Widespread Enhancer Activation via Erα Mediates Estrogen Response in Vivo During Uterine Development. Wendy N. Jefferson, H. Karimi Kinyamu, Tianyuan Wang, Adam X. Miranda, Elizabeth Padilla-Banks, Alisa A. Suen, and Carmen J. Williams

Little is known regarding how steroid hormone exposures impact the epigenetic landscape in a living organism. Here we took a global approach to understanding how exposure to the estrogenic chemical, diethylstilbestrol (DES), affects the neonatal mouse uterine epigenome. Integration of RNA- and ChIP-sequencing data demonstrated that ~80% of DES-altered genes had higher H3K4me1/H3K27ac signal in close proximity. Active enhancers, of which ~3% were super-enhancers, had a high density of estrogen receptor alpha (ERα) binding sites and were correlated with alterations in nearby gene expression. Conditional uterine deletion of ERα, but not the pioneer transcription factors FOXA2 or FOXO1, prevented the majority of DES-mediated changes in gene expression and H3K27ac signal at target enhancers. An ERα dependent super-enhancer was located at the Padi gene locus and a topological connection to the Padi1 TSS was documented using 3C-PCR. Chromosome looping at this site was independent of DES exposure, indicating that it was in place prior to ligand signaling. However, enrichment of H3K27ac and transcriptional activation at this locus was both DES and ERα-dependent. These data suggest that DES alters uterine development and consequently adult reproductive function by modifying the enhancer landscape at ERα binding sites near estrogen-regulated genes.

A Novel Atypical Centriole in Animal Spermatozoa Functions in the Zygote. Tomer Avidor-Reiss, Emily L. Fishman, Kyoung Jo, Quynh P.H. Nguyen, Dong Kong, Rachel Royfman, Anthony R. Cekic, Sushil Khanal, Ann L. Miller, Calvin Simerly, Gerald Schatten, Jadranka Loncarek, and Vito Mennella

One of the last major unidentified pathways during fertilization in humans, other mammals, and insects is the precise way in which the centrosome (the cell's dominant microtubule-organizing center) of the zygote is inherited. In contrast to the biparental merging of the sperm and egg nuclei at the onset of reproduction, as well as the unimaternal inheritance of the egg mitochondria, the exact mode of centrosome inheritance is yet not understood. Without DNA as reliable fiduciary markers, accurate tracking of the centrosome had not been possible, since many of its proteins are transient residents.

We previously found that insect (flies and beetles) sperm centrioles have in additions to their typical centriole that form the sperm tail also an atypical centriole (the Proximal Centriole Like or PCL) that lacks microtubules but is essential for fertility. We, therefore, hypothesized that animal spermatozoa have a combination of typical and atypical centrioles that are necessary for zygote function.

Recently, we found that the sperm centrosome of human and other mammal is composed of a novel atypical centriole made of splayed microtubules (distal centriole, DC) surrounding bars of centriole luminal proteins, a typical barrel-shaped centriole (the proximal centriole, PC), and the surrounding specialized matrix (the pericentriolar material, PCM). The atypical centriole is formed during spermiogenesis by differential reduction and enrichment of specific types of centrosomal proteins. In vivo and in vitro investigations found that the atypical DC is capable of recruiting PCM, forming a centrosome that nucleates a microtubules aster, tinplating a new centriole, and localizing to the spindle pole during the first mitosis. This now enables, for the first time, tracing of proteins contributed by the human sperm during fertilization, which both solves a fundamental mystery and affords new strategies for infertility diagnostics and male contraceptive approaches.

Punicalagin is Beneficial for Spermatogenesis in Male New Zealand White Rabbits. Mehmet Sukru Gulay, and Ozlem Yildiz Gulay

Taking into account that punicalagin (PUN) is a very powerful antioxidant; the current study evaluated the potential positive effects of oral PUN on spermatological parameters of male New Zealand White rabbits. A total of 24 male bucks was housed individually and trained for semen collection for 2 weeks before the experiment. After the training, rabbits were assigned into 4 groups and received daily gavages of 0, 1, 2, and 10 mg/kg PUN in tap water for 9 weeks. Doses were adjusted weekly according to the animals' weight. Libido was also evaluated during semen collection. Semen was collected once a week from each rabbit and samples at d 1 and 63 of the experiment were analyzed separately. The PROC GLM procedure and Dunnett post hoc analysis were used for statistical evaluations. At the end of the experiment, the rabbits were euthanized and weights of testes, epididymides and accessory sex glands as a whole was recorded. Initial values (ejaculate volume, ejaculate weight, ejaculate pH, sperm concentration, percent progressive motility, and seminal plasma protein levels) tested at d 1 were similar among the groups. There were also no differences in ejaculate volume, ejaculate weight, ejaculate pH and seminal plasma protein levels at the end of the experiment ($P > 0.1$). Libido and weights of reproductive organs were not affected by the treatments ($P > 0.1$). However, sperm concentrations ($P < 0.01$) and percent progressive motility ($P < 0.04$) were significantly improved, especially for bucks in 2 and 10 mg/kg PUN groups. Thus, the current study suggested that as low as 2 mg/kg PUN per day can be beneficial for spermatogenesis and motility in male rabbits.This project was supported by TUBİTAK (project no: 116O027).

