W142 Mathematical simulation to assess the validity of Bonnier's equation for estimating the frequency of monozygous twinning in a population of Holstein cattle. N. Silva del Río*[1], G. A. Broderick[2], and P. M. Fricke[1], [1]*University of Wisconsin*, *Madison*, [2]*US Dairy Forage Research Center*, *Madison, WI*.

Twin calving records (n = 96,069) were extracted from MN DHIA (1996 to 2004) to estimate the incidence of monozygous twinning (MZ) and to evaluate the impact of varying the twin sex ratio and frequency of same-sex twins (SST) on estimates of MZ calculated using Bonnier's equation. Bonnier's equation: m = 2npq-n2/2pq(n-n2), estimates the proportion of MZ among SST (m) based on total opposite-sexed twin (OST) pairs (n2) and the observed proportions of male (p) and female (q = 1-p) calves among all twin births. Bonnier's equation assumes the sex of one twin is independent of the other, therefore, similar proportions of SST and OST would be expected in the absence of MZ. We hypothesized a dramatic decrease in Bonnier's estimate of MZ if SST comprised a smaller proportion of a population than reported. Based on our study, 56.4% of twin calves were SST, and 51.9% were male. The estimates of MZ were calculated by simulating a reduction of SST of 5% (54.2% SST) or 10% (52.0% SST), whereas the proportion of male calves born as twins was 51.9% (observed) or simulated to be 50% (Table 1). Estimates of MZ were greater than expected based on observed outcomes of MZ (Silva del Río et al., Therio. 66:1292; 2006). We conclude that slight changes in the percentage of SST dramatically affect MZ estimates using Bonnier's equation, whereas the percentage of male calves born as twins has a minimal impact. Thus, if factors other than MZ affect the proportion of SST, such as oocyte maturity, vaginal and uterine pH, or hormonal estrous synchronization, then Bonnier's equation will inaccurately estimate the frequency of MZ.

Table 1. Estimation of monozygous twinning as a percentage of all twins using Bonnier's equation for observed and simulated percentages of same-sex twins (SST) and proportion of male calves born as twins.

	Male calves born as twins (%	
SST (%)	51.9 (observed)	50.0
56.4 (observed)	11.6	11.3
54.2	7.9	7.8
52.0	3.9	3.9

Key Words: Bonnier's Equation, Monozygous Twins, Holstein Cattle

W143 Activated caspase-3 activity in the bovine fetal ovary. N. M. Barkley*, M. F. Smith, and H. A. Garverick, *University of Missouri*, *Columbia*.

During early bovine fetal development there is a 170 fold increase in germ cells from d 50 to d 110 followed by a rapid decline of germ cells by d 170 and even fewer remaining germ cells by birth. The objective of this study was to determine if the decline in germ cell numbers in fetal bovine ovaries from d 110 to d 170 can be attributed to apoptosis by characterizing active caspase-3 (effector of apoptosis) positive cells in fetal bovine ovaries from d 60–260 of gestation. Ovaries were dissected from fetuses upon slaughter and fetal age was estimated based on crown-rump length. Following dissection, ovaries from each animal were fixed in 4% paraformaldehyde (pH 7.6) and embedded in paraffin. Ovaries were sectioned at a thickness of 5 μm. Activated caspase-3 protein localization was characterized by immunohistochemistry. Two serial sections from each animal were stained and all positive cells on each section were counted, classified as either germ cells or non-germ cells, and averaged. The mean positive cells for each time period (d 60-80, 100-120, 130-150, 170-180, 190-210, and 240-260; n≥4 animals per time period) were determined. Germ cells were distinguished based upon morphology, location within the tissue, and association with follicular cells. The mean values were transformed in SAS, due to heterogeneity in variance, to determine differences across the time periods of gestation. However, the reported values are the means prior to transformation. The mean numbers of active caspase-3 positive cells per section were 63^{ab}, 156^{a}, 38^{ab}, 16^{b}, 25^{b}, and 11^{b} for d 60-80, 100-120, 130-150, 170-180, 190-210, and 240-260, respectively (^{ab} $P<0.05$). Analysis of only germ cells showed 18.3^{abc}, 50.4^{a}, 13.9^{ab}, 3.3^{cd}, 6.1^{bcd}, and 0.1^{d} positively stained cells per section for d 60-80, 100-120, 130-150, 170-180, 190-210, and 240-260, respectively (^{abcd} $P<0.05$). The greatest amount of caspase-3 activity occurred prior to d 150 of gestation. Thus, the sharp decline in germ cell numbers from d 110 to d 170 can, in part, be attributed to apoptosis.

