587 Tamoxifen treatment affects morphological characteristics and gene expression within the reproductive tract of prepubertal Holstein heifers. A. Y. Wood*, H. L. M. Tucker, V. L. McCracken, S. E. Deaver, B. M. Brown, R. M. Akers, and M. L. Rhoads, *Virginia Polytechnic Institute and State University, Blacksburg.*

Reproductive responses to steroid hormones during the prepubertal period of heifers are poorly understood. This experiment was conducted as a first step toward understanding the significance of estrogen receptor signaling within the reproductive tissues during the prepubertal period. As such, tamoxifen (TAM) was administered to heifers (n = 8) daily (0.3 mg/kg subcutaneously) from approximately 28 to 120 d of age. Control heifers (CON; n = 6) received an equal volume of excipient. Gross measurements and samples of reproductive tract tissues, and plasma were collected upon sacrifice at 120.7 ± 0.3 d of age. TAM did not affect final body weight, hip height or plasma estradiol concentration. Irrespective of body weight, TAM dramatically decreased overall weight of the reproductive tract (42.04 ± 3.96 g vs 26.32 ± 3.43 g; $P = 0.01$). This difference was due to concomitant decreases in weight of the ovaries (5.89 ± 0.95 g vs 3.09 ± 0.58 g; $P < 0.05$) and uterus (34.45 ± 5.44 g vs 23.23 ± 3.33 g; $P = 0.11$). Interestingly, the number of ovarian follicles did not differ between CON and TAM animals. Expression of estrogen receptor (ER) α in the uterus of the CON animals was nearly double that of TAM animals (30359.60 ± 3053.27 cn vs 15794.12 ± 2644.21 cn; $P < 0.01$) whereas ERα merely tended to differ in the oviduct ($P < 0.15$) and did not differ in the ovary. Abundance of the β form of the ER did not differ in uterus, oviduct or ovary. Conversely, progesterone receptor (PR) expression in the uterus (43465.99 ± 5994.86 cn vs 59990.13 ± 5191.70 cn; $P = 0.06$) and oviduct (12495.22 ± 3433.85 cn vs 26570.15 ± 3179.13 cn; $P = 0.01$) was increased by TAM while ovarian expression of PR was similar between groups. In summary, the selective estrogen receptor modulator, TAM affected the morphological development of the entire reproductive tract. Effects of TAM on the sex steroid receptor expression, however, were most apparent in the uterus and oviduct. These results demonstrate that alterations in ER signaling influence reproductive tract characteristics of heifers during the prepubertal period.

Key Words: tamoxifen, prepubertal, estrogen

588 No evidence of a systemic mRNA biomarker of early pregnancy using RNAseq of whole blood on day 20 of pregnancy in dairy cattle. M. P. Mullen*[1,2], P. McGettigan[2], J. A. Browne[2], S. Scully[2], M. G. Diskin[1], A. C. O. Evans[3], and M. A. Crowe[2], [1]*Teagasc, Athenry, Co. Galway, Ireland,* [2]*School of Veterinary Medicine, University College Dublin, Dublin 4, Ireland,* [3]*School of Agriculture and Food Science, University College Dublin, Dublin 4, Ireland.*

Early and accurate pregnancy diagnosis in dairy cattle is a requirement for efficient herd management. However, most of the currently available methods involve pregnancy diagnosis at or after 28–30 d of gestation, which is too late to re-breed at the next cycle. Therefore, the objective was to evaluate if any potential biomarkers of early pregnancy in dairy cattle could be discerned in blood by d 20 post insemination (AI) at the mRNA level using RNaseq technology. Whole blood samples were collected on d 20 post-AI from 22 dairy cows. Plasma samples were also taken daily from d 16 to 21 and on the date of pregnancy determination to generate progesterone profiles. Pregnancy status was determined between Days 30 to 35 post-AI by transrectal ultrasonography and resulted in the classification of 14 pregnant and 8 nonpregnant cows. Total RNA was extracted from whole blood using the TEMPUS blood RNA stabilization and extraction protocol. Eighteen 100-bp paired-end strand specific RNA libraries were prepared for n = 10 pregnant and n = 8 non-pregnant cows and sequenced on the Illumina HiSeq2000 platform. After quality control, 432,693,725 million 100-bp paired-end reads were generated, of which 94.6% mapped to the bovine UMD 3.1 genome assembly. A total of 12,108 genes were deemed to be expressed (>4 reads per gene per animal). Despite 171 genes showing nominal differential expression ($P < 0.01$), none remained significantly differentially expressed between the pregnant and non-pregnant groups after correction for multiple testing (FDR $P < 0.05$). While it is possible that subtle changes in the systemic transcriptome may be occurring due to the presence of a developing conceptus, which may warrant further targeted investigation into the data set, no significant biomarker at the mRNA level could be discerned using RNaseq of the whole blood on d 20 of pregnancy in dairy cattle. Funded by Science Foundation Ireland 07/SRC/B1156.