Spatial and Temporal Resolution of ORC4 Fluorescent Variants Reveals Structural Requirements for Maintaining Higher Order Self-association and Pronuclei Entry. Hieu Nguyen, Brandon Nguyen, Thien P Nguyen, W. Steven Ward, and Nicholas G. James

DNA licensing is an essential step in replicating DNA during cell division. The Origin Replication Complex (ORC), which is composed of six proteins ORC1-6, are responsible for initiating licensing at replication origins and recruits of adaptor molecules necessary to form the blank. Previously, we reported that ORC4 polar body extrusion (PBE) in addition to DNA licensing. Herein we utilized lifetime and fluctuation microscopy on two fluorescent constructs of ORC4, ORC4-eGFP and ORC4-FlAsH, to examine its spatial dynamics during oocyte activation. Our results demonstrated that expression of ORC4-eGFP failed to form large, cage like structures around chromosomes during anaphase II while the FlAsH labeled variant clearly showed higher order self-association. Interestingly, both variants were found in the pronuclei indicating no loss in DNA licensing function. Our results demonstrate that fusion of ORC4 C-termini to eGFP prevents higher order oligomerization needed for cage formation during PBE; thereby we described a possible ORC4 construct, namely ORC4 with a FlAsH sequence at site Cys-Cys-Pro-Gly-Cys-Cys, which allows for site-specific labeling and minimal background signal from nonspecific binding within live embryos. This work also represents the first step towards establishing a transgenic mouse model expressing endogenous levels of a trackable variant of ORC4.

Expression Pattern of the Histone H3 Lysine 36 Methyltransferase, SETD2, in Porcine Oocytes and Embryos. Dong Il Jin, Tao Lin, Yun Fei Diao, Xiaoxia Li, Reza K. Oqani, Jae Eun Lee, and So Yeon Kim

SETD2 is a histone H3 lysine 36 (H3K36)-specific methyl-transferase that may play important roles in active gene transcription in human cells. However, its expression and roles in porcine oocytes and preimplantation embryos are not well understood. In this study, we determined SETD2 expression in porcine oocytes and preimplantation embryos derived from in vitro fertilization (IVF), parthenogenetic activation (PA) and somatic cell nuclear transfer (SCNT) by immunofluorescence using specific antibodies and laser scanning confocal microscopy. The SETD2 signal was observed in non-surrounded nucleolus (NSN)-stage oocytes, but not in surrounded nucleolus (SN)-, metaphase I (MI)- or metaphase II (MII)-stage oocytes. The SETD2 signal was detectable in sperm, but it was lost after in vitro fertilization. Thereafter, it became detectable at the 2-cell stage of IVF embryos, peaked at the 4-cell stage, when porcine embryonic gene activation occurs, and remained to the blastocyst stage. Similar to the pattern found in IVF embryos, the SETD2 signal was not detected in 1-cell-stage PA embryos, but it was detected at the 2-cell stage and maintained to the blastocyst stage. Unlike IVF and PA embryos, SCNT embryos did not lose the SETD2 signal at the 1-cell stage, and the signal was detectable throughout embryonic development. Overall, these data indicate that SETD2 could be related to embryonic gene activation in porcine preimplantation embryos, and that aberrant SETD2 expression in 1-cell-stage porcine SCNT embryos may contribute to the reprograming errors seen in these embryos and the low efficiency of somatic cell cloning.

Human Globozoospermia-Related Gene Spata16 is Required for Sperm Formation Revealed by CRISPR/Cas9-Mediated Mouse Models. Yoshitaka Fujihara, Asami Oji, Tamara Larasati, Kanako Kojima-Kita, and Masahito Ikawa

A recent genetic analysis of infertile globozoospermic patients identified causative mutations in three genes: a protein interacting with C kinase 1 (PICK1), dpy 19-like 2 (DPY19L2), and spermatogenesis associated 16 (SPATA16). Although mouse models have clarified the physiological functions of Pick1 and Dpy19l2 during spermatogenesis, Spata16 remains to be determined. Globozoospermic patients carried a homozygous point mutation in SPATA16 at 848G→A/R283Q. We generated CRISPR/Cas9-mediated mutant mice with the same amino acid substitution in the fourth exon of Spata16 to analyze the mutation site at R284Q, which corresponded with R283Q of mutated human SPATA16. We found that the point mutation in Spata16 was not essential for male fertility; however, deletion of the fourth exon of Spata16resulted in infertile male mice due to spermiogenic arrest but not globozoospermia. This study demonstrates that Spata16 is indispensable for male fertility in mice, as well as in humans, as revealed by CRISPR/Cas9-mediated mouse models.