Key Words: Bovine, Fetal Ovary, Caspase

W144 Multiple fibroblast growth factors stimulate interferon-tau production in bovine trophectoderm. K. A. Pennington* and A. D. Ealy, *University of Florida*, *Gainesville*.

Maintenance of early pregnancy in bovids depends on interferon-tau (IFNT) secretion from trophectoderm. Fibroblast growth factor 2 (FGF2) stimulates IFNT production in bovine blastocysts and a trophectoderm cell line (CT1). Several FGF receptors (FGFR) exist in the bovine conceptus, including FGFR2IIIb, a receptor subtype implicated in regulating placental development in several species. Multiple ligands for this receptor, notably FGF1, 7 and 10, are produced in bovine and ovine endometrium during early pregnancy. The objective was to determine if these uterine FGFs increase IFNT production in bovine trophectoderm. In study 1, CT1 cultures were incubated in medium containing 0, 0.05, 0.5, 5, 50 and 500 ng/mL of recombinant (r) bovine (b) FGF1, r human (h) FGF7, or rhFGF10. All cultures contained carrier protein (500 μg/mL BSA). RNA was extracted 24 h later and quantitative RT-PCR was used to determine IFNT mRNA abundance relative to an internal control (18s RNA). Four replicate experiments were completed. IFNT mRNA concentrations increased ($P<.05$) at 50 ng/mL rbFGF1 (5.9 ± 0.9 fold induction over non-treated controls), 50 ng/mL rhFGF7 (5.4 ± 0.2 fold induction) and 500 ng/mL rhFGF10 (8.4 ± 0.5 fold induction). In study 2, CT1 cultures were supplemented with various amounts of rbFGF1 or rhFGF10. rhFGF7 was not included in this study. Media were collected 48 h later and antiviral assays were completed to quantify IFNT protein concentrations. Five replicate experiments were completed. IFNT concentrations in conditioned medium increased ($P<.05$) in cells exposed to 50 ng/mL FGF1 (766.8 ± 266.1 pg/mL) and 500 ng/mL FGF10 (389.0 ± 185.5 pg/mL) compared with controls (56.3 ± 5.3 pg/mL). In conclusion, various FGFs can act on bovine trophectoderm to regulate IFNT mRNA and protein production. Further work is required to address the functional implications of these findings. Project supported

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Key Words: Placenta, Embryo, Conceptus

W145 Identification and characterization of three MX1 isoforms in sheep. D. S. Clark*[1], K Williams[2], and T. L. Ott[1], [1]*Pennsylvania State University*, *University Park*, [2]*University of Idaho*, *Moscow*.

Ruminant conceptuses produce interferon tau (IFN tau) during early pregnancy which increases expression of the myxovirus resistance gene, MX1, in the uterus. The role of MX1 in pregnancy is not known, but its role in the immune response is well-characterized. Kojima, et al. (2003) showed MX1 isoforms existed in cattle. In this study we characterized MX1 isoform expression in the sheep uterus. Sequence analysis of amplicons generated using a common set of MX1 primers in ovine endometrial cell lines revealed three isoforms that differed in their 5′ regions. Full-length sequences for each isoform were generated. Compared to the published sequence (Accession # X66093:MX1a) one isoform contained an 18 base deletion (MX1b) and the other an insertion of 186 bases (MX1c). Computer-assisted translation of MX1b resulted in a protein lacking 6 amino acids near the amino terminus. An early stop codon in MX1c caused a 27 amino acid exchange on the amino terminus. Sequence differences occurred at the boundary between putative exon 3 and exon 4, suggesting the isoforms may be splice variants. We developed a quantitative, real-time PCR assay to distinguish each isoform. All three isoforms were identified in ovine uterine luminal epithelial (oLE) and stromal (oSC) cell lines. Interestingly, only two of the isoforms, MX1a and MX1c, were detectable in glandular epithelial (oGE) cells and sheep endometrium. Levels of MX1c mRNA were roughly 1000-fold greater and did not change with the application of IFN tau in any cell line. However, levels of MX1a and MX1b strongly increased in response to IFN tau in oLE (> 300-fold at 6 hours). In endometrial RNA from pregnant ewes, MX1a mRNA levels increased approximately 17-fold, while MX1c levels did not change. We have yet to confirm the presence of proteins representing each putative splice variant in vivo, but those experiments are ongoing. Results suggest that three splice-variants of MX1 mRNA are present in sheep, and that, unlike MX1a and MX1b, MX1c is highly abundant and is not regulated by IFN tau.

