Physiology and Endocrinology I

T316 Effects of supplementation with different PUFA during the postpartum periods influence ovarian follicle size and number in lactating dairy cows. E. Dirandeh*[1], A. Towhidi[1], Z. Ansari Pirsaraei[2], M. Ganjkhanlou[1], S. Zeinoaldini[1], T. Saberifar[1], A. Rezaei Roodbari[1], M. A. Roodbari Shahmiri[3], and A. R. Zarenezhad[3], *[1]University of Tehran, Karaj, Alborz, Iran, [2]Sari Agricultural Sciences & Natural Resources University, Sari, Mazandaran, Iran, [3]Mahdasht Milk & Meat Company, Sari, Mazandaran, Iran.*

The objectives were to determine if a diet enriched in α-linolenic acid (n-3), or linolenic acid (n-6) would influence ovarian function in lactating dairy cows. Thirty high-yielding multiparous Holstein dairy cows without clinical illnesses were blocked according to calving date and parity. Cows were assigned randomly to be fed: (1) soybean whole roast (S, n = 10), or (2) linseed (L, n = 10), or 3) palm oil as a source of mostly saturated fatty acids (FA), (C, n = 10) from calving until d 60 postpartum (dpp). Supplementation of FA was at 1.5% of dietary dry matter, and there was no difference between groups (mean ± SEM) in parity (3.0 ± 1.90) or BCS at calving (3.2 ± 0.07). At 30 and 44 d postpartum cows received an injection of PGF2α for estrous synchronization Ultrasound measurements of follicle numbers were performed in 30 cows (n = 10 per group) on alternate days, from the day that second PGF2α inject until the d 10 of next estrous cycle (d 0 = day of estrus). The characteristics and fate of the first follicular wave was monitored using a real-time linear scanning ultrasound diagnostic system (B mode; Pie medical, Falco 100; 8 MHz transducer). Data were analyzed using the MIXED procedure of SAS. Results showed that the ovulatory follicle on the day preceding estrus synchronization (before the first AI, d 0) was larger in groups S (16.14 ± 0.91 mm) and L (15.24 ± 0.75 mm) compared with C (13.20 ± 0.86 mm; $P < 0.05$) but there were no significant differences between groups S and L. Mean number of small follicles (<5 mm), medium follicle (5-10 mm) and diameter of subordinate follicle were not affected by diets ($P < 0.05$). Cows offered diet S and L had a greater ($P = 0.037$) total number of follicles, number of large (>10 mm) follicles and tended to have a greater ($P = 0.064$) than cows offered diet C. In conclusion, these data demonstrate that n-3 and n-6 fatty acids increased follicle size and number but there was no difference among type of supplemental fatty acid.

Key Words: dairy cow, follicle, ultrasonography

T317 Feeding n-6 fatty acids during 40 dpp and shift to n-3 fatty acids from 40 to 120 dpp can improve fertility in lactating dairy cows. E. Dirandeh*[1], A. Towhidi[1], Z. Ansari pirsaraei[2], M. Ganjkhanlou[1], S. Zeinoaldini[1], A. Rezaei Roodbari[3], M. A. Roodbari Shahmiri[3], and A. R. Zarenezhad[3], *[1]University of Tehran, Karaj, Alborz, Iran, [2]Sari Agricultural Sciences & Natural Resources University, Sari, Mazandaran, Iran, [3]Mahdasht Milk & Meat Company, Sari, Mazandaran, Iran.*

The objectives were to determine if a diet enriched in α-linolenic acid (n-3), or linoleic acid (n-6) would influence reproductive performance in lactating dairy cows. Ninety high-yielding multiparous Holstein dairy cows with overtly clinical illnesses were blocked according to calving date and parity. Cows were assigned randomly to be fed: (1) soybean whole roast (S, n = 30), or (2) linseed (L, n = 30), or 3) palm oil as a source of saturated fatty acid (C, n = 30) from calving until first estrus after d 40 postpartum (dpp) and then half of the cows in each treatment group were switched to receive either L or C from first estrus after d 40 to 120 dpp. There was no difference between groups (mean ± SEM) in parity (3.0 ± 1.90) or BCS at calving (3.2 ± 0.07). Pregnancy was evaluated at 40 d after AI, and pregnant cows had their pregnancy reconfirmed at 60 and days after AI. All of the reproductive responses (binary responses) were analyzed by Glimmix. Result showed uterine involution in cows fed soybean whole roast occurred 1.2 earlier than the other groups ($P < 0.05$). Heat detection rate was not different between groups. For SL (% 44) and LL (% 45) groups compared with other groups pregnancy rate to first insemination was higher ($P = 0.001$); pregnancy rate to all inseminations was higher ($P = 0.019$) in SL (% 80) group and lower in LC (% 57) and CC (% 47) groups compared with other groups ($P = 00.1$). Conception rate tended to be higher ($P = 0.03$) in SL (% 84) group compared with other groups. There was a lower rate of early (d 0 to 24) embryo survival in SC (% 18) and CC (% 25) group compared with other groups ($P = 0.12$). In conclusion, feeding n-6 fatty acids during 40 dpp and shift to n-3 fatty acids from 40 to 120 dpp can improve fertility in lactating dairy cows.

Key Words: dairy cow, n-6 and n-3 fatty acids, pregnancy losses

T318 Presynchronization with PGF_{2a} and GnRH on the same day, 7 d prior to Ovsynch, allowed for similar pregnancies/AI compared with Presynch-10/Ovsynch. J. P. Martins*[1], M. J. T. Acevedo[1], T. O. Cunha[1], C. Piterini[1], M. R. Yousuf[1], K. Nobis[2], and J. R. Pursley[1], *[1]Department of Animal Science, Michigan State University, East Lansing, [2]Nobis Dairy Farm, St. Johns, MI.*

Fertility of dairy cows treated with Ovsynch is enhanced when the 1st GnRH of Ovsynch induces ovulation on d 6 or 7 of the estrous cycle, and PGF_{2a} (PG) 1 wk later, induces luteolysis. Current pre-synchrony programs that allow Ovsynch to be initiated on d 6 or 7 of the estrous cycle can be either logistically challenging for reproductive management personnel or can significantly lengthen duration of the entire program. The hypothesis of this experiment was that simplification of a Presynch program through the combination of PG (0.5 mg cloprostenol) and GnRH (0.1 mg gonadorelin diacetate tetrahydrate) on the same day, 7 d before Ovsynch, would allow for similar pregnancies/AI compared with Presynch-10. Lactating dairy cows (n = 444) 41 to 47 DIM were assigned in a random fashion to 2 treatments within 1st and 2nd+ parities for 1st service. Control cows received Presynch-10/Ovsynch consisting of the following: PG −14 d – PG −10 d – GnRH −7 d – PG −56 h – GnRH −16 h - AI. Treated cows received PG and GnRH - 7d – GnRH - 7 d – PG −56 h – GnRH −16 – AI. All cows received a 2nd injection of PG 24 h after the PG of Ovsynch to enhance complete luteolysis. All cows received AI between 75 and 81 DIM. Blood was collected to assess circulating concentrations of progesterone, and number of corpora lutea were recorded using ultrasonography, on d of PG of Ovsynch. Pregnancies/AI at 28 d post-AI were similar in controls compared with treated cows (45 vs. 45%). There was no effect of treatment on parity. There were no differences in pregnancy/AI between 1st and 2nd+ parities when treatments were combined (47 vs. 44%). Serum concentrations of progesterone were different in 1st parity, and similar in 2nd+ parity, control vs. treated cows (5.9 vs. 5.1 and 6.28 vs. 5.96 ng/mL). Percent of cows with >1 CL at time of PG was greater in 2nd+ parity treated vs. controls (89 vs. 75) and not different in 1st parity cows (65 vs. 70). In summary, administering both PG and GnRH on the same day, 7 d before the start of Ovsynch, appears to be a simple alternative that keeps pregnancies/AI to that of Presynch-10/Ovsynch.

Key Words: dairy, Ovsynch, fertility

T319 Effect of cloprostenol during early corpora lutea development on circulating concentrations of progesterone in breeding age dairy heifers. J. P. Martins*, M. J. T. Acevedo, C. Piterini, T. O. Cunha, and J. R. Pursley, *Department of Animal Science, Michigan State University, East Lansing.*

As part of an overarching objective to create a low progesterone environment during growth of the pre-ovulatory follicle in heifers to determine the effect of low progesterone on fertility parameters, we tested the effect of cloprostenol administration on early corpora lutea (CL) function. Dairy heifers between 12 and 13 mo of age were pre-synchronized to ensure all heifers were on d 6 of the estrous cycle at the start of the Ovsynch program. Only heifers that responded to the following strategy with CL regression and ovulation determined using ultrasound were utilized: 0.5 mg cloprostenol (PG)–2d - 0.1 mg GnRH – 6d - GnRH (G1; 1st GnRH of Ovsynch). Heifers (n = 164) that responded to the pre-synchrony injections were randomly assigned to 4 groups: high progesterone control (HPC), low progesterone control (LPC), and treatments P2 and P3. LPC, P2 and P3 received PG 1 d after G1 to regress all d 7 CL. Heifers from these groups had 1 new CL growing during the treatment period. Groups P2 and P3 received treatments of PG 2 and 3 d after G1, respectively. HPC did not receive PG on d 1 following G1, thus had a mature and a new CL growing during the treatment period. Blood samples were collected in all heifers on d 7 and in a subset of heifers on d 1, 2, 3, 4 (n = 88) after G1 to analyze circulating concentrations of progesterone. Pre-ovulatory follicle size (12.9 ± 0.1 mm; $P = 0.9$) at G1 and mean progesterone concentrations on d 1 (3.40 ± 0.15 ng/mL; $P = 0.9$) did not differ between randomized groups. Mean serum progesterone concentrations on d 7 were 8.23 (±0.43), 2.52 (±0.35), 1.53 (±0.20), and 1.24 (±0.15) ng/mL for HPC, LPC, P2, and P3, respectively. HPC had greater ($P < 0.001$) concentrations of progesterone compared with LPC, P2 and P3 on d 2, 3, 4 and 7 following G1. LPC had greater progesterone concentrations on d 7 than P3 ($P = 0.01$) and tended to be greater than P2 ($P = 0.08$). In summary, cloprostenol treatments during early corpus luteum development reduced circulating progesterone concentrations to sub-normal luteal levels compared with both high progesterone and low progesterone controls.