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Elastogrphic Assessment of Uterine Contractility and Subendometrial Vasculature as Prognostic Factors for Successful Pregnancy in IVF Patients: A Pilot Study. Han Moie Park, Hye Nam Lee, and Woo Sik Lee

Good-quality embryos and optimal intrauterine environment are the basic determinants of success for embryo transfer and the whole IVF–embryo transfer procedure. Implantation and pregnancy rates are inversely correlated with the frequency of uterine contractions. To evaluate whether uterine contractility elastography based on the analysis of tissues deformation induced by mechanical distortion, such as blood pulse at each heartbeat, subendometrial vasculature measurement by 3-dimensional Ultrasound with power Doppler angiography and clinicopathological factors are associated with successful pregnancy in IVF patients. Thirty-four volunteer women scheduled for embryo-transfer in IVF between August 2017 and December 2017. two-dimensional sagittal uterus elastography was recorded for 300 seconds, the elasticity index, defined as the mean ratio of elastographic measurements between endometrial area and subendometrial area was computed. Uterine contractility frequency, endometrial thickness, and volume were measured. Also, subendometrial 3-dimensional ultrasound and power Doppler angiography were performed to examine the vascularization index (VI), flow index (FI), and vascularization-flow index (VFI). The subendometrial FI was significantly increased in pregnant women on ET day (SMD, 0.30; 95% CI, 0.08, 0.52; P = .007). The elasticity index was higher in pregnant women (2.1 1.5 vs 1.0 0.5). Significantly, FI and elasticity index had relationship (p 1.5;OR=48.33), less echogenic endometrium, high endometrial thickness (>6mm; OR=21.1) on ET day were also predictive of pregnancy after ET. The present results suggest that Uterine contractility and subendometrial vasculature measurement by 2D and 3D ultrasound with power Doppler as non-invasive technique can used to assess to evaluate intrauterine environment for successful pregnancy in IVF patients.

Sildenafil Ameliorates Aging-Induced Changes in Rat Testis. Tatjana Kostic, Srdjan Sokanovic, Ivan Capo, Marija Medar, and Silvana Andric

NO-cGMP signaling pathway has been implicated in reduction of testicular steroidogenesis during aging. Here we analyzed the effect of PDE5 inhibition on old testicular phenotype formation. The old phenotype exhibited low testosterone and increased nitrite levels in circulation, increased cGMP accumulation in testicular interstitial fluid (TIF), progressive atrophy of testicular seminiferous tubules and enlargement of interstitial area followed by rise in blood vessel density and slight increase in the number of Leydig cells and macrophages. Leydig cells have reduced steroidogenic capacity, increased MAP kinases expression (MEK, ERK1/2, JNK) and antiapoptotic PRKG1 and AKT, suggesting increased proliferation/survival and accumulation of senescent Leydig cells in testis. In 12 month-old rats, a long-term treatment with sildenafil (PDE5 inhibitor) normalized testosterone/nitrite levels in circulation and cGMP accumulation in TIF; improved Leydig cell steroidogenic capacity; decreased MEK, ERK1/2 and PRKG1 expression; prevented an increase in the Leydig cells number and atrophy of seminiferous tubules leading to histological appearance of young rat testes. In 18 month-old rats, long-term PDE5 inhibition partially recovered testosterone and nitrite levels in serum; normalized PRKG1 expression without effect on MEK and ERK1/2; and slowed down Leydig cell and macrophage accumulation and regressive tubular changes. Culturing of primary Leydig cells from aged rats in presence of PDE5-inhibitor stimulated steroidogenic and MAPK gene expression. Taking together, results indicate that cGMP targeting alter both steroidogenesis and signaling pathways associated with cell

proliferation/survival. The long-term PDE5 inhibition improves testicular steroidogenesis and slows-down regressive changes in testes during aging.

