Physiology and Endocrinology I

M227 ACTH-induced stress impairs the expression of genes involved in steroidogenesis and angiogenesis in dairy cow preovulatory follicles. D. Biran[1], R. Braw-Tal[2], Y. Lavon[1], and Z. Roth*[1], *[1]Department of Animal Sciences, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University, Rehovot, Israel, [2]Institute of Animal Science, Agricultural Research Organization, Bet Dagan, Israel.*

Ovulation failure, follicular persistence and follicular-cyst formation are known to impair dairy cow fertility. While the mechanism is not entirely clear, stress-induced alteration in adrenal hormone secretion can cause such ovarian pathologies. We examined changes in genes involved in steroidogenesis and angiogenesis upon ACTH-induced stress. Six synchronized lactating cows were scanned daily by ultrasound (Aloka SSD-900) and plasma samples were taken throughout the experiment. Treated cows (n = 3) were administered ACTH analog (Synacthen Depot; 1mg, s.c.) every 12h from d 15 to d 21 of the cycle. Control cows (n = 3) were administered $PGF_{2\alpha}$ (Estroplan) on d 6 of the cycle to induce development of a preovulatory follicle. Ovaries from both control and treated cows were collected at the slaughterhouse 40h after the last $PGF_{2\alpha}$ administration and on d 23 of the cycle, respectively. Follicular diameter was measured and follicular fluids were aspirated to determine steroid concentrations. Slices of the follicular wall were taken for total mRNA isolation and sqRT-PCR. Administration of ACTH increased ($P < 0.02$) cortisol concentration in the plasma and reduced ($P < 0.01$) milk production, indicating stress. Androstenedione and estradiol concentrations in the follicular fluids were lower ($P < 0.05$) in ACTH-treated follicles relative to controls. The expression of mRNA for LH receptor, 3Î²-HSD and P450arom was lower ($P < 0.02$) in the ACTH-treated group than in controls but P450scc, StAR protein and P450c17 mRNA levels did not differ between the groups. mRNA expression for angiopoietin-1 and angiopoietin-2 did not differ between the groups but that for VEGF120 and VEGF164 was higher ($P < 0.01$) in controls than in ACTH-treated follicles. Findings indicate that ACTH induced stress, and impaired follicular steroidogenesis and angiogenic capacity, characterized by reduced follicular-fluid steroid concentration and low expression of genes involved in these processes. Such alterations might explain, in part, the mechanism underlying ovulation failure and the formation of persistent or cystic follicles under stress.

Key words: persistent follicle, cyst

M228 Comparison of different staining methods on sperm from Holstein bulls. A. Ata, M. E. Inanc, O. Kankavi, O. Yildiz Gulay*, and M. S. Gulay, *Mehmet Akif Ersoy University, Faculty of Veterinery Medicine, Burdur, Turkiye.*

The aim of the current study was to compare effectiveness of various semen staining methods in frozen-thawed and epididymal cattle semen. Frozen semen (American Breeder Service and Ege-Vet) were placed in a water bath first at 37°C for 30 s and thawed. Epididymal semen from cauda epidiymis was obtained from slaughter house (Güç-Birligi, Burdur, Turkiye) and diluted with phosphate buffer solution (PBS). After preliminary examination under the microscope, frozen-thawed and epididymal semen samples were smeared on glass microscope slides using another glass slide and air-dried. Sperm on the slides were stained with Coomassie Blue, Silver nitrate, May–Grünwald+Giemsa, Ponceau-S, Naphthol yellow-S+Eritrosin-B, Ponceau-S+Naphthol yellow-S+Eritrosin-B, Trypan Blue+Giemsa, Eosin+Coomassie Blue and modified Wright′s stain. Stained froty samples were examined under bright field microscopy (Nikon E-600). From all froties at least 100 spermatozoa were investigated. Different staining methods were compared by one way ANOVA. Differences between epididymal and frozen-thawed samples were analyzed by *t*-test procedure. Equatorial region of intact spermatozoa was well defined and acrosomal region was stained differently than other regions by some staining methods (Coomassie Blue, May-Grünwald+ Giemsa, Ponceau-S, Naphthol yellow-S+Eritrosin-B, Eosin+Coomassie Blue; $P < 0.05$). Equatorial regions of spermatozoa with corrapted acrosomes were not stained well. Epidiymal spermatozoa were stained better than frozen-thawed spermatozoa in all staining methods investigated in the present study ($P < 0.05$). This could be because of the substances used in frozen semen as cryoprotectans (proteins of animal origin, etc.). In conclusion, our results demonstrated that Eosin+Coomassie Blue staining method is simple, inexpensive, and reliable method for staining semen from Holstein bulls. The method works quickly, needs fewer methodological steps, and does not require complex laboratory conditions for assessment of establishing live-dead spermatozoa and acrosomal/morphological status in frozen-thawed and epididymal semen obtained from Holstein bulls

Key words: cattle, spermatozoa, staining

M229 Insulin sensitivity correlates with parameters of hepatic lipid metabolism, and is lower in older dairy cows. H. A. van Dorland[1], M. Graber[1,2], S. Kohler[2], T. Kaufmann[3], and R. M. Bruckmaier*[1], *[1]Veterinary Physiology, Vetsuisse Faculty, University of Bern, Bern, Bern, Switzerland, [2]Department of Animal Science, Swiss College of Agriculture, Zollikofen, Bern, Switzerland, [3]Clinic for Ruminants, Vetsuisse Faculty, University of Bern, Bern, Bern, Switzerland.*