Key Words: dairy heifer, corpora lutea, progesterone

T320 Comparison of 200 µg of GnRH versus 1000 IU hCG in Beefmaster and Brahman Cattle using an Ovsynch protocol. C. E. Ferguson*[1], G. Richey[1], A. McDuff[1], and D. J. Kesler[2], *[1]McNeese State University, Lake Charles, LA, [2]University of Illinois, Champaign-Urbana.*

Previous research has reported 200 µg dose of GnRH at time of AI can improve pregnancy rates in *Bos indicus*-influenced cattle. This experiment was designed to compare the effect of 1000 IU of hCG versus the increased dose of GnRH on pregnancy rate in *Bos indicus*-influenced cattle. In the study, 4 groups of cattle were used; pure-bred Brahman (BR) heifers (n = 64) between 14 and 18 mo of age from Texas, Beefmaster (BM) lactating mature cows (n = 29) ~60 d postpartum from Louisiana, BM lactating mature cows (n = 50) from Florida, and BM heifers (n = 50) between 14 to 18 mo of age from Florida. All females were synchronized using an Ovsynch + CIDR protocol. At 48 h following $PGF_{2\alpha}$ females were randomly selected to receive either 200 µg GnRH or 1000 IU hCG and then artificially inseminated (AI) 15 to 20 h later for BR heifers or ~12 h following treatment for BM heifers or cows. The BR heifers were bred later than BM heifers because they were inseminated with sex-sorted semen while BM heifers received conventional semen. Each group was evaluated via ultrasonography at ~30 d post-AI to determine pregnancy. Statistical analysis was performed in SAS and a chi-squared test was used to determine differences between pregnancy rates. There was no difference ($P > 0.05$) in pregnancy rates between 200 µg GnRH versus 1000 IU hCG in *Bos indicus*-influenced cattle. The pregnancy rates for BR heifers was (GnRH, 3/18, 8% versus hCG 7/39, 18%), for lactating BM cows (from LA) was (GnRH 17/30, 57% versus hCG 9/15, 60%), for lactating BM cows (from FL) was (GnRH 17/30, 57% versus hCG 11/20, 55%) and for BM heifers was (GnRH 12/25, 48% versus 13/25, 52%). There was a significant effect ($P < 0.05$) of time from GnRH or hCG to AI in BR heifers bred with sexed semen on pregnancy rates with 60% of all pregnancies occurring with AI 17 to 18 h post GnRH or hCG. These results indicate that 1000 IU hCG is a cost effective alternative to replace the use of 200 µg GnRH in timed-AI protocols for *Bos indicus*-influenced cattle.

Key Words: GnRH, hCG, Ovsynch

T321 Effect of prostaglandin $F_{2\alpha}$ on growth of *Mycoplasma bovis* associated with bovine mastitis. A. Ahmadzadeh*[1], L. Fox[2], M. McGuire[1], and K. Carnahan[1], *[1]University of Idaho, Moscow, [2]Washington State University, Pullman.*

Mycoplasma bovis (*M. bovis*) is a major pathogen that is inherently refractory to antibiotics. Certain fatty acids have been shown to inhibit the growth of mastitis pathogens such as *Staphylococcus aureus*. In vitro experiments were conducted to determine the effects of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) on growth of *M. bovis*. Five strains of *M. bovis* bovine origin were selected for the study. Two strains were reference strains (ATCC 25025 and 25523) and the other strains were isolated from diseased cattle. Isolates were cultured and suspended in saline to achieve an optical density of 0.2 at 520 nm, a suspension of approximately 10^7 to 2×10^8 cfu/mL. Subsequently, *M. bovis* suspensions were incubated in special culture media containing $PGF_{2\alpha}$ (dinoprost tromethamine) at final concentrations of 0 (control), 2, 4, and 8 mg/mL, for 8 h at 37°C in triplicate. A sample from each treatment group was obtained and cultured for 10 d and bacterial growth assessed as cfu. Data were analyzed by ANOVA and the model included the effect of treatment, strain, and their interaction. Treatment affected ($P < 0.01$) *M. bovis* growth and mean cfu decreased with concentrations of $PGF_{2\alpha}$ at 4 and 8 mg/mL (43.6, 42.1, 24.3, 7.8 [±1.1] for 0, 2, 4, 8 mg/mL, respectively). However, an effect of treatment × strain on mean cfu was detected ($P < 0.05$), indicating that the effect of $PGF_{2\alpha}$ on bacterial growth was not consistent across strains. Overall, the 2 mg/ml $PGF_{2\alpha}$ decreased ($P < 0.01$) cfu only in 2 strains compared with control whereas 4 and 8 mg/mL $PGF_{2\alpha}$ decreased ($P < 0.05$) cfu in all strains compared with control. These results provide evidence, for the first time, that $PGF_{2\alpha}$, in the form of dinoprost tromethamine, has inhibitory effects on growth of *M. bovis*, and this bacteriostatic effect appears to be strain and dose dependent.

Key Words: *Mycoplasma*, prostaglandin $F_{2\alpha}$, bacteriostatic

T322 Inclusion of bovine somatotropin in fixed-time AI protocols for *Bos indicus* beef cows. J. P. Albuquerque[1], R. F. Cooke[2], H. P. Dias[1], I. C. Bueno[1], A. D. P. Rodrigues[1], and J. L. M. Vasconcelos*[1], *[1]UNESP - Faculdade de Medicina Veterinária e Zootecnia, Botucatu, São Paulo, Brazil, [2]Oregon State University, Eastern Oregon Agricultural Research Center, Burns.*

Two experiments evaluated the effects of bovine somatotropin (ST) on pregnancy per AI of lactating Nelore (*Bos indicus*) cows assigned to the following estrus synchronization + fixed-time AI (FTAI) protocol: 2 mg injection of estradiol benzoate and insertion of intravaginal progesterone releasing device (CIDR) on d 0, 12.5 mg injection of $PGF2_{\alpha}$ on d

7, CIDR removal in addition to 0.6 mg of estradiol cypionate and 300 IU of eCG on d 9, and FTAI on d 11. Pregnancy status was verified via transrectal ultrasonography 30 d after FTAI. Data were analyzed with the PROC GLIMMIX of SAS. In Exp. 1, 896 cows (primiparous, n = 371; multiparous, n = 525) were randomly assigned to receive, concurrently with FTAI, 1 of 3 treatments: 1) 167 mg injection (s.c.) of sometribove zinc (bST167; n = 304), 2) 333 mg injection (s.c.) of sometribove zinc (bST333; n = 298), or 3) no injection (control; n = 294). Control cows had reduced pregnancy per AI compared with bST167 ($P = 0.08$) and bST333 ($P = 0.05$) cows (42.1, 49.0, and 49.8% of pregnant/inseminated cows, respectively; SEM = 4.0%), whereas no differences were detected ($P = 0.88$) between bST167 and bST333 cows. In Exp. 2, 290 cows (primiparous, n = 81; multiparous, n = 209) were randomly assigned to receive 333 mg of sometribove zinc (s.c.) at the beginning of the estrus synchronization protocol and at FTAI (bST, n = 111), or no injection (control; n = 179). Pregnancy per AI was similar ($P = 0.94$) between bST and control cows (37.6 and 38.1% of pregnant/inseminated cows, respectively; SEM = 4.8%). Hence, administration of bovine ST at FTAI enhanced pregnancy per AI in Nelore beef cows, independently of parity or dose evaluated. Conversely, 2 injections containing 333 mg of sometribove zinc and administered at the beginning and end of the estrus synchronization + FTAI protocol failed to improve pregnancy per AI in Nelore beef cows. The reason for this latter outcome is unknown, but may be attributed to increased milk production, as well as excessive circulating IGF-I and insulin concentrations that impaired, respectively, synchrony between embryonic/maternal tissues and oocyte quality in bST cows.

Key Words: bovine somatotropin, beef cows, artificial insemination

T325 Establishment of primary culture of omasal epithelial cells from newborn calves and detection of function for peptide absorption. Q. B. Xu*[1,2], H. Y. Liu[1,2], Y. M. Xie[1,2], Y. M. Wu[1,2], and J. X. Liu[1,2], *[1]Institute of Dairy Science, College of Animal Sciences, Hangzhou, China. [2]MoE Key Laboratory of Molecular Animal Nutrition, Zhejiang University, Hangzhou, China.*

It has been proved that small peptides can be absorbed in bovine forestomachs, especially omasum. However, little information is available on small peptide absorption by bovine omasal epithelial cells (OE cells). In this study, the bovine omasal tissues obtained from newborn Chinese Holstein calves were digested with 2.5% trypsin solution to obtain the OE cells. The isolated cells were then cultured in DMEM medium containing 5% fetal bovine serum, 5 μg/mL insulin, 10 ng/mL epidermal growth factor and 1% penicillin-streptomycin. Morphological observation revealed that the cultured cells displayed a homogeneous epithelial cell-like morphology as cobblestone with few fibroblasts visible. The OE cells possessed morphological features of desmosomes, tight junctions and microvilli. Immunocytochemistry assay showed that the cultured cells were cytokeratin 18 positive, indicating that they were omasal epithelial cells. These cells had normal growth properties with corresponding growth curve, and could be stably cultured for 10 passages. The transcriptional expression of peptide transporter 1 gene was detected in OE cells. To evaluate their function for absorption of small peptides, the OE cells were incubated with glycylsarcosine under different conditions. The glycylsarcosine could be absorbed intact into the OE cells in vitro and the absorption was dependent on concentration, time and temperature. From these results, it is inferred that peptide transporter 1 may play an important role in peptide absorption in the OE cells, and that the culture system of OE cells can be served as a useful in vitro model to study absorption of small peptides in bovine omasum.

Key Words: bovine omasal epithelial cells, primary culture, small peptide absorption

T326 Prepartum supplementation of beef cows: Hepatic and muscle gene expression of the offspring at weaning. M. Carriquiry*[1], J. Laporta[1], F. Pereyra[1], A. Astessiano[1], G. Quintans[2], and R. Perez-Clariget[1], *[1]Facultad de Agronomia, UDELAR, Montevideo, Uruguay, [2]Instituto Nacional de Investigaciones Agropecuarias, Treinta y Tres, Uruguay.*

The effect of supplementation during the last month of gestation on plasma IGF-I, muscle fiber characteristics and hepatic muscle gene expression was examined on multiparous beef cow offspring. Cows, ranked by body weight (BW) and body condition score, were blocked by calving day and assigned randomly to control (CON) or supplement (SUP) treatments. All cows were grazing native pastures (1200 kg DM/ha, 8% CP, 67% NDF) and supplemented cows were offered (1 kg/100 kg BW, 4.5 kg/d) whole rice-bran (15.2% CP, 31.7% NDF, 15.1%EE) during the last 40 d of gestation. Blood samples were obtained at 30, 90 and 180 (weaning) days and liver and muscle biopsies were collected at weaning in 10 crossbred (Angus/Hereford; n = 5/treatment) male calves. Gene expression was measured by SYBR-Green real time PCR using ACTB and HPRT as internal control genes. Data were analyzed in a mixed model with treatment as a fixed effect and block as a random effect. Data were considered to differ when $P < 0.05$ and are reported as lsmeans ± SEM. Prepartum supplementation did not affect calf BW at birth (40 ± 4 kg) or at weaning (183.3 ± 3 kg). Plasma IGF-I was greater in SUP than CON offspring (160.4 vs. 117.3 ± 10.4 ng/mL). At weaning, Semitendinous muscle fiber density was greater in SUP than CON offspring (2.9×10^{-4} vs. $2.4 \times 10^{-4} \pm 0.3 \times 10^{-4}$ fiber/μm^2) while muscle diameter did not differ among calves (69 vs. 72 ± 1.6 μm). Expression of IGFBP3 mRNA was greater in the liver (0.65 vs. 0.46 ± 0.06) and less in muscle (0.10 vs. 0.28 ± 0.04) in SUP than CON offspring. Neither liver nor muscle expression of GHR, IGF1 and INSR mRNA differed among treatments. Muscle expression of PPARγ mRNA was greater in SUP than CON offspring (0.11 vs. 0.04 ± 0.02) but SREBF1 mRNA did not differ among calves. Results of this experiment suggest that prepartum supplementation of beef cows in rangeland conditions would enhance growth and muscle adipogenesis potential in male calves.