Core Binding Factor β Expression in Ovarian Granulosa Cells is Essential for Female Fertility. Somang Lee-Thacker, Yohan Choi, Ichiro Taniuchi, Takeshi Takarada, Yukio Yoneda, CheMyong Ko, and Misung Jo

Core Binding Factor beta (CBFβ) is a non-DNA binding partner of all RUNX proteins and critical for transcription activity of CBF transcription factors (RUNXs/CBFβ). In the ovary, the expression of Runx1 and Runx2 is highly induced by the LH surge in ovulatory follicles and corpora lutea, while Cbfb is constitutively expressed. To investigate the physiological significance of CBFs in the ovary, the present study generated two different conditional mutant mouse models in which granulosa cell expression of Cbfb and Runx2 was reduced by Cre recombinase driven by an Esr2 promoter. Cbfbgc-/- and Cbfbgc-/- * Runx2 gc+/- mice exhibited severe subfertility and infertility, respectively. In the ovaries of both mutant mice, follicles develop normally, but the majority of preovulatory follicles failed to ovulate either in response to hCG administration in PMSG-primed immature animals or after the LH surge at 5 months of age. Morphological and physiological changes in the corpus luteum of these mutant mice revealed reduced size, progesterone production, and vascularization, as well as excessive lipid accumulation. In granulosa cells of periovulatory follicles and corpora lutea of these mice, the expression of Edn2, Ptgs1, Lhcgr, Sfrp4, Wnt4, Ccrl2, Lipg, Saa3, and Ptgfr was also drastically reduced. In conclusion, the present study provided in vivo evidence that CBFβ plays an essential role in female fertility by acting as a critical cofactor of CBF transcription factor complexes which regulate the expression of specific key ovulatory and luteal genes, thus coordinating the ovulatory process and luteal development/function in mice.

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Ubiquitin-Proteasome System (UPS) Regulates Spermadhesin Release During Boar Sperm Capacitation. Michal Zigo, Pavla Postlerova, Vera Jonakova, and Peter Sutovsky

The ubiquitin-proteasome system (UPS) is a complex enzymatic machinery responsible for protein degradation and turnover in all living organisms including plants. It is now well established that UPS participates in the events of mammalian sperm capacitation. To further elucidate the role of UPS in boar sperm capacitation, we studied the participation of UPS in spermadhesin release during in vitro capacitation (IVC). In vivo, the proteolytic cleavage of spermadhesins on the sperm surface is associated with the release of spermatozoa from the oviductal sperm reservoir.

At ejaculation, boar spermatozoa acquire low molecular weight (8-16 kDa) seminal plasma proteins, predominantly represented by spermadhesins, aggregated on the sperm surface. Due to their arrangement, such aggregates are relatively inaccessible to both antibody labeling and surface biotinylation. However, in IVC spermatozoa, both antibody and surface biotinylation are feasible as a result of de-aggregation and release of the outer layers of spermadhesins from the sperm surface. We took advantage of this de-aggregation to perform image-based flow cytometry studies of AQN-1, AWN,

PSP-I, and PSP-II spermadhesins, which induce higher fluorescent intensity in capacitated vs. ejaculated spermatozoa. Addition of proteasomal inhibitors during IVC (20 µM epoxomicin or 100 µM MG132), significantly reduced fluorescence intensity of both the AQN-1 and AWN (P 0.95).

Our results demonstrate that UPS participates in the de-aggregation and shedding of spermadhesins from the sperm surface during capacitation, with a possible implication in detachment from the oviductal sperm reservoir, though other proteolytic and signaling pathways likely contribute to spermadhesin processing during capacitation.

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Identification of a DNA-Binding Sequence for ZNFO, a Novel Oocyte-Specific Zinc Finger Transcription Factor in Cattle. Mingxiang Zhang, George W. Smith, and Jianbo Yao

ZNFO is a novel zinc finger transcription factor which is specifically expressed in bovine oocytes and early embryos. The protein contains a conserved KRAB domain at the N-terminus and nine zinc finger motifs at the C-terminus and it plays an important role during early embryonic development in cattle. Through a cyclic amplification and selection (CASTing) assay, we have identified a potential ZNFO binding element (ZBE), ATATCCTGNNNNNNNCCC. To further confirm the binding specificity of ZNFO to the identified element, an electrophoretic mobility shift assay (EMSA) was performed using IDye 700 labeled probes containing the target sequence and purified Halo-tagged ZNFO fusion protein. A competitive binding assay was also performed using 10-fold and 100-fold molar excess of unlabeled cold competitors containing the full length element and partial elements containing either ATATCCTG or CCC. The results confirmed the interaction between ZNFO and ZBE, and showed that both ATATCCTG and CCC are critical for the binding of ZNFO to ZBE. Further analysis of promoter regions of candidate bovine genes that contain ZBE may lead to the discovery of specific genes regulated by ZNFO.

Protein Profile During Placentome Maturation in Dairy Cows. Contreras D. A., Salgado-Hernandez E. G., González-Lozano M., Valdez-Magaña G., and Guerrero-Netro H. M.

