also justifies its symptomatology. A defective immune system in eutopic endometrium could have implications in endometrial cell survival and subsequent migration to the peritoneal cavity and/or in infertility. Our objective was to assess the differences in gene expression of macrophages-M1 (Mϕ1), macrophages-M2 (Mϕ2), regulatory T cells (Treg), and natural killer (uNK) cells in eutopic endometrium of women with and without endometriosis. Eleven endometrial biopsies were collected: 6 from donors and 5 from endometriosis patients. A cytometry panel of ten antibodies able to separate the populations of interest was designed. When analyzing each specific population, flow cytometry and statistical analysis of the sorted cells showed no significant differences in numbers between groups, except for Mϕ1 that were higher in control when compared to endometriosis (p=0.0087). After RNA-High-Sequencing and gene expression analysis, differentially expressed genes (FDR<0.05/LogFC≥2) in all comparisons were found. Preliminary results on biological significance analysis showed no differences between uNK of women with and without endometriosis. However, Treg molecular functions suggested alterations in endometriosis related to angiogenesis and viral functions. Analysis of Mϕ revealed that Mϕ1 are more pro-inflammatory in endometriosis than in controls and Mϕ2 have a pro-inflammatory phenotype in endometriosis. Mϕ are phenotypically plastic and their polarization state depends on their microenvironment. Mϕ2 could undergo polarization and adopt the Mϕ1 pro-inflammatory phenotype due to the altered environment in endometriosis. In conclusion, aberrant distribution of immune cells within the eutopic endometrium and the increase of inflammation in endometriosis may be related to the pathophysiology of the disease as well as having an effect in reproductive outcomes. (Work supported by NIH/NICHD2P50HD55765-11(LCG)).
P617 - Defining a proteomic biomarker panel for the noninvasive diagnosis of endometriosis. Genna Wilber, Gregory Burns, Irving Vega, Asgerally Fazleabas

Endometriosis is characterized by the presence and growth of endometrial tissue outside of the uterus. The standard diagnostic is pelvic laparoscopy to confirm the presence of disease by direct visualization. This invasive diagnostic combined with nonspecific symptoms like pelvic pain, dysmenorrhea, dyspareunia, and infertility, contributes to an average eight-year gap between the onset of disease to time of diagnosis. An accurate less invasive diagnostic method is a significant unmet need for women’s health. Uterine secretions contain an amalgam of proteins, lipids, amino acids, ions, and sugars which are dynamic throughout the menstrual cycle and pregnancy but studies on its changes associated with endometriosis are limited. Since there has been limited progress on identifying biomarkers of endometriosis, we have begun to investigate the proteome in uterine lavages utilizing a baboon model of induced endometriosis and label-free quantitative proteomic analyses. Briefly, proteins from uterine lavages from the same group of five animals obtained prior to disease induction and at three-months and fifteen-months following the induction of endometriosis were digested in 50% acetonitrile containing LysC and Trypsin and loaded on to a C18 column and eluted using a 2hr acetonitrile gradient into a Q-Exactive HF-X mass spectrometer. Each sample was run three times to account for technical variability. A total of 388 proteins were significantly differentially expressed in baboons with disease. We then performed an independent analysis of human uterine lavage samples from women with and without disease (n=6) to determine the differentially expressed protein profile in humans and compared it to the baboon protein profile. We found thirteen proteins that were significantly differentially expressed in both our baboon model and human patients with endometriosis. Validation of this proteomic profile of disease in a large cohort of patients with endometriosis will demonstrate its usefulness as a noninvasive diagnostic for endometriosis.

Support: NIH HD083273

P618 - Detection And Functional Analysis Of Hoxc8 As An Upstream Regulatory Gene In Ovarian Endometrioma. Ryo Maekawa

Objective: As demonstrated in recent direct reprogramming studies, cell specificity or pathogenesis of diseases is determined by a few key upstream regulators. We hypothesized that the aberrant expression of upstream regulatory genes is associated with the onset and development of ovarian endometrioma by altering the expression of the downstream genes.

Materials and Methods: In order to extract the upstream regulatory genes, Significance-based Modules Integrating the Transcriptome and Epigenome (SMITE) analysis was performed using the transcriptome data of ovarian endometrioma and the functional interaction gene network data which is publicly available. In SMITE analysis, HOXC8 was extracted as a candidate gene of upstream regulatory genes. To clarify the function of HOXC8, HOXC8-overexpressing cells were established using human endometrial stromal cells, and transcriptome analysis and functional assays (wound healing assay, cell migration assay and gel contraction assay) were performed.
Results: The expression statuses of 582 genes were altered by HOXC8-overexpression. Gene Ontology analysis revealed that genes associated with cell adhesion and composition of extracellular matrix were increased and several signaling pathways were altered. When these pathways were compared with the pathway that is actually activated in ovarian endometrioma, TGFβ-, MAPK- and TNF-signaling pathways were commonly activated. It is known that activation of TGFβ-, MAPK- and TNF-signaling pathways is characteristic of ovarian endometrioma and is involved in the invasion and adhesion. In the functional analysis, cell migration and cell adhesion activities were enhanced in the HOXC8-overexpressing cells.