Key Words: Sheep, Uterus, Interferon

W146 Effects of nutrient restriction during early gestation on postnatal calf growth. C. L. Bailey*, N. M. Long, M. J. Prado-Cooper, E. C. Wright, and R. P. Wettemann, *Oklahoma Agricultural Experiment Station*, *Stillwater, OK*.

Effects of prenatal nutritional restriction on postnatal growth of calves were evaluated in Angus ± Hereford heifers (15 mo). Heifers were AI with semen from a single Angus sire in each of 3 yr. At 32 ±0.5 d after insemination, pregnancy was verified and heifers were stratified by BW and BCS and allotted to low or moderate nutrition (L, n = 25, fed 55 %; M, n = 25, fed 100% NRC 1996 requirements). After 83 ± 3 d of treatment (115 d of gestation) heifers were commingled and received a common diet (100% NRC). Bulls were castrated at birth and calves were weaned at 229 ± 8 d of age. Data were analyzed using PROC MIXED procedures of SAS. There were no year x treatment effects. At treatment BW ($P = 0.16$) and BCS ($P = 0.21$) were similar for L and M heifers. After treatment, M heifers had greater BW ($P < 0.001$; 487 kg) and BCS (P = 0.006; 5.49) compared with L heifers (373 kg, SE = 10; 4.38, SE = 0.14). At calving, BCS did not differ ($P = 0.20$) for M and L heifers (5.05 vs. 4.64, SE = 0.24). Length of gestation was not influenced by treatment ($P = 0.50$; 274 and 275 d for L and M, respectively). Birth weights were similar ($P = 0.75$) for L and M calves (32 and 33 kg, respectively). Weight of calves at weaning (222 kg, SE = 5) was not influenced by treatment ($P = 0.81$). Glucose in plasma of calves at birth (80 and 79 mg/dL for L and M, respectively, SE = 7) was similar between treatments ($P = 0.93$). Cortisol in plasma of calves at birth (57 and 73 ng/mL for L and M, respectively, SE = 9) was not influenced by treatment ($P = 0.20$). Prenatal nutritional restriction of heifers that resulted in 1.1 BCS units difference at 115 d of gestation did not influence gestation length, birth weight, weaning weight, or plasma concentrations of glucose and cortisol in calves at birth.

Key Words: Prenatal Nutrition, Calves, Growth

W147 Effects of nutrient restriction during early gestation on carcass and organ weights of beef steers. N. M. Long*, M. J. Prado-Cooper, C. R. Krehbiel, U. Desilva, and R. P. Wettemann, *Oklahoma State University*, *Stillwater*.