Placenta retention is a problem that affects around 20% of dairy cattle. In ruminants, the placentome is responsible for connecting the uterous with the placenta. Each placentome is formed by fetal cotyledon and a maternal caruncula. During parturition, the placentome requires changes in protein expression, such as collagenases and metalloproteinases. These changes in protein expression are known as placentome maturation and result in the expulsion of the placenta; However, the mechanisms that take place during placentome maturation remain unknown. The objective of this study was to elucidate the mechanisms involved during placentome maturation.

The animal welfare and ethics committee of FMVZ UNAM approved all experiments. Placentomes were collected from Holstein cows that presented normal placenta expulsion (up to 12 hrs) or placenta retention. Samples were stored in RIPA buffer containing phosphatase inhibitor and protease inhibitor. Protein concentration was determined using Bradford assay, 50µg of protein was used for Mass

spectometry (Thermo Scientific Q Exactive Orbitrap Mass Spectrometer). The results were analyzed by Thermo Scientific Proteome Discoverer software 2.1 along FASTA UniProtKB/Swiss-Prot databases.

Mass spectrometry identified 5 significantly up-regulated and 5 downregulated proteins during placenta retention. The up-regulated proteins included fetuin-A, globin-1, apolipoprotein-a1, sterile α motif domain cloning 7, and tyrosine 3-monooxygenase. These proteins seem to play a role during inflammatory process and apoptosis. On the other hand, the down-regulated proteins included: globin C1 and A1, Serotransferrin, alpha 1 acid glycoprotein, alpha 2-HS-glicoprotein. These down-regulated proteins are involved in the formation of hemoglobin, oxygen transport and in the cholesterol reverse transport. These results to the best of our knowledge are the first to demonstrate different pathway activation during placenta retention, further work is required to assess the signaling pathways involved during placentome maturation.

This work was supported by FMVZ-UNAM-MEXICO.

MicroRNA-582-5p Suppresses Epithelial-Mesenchymal Transition via Down-Regulating Sorcin in Mouse Endometrium. Kanchan Gupta, Vijay K Sirohi, Rohit Kumar, and Anila Dwivedi

Our earlier studies have shown that sorcin is present in human endometrium and is also involved in the process of embryo implantation in mice. The present study was aimed to identify miRNAs regulating sorcin expression and playing role in endometrial receptivity and embryo implantation. Using bioinformatics target prediction tool target scan, we identified miR-23a-3p, miR-425-5p and miR-582-5p as predicted to target 3'UTR of sorcin mRNA. Among three identified miRNAs, miR-425 and miR-582 showed time specific changes during period of early pregnancy. We selected miR-582 for detailed exploration as its expression pattern was contrary to the sorcin expression on days -5 and -6 of pregnancy suggesting its probable role in pregnancy establishment. Over-expression of miR-582 caused significant down-regulation of sorcin in uterus and in mouse primary endometrial epithelial cells as observed by western blotting, quantitative real time PCR and immunofluorescence. In-vivo over-expression using mimic of miR-582 significantly reduced the implantation sites indicating its inhibitory role in embryo implantation. Also, the receptivity marker integrin β3 expression was found to be decreased in miR-582 over-expressed uterine horn as compared to control horn. In-vitro study on endometrium–trophoblast interaction further supported these findings. Upon transfection, the attachment and expansion of BeWo spheroids on RL95-2 endometrial cells were significantly reduced when these cells were transfected with mimic miR-582. Over-expression of miR-582 led to activation of epithelial marker E-cadherin and inhibition of mesenchymal markers N-cadherin, vimentin resulting into the suppression of migratory and invasive capacities of mouse primary endometrial epithelial cells. The study suggests that miR-582 causes suppression of epithelial to mesenchymal transition via targeting sorcin and hence plays important role during embryo implantation in mice uterus. This work was supported by Council of Scientific & Industrial Research (CSIR).

Cryopreservation Induces Mitochondrial Permeability Transition in the Bovine Sperm Model. Favian Treulen, María Elena Arias, Luis Aguila, Pamela Uribe, and Ricardo Felmer