Conclusion: HOXC8 can be an upstream regulatory gene in ovarian endometrioma. Aberrant overexpression of HOXC8 is associated with the onset and development of ovarian endometrioma by inducing the aberrant expression of the downstream genes which are associated with TGFβ-, MAPK- and TNF-signalings.

**P619 - Identification of a novel metastasis suppressor of mouse ovarian tumor cells.**
Naofumi Miwa, Mayu Hanaue

We have previously characterized a novel egg-coating envelope protein that suppresses fertilization in Xenopus lavis (SSR, San Diego, 2016). That protein (named dicalcin) binds to a glycoprotein, a constituent of the egg-coating envelope filament; regulates the orientation pattern of the filaments and the viscoelasticity of the entire envelope, thereby suppressing sperm-egg interaction. Metastasis is one of the major cellular processes involving glycoprotein and oligosaccharides. To contribute to the study of cancer metastasis, we investigated the action of dicalcin on the metastasis of mouse ovarian tumor cells. Extrinsically applied dicalcin remarkably binds to the cell surface as well as the cytoplasm of OV2944 cells. Pretreatment of OV 2944 cells with extracellularly applied dicalcin inhibited in vitro invasion using Matrigel chamber in a dose-dependent manner with IC50 of approximately 2 μM. It also suppressed the binding of OV2944 cells to Matrigel significantly; however, it unaffected the cell viability assessed by the MTT assay, which indicated that it suppresses in vitro invasion through its direct binding to OV2944 cells. Time-lapse imaging analyses discovered that the extracellular presence of dicalcin inhibited migration of OV2944 cells on the plastic plate. We next examined in vivo survival of mice that were injected with OV2944 cells. Concurrent injection with dicalcin-derived peptide significantly prolonged the survival days of the mice, indicating that dicalcin inhibited metastasis of OV2944 cells in vivo. In summary, our novel results elucidated the suppressive action of dicalcin on metastasis of mouse ovarian tumor cells through its binding to OV2944 cells. We believe that our present study will provide insight into molecular machinery of metastasis processes, and may lead to the development of potent bioactive compound that is capable of inhibiting cancer metastasis. Molecular mechanism of the action of dicalcin will also be discussed. There are no conflicts of interest to declare.

**P620 - Development of a new targeted therapy against hormone-dependent and chemo-resistant breast cancers based on estrogen-platinum hybrids and anti-inflammatory agents.**
Yassine Ouflqir, Valerie Boulanger, Isabelle Plante, Gervais Bérubé, Carlos Reyes-Moreno
Breast cancer (BRC) remains one of the leading causes of cancer-related mortality worldwide. Despite the rapid development of many chemical and immunological agents for the treatment of BRC, chemotherapy is still widely used today to fight resistant, recurrent and highly metastatic types of BRC. However, two major factors affect its efficacy: undesirable side effects due to lack of specificity and development of resistance mechanisms, which are mainly associated to cancer-related inflammation. In this context, we have been synthesized new anti-cancer hybrid estrogen-platinum (HEP) compounds, which exhibited high anti-proliferative activity and specificity toward hormone-dependent BRC. On the other hand, a small derivative of aminobenzoic acid, called DAB-1, was recently identified in our laboratory as potential drug showing anti-inflammatory and anti-cancer activities. The long-term goal of our research project is to evaluate the anti-cancer and anti-inflammatory effects of these molecules, when used in combination therapies for the treatment of hard-to-treat types of BRC. Our preliminary structure-function studies indicated that chemical modifications on functional groups composing DAB-1 provided novel derivatives with reduced cytotoxicity but enhanced anti-inflammatory activity. The therapeutic potential of these two molecules in combination is currently evaluated using human BRC cell lines, positive or negative for estrogen receptor, and sensitive or resistant to cisplatin. In particular, we evaluate their combined effect on the viability, proliferation, morphology, adherence and invasive and migratory properties of BRC cells. This work was supported by grants from the Réseau Québécois en Reproduction (RQR).

**P621 - An oocyte-derived biomaterial provides a “sperm safe” to preserve mammalian spermatozoa.** Francesca Duncan, Sergio Vaccari, Nam Tram, Hoi Chang Lee

Cancer treatments, while life preserving, can threaten fertility. Fertility preservation can be complex in males with conditions of extremely low germ cell numbers or in those who have undergone testicular or epididymal biopsy where there may be limited numbers of immature germ cells. Although fertility can be restored with only a single sperm via intracytoplasmic sperm injection (ICSI), a significant challenge is the storage and recovery of small numbers of sperm. We engineered an oocyte-derived biomaterial – the zona pellucida (ZP) – to function as a “sperm safe” for storing sperm. The ZP is a glycoprotein matrix that surrounds the mammalian oocyte. Using a piezo drill, we made a small hole in the ZP and mechanically separated it from the oocyte cytoplasm. In a subset, we further purified the ZPs through decellularization. Using a modified ICSI approach, between 1-6 sperm heads were injected into purified ZPs (N=31; average 2.7 ± 1.7 sperm heads/ZP), which were then placed in sperm cryoprotective medium, loaded into straws, and cryopreserved. An average of 2.5 ±1.4 sperm heads/ZP (N=29) were observed upon thawing, demonstrating negligible sperm loss. Sperm were recovered using micromanipulation methods, and in 11 of 12 thawed “sperm safes,” the recovery of between 2-4 sperm heads/ZP was 100%. “Sperm safe” derived sperm induced calcium transients when injected into eggs and supported fertilization and preimplantation embryo development following ICSI. 65% of the eggs injected with “sperm safe” sperm reached the 2 or 3-cell stage within 24 hours post-ICSI compared to 84% of controls using freshly isolated sperm. In addition, embryos generated with “sperm safe” sperm developed to the blastocyst stage. Thus, purified ZPs are an ideal natural biomaterial for the efficient preservation and recovery of small numbers of sperm, and efforts are ongoing to develop a synthetic ZP mimic that could be applied clinically.
An effect of platelet rich plasma (PRP) on autologous ovarian tissue transplantation on a rat model. Anna Niwinska, Ricardo Faundez, Edyta Juszczuk-Kubiak, Zdzisław Gajewski, Jarosław Kaczyński, Ewa Kautz

