**516 Membrane progesterone receptors (α, β, and γ) in early pregnancy.** R. L. Ashley,* S. M. Stanbrough, K. E. Quinn, J. D. Lindsey, and A. K. Ashley, *New Mexico State University, Las Cruces.*

Progesterone (P4) is required for maintenance of pregnancy and elicits physiological effects via binding to a nuclear receptor in various target tissues. Additionally, P4 can function through non-canonical membrane progesterone receptors (MPR) and numerous isoforms have been identified, including MPR α (MPRA), β (MPRB) and gamma (MPRG). P4 signaling through the MPRs has not been investigated in early pregnancy, and could effect initial maternal/fetal interactions. The objective of this study was to assess gene expression of MPRs in maternal and fetal ovine tissues during early pregnancy via real-time quantitative PCR (qPCR). Hypothalamus (HYP), pituitary (PIT), and caruncle (CAR) were collected from non-pregnant (NP) ewes on D10 of the estrous cycle, and from pregnant ewes on D20, 25, and 30 of gestation. Fetal placental tissue (FET) was also collected from pregnant ewes. RNA was isolated, cDNA synthesized and subjected to qPCR. Expression levels were normalized by standard methods, and subjected to ANOVA with Newman-Keuls post hoc test to determine significant differences ($P < 0.05$). Among the MPR isoforms in HYP, MPRA was highly expressed, while MPRG levels were the greatest in PIT. Both MPRA and MPRG were high in CAR, and MPRB were the most elevated in FET. In HYP, no gestation-induced alterations in any MPRs were observed. All 3 MPRs decreased with advancing pregnancy compared with NP in the PIT. MPRA decreased at early placentation (D20) and once attachment was established (D30) in CAR, whereas MPRG increased at early and mid-placentation (D25), but returned to basal levels by D30. MPRB decreased during mid-placentation in FET, with D20 and D30 expressing similar levels. MPRA and MPRG expression was similar across all times assessed in FET. During early gestation, P4 levels rise and are maintained in concert with critical fetal-maternal interactions for proper implantation and placentation. Our data indicate gestational status and stage effect MPR expression in numerous reproductive tissues, suggesting these proteins may be pivotal in P4-mediated establishment and maintenance of pregnancy. USDA-NIFA-AFRI 2009–65203–05717.

**Key Words:** implantation, placenta, membrane progesterone receptor

**517 Expression of PRSS, the plasminogen activator system and its activity in the ovine placentome during stage 2 of parturition.** A. K. McNeel,* R. A. Cushman, and J. L. Vallet, *USDA, ARS US Meat Animal Research Center, Clay Center, NE.*

The molecular mechanisms responsible for placenta separation are not completely understood. We know placentomes from cases of retained placenta possess limited matrix-metalloprotease (MMP) activity and retained placenta occurs at a greater incidence during induced parturition. The plasminogen activator (PA) system is a family of serine endoproteases capable of activating MMP. Two new serine proteases (PRSS) have been recently documented in multiple species, and in mice appear to be involved in follicular development and ovulation. We hypothesized that 1) the expression of PRSS and PA systems and PA activity increase as parturition approaches with maximal expression and activity during parturition, and 2) that both are reduced during induced parturition. To test these hypotheses, placentomes were collected at 17 and 10 d before projected parturition and during spontaneous and dexamethasone-induced parturition in ewes. Placentomes were separated into fetal and maternal tissues manually, snap frozen in liquid nitrogen, and stored at −80°C until protein and RNA extraction. Tissue replicates were homogenized and RNA and protein were extracted. Relative quantification for genes of interest was performed via RT-qPCR on purified RNA using the ΔΔCt method with GAPDH serving as the reference gene. Expression data were analyzed with the MIXED procedure of SAS with treatment as a fixed effect. Plasminogen-casein zymography was performed on protein isolates. There were no differences in expression of urokinase-type plasminogen activator (PLAU), SERPINB2, SERPINE1, or PRSS23 ($P \geq 0.05$). We were unable to detect expression of plasminogen, tissue-type plasminogen activator, or PRSS35. Plasminogen activator activity in placentomes was not detected using zymography. These data demonstrate that in the ewe, neither the PA nor PRSS systems change during Stage 2 of parturition compared with late gestation or induced parturition. USDA is an equal opportunity provider and employer.