Insulin sensitivity may be estimated by the “Revised Quantitative Insulin Sensitivity Check Index” (RQUICKI), which is based on plasma concentrations of insulin, glucose, and free fatty acids (NEFA). It was indicated before that RQUICKI might be used to identify lactating cows with disturbed insulin function. In the present study, data from a field study were used to investigate the association between RQUICKI and the parity of the dairy cows, and of parameters involved in hepatic metabolism. Blood and liver samples were obtained from 185 dairy cows in wk 3 ante partum (-wk3) and in wk 4 (+4wk) and 13 postpartum (+13wk). Blood plasma was assayed for concentrations of NEFA, glucose, and insulin. Liver was analyzed for mRNA expression levels by qRT-PCR encoding 27 enzymes and nuclear receptors related to carbohydrate and lipid metabolism. The results show that cows of >3 parities have a significant lower RQUICKI across sampling points than cows with <3 parities ($P < 0.001$). In addition, RQUICKI was lower in +4wk than in −3wk and +13wk ($P < 0.001$). Significant ($P < 0.01$) Spearman Rank Correlation Coefficients were observed between RQUICKI and mRNA abundance of liver X receptor α (LXRα), sterol regulatory element binding factor 1 (SREBF1), ATP citrate lyase, fatty acid synthase (FASN), Glycerol-3-phophate acyltransferase (GPAM), 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR), which may illustrate the pathological changes in the liver through infiltration of fat as observed to be associated with disturbed insulin function in man.

Key words: insulin resistance, metabolism, liver

M230 Intrauterine position and adjacent fetal sex status influences fetal and placental growth but not embryonic viability under crowded uterine conditions in pigs. B. A. Freking* and C. A. Lents, *USDA, ARS, U.S. Meat Animal Research Center, Clay Center, NE.*

Intrauterine position and sex of adjacent fetuses in litter bearing species have been implicated in physiological and behavioral differences in males and females. Our objective was to quantify influences of uterine position and sex status of flanking fetuses under crowded uterine conditions and test the impact on fetal and placental growth rate. Gilts were subjected to unilateral-hysterectomy-ovariectomy surgery at 160 d of age and mated at approximately 280-d of age. Gilts were assigned to be harvested at d 45, 65, 85, or 105 of gestation. A total of 297 pregnancies were evaluated in 4 contemporary groups. Position in the uterus relative to the cervix, fetal status (alive, dead, mummy), fetal weight, and placental weight were recorded at harvest. Data were coded to test when each fetus was adjacent to 0, 1, or 2 opposite sex fetuses. Data were analyzed by mixed-model ANOVA procedures fitting contemporary group, line, and flanking fetal sex code as fixed effects and sire as a random effect. Nonlinear functions were fitted to the fetal and placental weight data to establish unique growth curves for each flanking sex status code. When considering only observations that had an opportunity to be flanked by 2 adjacent fetuses, the fraction of live fetuses represented by each classification (0, 1, 2) were 26.4%, 50.1%, and 23.4%, respectively, indicating no impact on fetal survival. Fetal weight was not influenced by flanking sex status code at d 45, but was significant ($P < 0.05$) by d 65, and became highly significant ($P < 0.001$) by d 105. Least squares means at d 105 were 800.0 ± 20.3, 748.5 ± 17.8, and 672.7 ± 25.2 g, respectively for flanking sex status codes 0, 1, 2. Placental weight was also similarly influenced by flanking sex status code, but only apparent ($P < 0.01$) by d 105. Fetal growth development in pigs is influenced by sex status of adjacent fetuses, and could be a potential source of variation in behavioral and reproductive differences later in life.

Key words: fetal growth, pigs, survival

M231 The effect of teasing rams with a ewe stimulus prior to semen collection. A. G. Fahey*[1], P. Duffy[1], and S. Fair[2], *[1]University College Dublin, Belfield, Dublin 4, Ireland, [2]University of Limerick, Limerick, Ireland.*

The objective of this study was to determine if exposing rams to a ewe stimulus for 1 h before semen collection could improve the rams' libido and/or semen quality. The experiment was carried out during the breeding season. Rams (European breeds; 1.5 to 4 years of age) were allocated to one of 3 treatments according to breed and age: Treatment 1 (Control); rams (n = 5) were exposed to a ewe not in estrus for 1 h and were subsequently allowed to mount another ewe in estrus for semen collection. Treatment 2 (Non novel ewe); rams (n = 6) were exposed to a ewe in estrus for 1 h after which the same ewe was restrained on a ramp for semen collection. Treatment 3 (Novel ewe); rams (n = 6) were exposed to a ewe in estrus for 1 h after which the rams were allowed to mount a different ewe in estrus for semen collection. Rams did not have tactile contact with the teaser ewe during the 1 h exposure time and the experiment was repeated on each of 5 consecutive days. Libido was measured by the rams' reaction time (time from first foot on the ramp to when ejaculation into the artificial vagina had occurred) and the number of mounts taken before ejaculation. Each ejaculate was assessed for volume, concentration, wave motion and progressive linear motion after 1 h. Data were analyzed using ANOVA with repeated measures using the MIXED procedure of SAS. There were no significant effects of treatment, day or their interaction on reaction time, the number of mounts or on wave motion or progressive linear motion of sperm. There was no significant effect of treatment on semen volume, however, there was a significant effect of day ($P < 0.05$) and a treatment × day interaction ($P < 0.05$). Semen concentration and total sperm number were significantly affected by treatment ($P < 0.05$) and day ($P < 0.01$), however, there was no treatment × day interaction. Exposing rams to an estrus ewe before semen collection does not improve ram libido but does assist in improving the sperm number in the ejaculate.