Ovarian tissue cryopreservation and autotransplantation seems to be a fundamental support for oncological female patients undergoing oncofertility programs, serving not only as a fertility preservation technique but also as a chance for physiological processes restoration and prevention of premature menopause. Though this method has proven to be successful in some cases, it still needs to be ameliorated, as the transplanted tissue is subjected to 2-7 days long period of ischemia and oxidation stress, which causes the “burn out” of vesicles and follicular reserve loss.

In this preliminary study on rat model we examined the effect of introducing platelet rich plasma (PRP) to the grafted tissue and the transplantation site. PRP treatment has proven properties of vascularization enhancement and acceleration of wound healing. The treatment was conducted by transferring the tissue from simple culture media into a PRP-enriched media immediately before the procedure and an injection of PRP into transplant site after transplantation surgery.

Eighteen female WAG rats were ovariectomized. Ovaries were then sliced into pieces and slow-frozen with DMSO. After recovery and proven bereft of ovarian tissue residue (ELISA for estrogen levels), rats were subjected to autologous transplantation onto the uterine horn wall (n=2 slices per horn, right- treated, left- control). Samples were retrieved after 2, 7 and 30 days (group A,B,C respectively, each group n=6), and then analyzed with immunofluorescence under confocal microscopy for apoptosis levels and vascularization using specific antibodies (TUNEL-based assay, PECAM- platelet endothelial cell adhesion molecule antibody). Follicle count was performed with standard hematoxylin & eosin staining.

Result trends are consistent, but the statistical significance differs, depending on a group. Data suggests, that addition of PRP media immediately before transplanting the ovarian tissue decreases level of ischemia, improves neovascularization and reduces follicular reserve loss. These findings may open new treatment options. Research supported by KNOW2018/CB/ESR5/7

Rucaparib is not a simple chemotherapeutic panacea: findings of a graduate toxicology course. Kenneth Campbell

A 2018 graduate course in mammalian toxicology (Biology 666, 9 students) explored existing knowledge on Rucaparib (Rubraca, CO-338), a poly ADP-ribose polymerase (PARP) inhibitor of single strand DNA break repair. They extended this with new studies including a bioinformatics screen for environmentally susceptible organisms, a QSAR/QSPR (quantitative structure-activity/property relationship) screen for environmental risk, and a SwissTargetPrediction search for the parent molecule Rucaparib and its two major metabolites, M324 and M338. Up to 78% of dosed Rucaparib exits patients unchanged with a half-life of 17 hrs, indicating potential for movement into the environment. Metabolic studies in lab animals indicate clearance during 2 weeks of 600 mg/day of Rucaparib as 50% Rucaparib, 18% M324,
and 19% M338. PARP shows high cross-species conservation, including for Rucaparib binding domains; probable susceptible species include fish, birds, amphibians, and nematodes. QSAR modeling, however, predicts low bioaccumulation with a bioconcentration factor averaging 13.8 (range 0.16 to 30.9), well below the comparative standard of 1000. Still, target prediction indicates PARP only accounts for part of Rucaparib’s actions as it also may bind to at least 7 membrane receptors, 5 cyclin kinases, and 2 ion channels with probabilities of about 1/3 that of PARP1, its primary target. Moreover, the major metabolites M324 and M338, for which half-life studies are lacking, also bind with similar probabilities to similar potential target spectra. From these results, it is clear that Rucaparib, while demonstrating promise as a third generation chemotherapy for a variety of cancers, particularly BRCA 1/2 – driven homologous recombination deficient cancers, has clear potential to demonstrate side effects within patients driven both by binding to secondary target molecules and via the actions of its primary, and toxicologically uncharacterized, metabolites, M324 and M338. And, if released into the environment, it may compromise a wide spectrum of non-target species.