**Key Words:** placental separation, proteolysis, ruminant

**518 Physiological responses to repeated transportation of gestating Brahman cows.** D. M. Price*[1], A. W. Lewis[1], D. A. Neuendorff[1], J. A. Carroll[2], T. H. Welsh Jr.[3], R. C. Vann[4], and R. D. Randel[1], *[1]Texas Agrilife Research, Texas A&M University System, Overton, [2]USDA-ARS, Livestock Issues Research Unit, Lubbock, TX, [3]Texas Agrilife Research, Texas A&M System, College Station, [4]MAFES, Mississippi State University, Raymond.*

The transportation process acts as a stressor with adverse effects on animal health and performance. The purpose of this study was to examine physiological responses to repeated transportation of gestating Brahman cows; previously classified as mature cows into temperament groups of Calm, Intermediate, or Temperamental. Brahman cows (n = 48) were subjected to 2 h of transport (T) on d 60, 80, 100, 120, and 140 of gestation. Blood serum collected before and after each T was assayed for glucose and nonesterified fatty acid (NEFA) concentrations. Indwelling vaginal temperature (VT) monitoring devices were inserted before each T and VT were recorded before and every 5 min through 30 min after T. Statistical analysis of data used multivariate repeated measures and area under the curve methods. VT and serum glucose concentrations decreased in all temperaments ($P < 0.01$) with repeated T. Peak VT were greater ($P < 0.001$) in all cows regardless of temperament on d 60 of T, compared with all other T days. During T Temperamental cows tended ($P < 0.09$) to have higher peak VT (39.86 ± 0.15°C) compared with Calm (39.41 ± 0.16°C) and Intermediate cows (39.55 ± 0.08°C). Area under the VT curve decreased ($P < 0$ 0.003) by d 140 as the cows habituated to T. On d 60 post T, Calm cows had numerically lower VT (39.18 ± 0.18°C), compared with Intermediate and Temperamental cows (39.39 ± 0.10°C and 39.53 ± 0.17°C, respectively). Pre-T glucose concentrations on d 60 were greater ($P < 0.03$) for Temperamental cows (68.13 ± 4.31μg/dl) compared with Intermediate (53.42 ± 2.78 μg/dl) and Calm cows (52.76 ± 4.60 μg/dl). All cows had numerically increased NEFA concentrations post T, with temperamental cows showing the least numerical change due to T. However, NEFA concentrations did not increase with repeated T ($P > 0.10$). Glucose concentrations were affected ($P < 0.02$) by a time by temperament interaction with Temperamental cows having greater changes in serum glucose concentrations. These results reflect the partial acclimation of cows to a stressor over time, and demonstrate that both temperament and the T process influence physiological responses to stress in gestating cows.

**Key Words:** metabolites, transportation, vaginal temperature

**519 Reduced fertility in female progeny from beef heifers on dietary restriction during development.** S. E. Echternkamp,* D. R. Eborn, and R. A. Cushman, *USDA, ARS, U.S. Meat Animal Research Center, Clay Center, NE.*