Key Words: beef cattle, nutrition, fetal programming

T327 Morphometry of the tubular compartment in insulated boar testis. K. Yagoda*[1], F. Melo[2], and J. Parrish[1], *[1]University of Wisconsin-Madison, Madison, [2]Federal University of Goias Campus Jatai, Jatai, Goias, Brazil.*

Elevated ambient temperature is associated with summer infertility in boars. The aim of this study was to evaluate the effects on the testis of short-term scrotal insulation using morphometric analysis. One boar was used as a control and the other boar's scrotum was insulated in a sack for 48 h. The insulation procedure has been shown to increase scrotal temperature by 1.8°C. Testes sections were collected, fixed in Karnovsky solution, dehydrated, and embedded in Epon. Two-micrometer-thick sections from each sample were stained with 1% toluidine blue/sodium borate. All statistical methods were done using an ANOVA test and all values are presented as the mean ± standard deviation. We measured in the control and insulated boar, respectively, the volume density by counting 2880 points on a grid and found the percentages of interstitium (28.8 ± 0.1 vs. 33.7 ± 0.1, $P = 0.0705$), and seminiferous tubules (71.2 ± 0.2 vs. 66.3 ± 0.2, $P = 0.63$) did not differ. We measured the seminiferous tubule diameters (in microns) in 30 cross sections in each boar (214.9 ± 17.5 vs. 216.3 ± 16.2, $P = 0.747$) and found no difference. In contrast, the heights of epithelium in 30 cross sections (59.3 ± 6.2 vs. 53.5 ± 8.1, $P = 0.0029$) was different. We counted cell populations in 9 stage 1 cross sections and only found a difference in the preleptotene/

leptotene primary spermatocytes (13.1 ± 2.5 vs. 8.7 ± 1.6, $P = 0.0004$). In the insulated boar we observed a high density of cells in the lumen and small round vacuoles in the basal compartment. The lowered height of epithelium could be associated with greater cell loss during the thermal injury in the insulated boar. The preleptotene/leptotene primary spermatocytes appear to be the first cells affected by the heat, and had we allowed more time to pass after the insulation this effect would have been seen in the later cell stages. Morphometric analyses realized in this study showed that the scrotal insulation impaired the testicular function and affect the spermatic production.

Key Words: scrotal insulation, reproduction, spermatogenesis

T328 Impact of increased oxidative stress through excessive accumulation of adipose tissue on circulating adiponectin concentrations in dairy cows. S. Häussler*[1], S. P. Singh[1], L. Laubenthal[1], L. Locher[3], J. Winkler[2], U. Meyer[2], S. Dänicke[2], and H. Sauerwein[1], *[1]Institute of Animal Science, Physiology and Hygiene Group, University of Bonn, Bonn, Germany, [2]Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI), Braunschweig, Germany, [3]University of Veterinary Medicine, Foundation, Hannover, Germany.*

Adipokines play an important role in regulating energy metabolism and glucose homeostasis. Fat accumulation (FA) correlates with systemic oxidative stress that may dysregulate the production of adipokines. We are interested in the insulin-sensitizing adipokine Adiponectin (Aq); the objective of our present study was to investigate the effect of excessive FA on oxidative stress and on circulating Aq concentrations in cows. Non-pregnant, nonlactating, pluriparous German Holstein cows (n = 8) were gradually adapted to a high-energy ration (corn-grass-silage with increasing the proportion of corn silage), including a successive increase of the proportion of the concentrate feed (within 6 wk from 0% up to 60% of the dry matter of the daily ration). Within 20 wk, the mean body weight (BW) increased from 540 ± 56.8 kg to 792 ± 81.7 kg; body condition score (BCS, 5-point scale) rose from 2.31 ± 0.35 to 4.53 ± 0.39. Subcutaneous fat from the tail head region was biopsied every 8 wk. Cryosections (14 µm) were prepared, fixed in acetone and stained with hematoxylin. Adipocyte sizes (μm^2) were determined in 100 randomly selected adipocytes. Blood samples were collected monthly; serum Aq concentrations were measured by ELISA (Mielenz et al. 2013). Derivatives of reactive oxygen metabolites (dROM) were photometrically quantified in serum using *N*,*N*,diethyl-1,4-phenylendiamine as chromogen. Data (means ± SEM) were analyzed using ANOVA and Pearson correlations. Serum Aq concentrations (µg/mL) decreased gradually throughout the FA period (41.3 ± 1.76 to 31.1 ± 0.72; $P = 0.006$). Serum Aq concentrations were negatively correlated with BCS ($r = -0.724$, $P < 0.001$), BW ($r = -0.573$, $P = 0.004$), and adipocyte size ($r = -0.475$, $P = 0.022$) and tended to be related with dROM ($r = -0.372$, $P = 0.073$). Increased dROM concentrations (from 49.9 ± 9.24 up to 113 ± 14.5 µg H_2O_2 equivalents/mL) indicating mitochondrial dysfunction as a result of excessive FA, were accompanied by decreased concentrations of the insulin-sensitizing adipokine Aq.

Key Words: adiponectin, reactive oxygen species, dairy cows

T329 The effect of heat stress on lipolytic response of bovine primary adipocytes. M. P. Faylon*[1], L. H. Baumgard[1], R. P. Rhoads[2], and D. M. Spurlock[1], *[1]Iowa State University of Science and Technology, Ames, [2]Virginia Polytechnic Institute and State University, Blacksburg.*

Heat stress (HS) has an enormous economic impact on the global dairy industry, and recent research indicates lipid metabolism is altered in lactating cows experiencing HS. In particular, basal and stimulated lipolytic responses are severely blunted in multiple species during HS. Thus, we hypothesized that HS directly affects adipose tissue by diminishing its response to lipolytic signals. Bovine primary adipocytes were isolated from 5 multiparous Holstein cows in late lactation and cultured at either 42C (HS) or 37C (thermal neutral, TN). Isoproterenol (ISO) was administered at varying concentrations and glycerol release was measured as an indicator of lipolytic response. A dose response curve to ISO was determined under HS and TN conditions for adipocytes isolated from each cow, and differences in the curve-fitting parameters between HS and TN treatments were compared using paired-samples *t*-test. Likewise, the abundance of several lipolytic proteins in relation to HS was evaluated. Adipocytes exposed to HS had an elevated maximal response to ISO (106.9% increase, $P = 0.024$), and were more sensitive to lipolytic stimulation by ISO ($P = 0.02$) compared with cells cultured at TN. Basal lipolytic response was not different between HS and TN cells ($P > 0.05$). At a high ISO concentration, a significant decrease in lipolytic response was observed for HS but not TN cells, suggesting potential downregulation of β-adrenergic receptors in HS but not TN cells. Thermal treatment also increased phosphorylation of hormone sensitive lipase (HSL) at Ser^{563}, confirming increased activation of the PKA pathway under HS conditions. The increased sensitivity of HS cells to lipolytic stimuli was unexpected, as in vivo data previously reported a diminished response to lipolytic signals. Further investigation is warranted to understand the relationship between the in vitro results and the effect of HS on lipid metabolism in vivo.

Key Words: heat stress, adipose tissue, lipolysis

T330 Once-daily milking during a feed restriction does not alter transcription of key lipid metabolism genes in adipose tissue of grazing dairy cows. T. M. Grala[1], J. R. Roche[1], C. V. C. Phyn[1], A. G. Rius[1], R. H. Boyle[1], R. G. Snell[2], and J. K. Kay*[1], *[1]DairyNZ, New Zealand, [2]University of Auckland, New Zealand.*

Study objectives were to investigate the effect of once-daily (1×) milking, at 2 feeding levels, on transcription of key lipid metabolism genes in adipose tissue of grazing dairy cows. Multiparous cows (n = 120) were grazed on pasture and milked twice daily (2×) from calving until 34 ± 6 d in milk (mean ± SD). Cows were then allocated to one of 4 treatments in a 2 × 2 factorial arrangement. Treatments were imposed for 3 wk and consisted of 2 feeding levels (adequately fed; AF, consuming 14.3 kg DMI/cow per d, or underfed: UF, consuming 8.3 kg DMI /cow per d) and 2 milking frequencies (2× or 1×). After the 3-wk treatment period, all cows were AF and milked 2×. Adipose tissue was collected from 12 cows per treatment at wk −1, 3, and 5 relative to treatment start, RNA extracted and transcript abundance of genes involved in lipid metabolism quantified. At the end of the 3-wk treatment period, transcript abundance of genes involved in glyceroneogenesis, glycerolipid synthesis and fatty acid (FA) transport, synthesis, and oxidation were greater ($P < 0.05$) in AF cows milked 1× compared with all other treatments. There was no effect of feeding level on the expression of genes involved in FA oxidation. Additionally, there was no effect of milking frequency on the expression of genes involved in lipid metabolism in UF cows. At wk 5, when all cows had returned to AF and 2× milking for 2 wk, there were no consistent differences in transcript abundance of lipid metabolism genes between treatments. In conclusion, milking cows 1× during a 3-wk nutrient deficit did not alter lipid metabolism compared with 2× milking; however, 1× milking in AF cows upregulated genes associated with FA synthesis, storage and oxidation. These data indicate that by wk 3 of

a feed deficit, lipostatic mechanisms, such as reduced milk production and limited lipolysis, prevented excess body tissue mobilization in UF cows. Furthermore, lipostatic mechanisms, such as greater FA oxidation, prevented excessive adipose deposition in AF cows milked 1× that were in an improved energy state.

Key Words: once-daily milking, nutrition, lipid metabolism

T331 GPR109A mRNA abundance in two different fat depots of dairy cattle considering nicotinic acid and transition period related changes. P. Friedrichs[1], L. Locher[2], K. Huber[2], S. Dänicke[3], H. Sauerwein*[1], and M. Mielenz[1,4], *[1]Institute of Animal Science, Physiology & Hygiene Unit, University of Bonn, Bonn, Germany, [2]Department of Physiology, University of Veterinary Medicine, Hannover, Germany, [3]Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI), Federal Research Institute for Animal Health, Braunschweig, Germany, [4]Leibniz Institute for Farm Animal Biology (FBN), Department of Nutritional Physiology, Dummerstorf, Germany.*

The G-protein coupled receptor (GPR) 109A is predominantly expressed in adipocytes and mediates anti-lipolytic effects. We aimed to determine if nicotinic acid (NA), a known agonist of GPR109A influences the expression of this receptor in subcutaneous (SC) and retroperitoneal (RP) adipose tissue (AT) of dairy cattle. Likewise we studied time-dependent changes and differences of GPR109A mRNA abundance between both AT. For this, 20 pluriparous German Holstein cows were divided into a NA (n = 10) and a control group (n = 10). The animals from the NA group received a NA supplement (Lonza, Basel, Switzerland; 24 g/d) from d 1 until d 21 postpartum. The SCAT from tail head and RPAT were biopsied at d −21, 1 and 21 relative to calving. GPR109A mRNA abundance was quantified by qPCR. The statistical analyses were performed with SPSS 20.0 (SPSS Inc., Chicago, IL, USA) using ANOVA for analysis of the single tissues and Mann-Whitney U test for comparison of both AT. The mRNA abundance was not different between the NA versus control group neither in SCAT nor in RPAT. Thus, groups were pooled for further analyses. In both tissues GPR109A expression was independent of time. Comparing the mRNA abundance from pooled data of all sampling dates between both tissues yielded 1.51-fold higher values in RPAT than in SCAT ($P = 0.001$). When comparing both fat depots within the individual sampling times, RPAT showed 1.46-fold higher expression of GPR109A than SCAT on d −21 ($P = 0.014$) and 1.75-fold higher on d 21 ($P = 0.015$), but we detected no significant difference between both tissues at d 1. In conclusion, neither NA treatment nor time affected the mRNA expression of GPR109A in SCAT and in RPAT, respectively. The different GPR109A mRNA abundances between SCAT and RPAT confirmed our earlier works and indicate different importance for lipolysis and energy mobilization in both fat depots.