Key words: ram, libido, semen

M232 Effects of supplemental progesterone and timing of initiation of resynchronization on fertility in lactating dairy cows. T. R. Bilby*[1], R. G. S. Bruno[1], K. J. Lager[1], R. C. Chebel[2], J. G. N. Moraes[2], P. M. Fricke[3], G. Lopes[3], J. O. Giordano[3], J. E. P. Santos[4], F. S. Lima[4], J. S. Stevenson[5], and S. L. Pulley[5], *[1]Texas AgriLife Research and Extension, Texas A&M System, Stephenville, [2]Department of Veterinary Population Medicine, University of Minnesota, St. Paul, [3]Department of Dairy Science, University of Wisconsin, Madison, [4]Department of Animal Sciences, University of Florida, Gainesville, [5]Department of Animal Sciences and Industry, Kansas State University, Manhattan.*

Our objective was to determine the effect of exogenous progesterone (P_4) within a timed artificial insemination (TAI) protocol initiated at 2 different times post-AI on pregnancies per AI (P/AI) in lactating dairy cows. Lactating cows (n = 1,982) from 5 commercial dairy herds were assigned randomly at 31 ± 3 d post-AI, at non-pregnancy diagnosis, in a 2 × 2 factorial arrangement of 4 resynchronization treatments initiated early (EP; 31 ± 3 d) or late (LP; 39 ± 3 d) post-AI. Cows were either treated with a CIDR (C) insert or not (NC) as part of an Ovsynch-56 protocol (GnRH, 7 d later $PGF_{2\alpha}$, 56 h GnRH, 16 h TAI). Therefore, the 4 treatments were: 1) EPNC (n = 509), open cows at 31 ± 3 d post-AI and not receiving a CIDR; 2) EPC (n = 501), open cows at 31 ± 3 d post-AI and receiving a CIDR; 3) LPNC (n = 485), open cows at 39 ± 3 d post-AI and not receiving a CIDR; and 4) LPC (n = 487), open cows at 39 ± 3 d post-AI and receiving a CIDR. Cows were inseminated if observed in estrus before TAI. Pregnancies per AI were determined 31 and 60 d after TAI. In a subgroup of cows (n = 1,101), blood samples were collected and ovarian structures were examined at first GnRH (G1) and $PGF_{2\alpha}$ of the Ovsynch-56 protocol. Percentage of cows with CL at G1 was not affected by treatment but percentage of cows with CL at $PGF_{2\alpha}$ was greater ($P < 0.01$) for EP vs.. LP cows (87.9 vs.. 79.4%). In addition, percentage of cows with P_4 concentration > 1 ng/mL at G1 was not affected by treatment but was increased ($P < 0.01$) for EP vs.. LP cows at $PGF_{2\alpha}$ (86.5 vs.. 74.3%). Treatment did not affect ovulation to G1 and P/AI 31 d after resynchronized TAI (EPNC = 30.1, EPC = 28.8, LPNC = 27.5, LPC = 30.5%). An interaction was detected ($P < 0.04$) between timing of initiation of resynchronization and supplemental P_4 at d 60 with the CIDR tending ($P = 0.11$) to increase P/AI in late (LPNC = 23.7 vs.. LPC = 28.0%), but not in early (EPNC = 26.9 and EPC = 24.2%) cows. Embryo loss was unaffected by treatment. In conclusion, addition of a CIDR insert within the Ovsynch-56 protocol initiated late (d 39 ± 3) but not early (d 31 ± 3) post-AI improved P/AI.

Key words: dairy cows, resynchronization, CIDR

M233 Effect of circulating progesterone (P4) and two different GnRH doses on LH secretion in lactating dairy cows. J. O. Gior-

dano*, P. M. Fricke, J. N. Guenther, G. Lopes, M. M. Herlihy, and M. C. Wiltbank, *Department of Dairy Science, University of Wisconsin-Madison, Madison.*