Angus × Hereford heifers (15 mo of age) were used to evaluate the effect of prenatal nutritional restriction on tissues and organs of 22 mo old steers. At 32.0 ± 0.5 d after AI with semen from one sire, pregnancy was verified and heifers were stratified by BW and BCS and allotted to low (L, fed 56 % of NRC requirements) or moderate (M, fed 100% NRC requirements) nutrition. After 83 d of treatment, heifers were commingled and received a common diet (100% of NRC requirements). Bull calves were castrated at birth and maintained as a group before and after weaning. At 16 mo of age, L (n = 5) and M (n = 5) steers were fed a high concentrate diet and gained 2.2 ± 0.1 kg/d to an average BW of 550 kg. Steers were harvested and weights of the empty body, heart, lungs and trachea, spleen, kidney, liver, pancreas, and the gastrointestinal tract were recorded. Samples of organs, muscle (complexus), subcutaneous fat (SubQ), and KPH were stored at -80° C and DNA in tissue was quantified using Hoechst H33258 dye. RT-PCR was utilized to evaluate abundance of mRNA for genes involved in fatty acid metabolism in SubQ and KPH. Low steers had greater (2.41 ± 0.51 g; $P = 0.05$) total DNA in the pancreas compared with M steers (1.64 ± 0.51 g) and KPH from L steers tended ($P = 0.09$) to have increased concentrations of DNA compared with M steers. Concentrations of DNA in the other organs and SubQ fat were not influenced by treatment. Muscle fiber area was increased ($P = 0.04$) in L steers compared with M steers (1824 ± 76 vs 1336 ± 44 μm^2, respectively) and concentrations of DNA were greater ($P = 0.04$) in muscle from L steers than M steers. Abundance of RNA for fatty acid binding protein 4, fatty acid translocase and GLUT 4 was decreased in KPH from L steers compared with M steers. Nutritional restriction during early gestation increased muscle fiber area and decreased abundance of RNA for genes involved in fatty acid and glucose transport in KPH of steers at 22 mo of age.

Key Words: Steers, Organs, Gene Expression

W148 Effect of age at first calving on milk production and days open in first-parity Iranian Holstein dairy cows. A. Heravi Moussavi*, M. Danesh Mesgaran, and R. Noorbakhsh, *Ferdowsi University of Mashhad*, *Mashhad, Khorasan Razavi, Iran*.

Age at first calving (AFC) is one of the important factors contributing to economic return and is determined partially by farmer policy. The aim of this study was to evaluate the effect of age at first calving on milk production and days open in first-parity Iranian Holstein dairy cows. Calving data were collected during 1996 until 2006 in seven large commercial Holstein farms. During the period the median number of cows in the study herds was 500. Farms were located in northeastern Iran and were enrolled in the official milk-recording scheme. Each cow was characterized by demographic data (birth date, sire, first calving date), production data (cumulative first 60 and 200 days milk productions), and reproduction data (days open). The dependent variables analyzed were the cumulative first 60 and 200 days milk production, and days open. The AFC was included in the model with 23 levels (from 17 to 40 mo, one per month). Data were analyzed using General Linear Models using the statistical software package JMP. Age at calving averaged 27.23 ± 3.37 month. The median was 26 mo and 25% and 75% quartiles were 25 and 28 mo, respectively. Its distribution showed almost a bell shape. Age at first calving decreased from 1996 to 2006 ($P < 0.01$). The cumulative first 60 and 200 days milk production was impacted by AFC ($P < 0.001$) and the milk yields were increased in greater AFC. Days open was similar among different levels of AFC. The results of this study demonstrate that milk yields increased with increasing AFC, but the AFC had no apparent impact on the interval from calving to conception. The results also demonstrated that mean AFC is higher than the optimum age at first calving and can be decreased by 4 months.

Key Words: Dairy Cows, Age at First Calving, Days Open

W149 Changes in muscle proteome of dairy cattle with onset of lactation. P. J. Tyler*, K. A. Cummins, D. M. Carpenter, and R. Sabharwal, *Auburn University*, *Auburn, AL*.

An experiment was conducted to evaluate changes in the muscle proteome of dairy cows before and after the onset of lactation. Samples (n = 3 cows) of semi-tendinosus muscle were taken under local anesthesia either 7 d before expected calving date (mean 7.2 d) or 10 d after calving. Samples were subjected to two-dimensional gel electrophoresis in triplicate and stained with Sypro Ruby (Bio-Rad). Gel images were captured on a Typhoon 9410 (Amersham, Piscataway, NJ) and were processed with PD Quest software (Bio-Rad, version 8.0.1) for spot detection and expression quantification. The data were imported into the SAS statistical software (Version 9.1) for statistical differential protein expression analysis. There were a total of 613 spots detected in the union from all gels. Of these, 206 spots were matched in all 9 pre-calving gels, 251 matched in all 9 post-calving gels and 183 were matched across all 18 gels. Of the 183 commonly expressed spots, twenty-eight showed statistically significant ($P<0.05$) differential expression, with 9 down-regulated and 19 up-regulated between pre- and post-calving. Significant changes occur in the muscle proteome of lactating dairy cattle with the onset of lactation.

Key Words: Parturition, Muscle, Proteome