Key Words: adipose tissue, nicotinic acid, GPR109A

T332 Evaluation of the Idexx pregnancy detection assay for milk samples. B. Lawson*[1], D. Ray[1], K. Velek[2], E. Martel[2], R. Linscott[2], P. McCoy[1], M. Tate[1], J. Lawrence[2], and W. Silvia[1], *[1]University of Kentucky, Lexington, [2]Idexx Laboratories Inc., Westbrook, .*

The objective of this experiment was to assess the accuracy of the Idexx pregnancy detection assay for milk samples. Lactating Holstein cows (n = 17) were bred by timed artificial insemination. Pregnancy was evaluated by ultrasonography at 37, 65 and 93 d after breeding. Milk samples were collected on d 2 after insemination and at weekly intervals either 1) through d 58 post insemination if the cow was not pregnant on d 37 or 2) through d 93 if the cow was found pregnant. Plasma and milk samples were shipped to Idexx Laboratories Inc. to measure pregnancy associated glycoproteins (PAGs) using an ELISA. This ELISA classifies samples as (1) nonpregnant (NP; PAG concentration below a low critical threshold), (2) pregnant (P; PAG concentration above a high critical threshold) or (3) requiring recheck (RR; PAG concentration between the low and high thresholds). The Idexx staff was not informed of pregnancy status. Seven cows were pregnant by ultrasound on d 37, 65 and 93. All of these cows were classified as P using the milk PAG ELISA on d 30 and 37. Ten cows were found to be open by ultrasound on d 37. All 10 were classified as NP on d 30 and 37. A total of 70 samples were collected from the 7 pregnant cows from d 30 to 93 postinsemination. Using the milk PAG ELISA, 65 (93%) of these samples were classified as P, 5 as RR and 0 as NP. A total of 50 samples were collected from the 10 cows found not pregnant by ultrasound on d 37. In the milk PAG ELISA, 48 (96%) were classified as NP, 2 as RR and 0 as P. A total of 120 milk samples were collected on d 30 or after from these 17 cows. Sixty-five samples were classified P. All of these were from the 7 pregnant cows (positive predictive value: 100%). Forty-eight samples were classified as NP, all from nonpregnant cows (negative predictive value: 100%). Seven samples were classified as RR, 5 from pregnant cows and 2 from nonpregnant cows. We conclude that the milk PAG ELISA identifies pregnant and nonpregnant cows with a high degree of accuracy as early as 30 d after insemination.

Key Words: bovine, milk, pregnancy

T333 Gestational form of supplemental selenium (Se) affects gene expression in the newborn calf testis. II. Spermatogenesis. C. R. Skees*, P. J. Bridges, J. D. Patterson, and J. C. Matthews, *Department of Animal and Food Sciences, University of Kentucky, Lexington.*

In states with Selenium (Se)-deficient soils it is necessary for cattle producers to supplement Se in livestock feed rations. The objective of this study was to determine how inorganic versus organic forms of maternal dietary Se supplementation affect the expression of genes known to affect spermatogenesis in the newborn bull calf testis. Twenty-four Angus-cross cows managed under a standard forage-based cow calf production setting were randomly assigned (n = 8) to 1 of 3 treatment groups: sodium selenite (inorganic, ISe; Prince Se), Sel-Plex (organic, OSe; Alltech) or a 50/50 mix of ISe/OSe (mix). Cows were given ad libitum access to a commercial mineral mix that contained 35 ppm of Se beginning 4 mo before breeding through to calving. Thirteen bull calves were born (ISe n = 5, OSe n = 4, Mix n = 4) and castrated within 48 h of birth. Total RNA was extracted from small pieces of whole testis collected at castration. A microarray analysis was performed using bovine 1.0 ST arrays (Affymetrix) and the data set analyzed by one way ANOVA followed by a post hoc pairwise comparison (*t*-test). Overall, 1112 genes were affected ($P < 0.05$) by Se treatment, including 13 known to be involved with spermatogenesis: Ift52, Acvr1, Tlr3, Mapk14, Egf, Npr1, Raf1, Lep, Ldha, Meis1, Cdk5r1, Ccnd2 and Nphp1. When compared ($P < 0.05$) to ISe (the standard supplementation regimen) the expression of mRNA for Npr1, Lep, Nphp1 and Ccnd2 was increased, and Raf1 decreased, in testis of calves born to OSe supplemented cows, whereas mRNA for Egf and Ldha increased, and Tlr3, Mapk14 and Meis1 decreased, in testis from calves born to Mix supplemented cows. The relative ($P < 0.05$) content of mRNA for Ift52, Acvr1, Tlr3, Mapk14, Npr1, Lep, Meis1, Ccnd2 and Nphp1 was greater, whereas Raf1, Ldha and Cdk5r1 mRNA was lesser, in testis of calves born to OSe vs Mix supplemented cows. Our results provide evidence that the gestational source of dietary Se

affects development of the neonatal calf testis. Whether dam gestational form of supplemental dietary Se affects spermatogenesis and fertility of mature bulls requires investigation.

Key Words: selenium, spermatogenesis, testis

T334 Effect of implants on steroidogenic capacity of bovine granulosa cells. A. D. Stapp*[1], C. A. Gifford[1], K. B. Parker[1], B. I. Gómez[1], D. M. Hallford[2], and J. A. Hernandez Gifford[1], *[1]Oklahoma State University, Stillwater, [2]New Mexico State Universty, Las Cruces.*

Feedlot heifers are often implanted with steroids to increase growth efficiency thereby altering hormone profiles and changing the milieu in which ovarian follicles develop. Because granulosa cell (GC) culture is commonly used and often bovine ovaries are collected from abattoirs with no record of implant status, the objective of this study was to determine if the presence of anabolic and estrogenic steroids during bovine GC development affects FSH-regulated steroidogenesis. Sixteen feedlot heifers were assigned to 1 of 3 treatments: non-implanted (n = 5), and Revalor 200 for 30 d (30 d; n = 5) or 90 d (90 d; n = 6). At slaughter, paired ovaries were collected and small follicle (1 to 5 mm) GC were isolated from each pair and incubated with PBS (n = 16) or 100 ng/mL FSH (n = 16) for 24 h. Effects of FSH treatment on real-time PCR analysis of gene expression and hormone concentrations were analyzed using GLM procedure of SAS. Efficacy of treatment was confirmed by increased ($P < 0.01$) concentrations of medium estradiol in FSH-treated cells (62.31 ± 9.16 pg/mL) compared with PBS-treated controls (14.81 ± 9.16 pg/mL). Treatment with FSH tended to increase steroidogenic acute regulatory protein ($P = 0.09$) mRNA expression in GC of implanted heifers (−0.84 ± 0.56 and −1.79 ± 0.52) compared with non-implanted females (−0.21 ± 0.56). Similarly, P450 side chain cleavage ($P = 0.07$) tended to increase in response to FSH in GC of heifers implanted (2.89 ± 0.29 and 3.04 ± 0.27) compared with non-implanted heifers (1.99 ± 0.33). However, no difference in mRNA expression of 3-β-hydroxysteroid dehydrogenase ($P = 0.57$) and aromatase ($P = 0.23$) were demonstrated in implanted or non-implanted heifers. Medium estradiol concentrations of GC treated with FSH were similar ($P = 0.11$) between implanted (88.8 and 80 ± 21 pg/mL) and non-implanted heifers (80 ± 19.24 pg/mL). Similarly, FSH-mediated progesterone did not differ among treatment groups ($P = 0.22$). Results indicate follicles developing in the presence of high androgenic and estrogenic steroids tend to have an impaired ability to respond to FSH stimulation of the steroidogenic enzymes but not subsequent steroid production.

Key Words: granulosa cells, implant, steroidogenesis

T335 Effects of heat stress and plane of nutrition on liver insulin responsiveness in lactating cows. G. Xie*[3], M. V. Skrzypek[1], S. R. Sanders[1], L. H. Baumgard[2], and R. P. Rhoads[3], *[1]University of Arizona, Tucson, [2]Iowa State University, Ames, [3]Virginia Tech University, Blacksburg.*

Multiparous cows (n = 12; parity = 2; 136 ± 8 DIM, 560 ± 32 kg BW) housed in climate chambers were fed a TMR consisting primarily of alfalfa hay and steam-flaked corn. Cows were subjected to 2 experimental periods (P): (1) thermoneutral conditions (18°C, 20% humidity) with ad libitum intake (TN for group 1, WF for group 2) for 9d and (2) either heat-stress (HS) conditions (cyclical temperature 31.1–38.9°C, 20% humidity: min THI = 73, max THI = 80.5) fed for ad libitum intake (n = 6), or TN conditions, pair-fed (PF) with a HS animal (n = 6) for 9d. Rectal temperature (Tre) and respiration rate (RR) were measured thrice daily at 0430, 1200 and 1630h. Study objectives were to evaluate hepatic insulin responsiveness during HS and PF. Liver biopsies were obtained immediately before and after an insulin tolerance test (ITT) on the last day of each period. Insulin receptor β (IRβ), insulin receptor substrate 1 (IRS-1), Akt/protein kinase B (AKT) and phosphorylated AKT (P-AKT) were measured by Western blot analyses. During P2, HS increased Tre and RR by 1.48°C and 2.4-fold, respectively ($P < 0.01$). HS reduced ($P < 0.01$) DMI by 8 kg/d and by design PF cows had similar intake reductions. Milk yield was decreased similarly (30%) in HS and PF cows and both groups entered into a similar (−4.5 Mcal/d) calculated negative energy balance during P2. Compared with P1 ($P < 0.05$), basal glucose levels increased (5%) in PF cows, but decreased (5%) in HS cows during P2. The ITT caused a more rapid glucose disposal in P1 compared with P2 ($P < 0.05$), but glucose clearance did not differ between environments in P2. Protein abundance of IRβ remained constant during each period. The protein level of IRS-1 was lowered ($P < 0.05$) by insulin in WF only. Insulin increased P-AKT protein content in each period ($P < 0.05$) except PF. Abundance of AKT tended to decrease ($P = 0.057$) only in PF. Phosphorylation ratio of AKT increased 120% in each period ($P < 0.05$) after insulin infusion. These results indicate that liver insulin responsiveness remains unchanged despite mild systemic insulin resistance during HS and reduced nutrient intake.