Our objectives were: 1) to determine the effect of circulating P4 (high P4; HP4 vs.. low P4; LP4), and 2) increasing the GnRH dose from 100 (LD) to 200 (HD) μg on LH secretion in HP4 and LP4 in lactating cows (n = 24). Double-Ovsynch (Presynchronization; GnRH-7d-PGF-3d-GnRH; 7d later Breeding-Ovsynch; GnRH-7d-PGF-48h-GnRH-16h-TAI) was used to create the HP4 and LP4 environments. At the 1st GnRH of Breeding-Ovsynch (HP4) all cows having a CL ≥20 mm received either a LD or HD of GnRH. At the 2nd GnRH of Breeding-Ovsynch (LP4) cows randomly received a LD or HD of GnRH. Blood samples (BS) were collected every 15 min from −15 to 180 min after GnRH, and then hourly until 5 h after GnRH. As expected, mean P4 in HP4 was greater than LP4 (2.8 vs. 0.2 ng/mL; $P < 0.01$). Mean LH for LD cows (n = 12) was affected by P4 (1.7 vs. 7.4 ng/mL for HP4 and LP4; $P < 0.01$), time ($P < 0.01$) and treatment by time ($P < 0.01$). Circulating P4 in LD cows also affected the LH peak (3.4 vs. 17.7 ng/mL for HP4 and LP4; $P < 0.01$) and area under the curve (AUC; 488.0 vs. 2346.9 ng for HP4 and LP4 ng; $P < 0.01$). Mean LH for HD cows (n = 10) was also affected by P4 (3.5 vs. 9.6 ng/mL for HP4 and LP4; $P < 0.01$), time ($P < 0.01$) and treatment by time ($P = 0.04$). Circulating P4 in HD cows decreased LH peak (7.9 vs. 21.3 ng/mL for HP4 and LP4; $P = 0.02$) and AUC (1065.9 vs. 2933.3 ng for HP4 and LP4 ng; $P = 0.01$). In HP4 (n = 24), mean LH was affected by GnRH dose (1.7 vs. 3.7 ng/mL for LD and HD; $P < 0.01$), time ($P < 0.01$), and treatment by time ($P < 0.01$). Dose of GnRH affected LH peak (3.3 vs. 8.5 ng/mL for LD and HD; $P < 0.01$), time to LH peak (1.3 vs. 1.8 h for LD and HD; $P = 0.04$), and AUC (501.0 vs. 1177.8 ng for LD and HD; $P < 0.01$). In LP4 (n = 22), mean LH was affected by GnRH dose (6.9 vs. 10.7 ng/mL for LD and HD; $P < 0.01$), time ($P < 0.01$), and treatment by time ($P < 0.01$). Likewise, GnRH dose affected LH peak (15.7 vs. 23.6 ng/mL for LD and HD; $P = 0.01$) and AUC (2186.4 vs. 3443.2 ng for LD and HD; $P < 0.01$). We conclude that circulating P4 reduces GnRH-induced LH secretion, and a higher dose of GnRH can increase LH secretion both in a high and low P4 environment. Supported by Hatch project WIS01171.

Key words: LH secretion, progesterone, GnRH

M234 Assessment of an accelerometer system (Heatime) for detection of estrus and timing of insemination in lactating dairy cows. A. Valenza, G. Lopes*, J. O. Giordano, J. N. Guenther, and P. M. Fricke, *Department of Dairy Science University of Wisconsin-Madison, Madison.*

Two experiments were conducted on a commercial dairy in Wisconsin to evaluate an accelerometer system (Heatime) to manage reproduction. In the first experiment, lactating Holstein cows (n = 54) received an i.m. injection of GnRH (100 μg) from 35 to 49 DIM followed 7 d later by an i.m. injection of PGF2α (PGF; 25 mg). Beginning 48 h after PGF, blood samples were collected for progesterone (P4) analysis and ovaries were monitored using ultrasound at 8 h intervals for 120 h. Ovulatory response to GnRH treatment was greater ($P = 0.027$) for cows with <0.5 ng/mL P4 (96.2%, 25/26) than for cows with ≥0.5 ng/mL P4 (66.7%, 16/24). Cows were removed from the analysis if they were detected in estrus by the system before PGF (n = 6) or if they did not regress their CL after PGF (n = 6). For the 42 cows included in the analysis, 26% (11/42) underwent luteal regression but did not ovulate (5% were detected in estrus by Heatime; 21% were not), whereas 74% (31/42) regressed their CL and ovulated (67% were detected in estrus by Heatime; 7% were not). Peak accelerometer activity occurred 67.1 ± 2.5 h after PGF, and cows were inseminated 9.9 ± 2.3 h after peak activity. Ovulation occurred 85.9 ± 2.4 h after PGF, 20.9 ± 3.1h after peak activity and 10.7 ± 2.5 h after AI. In the second experiment, cows (n = 426) were assigned by odd or even ID number to receive an i.m. injection of GnRH (G, 100 μg) at AI detected by the Heatime system (n = 401 AI) or no treatment (control, C; n = 482 AI). Pregnancy diagnosis was performed by the herd veterinarian 30 d after AI using ultrasonography. Based on logistical regression analysis, pregnancies per AI (P/AI) was affected ($P < 0.001$) by parity (35.2 vs.. 22.3% for primiparous vs.. multiparous cows) and season (34.7 vs.. 22.6% for cool vs.. warm seasons); however, treatment with GnRH at AI did not affect P/AI (29.8 vs.. 26.8% for C vs.. G cows, respectively). We conclude that the Heatime system determined the correct timing of AI for most of the cows that displayed estrus and that treatment with GnRH at the time of AI determined by the Heatime system did not affect fertility in lactating dairy cows.