_P624 - Aggressive clear cell endometrial cancer with uterine-specific deletion of Pten and Dicer in a preclinical mouse model_. Xiyin Wang, Robert Emerson, Chi Zhang, Douglas Rusch, Shikha Khatri, Russell Broaddus, Francesco DeMayo, John Lydon, Shannon Hawkins

Endometrial cancer is the most frequent gynecologic malignancy, with 61,880 new cases diagnosed in 2019 in the US. Women with early stage disease are typically cured with simple hysterectomy. However, the prognosis for women with aggressive forms of the disease (i.e., high-grade endometrioid carcinoma, clear-cell carcinoma, or uterine carcinosarcoma) is dismal. These aggressive endometrial cancers account for only 15% of cases, but they account for 50% of deaths. Decreased DICER expression is associated with poor prognosis and recurrent disease. To study the role of DICER in endometrial cancer, we deleted Dicer in a mouse model of endometrial cancer, _Pgr^Cre;Pten^f/f;Dicer^f/f_ (dcKO). Gross uterine morphology consisted of areas of thin uteri with interspersed areas of enlarged gross tumor beginning at 8 weeks. As early as 3 weeks, a majority of dcKO uteri had endometrial hyperplasia with atypia and low-grade endometrioid adenocarcinoma. With age, dcKO uteri exhibited aggressive high-grade clear cell adenocarcinoma, with pale cytoplasm and nuclear atypia and uterine carcinosarcoma with malignant stroma. To determine the mechanism, we performed RNA and small RNA sequencing. A total of 1892 mRNAs and 51 miRNAs were differentially expressed between dcKO and control (log2 fold change >±1 and _P_ <0.05). Ingenuity Pathway Analysis revealed uniquely dysregulated pathways and TP53 as a key upstream regulatory factor (_P_ =7.77E-05). Our _in vivo_ model systems represent important tools to develop a novel therapy for rare, yet aggressive endometrial cancers.

_P625 - The Effect of Di (2-ethylhexyl) Phthalate (DEHP) on Incidence and Progression of Endometriosis_. Rachel Braz Arcanjo, Catherine Lawrence, Kailiang Li, Quanxi Li, Romana Nowak
Endometriosis is a common gynecological disorder affecting 6%–10% of women of reproductive age. The factors promoting establishment of endometriosis are not well understood but there is evidence that environmental toxicants may play a role. Ample evidence suggests that environmental exposure to DEHP, a known endocrine-disrupting chemical, adversely affects the female reproductive functions. In this study, our goal is to investigate the impact of DEHP exposure on establishment and progression of endometriosis and neuroinflammation using an immunocompetent mouse model of endometriosis. Intact C57/Bl/6 female mice were dosed with either 0, 30 or 60 mg/kg/day DEHP beginning 7 days before receiving tissue transplantation. Controls received no tissue transplantation. The burrowing assay was used to assess visceral pain/discomfort. Endometriotic lesions and brain tissues at days 4, 8, 16 and 32 post disease induction (n=5-7 animals per timepoint) were harvested and subjected to immunohistochemistry to assess lesion histology, glands presence (claudin-4), cell proliferation (Ki67), angiogenesis (CD31) and microglia activation (Iba-1). All lesions collected were confirmed by presence of stromal cells and glands. Our results showed that DEHP did not affect the incidence of lesions at any time point, however, DEHP exposure at 30 mg/kg/day increased lesion volume significantly (p<0.05). The exposed lesions exhibited more proliferation as indicated by the increased levels of Ki-67 (p<0.05). Burrowing activity was decreased in animals with lesions in comparison to the sham controls (p<0.05). In addition, Iba-1 expression and the number of hypertrophic microglial cells in the hippocampus was elevated in animals with endometriotic lesions (p<0.05), and these effects were further exacerbated by DEHP exposure. Collectively, our study revealed that DEHP enhances disease progression of endometriosis by increasing lesion size and cell proliferation. DEHP could also worsen endometriosis-associated pain by inducing neuroinflammation. Supported by NIH R21ES026388 (RAN) and R21ES024198 (QL).

P626 - Role of enhancer of zeste homolog 2 (EZH2) in endometrial cancer. Ana Mesa, Ricky Leung, Manjunatha Nanjappa, Theresa Medrano, Jiude Mao, Shuk-Mei Ho, Cheryl Rosenfeld, Paul Cooke

Enhancer of zeste homolog 2 (EZH2) is a histone methyltransferase that plays a fundamental role in silencing gene activity through trimethylation of lysine 27 on histone H3 protein subunit (H3K27). EZH2 dysregulation is associated with endometrial cancer (EC) and other uterine diseases but mechanism remains unclear. We created a uterine conditional knockout-mouse of EZH2 (EZH2cKO), which shows extensive uterine endometrial gland cell proliferation and heightened estrogen response. To further investigate EZH2’s uterine role, ovariectomized adult controls (EZH2floxflox) and EZH2cKO (EZH2floxflox, PgrCre+) mice (n = 4/group) were given either oil (V) or 1 ng/g BW of 17β-estradiol (E2), and 24h later, transcriptomic profiling of the uterus was performed. Bioinformatics was used to interrogate gene expression differences and potential translation to human EC as pathways enriched include cyclins and cell cycle regulation and molecular mechanisms of cancer. To understand the clinical relevance of differentially expressed genes (DEG) in EZH2cKO, survival analyses were performed based on data in the TCGA Uterine Corpus Endometrial Carcinoma cohort. In brief, cohort was divided into two groups according to DEG expression by an unbiased hierarchy-clustering method. Overall survival and recurrence-free survival based on time to new tumors after initial treatment was compared. Both vehicle (58 genes) and E2-treated (25 genes) EZH2cKO DEG are significantly associated with recurrence-free survival of patients in the TCGA cohort. Patients expressing
DEG in the uterus show two times or higher risk of cancer recurrence. Further analysis on vehicle-associated DEG uncovered 8 downregulated human orthologous genes, ACOT7, CITED4, GPX3, IGLON5, LRRc4B, NXPH3, TBX18, and TMEM200C, which could be crucial in cancer progression as reflected from TCGA survival analysis (Log-rank p-value = 0.03; hazard ratio = 1.64; p = 0.015). Current studies reveal EZH2cKO mice may be useful in understanding EC and other uterine diseases.