Key Words: pregnancy diagnosis, transcriptomics

589 Influence of post-insemination nutrition on embryonic development in beef heifers. S. G. Kruse*[1], B. J. Funnell[1], S. L. Bird[1], H. P. Dias[2], S. L. Lake[3], R. P. Arias[3], G. A. Perry[4], O. L. Swanson[4], E. L. Larimore[4], and G. A. Bridges[1], [1]*North Central Research and Outreach Center, University of Minnesota, Grand Rapids,* [2]*São Paulo State University, Botucatu, São Paulo, Brazil,* [3]*University of Wyoming, Laramie,* [4]*South Dakota State University, Brookings.*

Previous results have demonstrated that a reduction in nutrition immediately following AI reduces pregnancy rates. The objective of this experiment was to determine if nutrient restriction following AI affects early embryo development. Beef heifers in 3 replications (Rep; Rep 1; n = 44, Rep 2; n = 44, Rep 3; n = 50) were developed in a dry-lot and fed approximately 125% NRC requirements from weaning to timed-AI (d 0). Heifers were timed-AI to a single sire in all replications. Immediately following AI, heifers were assigned based on age and weight to one of 2 post-AI nutritional treatments. Half the heifers in each replication continued on the pre-insemination diet allowing weight gain (GAIN) and the remaining heifers were restricted fed to result in weight loss (LOSE). On d 6, embryos/ova were collected and recovered embryos/ova (LOSE; n = 42, GAIN; n = 46) were evaluated to determine quality (IETS standards; 1–5; 1 = excellent, 5 = degenerate) and stage (1–9; 1 = unfertilized, 9 = expanded hatched blastocyst). Embryos were then stained and evaluated to determine the number of dead cells (propidium iodide) and total blastomeres (Hoechst 33342). In Reps 1 and 2, concentrations of IGF-1 were assessed on d 0 and 6 and progesterone concentrations on d 4 and 6. Data was analyzed using the Mixed procedures of SAS. There were no treatment by Rep interactions for any data evaluated, thus all data were pooled. Embryo stage and quality were improved ($P < 0.05$) in the GAIN (4.6 ± 0.1, 2.0 ± 0.2, respectively) compared with LOSE treatment (3.8 ± 0.2, 2.8 ± 0.2, respectively). Embryos in the GAIN treatment had greater total blastomeres ($P = 0.03$; 70.6 ± 5.6) and percentage of live cells ($P = 0.01$; 83.3 ± 3.0%) compared with LOSE (48.9 ± 3.9; 71.1 ± 4.1%). Progesterone and IGF-1 concentrations did not differ between treatments nor were IGF-1 concentrations correlated with embryo parameters. In summary, nutrient restriction for 6 d immediately following AI resulted in poorer quality embryos that were retarded in stage, suggesting that immediate changes in nutrition can alter early embryonic development.

Key Words: embryo, nutrition, beef heifer

590 Effects of maternal nutrient restriction followed by realimentation on uterine blood flow during mid-gestation on beef cows. L. E. Camacho*[1], C. O. Lemley[2], L. Prezotto[1], K. C. Swanson[1], and K. A. Vonnahme[1], [1]*Department of Animal Sciences, North Dakota State University, Fargo,* [2]*Department of Animal and Dairy Sciences, Mississippi State University, Mississippi State.*