Key words: estrous detection, GnRH, Heatime

M235 Presynchronization with double-Ovsynch improves conception at first postpartum AI in primiparous lactating dairy cows. M. M. Herlihy*[2,3], J. O. Giordano[1], A. H. Souza[1], A. Keskin[1], A. B. Nascimento[1], J. N. Guenther[1], M. A. Crowe[3], S. T. Butler[2], and M. C. Wiltbank[1], *[1]Department of Dairy Science, University of Wisconsin-Madison, Madison, [2]Animal and Bioscience Research Department, Teagasc, Moorepark, Cork, Ireland, [3]School of Agriculture, Food Science and Veterinary Medicine, University College Dublin, Ireland.*

Lactating dairy cows (n = 739; 341 primiparous, 398 multiparous) were enrolled in a study to test the hypothesis that improved pregnancies per AI (P/AI) following Ovsynch can be achieved in first service, first parity cows through the use of Double-Ovsynch for presynchronization compared with Presynch-Ovsynch. Cows were randomly assigned to: Double-Ovsynch (DO, n = 366; GnRH-7d-PGF-3d-GnRH-7d-Ovsynch-56[GnRH-7d-PGF-56h-GnRH-16hTAI]) or Presynch-Ovsynch (PS, n = 373; PGF-14d-PGF-12d-Ovsynch-56). Progesterone (P4) was determined at GnRH1 of Ovsynch-56. Pregnancy was diagnosed by palpation per rectum at 39 d and late embryo loss rate was determined at 74 d. Treatment effects were analyzed by logistic regression using the GLIMMIX Procedure of SAS. Explanatory variables in the statistical model included treatment, parity (1, ≥ 2), body condition score (BCS) (low ≤2.50; high >2.50), treatment*parity and treatment*BCS interactions. Presynchronization with DO tended ($P = 0.06$) to improve P/AI (DO = 46.6%, 171/366 vs.. PS = 41.5%, 155/373). One-tailed contrasts revealed improved ($P = 0.04$) P/AI for first parity cows treated with DO (DO = 56.3%, 95/168 vs.. PS = 47.4%, 82/173), with no improvement ($P > 0.05$) observed for older animals (DO = 38.1%, 75/198 vs.. PS = 36.3%, 73/200). There was no effect ($P > 0.05$) of presynchronization treatment on incidence of late embryo loss after first service (9.1 vs.. 6.1%). Presynchronization at first service had no effect ($P > 0.05$) on P/AI at second service (33.1 vs.. 34.9%). DO increased the percentage of cows with P4 ≥ 0.5 ng/mL at GnRH1 of Ovsynch (DO = 93.7%, 343/366 vs.. PS = 75.3%, 281/373; $P < 0.001$) with similar effects in all parities. A treatment by BCS interaction ($P < 0.05$) was observed for serum P4 at GnRH1, with low BCS cows having greater P4 if assigned to DO than PS (1.90 vs.. 2.44ng/mL; $P < 0.05$). Thus, presynchronization with Double-Ovsynch induced cyclicity in cows of all parities; however, Double-Ovsynch increased fertility only in first parity cows and not older cows. The physiology underlying this parity difference is not yet clear.

Key words: presynchronization, Ovsynch, dairy cow

M236 Effect of GnRH and double AI (24h apart) on fertility of high-producing cows detected in estrus by professional tail chalk service. D. Cunningham[1], A. Fisher[1], A. H. Souza*[2,1], H. Rivera[1], A. Skidmore[3], and M. C. Wiltbank[2], *[1]Accelerated Genetics, Baraboo, WI, [2]Department of Dairy Science, University of Wisconsin, Madison, [3]Intervet/Schering-Plough Animal Health, Summit, NJ.*

The objective of this study was to evaluate the effects of double AI and supplemental GnRH given at AI in Holstein cows detected in estrus by daily tail chalk. Lactating cows from 2 commercial herds in WI (n = 1,101), DIM = 166 ± 3, in their 1st to 14th postpartum breedings were randomly assigned in a 2 × 2 factorial design as follows: SAI) Single AI when tail chalk was rubbed off at 0h (standard tail chalk procedure); DAI) Double AI (at 0h and 24h) with second AI done regardless of chalk been rubbed off again after 1st AI; SAI+GnRH) Single AI at 0h plus supplemental GnRH treatment simultaneously to AI at 0h; DAI+GnRH) Double-AI (at 0h and 24h) plus GnRH at 0h second AI performed regardless of further chalk reading after 1st AI. Estrus detection based on daily tail chalk reading was performed by 2 experienced technicians. Pregnancy was diagnosed by ultrasound at 30 ± 3d after AI. Factorial design comparison was analyzed with procedure Glimmix of SAS, with cow treated as a random effect and interaction between number of AI and GnRH forced into the statistical model. In addition, final model also took into account main effects and the interactions for farm, parity, DIM at AI, and month of breeding. There was no interaction ($P > 0.10$) between number of AIs and GnRH treatment on pregnancies per AI (P/AI). Similarly, main effects of number of AIs (SAI = 30%; DAI = 32%) and GnRH treatment (no-GnRH 31%; yes-GnRH = 30%) did not alter P/AI. Further analysis using only hard breeders (cows not conceiving until 200DIM; n = 239) also showed no significant interaction between treatments; or effect of number of AI (SAI = 23%; DAI = 22%) and GnRH at 1st AI (no-GnRH = 22%; yes-GnRH = 23%). In conclusion, blanket use of double breeding and/or GnRH at the time of AI failed to enhance fertility in lactating Holstein cows detected in estrus by professional AI technicians doing AI service based on tail chalk removal.