Key Words: heat stress, insulin, liver

T336 Progesterone, TNF-α, IGF-1, and $PGF_{2\alpha}$ concentrations in blood plasma of beef cows within 14 days after transfer of embryos. J. Copeland*[1], J. Batton[1], E. J. Cuadra[1], T. H. Elsasser[2], B. Johnson[3], J. E. Larson[4], M. C. Mason[1], and J. Yoonsung[5], *[1]Alcorn State University, Alcorn State, MS, [2]USDA ARS, Bovine Functional Genomics Laboratory, Beltsville, MD, [3]Coastal Plain Branch Experiment Station, Newton, MS, [4]Mississippi State University, Mississippi, [5]Prairie View A&M University, Prairie View, TX.*

Lactating beef recipient cows previously synchronized for estrus were randomly assigned to one of 4 treatments to assess the magnitude in progesterone (P_4) rise and its effects on TNF-α, IGF-1 and $PGF_{2\alpha}$ after transfer of embryos. Cows exhibiting estrus received an embryo in the uterine horn ipsilateral to CL 7 d post-estrus. Cows received either no treatment (Control, n = 16), a CIDR (Controlled Internal Drug Release) (CIDR, n = 16), an injection of 1000 IU of hCG (hCG, n = 15) or an injection of 100 µg of GnRH (GnRH, n = 15) at the time of embryo transfer. Blood samples were taken from all cows immediately on d 0 (day of transfer), 7, and 14 for analysis of P_4, TNF-α, and IGF-1. Blood samples for determination of 13,14-dihydro-15-keto $PGF_{2\alpha}$ (PGFM) were collected from half the animals in each treatment group on d 7 and the remaining half on d 14; samples were collected every 15 min for 2 h on both days. Data was analyzed using the SAS MIXED procedure. Pregnancy 60 d after embryo transfer was 56.2, 62.5, 46.7 and 6.7% for the control, CIDR, hCG and GnRH respectively. P_4 was higher ($P < 0.05$) in cows receiving hCG compared with other groups on d 7. Cows, whether pregnant or not at diagnosis, had an increase in P_4 from d 0 to 7 and a decline ($P < 0.05$) in P_4 from d 7 to 14. Non-pregnant cows had an overall decline in P_4 and TNF-α from d 0 to 14 ($P < 0.05$). Treatments did not affect TNF-α. No significant differences ($P > 0.05$) in IGF-1 were observed among treatment groups and between pregnant and non-pregnant cows. While PGFM increased ($P < 0.05$) from d 7 to 14 in cows receiving the CIDR and hCG, concentrations in the hCG group were only higher than those observed in the GnRH ($P < 0.05$) on d 14. Contrary to the non-pregnant cows, no significant differences were observed in concentrations of PGFM between samples collected

every 15 min in the pregnant cows on d 14 ($P < 0.05$). Regardless of pregnancy status, cows had a significant decline in P_4 during the second week after the transfer of an embryo; additionally, $PGF_{2\alpha}$ seems to be steadier in pregnant cows than in non-pregnant cows during that same period.

Key Words: embryo, P4, $PGF_{2\alpha}$

T337 Effect of an essential fatty acid (EFA)-deficient diet on luteal and uterine function in pseudopregnant (PSP) rats and prostaglandins (PG) E1, E2, and F2a (PGF2a, PGE1; PGE2) in nonpregnant (NP) and pregnant (P) ewes. C. W. Weems*[1], Y. S. Weems[1], and R. R. Magness[2], *[1]Dept. of Human Nutrition, Food, and Animal Sciences, University of Hawaii, Honolulu, [2]Dept. of Obstetrics and Gynecology, University of Wisconsin, Madison.*

PGF2a is the uterine luteolysin, but PGE1 or PGE2 are antiluteolysins and prevent luteolysis. PGE1 or PGE2 prevent a natural or induced luteolysis. The precursor for PGF2a and PGE2 is arachidonic acid (AA) and di-homo-gamma linolenic acid (DHGLA) for PGE1. The objective of EXPT 1 was to assess length of PSP and fatty acid (FA) profile by GLC in control 7 EFA deficient PSP rats. A control or EFA-free diet was fed to rats from weaning on d 21. EXP 2 objective was to measure uterine weight on d 9 in control and EFA-deficient PSP rats treated with Vehicle, PGE1, PGE2, or PGE1+PGE2 intrauterine at deciduomata (DCR) induction. PSP length was analyzed by a one-way ANOVA and data in Expt. 2 by a 2 × 2 × 4 CRD for ANOVA. EXPT 3 objective was to measure PGF2a, PGE1 and PGE2 in caruncles and uterine blood by HPLC and RIA on d 13 in NP and P ewes (n = 10 each). EXPT 3 data were analyzed by a one-way ANOVA. An EFA-deficient diet increased ($P < 0.05$) PSP length (12.1 ± 0.5 vs. 17.1 ± 0.6 d), decreased AA 78% ($P < 0.05$), and increased ($P < 0.05$) DHGLA (133%). Uterine weight of DCR rats was lower ($P < 0.05$) in EFA deficient PSP rats (3.2 ± 0.2 vs. 5.9+0.6 g). PGF2a in uterine blood was lower ($P < 0.05$) in EFA-deficient rats (1.9 ± 0.6 vs. controls 11.2 ± 0.21). Uterine weight in PGE1, PGE2, or PGE1+PGE2 at induction of DCR increased ($P < 0.05$) more in PGE2 than PGE1 in EFA-deficient PSP rats (78% vs. 1%). PGF2a in uterine blood was lower in EFA-deficient rats (1.9 ± 0.6 vs. controls 11.2 ± 0.21). PGE1 and PGE2 in caruncles increased ($P < 0.05$) in P ewes on d 13 NP PGE1 (4.7 ± 0.5 ng/g); NP PGE2 (11.7 ± 1.4 ng/g); P PGE1 (12.5 ± 2.3 ng/g), and P PGE2 (24.3 ± 5.1 ng/g). PGF2a, PGE1, and PGE2 in uterine blood of NP ewes averaged 7.3 ± 0.2, 1.2 ± 0.4, and 2.6 ± 0.5 and PGF2a, PGE1, and PGE2 in P ewes averaged 9.9 ± 1.2, 11.6 ± 1.6 and 16.5 ± 2.1. In summary, an EFA deficiency increased PSP and decreased the DCR response in PSP rats. Both PGE1 and PGE2 increases in P ewes support PGE1 and PGE2 as antiluteolysins. Deceased contributors: Hal Behrman and Dorothy Cope.

Key Words: EFA, PSP rats, ewe

T338 Effects of FSH stimulation on β-catenin accumulation in bovine granulosa cells. K. B. Parker*[1], C. A. Gifford[1], A. D. Stapp[1], B. I. Gomez[1], D. M. Hallford[2], and J. A. Hernandez Gifford[1], *[1]Department of Animal Science, Oklahoma State University, Stillwater, OK, USA, [2]Department of Animal and Range Sciences, New Mexico State University, Las Cruces, NM, USA.*

Regulation of estradiol (E_2) biosynthesis by FSH requires the transcriptional co-factor β-catenin (CTNNB1). Increased abundance of CTNNB1 is demonstrated in large antral follicles with greatest concentrations of intra-follicular E_2. In bovine granulosa cells (GC), FSH increases CTNNB1 and protein kinase B (AKT) protein, and WNT2 mRNA expression. These data indicate FSH regulates CTNNB1 through the canonical WNT or AKT signaling pathways. The objective of this study was to elucidate AKT's role in CTNNB1 accumulation. Bovine GC were pre-incubated with AKT inhibitor (LY294002; LY) for 30 min, then cultured with or without FSH for 24 h (n = 4). Total protein was collected for analysis by Western blot. Relative abundance of protein was analyzed using one-way ANOVA procedure of SAS. Expectedly, LY reduced total AKT abundance ($P = 0.05$) and ablated phosphorylated AKT protein. Inhibition of AKT signaling with LY alone ($P = 0.13$) or in combination with FSH ($P = 0.16$) tended to reduce CTNNB1 protein compared with control and FSH-treated GC. Progesterone (P_4) media concentrations did not differ among treatment groups ($P = 0.52$); however, numerical reduction in P_4 was demonstrated in LY compared with FSH-treated GC. Inhibition of AKT reduced ($P < 0.01$) production of FSH-mediated E_2 production. A subsequent study was conducted to investigate whether a known AKT stimulator, IGF-I, could also mediate CTNNB1 accumulation (n = 4). Bovine GC treated with FSH had greater CTNNB1 compared with control and IGF-I groups ($P = 0.01$). Though, FSH did not increase P_4 ($P = 0.42$), IGF-I alone and in combination with FSH increased ($P < 0.01$) P_4 to 161.8 and 185.9 (±13) ng/mL, respectively. Estradiol concentrations were 93 pg/mL for control, and addition of IGF-I alone or in combination with FSH increased E_2 to 280 and 343 (±65) pg/mL, respectively. Data demonstrate that AKT is required for FSH-induced accumulation of CTNNB1 and steroid synthesis. However, IGF-I stimulation of AKT increased steroid production but not CTNNB1 accumulation indicating AKT is necessary for FSH-induced accumulation of CTNNB1, and CTNNB1 is not required for IGF-I induction of steroidogenesis.

Key Words: FSH, granulosa cells, β-catenin

T339 Combined effect of cytological endometritis and cyclicity on fertility of dairy cows. A. Vieira-Neto*[2], W. R. Butler[3], R. O. Gilbert[3], and K. N. Galvão[1], *[1]University of Florida, Gainesville, [2]Universidade do Estado de Santa Catarina, Lages, SC, Brazil, [3]Cornell University, Ithaca, NY.*

Anovulation is associated with cytological endometritis (CTE); therefore, the negative association of either condition with fertility could be confounded by the other. The objective was to evaluate if both cyclicity and CTE would be negatively associated with fertility. Holstein cows (n = 403) from 5 dairies in upstate New York were used. Cyclicity was characterized by serum progesterone concentration ≥1.0 ng/mL at 21, 35, or 49 DIM. CTE was characterized by the presence of ≥4% neutrophils on the uterine cytology performed at 49 DIM. Time to pregnancy up to 300 DIM was analyzed using the PHREG and LIFETEST procedure of SAS. The PHREG model included the effects of cyclicity, CTE, interaction between cyclicity and CTE, parity, body condition score, calving season, PGF treatment, and herd. An interaction between cyclicity and CTE was observed ($P = 0.02$), and 4 dummy variables were created based on the permutation of cyclicity and CTE: Cyclic with CTE (CycCTE; n = 118), cyclic without CTE (CycHealthy; n = 205), anovular with CTE (AnovCTE; n = 34), and anovular without CTE (AnovHealthy; n = 46). Compared with CycHealthy cows, AnovCTE [hazard ratio (HR) = 0.54; CI = 0.34–0.88; $P = 0.01$] and AnovHealthy (HR = 0.69; CI = 0.47–1.00; $P = 0.05$) cows had decreased hazard of pregnancy, and CycCTE tended (HR = 0.79; CI = 0.60–0.1.02; $P = 0.07$) to have decreased hazard of pregnancy. CycHealthy cows were the most fertile as evidenced by the fact that they had the shortest median time to pregnancy (122 d). On the other hand, AnovCTE had the longest median time to pregnancy

(180 d), while CycCTE and AnovHealthy were intermediate (159 and 171 d, respectively). In summary, cyclicity and uterine health statuses are both important for fertility in dairy cows. Both, anovulation and presence of CTE were negatively associated with fertility, and when combined, they had an additive negative effect.