**P627 - Progesterone Receptor is a Physiologically Relevant Metformin Target in Endometrial Cancer Cells.** Sofia Jade Saguyod, Rosalia Simmen, Michael Velarde

The anti-diabetes drug Metformin (1,1-dimethylbiguanide hydrochloride; MET) has been increasingly shown to exhibit anti-tumorigenic effects in many cancer cell types but the signaling mechanisms of MET on endometrial cancer (EC) are poorly understood. Nonetheless, among EC patients, MET-users had a higher overall survival compared to non-MET users. Our recent studies show that short-term MET promotes progesterone receptor (PGR) signaling in non-diabetic, post-menopausal women with EC. Because higher levels of PGR are predictive of better outcome in EC patients, the ability of MET to regulate PGR under distinct cellular contexts may provide insights into its mechanism of action in EC. Here, we tested whether MET regulates PGR expression in the human EC cell line Ishikawa treated with physiological (0.1 nM) and high (10 nM) estradiol (E2) concentrations, when grown in media containing standard (5 mM) and high (25 mM) glucose levels, the latter to approximate the environment of EC cells of non-diabetic and diabetic women, respectively. MET (10 and 100 µM) comparably increased PGR and PGR-B transcript levels, in E2-treated cells exposed to glucose (5 and 25 mM). MET effects on estrogen receptor (ESR)-alpha and ESR-beta transcript levels varied with E2 and glucose concentrations. Ishikawa cells were grown in low attachment plates for endosphere formation, and MET effects on the growth (Passage 1) and expansion (Passage 2) of this epithelial sub-population were evaluated. MET treatment (10 µM) of cells grown in media containing 5 mM glucose reduced Ishikawa cells’ endosphere forming-ability and regenerative capacity; interestingly, E2 treatment (10 nM) abrogated these MET effects. Our findings show that MET acts directly on EC cells to alter PGR/ESR signaling and that MET effects are influenced by the metabolic and estrogenic cellular milieu. These collective studies suggest novel strategies for EC prevention and treatment involving MET.

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**P628 - Waltheria indica root exhibits male contraceptive effect through inhibition of androgenesis in male Wistar rats.** Afisu Basiru, Jimoh Akorede, Funsho Olayemi, Kehinde Soetan

There is need for culturally acceptable and male-centered birth control methods in rural parts of developing countries. Traditional use of *Waltheria indica* as male contraceptive has been reported in Nigeria. This study was designed to evaluate the contraceptive potential of *Waltheria indica* root (WIR) in male rats. Fifteen males Wistar rats and nine female rats of proven fertility were used. The males rats were divided into three groups (n=5). Group I served as the control (distilled
water). While groups II and III were given synthetic quinoline alkaloid (13.5mg/Kg) and bioactive fraction of WIR (1000µg/Kg) respectively. The treatment was done for 28 days after which three males from each of the groups were paired with three female rats. After the mating, ethyl ether anaesthetized male rats were sacrificed. Epididymal sperm, testicular and serum samples were assayed for sperm parameters, testicular androgenic enzymes [Δ^5, 3β-Hydroxysteroid dehydrogenase and 17β Hydroxysteroid dehydrogenase (17β-HSD)] and testosterone respectively. Fertility index of the male rats were also determined. Data obtained were subjected to one way analysis of variance with Dunnett's Multiple Comparison Test used to compare means at p<0.05 using GraphPad Prism. The bioactive fraction of WIR significantly reduced the sperm count (p<0.05) and motility (p<0.001). There was also significant reduction in serum level of testosterone for the crude extract of WIR (p<0.01) and the compound isolated from WIR (p<0.05). The serum levels of Δ^5, 3β-HSD and 17β-HSD enzymes were significantly reduced by the bioactive fraction of WIR (p<0.001). The bioactive fraction of WIR reduced the fertility index of male rats from 100% (control) to 0%. These findings support the traditional use of Waltheria indica as male contraceptive agent. More so, reduced level of testosterone and subsequent reduction of key androgenic enzymes indicate inhibition of androgenesis.