Key words: artificial insemination, GnRH, dairy cow

M237 Paraoxonase expression and activity in bovine granulosa cells and follicular fluid. A. Schneider[1,2], V. A. Absalon-Medina[2], G. Esposito[3,2], M. N. Corrêa[1], and W. R. Butler*[2], *[1]Universidade Federal de Pelotas, Pelotas, RS, Brazil, [2]Cornell University, Ithaca, NY, [3]University of Naples Federico II, Naples, Italy.*

The aim of this work was to evaluate expression of paraoxonase (PON) 1, 2 and 3 in bovine granulosa cells and activity in follicular fluid (FFL). The PON enzyme family possesses anti-oxidant and anti-inflammatory effects and is highly expressed in liver. In plasma PON1 is bound to HDL and is reduced during pathophysiological challenges during the peripartum transition period. Ovaries were collected from cows during slaughter and follicles were used for expression analysis (7 estrogen-active follicles [EAF] and 7 atretic follicles [ATF]) and enzyme activity in FFL (10 EAF and 21 ATF). Follicles were dissected from the stroma, FFL was aspirated and the follicle walls immersed in RNALater. To recover granulosa cells, follicular walls were removed from the RNALater, halved, scraped and washed with cold saline into a Petri dish. Granulosa cells were recovered by centrifugation at 2000 x g for 3 min. Total RNA was isolated and real-time PCR used to evaluate PON 1, 2 and 3 mRNA expression according to the ΔΔCt method. Estradiol (E2), progesterone (P4), HDL, LDL, cholesterol and PON1 were evaluated in FFL. Additionally, FFL from 15 EAF was aspirated in Holstein cows to compare PON1 activity in FFL and plasma. In granulosa cells PON2 and 3 mRNA expression was not different between EAF and ATF, PON1 being undetectable. In EAF and ATF, FFL E2 concentration was 133 ± 33 and 12 ± 3 ng/mL, with E2/P4 ratio of 2 and 0.2, respectively. PON1 activity was higher ($P < 0.05$) in EAF (83 ± 8 kU/L) than ATF (62 ± 5 kU/L), as well as HDL, LDL and cholesterol concentrations ($P < 0.05$). E2 concentration in FFL was correlated to PON3 expression ($r = 0.59$, $P < 0.05$) and PON1 activity ($r = 0.50$, $P < 0.01$). PON1 activity in FFL (61 ± 5 kU/L) was lower ($P < 0.01$) than in plasma (123 ± 11 kU/L), but correlated ($r = 0.69$, $P < 0.01$). In summary, although PON1 activity in FFL increases with E2 concentration, its origin appears to be from plasma since it is not expressed in granulosa cells. Moreover, increased PON1 activity in FFL in association with increased concentration of HDL and cholesterol indicates that this is due to a higher transfer rate of the protein from plasma in EAF.

Key words: granulosa cells, PON, HDL

M238 Development of a lentiviral RNA interference (RNAi) system for interleukin–1 beta (IL1B) expressed in elongating porcine embryos. D. J. Mathew*, E. M. Newsom, R. D. Geisert, and M. C. Lucy, *University of Missouri, Columbia.*

Most embryonic loss in pigs occurs during conceptus elongation and attachment. During this time, pig conceptuses increase expression of IL1B but the function of this molecule in embryonic development is unknown. There appear to be at least 2 IL1B genes – the prototypical IL1B cytokine (secreted by macrophages and other immune cells) and an embryonic IL1B (IL1BE) expressed by the pig conceptus. Our ultimate objective is to assess the function of IL1BE by developing an in vivo RNAi lentivirus-based system that specifically knocks down IL1BE in porcine embryos. As a first step, we screened oligonucleotides for their capacity to knock down IL1BE but not IL1B in vitro. Full-length cDNA for IL1B and IL1BE were cloned into an expression vector that contained a luciferase reporter for monitoring RNAi (psi–CHECK1; Promega, Madison, WI). We then identified 8 19 bp oligonucleotides that were complimentary to IL1BE but not IL1B mRNA using the siRNA Target Designer program (Promega). Based on these original sequences, longer oligonucleotides that were designed to form short hairpin RNA (shRNA) were annealed to their complimentary oligonucleotides and inserted into the pGeneClip U1 expression vector (Promega). Baby Hamster Kidney (BHK–21) cells were simultaneously transfected with the psi–CHECK1 vector containing either IL1B or IL1BE and one of 8 shRNA vectors. After 48 h, cells were lysed and assayed for luciferace activity. In cells transfected with the IL1BE, 7 of 8 shRNA decreased ($P < 0.01$) luciferase activity compared with the positive control. Two of the shRNA that knocked down IL1BE did not knockdown IL1B (i.e., they were specific for IL1BE). Luciferase activity was reduced by >90% ($P < 0.001$) by the 2 shRNA specific for IL1BE. Luciferase activity in cells transfected with IL1BE and a vector containing scrambled shRNA was not different ($P > 0.10$) from the positive control. We conclude that IL1BE can be specifically knocked down in vitro by using RNAi. This project was supported by National Research Initiative Competitive Grant no. 2007–35203–17836 from the USDA National Institute of Food and Agriculture.