The objective of this study was to examine the effect of maternal nutrient restriction followed by realimentation during mid-gestation on uterine blood flow (BF). Lactating, multiparous Simmental beef cows (n = 10) were placed in a pen equipped with Insentec B. V. roughage individual intake control system feeders. On d 30 of pregnancy, cows were randomly assigned to treatments: control (CON; 100% NRC; n = 6) and nutrient restriction (RES; 60% NRC; n = 4) from d 30 to 140 (period 1) and thereafter being realimented to CON until d 198 of gestation (period 2). Calves were weaned at d 90 of dam gestation. Uterine artery measurements ipsilateral (i) and contralateral (c) to the conceptus were obtained on d 30, 58, 86, 114, 140, 152, 159, 166, and 198 of gestation via Doppler ultrasonography and included BF, pulsatility index (PI), and resistance index (RI). There was a treatment × period interaction ($P = 0.02$) on iBF where BF was similar ($P = 0.77$) between groups during period 1 but during period 2 iBF was greater ($P = 0.03$) in RES vs. CON. There were no treatment × day × period interactions ($P \leq 0.36$) for iPI and iRI but both decreased ($P < 0.01$) as gestation proceeded. There was a treatment × day × period interaction ($P = 0.06$) tendency for cBF. From d 30 to 114 cBF was similar between groups and by d 140 until 198 CON had greater cBF vs. RES. For cPI, there was a treatment × day × period interaction ($P = 0.02$) where both groups were similar from d 30 until 114, but on d 140 cPI was greater in RES vs. CON. From d 152 to 159 cPI was similar in both groups and by d 166 cPI was greater in RES vs. CON. Contralateral RI was not affected by treatment or period ($P = 0.60$). Both cPI and cRI decreased as gestation proceeded. There was no treatment × day × period interaction ($P = 0.98$) for total BF; however, there was an exponential increase in BF through gestation. Nutrient restriction during mid-gestation followed by realimentation affects uterine BF in pregnant beef cows without affecting resistance indices. Further investigations in uterine and placental vascular reactivity are warranted.

Key Words: nutrient restriction, pregnancy, uterine blood flow

591 Nutritional genomics: Effect of maternal methionine supplementation on the transcriptome of day 7 embryos from superovulated lactating dairy cows. F. Peñagaricano*[1], A. H. Souza[1], P. D. Carvalho[1], A. Driver[1], R. Gambra[1], J. Kropp[1], K. S. Hackbart[1], D. Luchini[2], R. D. Shaver[1], M. C. Wiltbank[1], and H. Khatib[1], [1]*University of Wisconsin-Madison, Madison,* [2]*Adisseo, Alpharetta, GA.*

The aim of this study was to assess the effect of maternal methionine supplementation on the transcriptome of d 7 embryos. Holstein cows were assigned to 1 of 2 treatments differing in level of dietary methionine from calving until embryo flushing (around 70 DIM). The treatments were (1) Methionine; diet formulated to deliver 2875 g MP with 6.8 Lys %MP and 2.43 Met %MP; (2) Control; same basal diet but formulated to deliver only 1.89 Met %MP. Cows were superovulated with a modified 5 d-Double Ovsynch with 4d of decreasing FSH (400 mg/cow) doses and flushed 6 d after synchronized ovulations. Cows with at least 4 grade 1 or 2 embryos were selected for the study (4 cows per group). Embryos from an individual cow were pooled (2–4 embryos per pool, 2 pools per cow) and analyzed by RNA sequencing. Total RNA extraction, amplification, library preparation, and sequencing were performed following Illumina mRNA-Seq protocol. Sequencing reads were mapped to the bovine reference genome (bosTau7) using Tophat. The resulting alignments were used to reconstruct transcript models by Cufflinks. Differential gene expression was analyzed using the edgeR package in R. In addition, gene set enrichment analysis was performed using a test of proportions based on the cumulative hypergeometric distribution. A total of 276 genes out of 10,662 showed differential expression between treatments (q-value <0.10). Some of the most significant genes are related to embryo development (e.g., VIM and TBX15), regulation of apoptosis (e.g., IFI6, BCL2A1), and immune system (e.g., BLA-DQB, LCP1, TYROBP). Some uncharacterized genes and novel transcripts also had differential expression. Pathway analysis revealed that several Gene Ontology terms (n = 33), InterPro entries (n = 12), and one KEGG pathway were enriched (q-value <0.05) with differentially expressed genes. Interestingly, many pathways closely related to the immune system were found to be significant. Overall, our results support the hypothesis that maternal methionine supplementation affects the transcriptome of bovine preimplantation embryos.

Key Words: methionine, embryo transcriptome, dairy cow

592 Activation of the transcription factor nuclear factor kappa B (NFKB) by recombinant porcine cytokines in the uterine epithelium. D. J. Mathew*, R. D. Geisert, and M. C. Lucy, *University of Missouri, Columbia.*