Key Words: cyclicity, cytological endometritis, fertility of dairy cows

T340 Effect of propionate, palmitate and insulin on chemerin gene expression in monolayer cultures of bovine hepatocytes. S. G. Roh*[1], S. Kitayama[1], Y. Suzuki[1], K. H. So[1], K. J. Yi[1], E. Yamauchi[1], S. Haga[2], and K. Katoh[1], *[1]Lab of Animal Physiology, Graduate School of Agriculture Science, Tohoku University, Sendai, Miyagi-ken, Japan, [2]NARO Institute of Livestock and Grassland Science, Japan.*

Chemerin, an adipokine, gene was highly expressed in adipose and liver tissues of Japanese Black cattle, and TNF-α increased the expression of chemerin and chemerin receptor in cultured bovine adipocytes. However, it is not known about the regulatory factors on gene expression of chemerin in cultured bovine hepatocytes. The objective was to investigate the effect of propionate, palmitate and insulin on chemerin mRNA expression in in vitro cultured bovine hepatocytes prepared from preweaning and postweaning cattle. The caudate lobes of liver tissues were sampled from Japanese Black cattle (3-week, 3- and 5-mo-old). The collagenase solution was recirculated through the caudate lobe to collect cells in the medium. The cells were seeded and cultured for 48 h. The cells were washed 3 times with fresh medium and cultured for an additional 6 h in serum-free medium. Then the medium was exchanged with fresh serum-free medium in the presence or absence of propionate (0.1, 1 m*M*), palmitate (50, 100, 250 μ*M*) and insulin (1, 10, 100 n*M*) for 24 h and total RNA was extracted from the cells for analysis of chemerin mRNA. Data were analyzed by 2-way ANOVA using PROC GLM (SAS Inst. Inc.). Values are representative of at least 2 separate series of cultures (5 to 6 replicates per each treatment). Propionate treatment (1 m*M*) reduced ($P < 0.05$) the expression levels of chemerin gene in cultured hepatocytes of 3-wk-old cattle by 40%, while it increased ($P < 0.05$) the expression in 5-mo-old cattle by 1.3-fold. Palmitate (100 μ*M*) did not change the expression level of chemerin mRNA in hepatocytes of 3-week-old cattle ($P > 0.1$), while it increased ($P < 0.05$) the expression in 3-mo-old cattle by 1.4-fold. Insulin (100 n*M*) decreased ($P < 0.05$) the levels of chemerin gene expression in 3-wk- and 3-mo-old cattle by 65% and 20%, but not in 5-mo-old cattle. The expression of chemerin gene was differently controlled by propionate, palmitate and insulin treatments in hepatocytes, and between preweaning and postweaning animals, indicating that chemerin may play a role on nutrient metabolism associated with development.

Key Words: bovine, hepatocyte, weaning

T341 Gene expression in Holstein bull testicular testis after scrotal insulation. J. R. Schindler* and J. J. Parrish, *University of Wisconsin, Madison.*

Holstein bulls (n = 9, approximately 5 years old) were used to study the effects of heat stress on testis gene expression. Bulls were split into a control, insulated, and insulated and recovered groups. Tissue from the control group was harvested without insulation and from the insulated group immediately following insulation for 48 h. Semen was collected from the recovered group for 2 weeks prior, and for 45 d after insulation (3 d per week). Semen was analyzed for nuclear shape (Fourier Harmonic Analysis) to confirm that a heat stress event occurred. The mean harmonic amplitude 1 increased on d 19 post insulation compared with pre-insulation (mean ± SEM, 0.196 ± 0.017 vs. 0.171 ± 0.003; $P < 0.05$). The mean harmonic amplitude 3 increased on d 19, 21, 23, 26, and 28 post insulation compared with pre-insulation (mean ± SEM, 0.173 ± 0.012, 0.170 ± 0.012, 0.179 ± 0.016, 0.185 ± 0.017, and 0.167 ± 0.013 vs. 0.145 ± 0.003; $P < 0.05$). The mean harmonic amplitude 5 increased on d 19 post insulation compared with pre-insulation (mean ± SEM, 0.153 ± 0.012 vs. 0.132 ± 0.003; $P < 0.05$). A mixed effects model was used to compare the control days to each day, post-insulation. Changes in harmonic amplitudes are consistent with previous results. Total RNA from the testis of the control and insulated groups was extracted and microarray analysis was done using a Nimblegen 385K custom bovine expression array. Using a targeted approach, a subset of 200 genes in the array was investigated. The subset was constructed using a National Center for Biotechnology Information search of genes involved in heat stress, apoptosis, DNA repair, and hypoxia. Six genes in the insulated group were statistically different from the control: Cytochrome C-testis, BCL2L13, Dynein, RPS6KB1, OBFC2A, and TIMP-2. These 6 genes were subjected to a validation experiment using qRTPCR. Only TIMP-2 showed a trend for a difference with a 122% decrease in expression from the control ($P = 0.06$) using a mixed effects model. Decreased TIMP-2 expression in the testis would lead to an increase in remodeling of the extracellular matrix in interstitial space and/or the seminiferous epithelium.

Key Words: bull, heat stress, gene expression

T342 Effect of induction of ovulation, early in lactation, on uterine health and fertility in dairy cows. J. H. Bittar*[1], P. Pinedo[3], K. E. Hencken[1], C. C. Barbosa[1], M. Gobikrushanth[1], S. Croyle[1], C. A. Risco[1], A. Vieira-Neto[2], J. E. Santos[1], and K. N. Galvão[1], *[1]University of Florida, Gainesville, [2]Universidade do Estado de Santa Catarina, Lages, SC, Brazil, [3]Texas A&M University, Amarillo.*

Objective was to evaluate the effect of GnRH early postpartum on induction of ovulation, uterine health and fertility in dairy cows. Holstein cows without a CL at 17 ± 3 DIM (n = 255) were randomly assigned to receive an injection of GnRH (n = 128) at 17 ± 3 DIM and at 20 ± 3 DIM or to remain as controls (n = 127). Cows had their ovaries scanned by ultrasonography (US) twice a week for a total of 4 US. Ovulation was characterized by the appearance of a corpus luteum (CL) ≥ 20mm in any US or when a CL < 20mm appeared in 2 consecutive US. Clinical (CE) and cytological endometritis (CTE) were diagnosed at 35 ± 3 DIM. Data was analyzed using the LOGISTIC and PHREG procedure of SAS adjusting for the effect of parity, calving related problems, metabolic problems, or metritis. Cows receiving GnRH had increased ovulation (71.1 vs. 43.3%; $P < 0.001$). GnRH treatment (GTRT) did not affect the prevalence of CE (26.2 vs. 20.8; $P = 0.41$) or CTE (30.9 vs. 32.8; $P = 0.29$). Cows having calving problems (39.7 vs. 17.5%; $P = 0.004$) and metritis (39.7 vs. 16.2%; $P < 0.001$) had increased prevalence of CE. Metritis (39.7 vs. 16.2%; $P < 0.001$) also increased the prevalence of CTE (50.7 vs. 23.5%; $P < 0.001$). An interaction between GTRT and ovulation after GTRT (GTRT × Ov) showed that treated cows that ovulated had decreased CTE compared with cows that did not ovulate (25.6 vs. 43.2%; $P = 0.05$), but ovulation did not affected ($P = 0.88$) CTE in control cows. GTRT did not affect conception rate (CR) at 32 (42.2 vs. 43.3%; $P = 0.74$) or 74 d after AI (37.5 vs. 35.4%; $P = 0.26$), or pregnancy loss (11.1 vs. 18.2%; $P = 0.30$). The interaction GTRT × Ov showed that treated cows that ovulated had increased CR at 74 d compared with cows that did not ovulate (42.9 vs. 24.3%; $P = 0.05$), while there was no difference ($P = 0.57$) in control cows. The interaction GTRT × Ov showed that treated cows that did not ovulate had decreased hazard of pregnancy up to 300 DIM compared with cows that ovulated (HR = 1.9; $P = 0.01$), or control

cows that did (HR = 1.8; $P = 0.03$) or did not ovulate (HR = 1.9; $P = 0.01$). GnRH treatment early postpartum increased ovulation; however, it failed to improve uterine health or fertility.

Key Words: ovulation, uterine health and fertility, dairy cow

T343 Temporal gene expression profiling of liver from peripartal dairy cows during spring and summer. H. Akbar[1], U. Bernabucci[2], L. Basiricò[2], P. Morera[2], and J. J. Loor*[1], *[1]University of Illinois, Urbana, [2]Università degli Studi della Tuscia, Viterbo, Italy.*

Thermal stress during hot seasons renders dairy cattle more susceptible to metabolic disease, including liver lipidosis. Hepatic transcriptome-wide changes during heat stress remain unknown. We examined temporal gene expression profiles during the dry period and early lactation in liver of 12 Holstein cows that calved in the spring (SP, March to April) or summer (SU, June to July) using a whole-transcriptome bovine microarray (Agilent) and quantitative RT-PCR (qPCR). Liver tissue was harvested at −30, 3, and 35 d relative to parturition. The 23 target genes selected for qPCR were associated with heat shock response (HSP70A1A, HSTF1), fatty acid oxidation (CPT1A, PPARA, ACOX1), hepatokines (FGF21, ANGPTL4), esterification and VLDL assembly (MTTP, APOB100, DGAT1, SREBF2), glucose metabolism (PC, PCK1, PDK4), and inflammation and stress (TNF, GPX1, SOD1, SOD2, SAA3, HP, HAMP). The ANOVA model included day, season, and day × season as fixed effects, and cow within season as the random effect. Statistical difference for interaction and main effects was declared significant at $P < 0.05$. Results from qPCR revealed a 2-fold increase in expression of HSP70A1A in SU between −30 and 3 d. In contrast, expression of HSTF1 between −30 and 3 d increased 20-fold in SP and 7-fold in SU. Despite the 6- to 11-fold increase in CPT1A between −30 to 3 d in SU and SP, SU was associated with lower overall expression (~2-fold) of CPT1A and also PPARA and ACOX1. Except for MTTP, expression of APOB, DGAT1, and SREBF2 was 2- to 6-fold greater overall in SP than SU. The gluconeogenic enzyme PCK1 increased 14-fold from −30 and 3 d in SP but decreased 2-fold in SU. Expression of acute-phase proteins increased > 60-fold between −30 and 3 d regardless of season, but there was greater overall expression (~40-fold) in SU. Overall, results revealed that during hot seasons the liver transcriptome in peripartal cows is markedly altered and likely contributes to the susceptibility of those cows to develop disease.