Developing replacement heifers on lower energy diets does not affect fertility but may impart epigenetic effects on their progeny. Objective was to determine whether ovarian development or fertility is impeded in female progeny of replacement beef heifers developed on a lower energy diet. At 8 mo of age, Angus (n = 40) or composite MARC II (n = 40) heifers were assigned by BW and genetic line to be fed either a high (HE) or low (LE) energy diet for 180 d plus the first 22 d of the 47-d breeding period to achieve an ADG of either 0.45 or 0.9 kg/d or 55 vs. 65% of mature BW. Heifers within a line were mated to the same bulls. After breeding, treatment groups were combined and managed on pasture. Female progeny were developed on a standard heifer management protocol and bred at 14 mo of age. Total number of antral follicles (AFC), length and height of both ovaries, and uterine diameter were measured by transrectal ultrasonography in treated heifers (n = 72), and subsequently in female progeny (n = 32), at first breeding; pregnancy was diagnosed at about 75 d of gestation. Data were analyzed by ANOVA with diet and line as independent variables and their 2-way interaction. At 14 mo of age, LE heifers were lighter ($P < 0.01$) than HE heifers (344.8 vs. 429.4 ± 7.1 kg). Progeny birth weight (36.4 kg) was not affected by diet or line but Angus progeny were lighter ($P < 0.01$) at breeding (362.1 ± 7.2 vs. 387.2 ± 5.6 kg). Progeny of LE dams had fewer antral follicles than HE progeny (19.0 ± 1.9 vs. 26.0 ± 1.7; $P < 0.01$), whereas AFC (22.5 ± 2.8) did not differ between LE and HE dams. Ovarian size (length x height) was not affected by diet but was greater ($P < 0.05$) for MARC II vs. Angus heifers in both generations (dams, 301.3 vs. 351.9 ± 3.5 $mm^2$; progeny, 454.4 ± 31.8 vs. 586.3 ± 26.3 $mm^2$, respectively). Uterine diameter (12.1 mm) was not influenced by diet. Pregnancy rate was less ($P < 0.05$) for progeny from LE vs. HE dams (42.6 ± 15.3 vs. 80.3 ± 10.1%) and for Angus vs. MARC II progeny (39.3 ± 14.3 vs. 80.2 ± 12.1%). Results indicated that environmental effects imposed during early embryonic development in first-parity heifers may affect embryonic gametogenesis and fertility of progeny. USDA is an equal opportunity provider and employer.

**Key Words:** diet, progeny, fertility

**520 The impact of cow nutrient status during the second and third trimester on development of the reproductive axis and fertility of daughters.** R. A. Cushman,* A. K. McNeel, and H. C. Freetly, *USDA, ARS, U.S. Meat Animal Research Center, Clay Center, NE.*

Fluctuating feed resources to beef cows across the production cycle is a proven method for decreasing input costs; however, altering nutrients during gestation can decrease ovarian follicle numbers in female offspring. We hypothesized that limiting nutrients to mature (≥3 years) crossbred beef cows during the second and third trimester would result in daughters with decreased follicle numbers detectable by ultrasonography as yearlings. Over 4 breeding seasons, pregnant beef cows (n = 397) were assigned to Low (L), Moderate (M) or High (H) nutrient intake during the second and third trimesters to make 4 dietary treatment groups (L-H, L-L, M-H, and M-M). Heifers (n = 416) born to these cows were weighed at weaning and moved to a dry lot where they were monitored for behavioral estrus. Two weeks before their first breeding season, heifers were submitted for ultrasonographic examination of their ovaries to determine antral follicle numbers. Heifers were placed with bulls for 60 d and pregnancy status was determined 45 d after the bulls were removed. Growth and reproductive traits were analyzed using the MIXED Procedure of SAS with maternal diet, year, and the interaction as the independent variables and dam as a random effect. Maternal dietary intake did not affect heifer growth rates, age at puberty, or antral follicle counts ($P \geq 0.14$). However, heifers born to dams fed a high nutrient level during the third trimester (L-H or M-H) had a decreased number of days to calving ($P = 0.04$). From this we conclude that limiting nutrient intake during late gestation in mature (≥3 years) beef cows does not influence the ovarian reserve or maturation of the reproductive axis in daughters. However, high maternal nutrient levels during the third trimester improve the first service conception rates of their daughters when they go to their first breeding season. USDA is an equal opportunity provider and employer.