_P629 - Potential use of 17BIPHE2 as a vaginal contraceptive/microbicide._ Seung Gee Lee, Wongsakorn Kiattiburut, Mark Baker, Deborah Anderson, Guangshun Wang, Jonathan Angel, Nongnuj Tanphaichitr

We have previously demonstrated that antimicrobial peptide LL-37 and its truncated form, GF-17, have spermicidal activity on both human and mouse sperm. LL-37 and GF-17 at their spermicidal concentration (10.8 µM) also exert microbicidal activity against Neisseria gonorrhoeae, an important cause of sexually transmitted infections (STI) (Kiattiburut et al., Human Reprod 2018). LL-37 and GF-17, therefore, have the potential to be developed into vaginal contraceptives/anti-STI agents. Our recent findings, however, indicated that both peptides were susceptible to degradation in human cervico-vaginal fluid (CVF); >60% of LL-37/GF-17 was degraded after 48 h incubation in human CVF. Finding LL-37/GF-17 mimetics that are resistant to CVF proteases is one avenue to circumvent this challenge. 17BIPHE2 is a mimetic of GF-17 (GFKRIVQRIKDFLRLNV) with the two isoleucines and leucine replaced with corresponding D-amino acids, and the two phenylalanines substituted with biphenylalanines. Our results indicated that 17BIPHE2 was much more resistant to degradation in CVF; <20% degradation was observed in 2 of 3 CVF samples after 48h incubation. The results corroborate its reported stability against V8 protease and protease K (Wang et al., ACS Chem Biol, 2014). Our study further indicated that 17BIPHE2 was bactericidal to _N. gonorrhoeae_ with both MIC and MBC at 1.8 µM, consistent with its microbicidal activity against several pathogenic bacteria (Wang et al., ibid). Furthermore, treatment of mouse sperm with 17BIPHE2 induced immotility and membrane permeabilization in a concentration-dependent manner. At 10.8 µM 17BIPHE2, 100% of sperm became immotile and 80% showed membrane permeabilization. No mouse eggs (n = 31) were fertilized by sperm treated with 10.8 µM 17BIPHE2. Although studies on spermicidal properties of 17BIPHE2 need to be performed _in vivo_ using mouse sperm and _in vitro_ in human sperm, our results suggest that 17BIPHE2 is a promising candidate for development into a vaginal contraceptive/microbicide.
**P630 - High-throughput screen identifies new hormone alternative contraceptive.**
Alaknanda Alaknanda, Rebecca Robker, Krzysztof Mrozik, Andrew Zannettino, Darryl Russell

Despite contraceptive availability and refinement, unplanned pregnancy remains a troubling global reproductive health issue. Global estimates suggest that of the 208 million pregnancies per year, roughly half (41%) are unplanned, leading to millions of unsafe abortions and over 500,000 maternal deaths\(^1\). A major reason for this are the serious side-effects of hormone contraceptives, including cardiovascular and breast cancer risks, which lead to their non-use or discontinued use. There is an acute need for new safer contraceptives that can overcome the systemic side-effects of hormone therapy and offer wider contraceptive choice to women.

We developed a high-throughput approach for screening broad classes of drugs for potential ovulation blocking capacity using automated assessment of cumulus oocyte complex (COC) adhesion to ECM \(\text{in vitro}\). One “hit” compound from the drug library screen that potently and dose dependently inhibited COC adhesion \(\text{in vitro}\) caused significantly reduced ovulation (11 vs. 26 oocytes/ovary; \(p=5.8\times10^{-6}\)) compared to controls in mice \(\text{in vivo}\). There was no difference in the growing follicular count but ovulated structures were significantly reduced in drug treated group. This was not due to LH-pathway downregulation as \(\text{Lhcgr}\) and downstream signalling remained intact. Importantly, no difference in proliferative (Ki-67) or apoptotic (cleaved caspase-3) counts was detectable between groups, suggesting minimal drug toxicity. During COC \(\text{in vitro}\) maturation, the drug severely inhibited COC expansion and oocyte meiotic resumption. Both ovarian histology and immunofluorescence revealed structural dysgenesis of COCs with an apparent loss of contact between oocyte and cumulus cells. Overall, this study is the first to 1) develop a unique high-throughput model for screening drugs for contraceptive potential; 2) identify, evaluate and validate a new class of drugs with potent \(\text{in vitro}\) and \(\text{in vivo}\) potential; and 3) demonstrate a critical role of oocyte-cumulus signalling by this target during folliculogenesis and ovulation.

**P631 - Contraceptive Efficacy and Injection Safety Profile of Porcine Zona Pellucida and Recombinant Zona Pellucida Vaccines Formulated with Pet Gel A and Poly (I:C) in Caribbean Donkeys.** Brittany Middlebrooks, Erik Peterson, Darryn Knobel, Martin Schulman, Henk Bertschinger, Hilari French, Michael Crampton, Robyn Roth, P. Van Zyl

With the increasing population of wild horses (\(\text{Equus ferus}\)) and feral donkeys (\(\text{Equus asinus}\)) globally there is a need for practical, effective and humane population control methods. Previously, administration of a native porcine zona pellucida (pZP) and a recombinant zona pellucida (recZP) vaccine, both formulated with Freund’s adjuvants, was investigated in Caribbean donkeys. Despite successful immunocontraception, injection site reactions associated with Freund’s adjuvants were observed. This refined study aimed to achieve similar contraceptive effects without the adjuvant-associated adverse reactions.