Key words: embryo, pig, expression

M239 Differential gene expression in liver of lactating (L) and non-lactating (NL) primiparous Holstein cows during early pregnancy. J. Green*, E. Newsom, C. Okamura, and M. Lucy, *University of Missouri, Division of Animal Science, Columbia.*

The objective was to determine the physiological effect of lactation on hepatic mRNA expression in primiparous Holstein cows during early pregnancy. Liver was collected from cows that were either L (n = 22) or NL (n = 18) and were either d 28 (n = 6 L and 6 NL), d 35 (n = 8 L and 6 NL), or d 42 (n = 8 L and 6 NL) of gestation. Hepatic RNA was submitted to the University of Missouri DNA core for microarray analysis (Bovine Genechip; Affymetrix, Santa Clara, CA). Data were analyzed by using JMP Genomics 4.1 (SAS Inst., Cary NC). Data Analyses identified 299 targets that were differentially expressed ($P < 0.001$) between L and NL. Gene lists were analyzed for functional significance by using the iPATH software (Letunic et al. 2008, Trends Biochem Sci. 33:101–3). The L cows had greater hepatic mRNA expression for enzymes involved in gluconeogenesis, cholesterol synthesis, lipid synthesis, and cholesterol metabolism compared with NL cows. Quantitative real time reverse transcription PCR (qRTPCR) assays were used to confirm microarray results. The qRTPCR assays were validated by DNA sequencing of the amplified product and by performing serial dilutions of a single sample to assess efficiency. Based on qRTPCR, there was an effect of lactation because L cows had greater expression of ATP citrate lyase (Acly; $P < 0.02$), acyl-CoA synthetase long-chain family member 1 (Acsl1; $P < 0.06$), acyl-CoA synthetase short-chain family member 2 (Acss2; $P < 0.03$), apolipoprotein A-1 (Apoa1; $P < 0.003$), cytochrome P450scc (Cyp11a1; $P < 0.05$), fatty acid synthase (Fasn; $P < 0.05$), HMG-CoA reductase (Hmgcr; $P < 0.002$), pyruvate carboxylase (Pc; $P < 0.08$), phosphoenolpyruvate carboxykinase 1 (Pck1; $P < 0.001$), stearoyl-CoA desaturase-1 (Scd; $P < 0.01$), and suppressor of cytokine signaling 2 (Socs2; $P < 0.001$) while NL cows had greater mRNA transcript levels of protein phosphatase 1 regulatory subunit 3C (Ppp1r3c; $P < 0.001$). There was no effect of lactation for cyclophilin (housekeeping gene). There were no effects of d of pregnancy on gene expression. In conclusion, lactation had a large effect on gene expression in liver that was not affected by d of pregnancy.

Key words: lactation, metabolism, Holstein

M240 Immunohistochemical evidence for the presence of G protein-coupled receptor 43 in cattle rumen epithelium but not in the pancreatic islets of Langerhans. A. Wang[1], R. M. Akers[2], and H. Jiang*[1], *[1]Department of Animal and Poultry Sciences, Virginia Tech, Blacksburg, [2]Department of Dairy Science, Virginia Tech, Blacksburg.*

Volatile fatty acids (VFAs) are the major products of microbial fermentation in the rumen. Besides serving as substrates for energy generation, VFAs are also known to stimulate rumen development, increase serum insulin and glucagon concentrations, and regulate gene expression in cattle and sheep. The mechanisms underlying these regulatory effects of VFAs are unknown, but recent discovery that VFAs can bind to G protein-coupled receptor 43 (GPR43) and 41 (GPR41) suggests that the regulatory effects of VFAs may be mediated by these receptors in VFA target tissues. As a step toward testing this possibility, we determined whether GPR43 was expressed in bovine rumen wall and the pancreatic islets of Langerhans. Rabbit antiserum against a bovine GPR43 peptide was generated. The specificity of the antiserum for binding to GPR43 was confirmed by Western blotting analysis of recombinant bovine GPR43 protein. Immunohistochemical analyses using this antiserum revealed the presence of GPR43-immunoreactive cells in the epithelium of both adult and newborn cattle rumen, but not in the mucosa, submucosa, or muscle layer. The same immunohistochemical analyses did not reveal any GPR43-immunoreactive cells in the bovine islets of Langerhans or the surrounding exocrine tissue. These data suggest that the effect of VFAs on rumen development in cattle may be mediated by GPR43 in the rumen epithelial cells and that the effects of VFAs on serum insulin and glucagon concentrations, however, unlikely involve binding to GPR43 in the pancreas.