When the mitochondria of somatic cells are exposed to pathological calcium overload, these trigger the mitocondrial permeability transition (MPT) leading to mitochondrial dysfunction and cell death. On the other hand, cryopreservation procedures expose mammalian spermatozoa to physical and chemical stressors, which affect plasma membrane integrity and induce a pathological calcium overload that gradually promote loss of sperm quality and eventually function. Although several studies highlight the role of calcium in many physiological and pathological processes, the MPT induced by an intracellular calcium increase and its effect on cell quality of mammalian spermatozoa are unknown. The aim of this study was to evaluate the effects of cryopreservation on MPT and its relationship with the deterioration of sperm quality in the bovine model. To do this, frozen bovine spermatozoa were thawed and adjusted to 2 x 106 mL-1 and incubated for 4 h at 38ºC. By using flow cytometry we evaluated MPT by the calcein-AM and cobalt chloride method, intracellular Ca2+ level using FLUO3-AM, plasma membrane integrity by exclusion of propidium iodide, mitochondrial membrane potential (DYm) with tetramethylrhodamine methyl ester perchlorate and intracellular ROS production with dihydroethidium. ATP levels were assessd by a quimioluminiscent method. The results showed that thawed spermatozoa trigger MPT associated to an intracellular calcium increase and this was accompanied by DYm dissipation, decrease of ATP levels and ROS production, and deterioration of the plasma membrane integrity. In conclusion, the cryopreservation induces MPT and this is associated with loss of sperm quality.

Dietary Supplementation with L-Arginine to Gilts Between Days 14 and 25 of Gestation Enhances Placental Expression of Angiogenic Proteins. Mohammed A. Elmetwally, Xilong Li, Gregory A. Johnson, Robert C. Burghardt, Cassandra Herring, Fuller W. Bazer, and Guoyao Wu

Dietary supplementation of gilts with 0.4 or 0.8% L-arginine (Arg) between Days 14 and 25 of gestation enhances placental angiogenesis and embryonic survival. However, the underlying mechanisms are largely unknown. This study tested the hypothesis that Arg supplementation stimulated placental expression of proteins related to angiogenesis: endothelial nitric oxide synthase (eNOS), vascular endothelial growth factor (VEGF), placental growth factor (PGF), GTP cyclohydrolase-I (GTP-CH), and ornithine decarboxylase (ODC). Gilts were checked daily for estrus with boars and bred at onset of their second estrus and 12 h later (the time of breeding = day 0 of gestation). Between Days 14 and 25 of gestation, 10 gilts/treatment were housed individually and fed twice daily 1 kg of a corn-soybean meal-based diet supplemented with 0.0, 0.4, or 0.8 % Arg. All diets were made isonitrogenous by addition of L-alanine. On Day 25 of gestation, gilts were hysterectomized to obtain conceptuses for histochemical and biochemical analyses. eNOS and VEGF receptor-1 proteins were localized in the wall of blood vessels at the maternal placental interface. Compared with the control, dietary supplementation with 0.4 or 0.8% Arg increased ($P < 0.05$) the amounts of NOx (nitrite plus nitrate) and polyamines (putrescine plus spermidine plus spermine) in allantoic and amniotic fluids; placental concentrations of PGF, NOx, polyamines and BH4; placental syntheses of NO and polyamines; and placental activities of GTP-CH and ODC1. Collectively, these results indicate that dietary Arg supplementation to gilts between Days 14 and 25 of pregnancy increase placental angiogenesis by increasing the expression of genes for angiogenic proteins.

Disruption to O-GlcNAc Cycling Promotes Epithelial-Mesenchymal Transition in Endometrial Cancer Cells in Vitro. Nicole Morin Jaskiewicz and David H. Townson

Diabetic women have a 2-3 fold increased risk of developing endometrial cancer, however, the molecular aspects of this risk are not fully understood. This study investigated the alteration of cellular O-GlcNAcylation of proteins as the potential mechanistic connection between these two conditions. Aberrant O-GlcNAcylation is a potential cause of insulin resistance and is a hallmark of many cancers. For instance, O-GlcNAc cycling enzyme mRNA (OGT, MGEA5) is upregulated at the myometrial edge of endometrial tumors, suggesting a role in cancer metastasis. In the current study, in vitro analysis of the endometrial cancer cell line (Ishikawa) indicated an increase in epithelial mesenchymal transition (EMT), especially under Hyper-O-GlcNAc conditions (1µM Thiamet-G). Hyper-O-GlcNAcylation resulted in an upregulation of EMT associated genes (WNT5B and FOXC2), a decrease in epithelial protein (ZO-1), and an increase in mesenchymal proteins (Snail and Claudin-1). Reorganization of actin filaments into thick stress filaments, consistent with EMT, was also noted in Thiamet-G-treated cells. Interestingly, Hypo-O-GlcNAcylation (via inhibition of the OGT enzyme; 50 µM OSMI-1) also upregulated WNT5B and FOXC2, inferring that any disruption to O-GlcNAc cycling impacts EMT. However, Hypo-O-GlcNAcylation also reduced cellular proliferation/migration. In summary, disruption of O-GlcNAc cycling (via OGT or OGA inhibition) promoted EMT at both the mRNA and protein levels. Additionally, Hypo-O-GlcNAcylation specifically had a negative impact on cellular proliferation/migration, and cytoskeletal organization. This material is based upon work supported by the National Science Foundation Graduate Research Fellowship under Grant No. (DGE 1450271).