Key Words: heat stress, transition cow, transcriptomics

T344 Chronic uterine infusion of melatonin or melatonin receptor antagonist during mid-gestation alters ovine placental nitrites and superoxide dismutase activity. K. E. Brockus*[1], L. E. Camacho[2], K. A. Vonnahme[2], and C. O. Lemley[1], *[1]Mississippi State University, Mississippi State, [2]North Dakota State University, Fargo.*

Previous data from our laboratory showed an increase in umbilical artery blood flow in ewes infused with melatonin (MEL), while in contrast MEL receptor antagonist (luzindole, LUZ) infusion decreased blood flow. The objectives of the current experiment were to determine maternal and fetal concentrations of total nitrites (an index of nitric oxide production) and placental superoxide dismutase (SOD) activity following a 4 week uterine infusion of MEL (n = 5), LUZ (n = 5), or vehicle control (CON; n = 4). Singleton pregnant ewes were implanted with Alzet osmotic pumps (Durect Co; 2.5 μL/h infusion of 1 mg/mL MEL or LUZ) under the perimetrium of the gravid uterine vascular network on d 62 of gestation. This infusion model resulted in local delivery of MEL to the fetus as evident by a 25% increase in maternal MEL concentrations vs. a 125% increase in fetal MEL concentrations. On d 90 of gestation maternal blood samples were collected, ewes were euthanized, umbilical cord blood samples were collected (combination of umbilical artery and vein), and placentomes were separated and frozen for later determination of tissue levels of nitrites or SOD activity. Total nitrites in maternal serum were not different ($P > 0.50$) across all treatments; however, total nitrites in umbilical cord serum were increased ($P < 0.03$) in both LUZ and MEL vs. CON dams. Placental concentrations of nitrites in the caruncle were increased in LUZ and MEL vs. CON dams, while cotyledon nitrites were not different ($P > 0.10$) across all treatments. Caruncle SOD activity was increased ($P < 0.05$) in MEL dams vs. CON and LUZ; however, cotyledon SOD activity was not different ($P > 0.30$) across all treatments. The results from the current study show an increase in placental antioxidant enzyme activity following chronic uterine MEL infusion, which may be mediating the previously observed increase in umbilical artery blood flow. Moreover, placental nitric oxide production may be increased in both LUZ and MEL infused dams irrespective of the previously observed differences in umbilical artery blood flow.

Key Words: melatonin, nitrites, superoxide dismutase

T345 Follicular dynamics in Holstein heifers subjected to 5-d protocols to synchronize ovulation. H. Ayres*[1,3], L. M. Vieira[1], R. M. Ferreira[1], E. O. S. Batista[1], R. V. Sala[1], J. P. Barbuio[3], F. P. Rennó[1], J. E. P. Santos[2], and P. S. Baruselli[1], *[1]Department of Animal Reproduction, University of São Paulo, São Paulo, Brazil, [2]Department of Animal Sciences, University of Florida, Gainesville, [3]MSD Animal Health, São Paulo, Brazil.*

Three experiments (Exp) were designed to evaluate follicular dynamics in Holstein heifers treated with 5-d protocols to synchronize ovulation. Protocols differed in the source of progesterone (P4), the use or not of GnRH on d 1 of the protocol and type of ovulatory stimulus. In Exp1, heifers received a P4-releasing intravaginal device on D0 and were assigned to receive (n = 12) or not (n = 12) 0.1mg gonadorelin (GnRH; Fertagyl, Merck). On D5, the P4 device was removed and 0.53 mg cloprostenol (PGF; Ciosin, Merck) were administered followed by 0.1 mg GnRH 48 h later. In Exp2, heifers received a protocol similar to Exp1, except that on D0 they were assigned to receive a P4 device and no GnRH (n = 13), a Norgestomet implant (Crestar, Merck) and no GnRH (n = 11), or a norgestomet implant combined with GnRH (n = 12). In Exp3, heifers received a norgestomet implant on D0, which was removed on D5. PGF was administered on D5 and heifers were assigned to receive either 1mg estradiol benzoate (EB, Gonadiol, Merck AH; n = 11) 48 h or GnRH (n = 12) 72 h later. Ultrasonographic evaluations of the ovaries were performed using a 5-MHz linear transducer. Continuous data were analyzed by ANOVA and for binary data by logistic regression (GLIMMIX, SAS). In Exp1, the use of GnRH on D0 did not affect follicular growth and synchronization of ovulation. In Exp2, the use of norgestomet implant without GnRH on D0 anticipated ovulation (76.4 ± 4.3) compared with P4 device (98.5 ± 4.1; $P = 0.003$) and norgestomet implant with GnRH was similar a both. Regardless the use of GnRH on D0, norgestomet implant-treated heifers had larger diameter of the ovulatory follicle (OF) (13.6 ± 0.4) than P4 device-treated heifers (12.3 ± 0.4; $P = 0.01$). As for the ovulatory stimuli, both GnRH and EB had similar ovulation rates (100 and 91.7%, respectively; $P = 0.90$). When P4 was administered via intravaginal insert use of GnRH at the beginning of the protocol did not improve synchronization of follicle growth. However, when norgestomet was the source of P4, administration of GnRH increased the diameter of the OF and synchronized ovulation compatible with insemination at 72 h after implant removal.

Key Words: follicle dynamic, Holstein heifer, progesterone

T346 Associations between plasma anti-Müllerian hormone (AMH) and fertility responses of seasonally calving grazing dairy cows. E. S. Ribeiro*[1], R. L. A. Cerri[2], R. S. Bisinotto[1], F. S. Lima[1], L. F. Greco[1], A. Morrison[3], A. Kumar[3], W. W. Thatcher[1], and J. E. P. Santos[1], *[1]Department of Animal Sciences, University of Florida, Gainesville,, [2]Department of Land and Food Systems, University of British Columbia, Vancouver, BC, Canada, [3]Ansh Labs Inc., Webster, TX.*

The objective was to investigate associations between plasma AMH and fertility of grazing dairy cows subjected to synchronized AI on the first day of a 100-d breeding season. Lactating cows (n = 1,237) in 2 farms had estrous cycles presynchronized and were enrolled in a timed AI (TAI) protocol (GnRH on d −8, $PGF_{2\alpha}$ on d −3 and −2, and GnRH + AI on d 0). All cows received the first AI on the same day (study d 0). Blood was sampled on d −8 and analyzed for AMH using a chemiluminescence immune assay (AnshLite Bovine AMH CLIA). From d 19 to 34, detection of estrus was performed daily and cows were re-inseminated. On d 35, bulls were placed with cows for 65 d of breeding. Concentrations of AMH were affected ($P < 0.01$) by breed and lactation number. Concentrations were higher for Jersey cows, followed by crossbreds and then Holsteins (337 vs. 298 vs. 264 pg/mL). Cows on lactations 2 and 3 had higher ($P < 0.05$) AMH than those on lactations 1 and > 3 (342 and 328 vs. 257 and 273 pg/mL). Plasma AMH was related negatively (AOR = 0.93; $P = 0.01$) with estrous expression at TAI. The odds of detecting a cow in estrus at timed AI reduced 7% per every 100 pg/mL increase in plasma AMH. Nevertheless, AMH was not associated with detection of estrus and re-insemination after first AI. Plasma AMH was not associated with pregnancy per AI (P/AI) for the TAI, but tended ($P = 0.10$) to be positively related to P/AI for the second AI performed after estrous detection (AOR = 1.07) and pregnancy for natural service (AOR = 1.07). Non-pregnant cows at the end of 100-d breeding period had lower ($P = 0.05$) plasma AMH than those that became pregnant (252 vs. 310 pg/mL). In conclusion, concentrations of AMH in plasma were associated with reduced expression of estrus at TAI but not later in the breeding period. Plasma AMH was not associated with fertility of cows subjected to synchronized ovulation, but was associated with fertility of cows that failed to become pregnant to TAI and were subsequently bred on estrus (AI on estrus + natural service). Synchronization of ovulation might override associations of AMH and fertility of lactating grazing cows.

Key Words: AMH, dairy cow, reproduction

T347 Comparison of four methods to determine pregnancy success in beef cattle. G. A. Perry*[1], M. F. Smith[2], and K. G. Pohler[2], *[1]Department of Animal Science, South Dakota State University, Brookings, [2]Division of Animal Science, University of Missouri, Columbia.*

Transrectal ultrasonography has been available for several years, but accuracy is dependent upon a well-trained technician. More recently, blood tests to determine pregnancy have become commercially available. Therefore the objective of this study was to compare the accuracy of determining pregnancy status on d 30 after fixed-time AI among: transrectal ultrasonography, visual observation for return to estrus, and 3 different blood tests. Beef heifers (n = 42 and 69 at location 1 and 2, respectively) and cows (n = 102 at location 1) were synchronized using the PG 6-d CIDR protocol with FTAI at 66 to 72 h after CIDR removal concurrent with an injection of GnRH. EstroTect estrus detection patches were placed on animals (6 or 19 d after AI at location 1 and 2, respectively) and estrus was monitored twice daily until d 30. On d 30, pregnancy status was determined by transrectal ultrasonography and blood samples were collected. Blood samples were sent to Genex, University of Missouri (UM), and BioTracking for determination of pregnancy status. Data were analyzed using the GLIMMIX procedures of SAS with herd as a random effect. When compared with transrectal ultrasonography there was no difference ($P = 0.14$) for assay sensitivity (ability to correctly identify pregnant animals; 99, 98, 94, and 95% for estrous detection, Genex, UM, and Biotracking). There was a tendency ($P = 0.09$) for decreased specificity (ability to correctly identify non-pregnant animals; 82, 67, 82, and 87% for estrous detection, Genex, UM, and Biotracking). There was no difference in the positive predictive value ($P = 0.20$; likelihood a pregnant animal was called pregnant) or negative predictive value ($P = 0.29$; likelihood a non-pregnant animal was called not pregnant) among tests (90 and 99%, 83 and 97%, 89 and 89%, and 92 and 91% for estrous detection, Genex, UM, and Biotracking). Furthermore, there was no difference ($P = 0.25$) in the overall accuracy of the test (percent of time correctly identified; 92, 86, 89, and 92%, for estrous detection, Genex, UM, and Biotracking, respectively). Therefore, several options are available to determine pregnancy status in cattle.

