Abstract # 2164

Artificial Intelligence Analysis of the Mammalian Sperm Zinc Signature Predicts Male-factor Subfertility. Karl Kerns, University of Missouri, USA

Analysis of both the U.S swine and bovine herds show variation in pregnancy rate is more attributable to male-factor subfertility and infertility than the dam. To date, a limited degree of correlations is observed between conventional semen analysis parameters and actual fertility after standard quality cutoffs are met. Thus, a clear ability to predict male-factor fertility is lacking. Building on our recent discovery of the sperm zinc efflux on the pathway to fertilization competency present in boar, bull, and human spermatozoa published in Nature Communications (DOI:10.1038/s41467-018-04523-y), we hypothesized in vitro capacitation-induced changes to the sperm zinc signature would be indicative of male-factor sub- and infertility. The ongoing fertility trial currently includes 108 boar ejaculates inseminated to over 1,917 sows in a single, fixed-time artificial insemination setting, with pregnancy results ranging from 56.4% - 96.8%. Each ejaculate underwent in vitro capacitation with 10,000 spermatozoa imaged at 0, 1, and 4 hours utilizing high-throughput, image-based flow cytometry. We calculated over 6,550 bioimage values for each of the time points analyzed. Mutual information analysis found 27 sperm bioimage features with scores greater than 0.1 mutually informative to the pregnancy rate. Linear regression analysis was performed on these features and tested with a nested model. ANOVA of the linear regression model identified four features significant with high fertile males within the nested model and eight features for the full model. Next the data was randomly split (4:1) into training and testing sets and classification trees were calculated to predict the pregnancy rates after being discretized into fertile (above 85% pregnancy rates) and subfertile classes (below 80% pregnancy). One tree was trained with 17 features found in traditional semen analysis related strictly to sperm morphology and computer-assisted sperm analysis (CASA) motility outputs, and a separate tree was trained with 170 features related to differences in zinc signature subpopulation changes after in vitro capacitation, significant features found by mutual information analysis, and motility. The traditional semen analysis feature set yielded respective training and testing accuracies of 100% and 53.8%, whereas the later feature set yielded respective training and testing accuracies of 100% and 76.9%. Artificial neural network analysis of zinc, acrosome, and plasma membrane integrity bioimages along with litter size are currently underway. In summary we identified the ability for sperm to transition from a zinc signature 1 and 2 to a capacitated-state signature 3 and 4 along with acrosomal modification and changes to the plasma membrane integrity excels in

predictive value of male factor fertility compared to traditional motility and morphology scores alone. Altogether, our findings establish a new paradigm on the role of zinc ions in sperm function and pave the way for accurate sperm biomarker identification of male-factor sub/infertility in future precision agriculture and medicine applications. Supported by the National Institute of Food and Agriculture (NIFA), U.S. Department of Agriculture (USDA) Postdoctoral Fellowship award number 2019-67012-29714 (KK), USDA NIFA grant number 2017-67015-26760 (PS), NIH BD2K Training Grant T32HG009060 (SK), and funding from the MU F21C Program (PS).