Production of Knock-In Embryo By CRISPR/Cas9-Mediated Homologouse Recombination to Produce Bovine Lactoferrin on Bovine B-Casein Gene Locus. Da Som Park, Se Eun Kim, Deog-Bon Koo, and Man-Jong Kang

The production of pharmaceutical proteins by transgenic animals is one of the major successes of biotechnology. Knock-in system is a more powerful method to produce mammary gland bioreactor. To date, zinc-finger nuclease(ZFNs), transcription activator-like effector nuclease(TALENs), and clustered regularly interspaced short palindromic repeats(CRISPR)/Cas9 systems have been developed for gene targeting. The objective of this study was to develop a knock-in embryo for expression of bovine lactoferrin in the bovine β-casein gene locus by microinjection of knock-in vector with CRISPR/Cas9 into bovine zygote. The three replacement knock-in vectors containing a different length of homologous arm were constructed. These targeting vectors were used enhanced green fluorescent protein(eGFP) as a positive selection marker. These knock-in vectors with CRISPR/Cas9 were microinjected into the pronuclear bovine embryo. And the embryos were cultured to blastocyst in the culture medium. These blastocysts were analyzed by PCR to confirm gene targeting by homologous recombination. As a result, when bLF_1kbHR and 40HR_GFP knock-in vector with CRISPR/Cas9 was microinjected into cytoplasm of bovine zygotes, the efficiency of gene targeting was 22.2-26.7% but gene targeting by homologous recombination was not detected when bLF_100HR_GFP knock-in vector was microinjected into cytoplasm of bovine zygotes. The precise bovine lactoferrin gene integration of knock-in embryos was confirmed by DNA sequencing analysis. Our knock-in system may help to create transgenic dairy cattle

expressing enhanced bovine lactoferrin protein in the mammary gland via the endogenous expression system of the bovine β-casein gene.

Sperm Epimutation Biomarkers of Obesity and Pathologies following DDT Induced Epigenetic Transgenerational Inheritance of Disease. Stephanie E. King, Margaux McBirney, Daniel Beck, Ingrid Saddler-Riggleman, Eric Nilsson, and Michael K. Skinner

Dichlorodiphenyltrichloroethane (DDT) has previously been shown to promote the epigenetic transgenerational inheritance of adult onset disease in rats. The current study investigated the potential that sperm epimutation biomarkers can be used to identify ancestral induced transgenerational disease and associated pathologies. Gestating F0 generational rats were transiently exposed to DDT during fetal gonadal sex determination and the incidence of adult onset pathologies was assessed in the subsequent F1, F2, and F3 generations. In addition, sperm differential DNA methylation regions (DMRs) that were associated with specific pathologies in the F3 generation males were investigated. No pathology was observed in the F1 generation DDT lineage males or females compared with F1 generation controls (vehicle exposure). There was an increase of testis disease and early onset puberty in the F2 generation DDT lineage males. The F3 generation DDT males had significant increases in testis disease, prostate disease, and late onset puberty. The F3 generation DDT females had significant increases in ovarian and kidney disease. Both the F3 generation males and females had significant increases in the frequency of obesity and multiple disease. Germline mediated epigenetic alterations are required for the transgenerational inheritance of disease. The F3 generation sperm was collected to examine differential DNA methylation regions (DMRs) for the ancestrally exposed DDT male populations. Unique sets of sperm DMRs were associated with late onset puberty, prostate disease, kidney disease, testis disease, obesity and multiple disease pathologies. Gene associations with the DMR were also identified. The male germline epigenetic DMR signatures identified for these pathologies provide potential biomarkers/diagnostics for transgenerationally inherited disease susceptibility. Observations demonstrate that DDT can promote the germline mediated epigenetic transgenerational inheritance of reproductive diseases and other pathologies. The DMRs or epimutations observed in the F3 generation sperm provide potential biomarkers for transgenerational disease and ancestral environmental exposures.

The Effect of Thymic Nurse Cell (TNC) Exposure on the Uterus in NZBWF1 Mice. Eboni Price, Michael Henderson, Maria Martinez, Chastity Bradford, Donunique Fine, Terrance Platt, and Olga Bolden-Tiller