Twenty-five reproductively sound jennies were randomly distributed between three separately maintained vaccine treatment groups. All treatments were administered at 5-week intervals: the
recZP group (n=8; 250 µg ZP3 and 250 µg ZP4, CSIR Biosciences, South Africa formulated with Poly (I:C) and Pet Gel A adjuvant) at week 0 and boosters at week 5 and 10, the pZP (n=9; 100 µg pZP, Trumpeter Farms and Veterinary Service, USA, formulated with Poly (I:C) and Pet Gel A adjuvant) and control group (n=8; Poly (I:C) and Pet Gel A adjuvant alone) at week 5 and booster at week 10. A reproductively sound jack was added into each group during week 15 to test contraceptive efficacy. Jennies are monitored for ovarian cyclic activity and early pregnancy by weekly transrectal ultrasonography.

Currently, at 150d post final treatment, pregnancy has been diagnosed in 8/8 control, 1/9 (11%) pZP and 0/8 recZP jennies, respectively. All controls have continued showing normal cyclicity, whereas 3/8 (38%) recZP and 4/9 (44%) pZP jennies have shown suppressed ovarian activity. No vaccine reactions were noted throughout all groups.

This preliminary data demonstrates effective and safe immunocontraception and will inform future development of a practical and effective population control plan for other feral and wildlife species.

**P632 - Investigation of novel male reproductive tract-specific genes as contraceptive targets.** Kaori Nozawa, Qian Zhang, Haruhiko Miyata, Zhifeng Yu, Darius Devlin, Ryan Matzuk, Masahito Ikawa, Martin Matzuk

There has been a demand for a male contraceptive for long time to provide more diversity to the contraceptive market and balance the responsibility of contraception between women and men. Male reproductive tract-specific proteins would be ideal targets for non-hormonal contraception, as they will likely have minimal side effects. In this study, we focused four epididymis or testis-specific genes [epididymal protein 3B (*Eddm3b*), colipase-like 2 (*Clpsl2*), Leucine rich colipase like1 (*Lrcol1*), and serine-rich single-pass membrane protein 1 (*Ssmem1*)] and validated the suitability of these proteins as contraceptive targets. We have generated knockout (KO) mice using the CRISPR/Cas9 system to evaluate the *in vivo* significance of these genes by phenotypic analysis. Mating experiments revealed that fecundity was unaffected in KO males for *Eddm3b*, *Clpsl2*, and *Lrcoll* whereas *Ssmem1* KO males were sterile. *Ssmem1* KO mice showed abnormal sperm head morphology (globozoospermia) and significant reduction in testis size and sperm motility. We found that SSMEM1 is indispensable but EDDM3B, CLPSL2 and LRCOL1 are not essential for male reproduction in mice although they are all expressed predominantly in male reproductive tissues. Further analysis of *Ssmem1* KO males will potentially reveal a new contraceptive target and provide new information to improve the diagnosis of male infertility.

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**P633 - The Synaptonemal Complex Protein 3 (SCP3) is a Potential Biomarker of CDK2 as Target for Male Contraceptive Drug Development.** Lesya Holets-Bondar, Sudhakar Jakkaraj, Gunda Georg, Joseph Tash, Vargheese Chennathukuzhi
Cyclin-dependent kinases (CDKs) control the eukaryotic cell cycle by phosphorylating serine and threonine in key regulatory proteins. Expression of CDKs is dependent on the stages of spermatogenesis during testis development and maturation. CDK2-knockout mice are viable, but sterile due to block at meiotic prophase-I. Thus, CDK2 is essential for meiosis but not for mitosis, is a promising target for the development novel non-hormonal male contraceptives. The biomarkers of CDK2 inhibition, phospho-RB and p53, express in testis and regulate of transition through the spermatogenic cell cycle. SCP3 expressed in spermatocytes nuclei, is essential for meiotic progression and spermatoozoon formation, and has utility as novel biomarker for rapid screening of CDK2 inhibitors as potential contraceptive agents. We used dinaciclib and several recently synthesized CDK2 inhibitors, to evaluate the changes of biomarkers of CDK2 activity in testis. For testis explant assay, pieces of testes were cultured for 6h in culture medium with dinaciclib or novel CDK2 inhibitors. Quantitative analysis of biomarkers indicated attenuating pRB, IC\textsubscript{50} 270pM, and stimulation of p53, IC\textsubscript{50} 43pM. After 6h culture of testis with several novel CDK2 inhibitors we similarly found that decrease of SCP3 is strongly correlated with pRB reduction (P<0.05). In vivo, to detect whether a single dinaciclib dose may decrease the meiotic progression in testis, mice were treated with 25% maximum tolerated dose of dinaciclib. SCP3 protein expression was analyzed at 24h and 120h. We observed a decline of SCP3 at 24h post dinaciclib treatment, and recovery SCP3 expression at 120h. Our data is the first demonstration that pharmacologic inhibition of CDK2 in adult males is a potential strategy to develop non-hormonal male contraceptives. CDK2 inhibitors show reduction in SCP3, thus supporting its potential as a promising meiosis specific biomarker for rapid screening of new CDK2 compounds. Supported by NIHHD080431 to VC

**P634 - Investigating Heritable Impacts of Germ Cell Toxicant Exposures.** Jill Escher

Drug exposures to germ cells during vulnerable periods of germline synthesis can result in perturbation of epigenetic programming. These molecular glitches can confer a non-genetic heritable risk for pathology on the offspring borne of the affected germ cell. Unfortunately, despite growing evidence for this biological phenomenon in mammal models, and to some extent, in human studies, current approaches in toxicology overlook this important dimension of risk.