**Key Words:** beef heifers, puberty, antral follicle count

**521 Feeding distillers grains as an energy source to gestating and lactating beef heifers: Impact on growth, puberty attainment and reproductive processes in female progeny.** P. J. Gunn*[1], J. P. Schoonmaker[1], R. P. Lemenager[1], and G. A. Bridges[2], *[1]Department of Animal Sciences, Purdue University, West Lafayette, IN, [2]North Central Research and Outreach Center, University of Minnesota, Grand Rapids.*

Angus-cross beef heifers pregnant to a single sire (n = 80) were used to assess the effects of feeding dried distillers grains with solubles (DDGS) as an energy source during late gestation and early lactation on female progeny growth, puberty attainment and reproductive processes. From 192 d of gestation through 118 ± 0.2 d of lactation, dams were fed either a control diet of corn silage and haylage (CON; 10% CP prepartum; 11.8% CP postpartum) or corn stover and DDGS (DG; DDGS at 1.2% BW per d; 15.7% CP). Heifer progeny (n = 33) were weaned and commingled at 18 ± 0.4 d of age. Heifers were managed similarly for the remainder of the project. Heifer wt and blood samples for progesterone were collected weekly to assess wt and age at puberty. At 242 ± 0.4 d of age, 1 follicular wave was mapped via ultrasonography in 10 prepubertal heifers per treatment. Prepubertal antral follicle count and ovarian size were determined at 240 and 330 ± 0.4 d of age. Hip ht was recorded at 213, 297, and 436 ± 0.4 d of age. Estrous synchronization and AI was initiated at 447 ± 0.4 d of age. Categorical and continuous data were analyzed with the GLIMMIX and MIXED procedures of SAS, respectively. The DG progeny were heavier than CON ($P = 0.03$) at weaning (241 ± 5 kg vs. 223 ± 6 kg), but not at breeding (416 ± 7 vs. 401 ± 7 kg). Also, DG progeny had a greater ($P < 0.01$) frame score than CON throughout the developmental period. Ovarian size, antral follicle count, and follicular growth parameters did not differ between treatments. Age at puberty did not differ between CON (303 ± 10 d) and DG (320 ± 10 d) treatments; however, wt at puberty was heavier ($P = 0.01$) for DG (326 ± 7 kg) than CON (298 ± 8 kg) progeny. In addition, DG progeny (70.6%) had greater ($P = 0.05$) AI pregnancy rates than CON (33.3%). However, overall breeding season pregnancy rate did not differ. In summary, feeding DDGS at 1.2% of BW per d to first-parity heifers resulted in female progeny with greater skeletal growth that were heavier at onset of puberty, but had increased AI pregnancy rates.

**Key Words:** beef heifer, DDGS, developmental programming

**522 Chronic uterine infusion of melatonin or melatonin receptor antagonist alters ovine placental efficiency and fetal blood flow during mid-gestation.** C. O. Lemley,* L. E. Camacho, and K. A. Vonnahme, *North Dakota State University, Fargo.*

Objectives of the current experiment were to determine fetal descending aorta blood flow (BF), umbilical artery BF, and placental and fetal development following a 4 week uterine infusion of melatonin (MEL; n