Systemic lupus erythematosus (SLE) or lupus is a chronic autoimmune disease that attacks many organs of the body and has been predominately characterized as a female disease, as it is estimated that four to 12 females for every one male is at risk for SLE within the child bearing ages of 15 to 44 in humans with a significance in African-American women. SLE is known to cause considerable damage to the reproductive system in females. Autoimmune oophortis, a rare cause of primary ovarian insufficiency, has been associated with lupus and estrogen and occurs when the body's immune system attacks the ovaries and uterus causing inflammation, fibrosis, and atrophy. Recent studies suggest that a reduction

in the number of TNC correlates with the aggressiveness of SLE reported in the NZBWF1 mice, which displays human like symptoms. We hypothesize that TNC exposure can mitigate the effects of SLE on the female reproductive system. The objective of the current study was to utilize the NZBWF1 mouse model to determine the effect of SLE and the exposure of TNC on the uterus. NZBWF1 mice were assigned to one of the following groups: A = No Treatment (n=6), B = Saline (n=7), and C = TNC Injected (n=9), and BALB/c mice were used as a control (group D; n=8). Groups A and B were pooled for the statistical analysis ANOVA. Animals were treated at 12 weeks of age and sacrificed at 16 weeks at which time the uteri and ovaries were harvested and weighed. The uterine weight for groups A (0.095 ± 0.017g) and group B (0.102 ± 0.106g) were not significantly different, and when pooled, the uterine weights for these animals (0.098 ± 0.008g) were significantly lower (P This research was supported by USDA/NIFA80-22090210 and the NIH RCMI Grant# G12RR003059-21A.

Identification of Potential Novel Protein Coding Genes in the Post-Meiotic Male Germ Cell. Elizabeth Snyder, Jaya Gamble, Steve Gygi, and Robert Braun

The male germ cell has one of the most complex transcriptomes in the mammalian body, yet only limited descriptions of the male germ cell transcriptome have been reported and very little work has addressed whether germ cell transcriptome complexity impacts proteome diversity. Using a set of novel analysis tools, RNA-sequencing (RNA-seq) data from late juvenile testis was used to define a complete testis transcriptome. This transcriptome was compared to the annotated mouse transcriptome and found to include a large number of unannotated transcripts, from both known and novel loci. Extensive molecular confirmation demonstrated the efficacy of the transcript-building pipeline for both unannotated transcript types. This analysis further found many novel transcripts were testis specific or enriched. Additionally, RNA-seq data suggested most were expressed primarily in meiotic and post-meiotic germ cells. In silico coding potential analysis identified a subset of novel transcripts with high protein coding potential (putative mRNAs - pmRNAs), suggesting that germ cell transcriptome complexity might indeed increase proteome complexity. The majority of pmRNAs examined showed testis enriched or specific expression similar to other novel transcripts. Nearly all pmRNAs were associated, to some degree, with actively translating ribosomes and a number were found to have non-mouse homologs with known or provisional protein-coding status. To test whether pmRNAs could be generating protein, FACs isolated post-meiotic germ cells were analyzed by LC-MS/MS. This analysis identified a large number of peptides that could be derived exclusively from pmRNAs, further supporting the idea that pmRNAs represent genuine protein coding transcripts. Surprisingly, the LC-MS/MS analysis also identified a set of peptides that appeared to be derived from annotated long non-coding RNAs (lncRNAs). Ongoing and future efforts will focus on molecular confirmation of peptides generated from pmRNAs and protein coding lncRNAs.

Microbiome Profile of Mouse Female Reproductive Tract During Embryo Implantation. Yuehuan Li, Christian Lee Andersen, Zidao Wang, and Xiaoqin Ye

Emerging clinical studies have implicated altered distribution of bacteria within women's reproductive tract in reproductive diseases, such as miscarriage and infertility. The microbiome in the female reproductive tract can potentially be used to predict the overall health status of the female reproductive tract. Because of ethical issues, mouse models have been widely used in vivo models for studying mechanisms involving physiology (e.g., embryo implantation) and pathology (e.g., embryo implantation failure) of the female reproductive system. Embryo implantation is a critical step in mammalian reproduction. It initiates around gestation day 4.0 (D4.0) in mice. There is a lack of information available about the microbiome in the mouse female reproductive tract during embryo implantation. We hypothesize that the microbiome profile in the female reproductive tract undergoes changes to facilitate embryo implantation and mouse models with defective embryo implantation have altered microbiome profiles in their reproductive tracts. To test this hypothesis, we will determine the whole microbiome profiles in different parts (vagina, uterus, and oviduct) of the female reproductive tract at different time points of early pregnancy leading to embryo implantation, from D0.5 to D4.5. We have collected vaginal, uterine, oviductal, and uterine flush samples from the wild type mice on D0.5, D2.5, D3.5, and D4.5, and are collecting comparable samples from our mouse models with defective embryo implantation. Preliminary quantitative PCR data from wild type uterine samples suggest differential expression of Lactobacillus genus during early pregnancy. Our ongoing study will determine the spatiotemporal microbiome composition and distribution in the mouse female reproductive tract with normal or failed embryo implantation. The obtained data will provide valuable information for future investigation on the functions of female reproductive tract microbiome in embryo implantation.