The Escher Fund for Autism (EFA) is a small science philanthropy based in San Jose, California, that funds pilot projects examining the heritable impacts of germ cell toxicant exposures, such as synthetic steroid drugs, general anesthetics, and tobacco. EFA is also devoted to science advocacy to change the way we consider potential long-term consequences of pregnancy and early life drug exposures, expanding past toxicology’s standard somatic paradigm to incorporate germ cell risks. Further, EFA argues that pregnancy drugs common in the post-war decades should be investigated as potential contributors to the “missing heritability” of many pathologies now surging in prevalence, including autism, ADHD, asthma and allergies.

EFA-related studies have appeared in Scientific Reports (grandmaternal smoking linked to grandchild’s autism risk), JAMA Pediatrics (grandmaternal DES linked to grandchild ADHD risk), PLoS Biology (paternal nicotine linked to ADHD type traits in offspring, mouse model),
and other publications. Founder Jill Escher has published in Environmental Epigenetics and has a manuscript accepted at Environmental and Molecular Mutagenesis, and is a councilor-elect of the Environmental Mutagenesis and Genomics Society. The EFA website also lists more than 100 mammal and human studies finding heritable impacts of drug and chemical exposures.

EFA periodically announces grant programs for research but also funds conferences and meeting and other efforts aimed at furthering understanding of the generational legacy of drugs and chemicals. For more information please visit GermlineExposures.org.

P635 - Revisiting third cerebroventricle access in cattle for study of hypothalamic function: technical approach, experimental applications, and physiological pitfalls. Meaghan O'Neil, Sarah West, Rodolfo Cardoso, Gary Williams

Cannulation of the third cerebroventricle (IIIIV) in cattle represents a valuable tool for investigating neuroendocrine mechanisms governing the central control of reproduction and its interface with metabolism and behavior. First utilized in sheep and later in cattle, measurement of pulsatile release of GnRH in IIIIV cerebrospinal fluid (CSF) has been shown to be an effective surrogate for its detection in the hypothalamic-hypophyseal portal vasculature. The procedure also enables long-term sampling of CSF for measurement of other neuropeptides and as a route for delivery of biological agents. Objectives here are to revisit the complexities and value of this technique, including 1) a description of the current neurosurgical approach in cattle, 2) specific examples of efficacy for both sampling and treatment, and 3) assessment of historical and potential pitfalls. Following induction, intubation and stabilization under isoflurane gas anesthesia, cattle are placed in sternal recumbency and the head is placed in a head holder. Using both external cranial landmarks and lateral radiography, the head is aseptically prepared, a circle of skin and fascia centered over the sagittal suture is removed, and a 5-mm hole is drilled through the frontal bone and cranium. Using a stereotaxic manipulator, a 14-g guide cannula of the appropriate length is lowered to the target. Polytetrafluoroethylene tubing (28-g) is inserted through the guide cannula to aid in establishment of CSF flow. The guide cannula is raised approximately 5 mm above the ventricle, cemented in place, and the tubing is removed until reinsertion for use. Data presented reiterate the close association of GnRH pulses with both tonic and surge modes of LH release, and the effects of several physiological and pharmacological agents. Current applications also include assessment of developmental changes in leptin transport across the blood-brain barrier. Asepsis, antibiotic prophylaxis, behavior training, and minimizing stress reduce technical and physiological pitfalls.

P636 - Impact of tutor and monitor-assisted model and active methodologies to increase performance of health professional students in embryology and physiology of a public University. Danielle de Souza, Rosangela Rodrigues, Huryel de Oliveira, Valerie Cristine Sandes, Mariany Maria Vasques, Amanda Beatriz Nunes, Andressa Maria Neri, Julia Dias, Karine Tatiana Fernandes, Karoliny Matos, Lucas da Silva, Vivian Oliveira

Health professional students and health care requires an integrative view of biological and field-specific knowledges. Disciplines of the first year of graduation present high level of reprobation
(around 30 to 40%) in our institution because of the complexity of contents related to embryology and physiology. Active methodologies and tutor and monitor-assistance improved the performance of students as described in literature. Furthermore, we hypothesize that the combination of both models will improve the performance of our students. Second semester students who were coursing an integrative discipline of embryology and endocrine and reproductive physiology (n = 192) were assisted by 3 tutors and 7 monitors. All tutors and monitors cours ed the same discipline and had very good or excellent performances. Each tutor was responsible for one group of monitors (2 to 3 monitors), which were always present in class (62 to 65 students/ class). The contents were presented in-class by the professor and the students completed the assignments before the lectures. The assignments contained questions of the proposed theme of each lecture and case studies to improve the integration between biological and field-specific knowledges. The tutors and monitors could help the students in extra-class meetings in the practical laboratory using anatomic models and histological materials. In addition, embryology was also explored using published case studies during the class and the tutors helped the students to develop videos about embryonic or neonatal congenic diseases as an extra-class assignment. The students needed 50% of performance in three theorical tests and 2 practical tests to be approved. Increase of grades was observed in theoretical tests (33,23%), practical tests (29,04%) and final grades (26,4%) compared to previous students (of 2017). The reprobation decreased to 18,75%. The combination of tutor and monitor assistance and active methodologies improved the grades of health professional students.