= 5), melatonin receptor antagonist, luzindole (LUZ; n = 5), or vehicle (CON; n = 4). Multiparous singleton pregnant ewes were surgically implanted with mini osmotic pumps (2.5 µl/hr infusion of 1 mg/mL MEL or LUZ) on d 62 of gestation. Pumps and catheters were sutured under the perimetrium and catheters were advanced to the gravid uterine vascular network of the mesometrial region. On d 90 of gestation fetal BF was determined via Doppler ultrasonography followed by euthanasia. Data were analyzed using the mixed procedure of SAS. The model statement included infusion treatment and fetal sex. Placentome weight, cotyledon weight, and placentome number were not different ($P > 0.10$) among treatments. Caruncle weight was decreased ($P < 0.05$) in MEL and LUZ vs. CON treated ewes. Placental efficiency (fetal weight: placentome weight ratio) was increased ($P = 0.05$) in MEL vs. LUZ treated ewes with CON being intermediate. Fetal weight, biparietal distance, ponderal index, and crown rump length were not different ($P > 0.10$) among treatments, while thoracic girth was increased ($P < 0.02$) in MEL vs. LUZ treated ewes with CON being intermediate. Umbilical artery BF and umbilical artery BF relative to placentome weight were increased ($P < 0.05$) in MEL vs. CON and LUZ treated ewes. Fetal descending aorta BF was increased ($P < 0.05$) in MEL vs. LUZ treated ewes, while fetal descending aorta BF relative to fetal weight was increased ($P < 0.005$) in MEL vs. CON and LUZ treated ewes. The ratio of fetal descending aorta BF to umbilical artery BF was not different ($P > 0.95$) among treatment groups. The increase in umbilical artery BF due to chronic uterine melatonin infusion is potentiated by an increased fetal cardiac output through the descending aorta. Moreover, melatonin receptor antagonism did not alter umbilical artery blood flow compared with CON ewes. Therefore, mechanisms apart from melatonin receptor activation may mediate the observed increase in fetal BF following melatonin treatment.

**Key Words:** fetal blood flow, melatonin, placental efficiency

**523 Influence of metabolizable protein supplementation during late gestation on vasoreactivity of maternal placental arteries in sheep.** L. A. Lekatz*[1], A. Reyaz[1], M. S. Sane[2], F. Yao[2], S. T. O'Rourke[2], C. Schwartz[1], M. L. Van Emon[3], C. S. Schauer[3], K. M. Carlin[1], C. O. Lemley[1], and K. A. Vonnahme[1], *[1]Center for Nutrition and Pregnancy, Department of Animal Sciences, North Dakota State University, Fargo, [2]Department of Pharmaceutical Sciences, North Dakota State University, Fargo, [3]Hettinger Research Extension Center, North Dakota State University, Hettinger.*

To examine the effects of metabolizable protein (MP) intake during late gestation on the vasoreactivity of placental arteries, 18 pregnant ewes received isocaloric diets containing 60% (MP60), 100% (MP100), or 140% (MP140) of MP requirements from d 100 to 130 of gestation. On d 130, several caruncular (CAR) arteries from placentomes of similar size and in close proximity to the umbilicus were selected for vasoreactivity studies using wire myography. Arterial rings (n = 4/ewe) were suspended in organ chambers with a wire myograph filled with 5 mL of physiological salt solution aerated with a mixture of 95% $O_2$ and 5% $CO_2$ and kept at 38.6°C. Optimal tension was found by progressively stretching the rings until the contractile response to KCl (20 mM) was maximal. The presence or absence of endothelium was verified by testing the ability of bradykinin (BK; $10^{-7}$ M) to produce endothelium-dependent relaxation during contraction evoked by norepinephrine ($10^{-6}$ M). Each ring underwent a dose response curve (DRC) to BK in the presence or absence of an inhibitor. Rings were contracted with either U46619 ($10^{-6}$ M) or 5-HT ($10^{-6}$ M), and the DRC to BK in the absence of an inhibitor was obtained. In addition, BK DRC were obtained for rings which were incubated for 30 min with one of the following inhibitors: iberiotoxin (IBTx; an inhibitor of $Ca^{2+}$-activated $K^+$ channels; $10^{-7}$ M), indomethacin (INDO; a cyclooxygenase inhibitor; $10^{-5}$ M), or *N*(omega)-L-arginine methyl ester (NLA; a nitric oxide synthase inhibitor; $10^{-5}$ M) before the BK DRC was obtained. There was no difference ($P = 0.97$) in the response to BK among the 3 groups. While there was no difference ($P = 0.67$) in relaxation due to BK in the presence of IBTX, there was a treatment by dose interaction ($P = 0.02$) when arteries were incubated with NLA where pattern of relaxation differing slightly among treatments. The response to BK is inhibited similarly by IBTx and NLA in across all treatments indicating vasorelaxation being partially dependent on NO and BKCa channels. In MP140, inhibition of cyclooxygenase by INDO enhances the response to BK ($P = 0.09$) compared with MP100 and MP60. This could suggest that contractile arachidonic acid metabolites are produced in MP140 CAR arteries.