Key Words: fixed-time AI, pregnancy status, blood test

T348 The relation of two apoptosis-related proteins (bax and bcl-2) to adipocyte cell size in bovine adipose tissue. D. Germeroth[1], M. Steyer[2], T. Ettle[3], M. Rodehutscord[2], H. Sauerwein[1], and S. Häussler*[1], *[1]Institute of Animal Science, Physiology and Hygiene Group, University of Bonn, Bonn, Germany, [2]Institute of Animal Nutrition, University of Hohenheim, Hohenheim, Germany, [3]Bavarian State Research Center for Agriculture, Institute of Animal Nutrition and Feed Management, Grub, Germany.*

Programmed cell death (apoptosis) in adipose tissue (AT) is related to adipocyte size. The initiation and regulation of apoptosis can be mediated by the intrinsic pathway through mitochondria. Members of the Bcl-2 protein family, e.g., bcl-2 (anti-apoptotic) and bax (pro-apoptotic) are key regulators involved in this mitochondrial pathway. Our objective was to evaluate whether adipocyte size and the mitochondrial pathway are related in bovine AT. Therefore, Simmental heifers (n = 39) from 2 feeding trials with a mean BCS of 4.0 ± 0.13 (scale 1–5) were examined and AT from the tail head region was biopsied. Cryosections were cut (12–14 µm) and stained with Mayer's hematoxylin, adipocyte area (μm^2) of 100 randomly selected cells per section was evaluated. For immunohistochemistry, sections were fixed in ice-cold acetone at room temperature (RT). Endogenous peroxidase activity and unspecific binding sides were blocked with 0.3% H_2O_2 (15 min, RT) and normal goat serum (1:10, 20 min, RT), respectively. Sections were incubated with monoclonal mouse antibodies against human bax (1:30, RT) and human bcl-2 (1:30, 4°C) over night. Afterward, sections were incubated with biotinylated polyclonal goat antibodies against mouse IgG (1:200) and peroxidase-labeled streptavidin (1:1000) both for 30 min at RT. Staining was achieved by 3-amino-9-ethylcarbazol, counterstaining was done by Mayer's hematoxylin. Bovine placenta (6 µm) served as control. Data (means ± SEM) were evaluated using Spearman correlations. The mean adipocyte size was 6,833 ± 295 μm^2. The mean portions of bax and bcl-2 positive cells were 6.45 ± 0.67% and 12.74 ± 1.31%, respectively. Adipocyte size was positively correlated with bax ($r = 0.571$, $P < 0.001$) and bcl-2 ($r = 0.654$, $P < 0.001$) as well as the portion of bax to bcl-2 ($r = 0.632$, $P < 0.001$). The presence of both apoptosis-related proteins indicates that the mitochondrial pathway of apoptosis functions in bovine AT. Both bax and bcl-2 were related to adipocyte size. The amount of anti- and pro-apoptotic parameters was similar. Therefore, we assume that the mitochondrial pathway leading to apoptosis is not initiated.

Key Words: adipocyte size, dairy cows, Bcl-2 family

T349 Niacin increases adiponectin secretion in differentiated bovine preadipocytes in vitro via G-protein coupled receptor 109A. C. Kopp[1], S. P. Singh[1], H. Sauerwein*[1], and M. Mielenz[2], *[1]Institute of Animal Science, Physiology & Hygiene Unit, University of Bonn, Bonn, Germany, [2]Leibniz Institute for Farm Animal Biology (FBN), Department of Nutritional Physiology, Dummerstorf, Germany.*

Adiponectin (AdipoQ), an insulin-sensitizing adipokine, is involved in regulating energy homeostasis and lipid metabolism by increasing fatty acid oxidation in liver and glucose uptake in adipocytes of monogastrics. The G-protein coupled receptor 109A (GPR109A) is involved in downregulating lipolysis not only by its endogenous ligand β-hydroxybutyrate but also by pharmacological doses of Niacin (NA). In rat adipocytes, increased AdipoQ secretion in response to stimulation with NA was attributed to GPR109A signaling. In cattle, corresponding data are missing. Our objective was thus to examine the effect of NA on AdipoQ secretion and the involvement of GPR109A in bovine adipocytes. A primary cell culture system using differentiated preadipocytes was established. Subcutaneous adipose tissue was collected from 5 Holstein-Friesian dairy cows. Stromal-vascular cells were isolated, pooled and seeded at 2500 cells/cm^2. Preadipocytes after 12 d of differentiation were used. After starvation, cells were incubated either with 100 ng/mL pertussis toxin (PTX), a nonselective G-protein uncoupling agent, or PBS for 16 h to characterize the NA mediating pathway. Afterward cells were treated with NA (10 or 15 μ*M*) for 12 or 24 h or with PBS as controls, respectively. The AdipoQ concentrations in the cell culture supernatants were quantified by ELISA (Mielenz et al., 2013, doi:10.1016/j.domaniend.2012.10.004). Statistical analyses were performed using ANOVA with Bonferroni post hoc tests. Data are given as means ± SEM. The concentrations of AdipoQ for both NA doses and durations of NA treatment were increased ($P \leq 0.001$) to maximal 306 ± 11 ng/mL compared with controls (48 ± 2 ng/mL). Pre-incubation with PTX reduced ($P \leq 0.001$) the response to NA to maximally 2.2 fold higher concentrations than in the controls. Our results of NA stimulated AdipoQ secretion from differentiated bovine adipocytes together with the dampened increase after PTX treatment point to GPR109A as mediating at least partially the NA stimulated increase of AdipoQ secretion in cattle.

Key Words: bovine adiponectin, niacin, G-protein coupled receptor 109A

T350 Effect of storage time on the viability of cryopreserved bovine spermatozoa. A. I. Gallegos*[1], S. A. Ericsson[1], H. D. Blackburn[2], S. F. Spiller[2], B. J. Warnock[1], M. K. Meador[1], M. W. Smith[1], and P. H. Purdy[2], *[1]Sul Ross State University, Alpine, TX, [2]USDA-ARS-National Animal Germplasm Program, Fort Collins, CO.*

Long-term cryopreserved semen viability can affect the National Animal Germplasm Program's (NAGP) sampling strategy and ability to reconstitute livestock populations. Therefore, the purpose of this project was to determine if prolonged storage of cryopreserved sperm affects cell viability. Cryopreserved sperm samples from 12 Hereford bulls were utilized from the NAGP repository. These samples were separated into groups based on storage time of 40–50, 30–39, 20–29 or 10–19 yr. The percentage of progressively (PMS) and total motile sperm (TMS), curvilinear velocity (VCL), and beat cross frequency (BCF) was obtained using computer assisted sperm analysis. Flow cytometric analysis and specific fluorescent stains were utilized for the following viability assessments: FITC PNA – percent live non-acrosome reacted sperm (LNARS); propidium iodide – percent membrane intact sperm (MIS); Yo-Pro-1 - cell membrane integrity/ non-apoptotic sperm (NAS); merocyanine 540 (M540) - percent of sperm with relatively ordered membranes (ROM); and the combination of Yo-Pro-1 with M540 for assessment of membrane phospholipid order (MPO). Differences in measures of sperm viability were assessed using ANOVA with a fixed effect model that included the effects of storage time, type of cryopreservation diluent (milk or egg yolk), and their interaction. Significant differences were not observed for the main effects or for the interaction term (Table 1). These results demonstrate that bovine sperm viability was not affected as a result of the storage time in liquid nitrogen. These results suggest sample deterioration is not occurring and need not be a factor for consideration in collection development and sample utilization.

Table 1. Mean values of stored sperm viability

	Storage (yr)				
Measure	40+	30–39	20–29	10–19	SEM
PMS (%)	6	15	12	11	1.7
TMS (%)	26	45	48	39	3.3
VCL (μm/s)	134	167	164	148	7.9
BCF (Hz)	36	35	32	31	1.3
LNAR (%)	62	81	47	35	3.5
MIS (%)	63	63	57	57	5.1
NAS (%)	66	57	55	47	5.3
ROM (%)	84	77	57	83	5.2
MPO (median)	92	9	22	8	14.7

Key Words: sperm, viability, cryopreservation

T351 Effect of exogenous eCG during the first or second service during the Ovsynch protocol upon the pregnancy per insemination of lactating Holstein cows. K. G. Gonzalez-Garcia*[1], C. Leyva[1], C. A. Cancino[1], J. L. Morales[1], M. Mellado[2], F. G. Veliz[2], and C. A. Meza-Herrera[3], *[1]Universidad Autonoma Agraria Antonio Narro Unidad Laguna, Torreon, Coahuila, Mexico, [2]Universidad Autonoma Agraria Antonio Narro, Saltillo, Coahuila, Mexico, [3]Universidad Autonoma Chapingo, URUZA, Bermejillo, Durango, Mexico.*

The aim of this study was to evaluate the effect of eCG during Ovsynch protocol upon the pregnancy per insemination in dairy cattle. Lactating Holstein cows (n = 200) from a dairy herd in northern Mexico, were divided into 2 groups (n = 100 each). Females from both groups were treated according to the Ovsynch protocol (d0 GnRh, d7 PgF2α, d9 GnRh, d10 AI). However, while one group received 400 IU of eCG on d 7 of the protocol, the other group was defined as the control group; all animals in both groups were inseminated at first service. In the second part of experiment, those cows diagnosed as non-pregnant (n = 48), were used to evaluate the effect of a second Ovsynch protocol with (n = 21) and without (n = 27) application of eCG. Those cows injected with eCG but diagnosed as non-pregnant, served as control to the non-pregnant, non-eCG-injected in the first part of treatment. All cows were inseminated at fixed time. Pregnancy diagnosis was performed by rectal palpation at d-39. The pregnancy per insemination of both groups was compared using a chi-squared test (SYSTAT 10 (Evenston, ILL, USA, 2000). The results of the pregnancy per insemination of cows subjected to Ovsynch with and without eCG are included in Table 1. Results suggest that injection of eCG in the first or second service does not improve the pregnancy per insemination in lactating Holstein cows in northern Mexico.

Table 1. Effect of eCG injection at first or second service during Ovsynch protocol on the pregnancy per insemination in Holstein cows

Group	First service (no.)	Pregnancy per AI (%)	Second service (no.)	Pregnancy per AI (%)
eCG	100	21[a]	21	23.8[a]
Control	100	17[a]	27	40.7[a]

[a]Means with different superscripts in each column differ significantly ($P > 0.05$).

Key Words: eCG, Ovsynch protocol, pregnancy

T352 Visual analytics of bovine nutriphysiogenomics datasets. M. J. Khan*[1], M. Welge[1,2], C. Bushell[1,2], M. Berry[1,2], L. Gatzke[1,2], and J. J. Loor[1], *[1]University of Illinois, Urbana, [2]National Center for Supercomputing Applications, Urbana, IL.*

High-throughput microarray technology has provided a wealth of information on the dynamism of the transcriptome in key tissues of dairy cattle during key life stages such as the transition from pregnancy to lactation. With the advent of next-generation sequencing technology and its application in bovine bioscience, high-dimensional data are becoming more prevalent. The research of transcriptome data mining has relied on the use of software which places strict limits of the feature dimensionality, utility, and comprehensibility of analysis. The objective of this study is to develop new, innovative visual and interactive techniques for effectively studying, exploring and experimenting with the data to help form and confirm hypothesis. Technique development is focused on the use of statistical and machine learning tools and approaches in support of data driven biology that are required to underpin and enable modern nutriphysiogenomics research. Five different data sets including cow performance and transcriptomics of mammary gland, adipose tissue and liver were used. Mammary data encompass d −30, −15, 1, 15, 30, 60, 120, 240 and 300 relative to parturition. Adipose data encompass −65, −42, −14, 1, and 14 d in cows fed control or a moderate-energy diet prepartum. Liver data encompass −65, −30, −14, 1, 14, 30, and 49 d in cows fed control, moderate-energy, or underfed energy prepartum. Specifically we are applying approaches in the following areas 1) analyzing and interrogating microarray and next-generation sequencing data sets; 2) extracting quantitative features from large and complex data sets; 3) capturing variation and linking biological processes to phenotypic traits; 4) supporting knowledge-discovery in biological data using visualization approaches. These approaches will allow the researcher to extract relevant features to explain phenotypic behavior. These include the use of "small-multiples" each visually representing the distribution of the data, the display of gene expression amplification for comparison over time and tissues, and techniques that support the exploration of "what if scenarios" to produce alternative phenotypic outcomes.

Key Words: bioinformatics, nutrition, transcriptomics