Embryonic mortality in the pig is temporally associated with conceptus elongation. Embryos undergoing elongation secrete estrogen for maternal recognition and interleukin 1 β (IL1B) to induce an inflammatory reaction in the uterus. There are 2 interleukin 1 β (IL1B) genes in the pig. IL1B is expressed by macrophages and the novel embryonic IL1B (IL1BE) is expressed by the pig conceptus. Within the uterus, IL1BE is believed to activate NFKB, a transcription factor needed for establishment of pregnancy. NFKB, in turn, controls cyclooxygenase-2 (COX2) expression. The objective was to test the capacity of IL1BE to activate NFKB and increase COX2 mRNA expression in porcine endometrium. Endometrium was dissected from 3 gilts and cultured in MEM for 4 h at 37°C. The tissues were then left untreated (negative control) or treated with a low, medium, or high dose of LPS (positive control; 1, 10, or 100 μg/mL), or recombinant β galactosidase (negative control), human IL1B or immature and mature forms of porcine IL1B and IL1BE (10, 100, or 1000 ng/mL). Tissues were frozen for mRNA expression analyses (RTPCR) or fixed and stained for NFKB localization. For NFKB, luminal epithelial cell nuclear vs cytoplasmic NFKB ratio was calculated. Within the uterine epithelium, there was an effect of treatment ($P < 0.001$) and concentration ($P < 0.05$) on NFKB activation. Untreated and negative control-treated epithelium had the least (0.48 and 0.51, respectively; SEM = 0.01) and mature pig IL1B, LPS, mature human IL1B and mature pig IL1BE treated had the greatest (0.82, 0.72, 0.69, and 0.69, respectively; SEM = 0.01) NFKB activation. For COX2 mRNA expression, there was an effect of treatment ($P < 0.05$). Untreated, immature pig IL1B and IL1BE treated had the least COX2 expression (0.70 ± 0.45, 0.99 ± 0.30, and 1.00 ± 0.30, respectively) and mature pig IL1B, human IL1B and pig IL1BE treated had the greatest COX2 expression (2.42 ± 0.35, 1.51 ± 0.30, and 1.43 ± 0.35, respectively). IL1BE may be involved in the establishment of pregnancy in the pig through its capacity to activate NFKB and increase COX2 expression in the uterine endometrium.

Key Words: pig, pregnancy, expression

593 Effects of prenatal transportation stress on preweaning temperament and growth of Brahman calves. B. P. Littlejohn*[1,2], D. M. Price[1,2], J. P. Banta[2], A. W. Lewis[2], D. A. Neuendorff[2], J. A.

Carroll[3], R. C. Vann[4], T. H. Welsh Jr.[1], and R. D. Randel[2], [1]*Texas A&M Department of Animal Science, College Station,* [2]*Texas A&M AgriLife Research and Extension Center, Overton,* [3]*Livestock Issues Research Unit, USDA-ARS, Lubbock, TX,* [4]*MAFES- Brown Loam, Mississippi State University, Raymond.*

The objective of this experiment was to examine the effects of prenatal stress on preweaning temperament and 180-d adjusted weaning weight of Brahman calves. Mature cows were assigned to receive 1 of 2 treatments, which consisted of a control group (n = 42) and a prenatally stressed group (n = 43). Cows in the prenatally stressed group were subjected to 2 h of transportation at 60, 80, 100, 120, and 140 d of gestation. Relative to weaning (d 0 = weaning), pen score (PS; 1 = calm and 5 = excitable), exit velocity (EV; m/s) and temperament score [TS = (PS+EV)/2] were recorded for each calf at d −112, −84, −56, −28, and 0. All data were analyzed using Mixed Models procedures of SAS. Treatment, sex, and day, were included as fixed effects; sire and weaning group were included as random effects. The 180-d adjusted weaning weight was not influenced by treatment ($P = 0.17$) or the sex × treatment interaction ($P = 0.28$). However, male calves (220.35 ± 5.14 kg) were heavier than female calves (201.13 ± 5.27 kg; $P < 0.01$). No interactions were significant for EV, PS, or TS. Pen score tended ($P = 0.06$) to be greater for prenatally stressed (2.83 ± 0.40) as compared with control calves (2.37 ± 0.40). Exit velocity was greater ($P = 0.02$) for prenatally stressed (2.24 ± 0.19 m/sec) as compared with control calves (1.76 ± 0.20 m/s). Temperament score was also greater ($P = 0.03$) for prenatally stressed (2.53 ± 0.28) relative to control calves (2.07 ± 0.28). Of these 3 measures of temperament, there was no effect of calf sex. However, there was an effect of day for each measure of temperament, as shown in the table below. In general, temperament decreased with time. Prenatal stress resulted in increased excitability of calves; however, prenatal stress did not influence preweaning growth rate or weaning weight.

Table 1.

	d −112	d −84	d −56	d −28	d 0	SE	*P*-value
Exit velocity	2.36 [a]	2.04[b]	1.95 [b]	1.81 [b]	1.82 [b]	0.18	0.0007
Pen score	2.80 [a]	2.64 [a]	2.60 [a]	2.43 [b]	2.53 [ab]	0.39	0.0455
Temperament score	2.58 [a]	2.34 [b]	2.28 [b]	2.12 [c]	2.17 [bc]	0.26	<0.0001

Key Words: prenatal stress, calf, temperament