ablation of canonical AHR activity (CYP1A1) in maternal tissues, while placental and fetal tissues retained canonical AHR activity (CYP1A1). Disruption of AHR activity in maternal tissues interfered with TCDD-activated placentation site adaptations. Taken together, these findings indicate that at least some of TCDD effects on placental development are mediated through its actions on the maternal environment. In summary, we have identified a developmental window of sensitivity to environmental pollutants affecting hemochorial placentation with the potential of impacting fetal and postnatal health. (Supported by NIH grants: HD20676, HD079363.)

**P94. Transforming Growth Factor-Beta1 Induced Activation of Rho-Kinase Pathways Regulate Bovine Trophoblast Differentiation.**

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Trophectodermal cells of blastocysts develop to generate the matrotrophic interface between the uterine epithelium and embryo. Pre-implantation development of the bovine embryo is characterized by a phase of rapid undifferentiated trophoblast proliferation resulting in an elongated conceptus. Implantation corresponds to end of the elongation phase that leads to functional and structural differentiation of some mononucleate trophoblast cells (MTCs) into binucleate cells (BNCs). With the least invasive epitheliochorial placentation, implantation is thought to be achieved by adhesion and subsequent fusion of these BNCs to the uterine caruncular epithelial cells. However, the molecular mechanisms driving early undifferentiated expansion of MTCs, their subsequent differentiation into BNCs and events leading to implantation remain poorly understood. Using both shotgun proteomics and transcriptome sequencing we identified Rho-kinase signaling as an overrepresented pathway in bovine blastocyst-derived trophoblast cells. Inhibition of Rho-kinase signaling using Y27632 revealed its critical role in regulation of undifferentiated MTC proliferation. Investigating upstream mechanisms led to identification of transforming growth factor-β (TGF-β) as an activator of Rho-kinase in MTCs. Treatment with SB431542, a type I TGF-β receptor inhibitor, significantly increased MTC proliferation, identical to Rho-kinase inhibition. Conversely, treatment with recombinant TGF-β1 resulted in significant reduction of MTC proliferation and induced morphological differentiation into BNCs. This study provides new mechanistic information on signaling that directs bovine trophoblast proliferation and differentiation, and advances understanding of implantation in cattle. This research was supported by USDA-NIFA 2013-67015-21223 (VS) and 2011-67015-30688 (PJH).

**P97. Effect of Size and Position of the Reproductive Tract on Concentrations of Bovine Pregnancy Associated Glycoproteins (PAGs) and the Relationship with Fertility.**

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Reproductive failure leads to significant economic consequences in both beef and dairy animals. Variables associated with these reproductive losses during early gestation are multifaceted including contributions from the oocyte, uterus, sperm, embryo and the placenta. Bovine pregnancy associated glycoproteins (PAGs) produced by the binucleate cells of the ruminant placenta are used to diagnosis pregnancy and may serve as a potential marker of embryonic mortality in cattle. Increased circulating concentrations of PAGs at day 28-31 of gestation have been correlated with pregnancy and predictive of impending embryonic mortality in both beef and dairy cattle. The aim of this study was to evaluate the effect of uterine size and position on concentrations of bovine pregnancy associated glycoproteins (PAGs) and its relationship with pregnancy per AI (P/AI) and pregnancy losses in lactating dairy cows. A total of 662 lactating Holstein cows (average milk production of 37.5 ± 7.1 kg/day) received an estradiol/progesterone based timed AI protocol to induce ovulation and TAI occurred on day 0. Reproductive tract was classified according to size and position score (SPS) at the moment of TAI as follows: SPS1 – small and compact uterine horns and cervix resting within the pelvic cavity; SPS2 – moderate size of uterine horns and cervix, partially resting at/over the pelvic brim and SPS3 – large and thick uterine horns and cervix resting beyond the pelvis. Blood samples were taken at 24 and 31 days after AI to measure circulating PAG concentrations. Pregnancy diagnosis was performed at days 31 and 120 after AI by ultrasonography. Data were analyzed using the GLIMMIX procedure of SAS. SPS was impacted by parity ($P < 0.01$), where primiparous cows had a higher frequency of SPS1 and lower frequency of SPS3 when compared with multiparous cows (SPS1 - 39.9% vs. 19.3%; SPS3 – 2.9% vs. 15.5%). Average circulating concentrations of PAGs in the pregnant cows were 0.93 ± 0.56 ng/ml and 3.56 ± 1.76 ng/ml on d24 and 31, respectively. Concentration of PAGs were not different at d24 between SPS or parity; however, primiparous cows had higher concentration of PAGs at d31 than multiparous cows (4.51 ± 0.27 ng/ml vs. 3.43 ± 0.8 ng/ml; $P < 0.01$). Independent of parity, PAG concentration on d31 was higher in animals with SPS1 when compared with SPS2 and SPS3 (5.08 ± 0.36 ng/ml vs. 3.66 ± 0.25 ng/ml vs. 3.04 ± 0.57 ng/ml respectively; $P < 0.01$). Animals that had SPS1 also had higher fertility when compared to SPS2 and SPS3 (42.4% vs. 32.6% vs. 23.9%, $P < 0.01$) even when parity was held constant. Pregnancy loss between d31 and d120 was increased in cows with SPS3 compared to SPS2 and SPS1 (14.9% vs. 9.6% vs. 2.9% for SPS3, SPS2 and SPS1, respectively; $P = 0.04$). In conclusion, cows with a larger reproductive tract resting beyond the pelvic cavity (e.g. SPS3) had lower fertility, lower circulating concentration of PAGs at d31 post-AI and higher pregnancy losses by d120 post-AI.

**P100. WITHDRAWN.**