**Key Words:** bradykinin, placental vasoreactivity, sheep

**524 Transgenerational effects of n-3 and n-6 supplementation under the control of transcription factors related to lipid metabolism.** C. B. Jacometo[1], S. Halfen[1], F. T. da Rosa[1], A. Schneider[1], C. C. Brauner[1], F. A. B. Del Pino[1], J. J. Loor[2], N. J. L. Dionello[1], L. F. M. Pfeifer[3], E. Schmitt*[1], and M. N. Corrêa[1], *[1]Federal University of Pelotas, Pelotas, Rio Grande do Sul, Brazil, [2]University of Illinois, Urbana, [3]Embrapa Rondônia, Porto Velho, RO, Brazil.*

Our objective was to determinate the influence of omega-3 and omega-6 consumption throughout generations under transcription factors expression related to lipid metabolism. Wistar rats females, 8 week old, (G0, n = 18/group) were divided in: diet containing flaxseed oil (G3) and diet containing soybean oil (G6). The diets were elaborated in accordance with AIN-93G recommendations (AIN-93G). After 30 d of acclimatizing the rats were mated for 3 d in 1:3 male:female ratio. The Males received only the control diet. The F1 female were selected at weaning (n = 16/group) and was divided into 3 groups: females from G3 group that continued receiving diet containing flaxseed oil (G3–3); females from G3 group that began to receive diet containing soybean oil (G3–6); and females from G6 group that continued to receive diet containing soybean oil (G6–6). These animals were mated with 60 d old as described above (G0). The F2 (n = 16/group) was selected like F1 and the diet groups were maintained: G3–3-3; G3–6-6 and; G6–6-6. The F2 was mated like the F1. Prepartum euthanasia (19 ± 1 d) was conducted in each generation (n = 4/group) to collect liver samples. The Real-Time quantitative PCR was performed to evaluate the peroxisome proliferator-activated receptor α (PPARα), retinoid X receptor α (RXRα), liver X receptor α (LXRα), sterol regulatory element binding protein 1c (SREBP-1c), carbohydrate regulatory element binding protein (ChREBP) and nuclear corepressor receptor 1 (NCoR1) expression. Actin β (ACTB), Glyceraldehyde-3-phosphate (GAPDH) and 18S subunit ribosomal RNA (18S) were used as internal control. NCor1 and ChREBP did not differ between groups and so throughout generations, indicating a PUFA independent control. Differences between groups were not observed in G0 generation. In the F1, the PPARα were higher expressed in the G6–6 group than the G3–6 group ($P = 0.03$) and LXRα were lower in the G3–6 group than the G3–3 group ($P < 0.01$) and G6–6 group ($P < 0.05$). In the F2 generation LXRα had a lower expression in the G3–3-3 group compared with the G3–6-6 ($P < 0.01$) and G6–6-6 ($P < 0.01$). There were cumulative effects throughout generations in all groups with crescent RXRα (G0-F2:$P < 0.01$ and F1-F2:$P < 0.02$) and LXRα expression (G0-F2: $P < 0.01$ and F1-F2:$P < 0.01$) along generations. In the F2 generation the G3–3-3 group had a higher PPARα expression and a lower LXRα expression indicating lipolytic and anti-lipogenic effect. Our results indicate that the PUFAs effect on the control of lipolysis and lipogenesis is cumulative throughout generations.

**Key Words:** lipid metabolism, epigenetic, nutriphysiogenomics