Key words: receptor, rumen, VFA

M241 Effects of protein supplementation during heifer development on reproductive characteristics and success in beef heifers. A. S. Summers*[1], R. A. Cushman[2], S. P. Weber[1], M. L. Spangler[1], and A. S. Cupp[1], *[1]University of Nebraska-Lincoln, Lincoln, [2]USDA-ARS Roman L. Hruska U.S. Meat Animal Research Center, Clay Center, NE.*

A 2-yr study was conducted to determine the effects of feeding different protein supplements during heifer development on reproductive traits and performance. Our hypothesis was that protein supplementation would enhance reproductive performance in heifers with below average reproductive characteristics. Heifers from 2 herds at the University of Nebraska Animal Development and Research Center were used with heifers (Angus and Angus x Simmental hybrids) from the teaching herd (n = 56) being fed a modified dried distillers grain (MOD) supplement at 1.36 kg/d from weaning (mid September) through May. Heifers (MARC III x Red Angus) from the physiology herd (n = 173) were randomly assigned to groups and fed dried distillers grain-based (DDG) or corn gluten feed-based supplement (CFG) offered at 0.59% and 0.78% BW, respectively, from mid-November through May. Supplements were formulated to be isocaloric but differed in undegradable protein. All heifers were fed ad libitum meadow hay through winter while grazing dormant pasture. Prior to breeding, heifers were transrectally ultrasounded to determine antral follicle count (AFC), uterine horn diameter (UHD), ovarian size, presence of a CL, and to determine reproductive tract score (RTS). Heifers developed on MOD diet were 23 d older ($P < 0.01$) and had greater ($P < 0.01$) ovarian area, total AFC, and percent of CL present compared with other groups. However, MOD heifers had lower ($P < 0.01$) UHD compared with other groups. There was no difference ($P = 0.19$) in proportions of heifers bred to A.I.; however, overall pregnancy rates were lower ($P < 0.01$) for MOD compared with other groups. There was a positive effect of small follicle counts on RTS [RTS = 3.9 + 0.01(small follicles); $P < 0.01$, r2 = 0.04] and AFC [AFC = 4.9 + 0.8(small follicles); $P < 0.01$, r2 = 0.86]. Although MOD and DDG diets were similar, results from these groups varied, suggesting that age led to some variation in response to these supplements. We also conclude that RTS and AFC are influenced by small follicle counts. USDA is an equal opportunity provider and employer.

Key words: antral follicle counts, beef cattle, reproduction

M242 Effect of parity on thermal response and energy balance (EB) of sows housed at 24-27°C during lactation. W. R. Martin*, T. J. Safranski, D. E. Spiers, and M. C. Lucy, *University of Missouri, Columbia.*

An earlier study showed that parity 1 sows (P1) were more sensitive to heat stress compared with greater parity sows as indicated by rectal temperature (RT) and respiration rate (RR). To confirm this relationship, a second trial was designed to measure RT and RR of P1 (n =

7), parity 2 (P2; n = 4) and parity 6 (P6; n = 2) sows in one farrowing room. Sows entered 1wk before farrowing and remained until 4 d after weaning. Ear temperature (ET), shoulder temperature (ST), RT and RR were measured daily at 1400 h. Room temp was 24–27°C. Pregnant sows housed in the room served as a non-farrowed control group. Sow BW and litter wt were measured weekly. Feed offered and refused (kg) were recorded. EB (Mcal ME) was estimated from BW, litter wt, and feed consumed. There were effects of parity ($P < 0.05$) and d of lactation (DOL; $P < 0.001$) on RT. The RT increased after farrowing (38.2 ± 0.1°C on d −1 to 39.1 ± 0.1°C on d 0) and remained elevated during lactation (38.8 ± 0.1 to 39.3 ± 0.1°C). Before farrowing, RT was unaffected by parity ($P > 0.1$; 38.3 ± 0.1°C) but during lactation, RT was greatest in P1 sows (39.4 ± 0.1, 39.0 ± 0.1, 38.8 ± 0.2°C for P1, P2, and P6). Despite greater RT during lactation, P1 sows did not have greater RR (50 ± 1 breaths per min; BPM) or ET (37.3 ± 0.1°C), but ST was greater (36.5 ± 0.1, 36.2 ± 0.1, and 36.2 ± 0.2°C for P1, P2, and P6; $P < 0.05$). Control sows (pregnant, not lactating) had lesser RT (38.3 ± 0.1°C; $P < 0.001$), RR (41 ± 2 BPM; $P < 0.005$), ET (35.9 ± 0.1°C; $P < 0.001$), and ST (34.8 ± 0.2°C; $P < 0.001$) compared with lactating sows. There was an effect of DOL on EB during lactation, but parity had no effect. Sow EB increased from d 0 (−8.5 ± 1.2 Mcal ME) to d 5 (−1.9 ± 1.2 Mcal ME), but then decreased to d 9 (−3.8 ± 1.2 Mcal ME) and then achieved neutrality by d 11. Previous trial results showing greater RT in younger sows were confirmed. Greater RT in younger sows may be due to thermal insensitivity as result of metabolic heat production for growth. This may partially explain summertime infertility in P1 sows. This project was supported by National Research Initiative Competitive Grant no. 2007–55203–18261 from the USDA National Institute of Food and Agriculture.

Key words: sow, heat stress, parity

M243 Effects of progesterone concentrations at the end of a fixed-time AI protocol and time of administration of PGF2α in fixed-time AI and ET protocols in lactating dairy cows. M. Pereira[1], A. Rodrigues[1], T. Martins[1], F. Aono[1], P. Borges[2], T. Guzella[1], C. Sanchez[1], M. Veras[2], F. Aragon[2], and J. L. M. Vasconcelos*[1], *[1]FMVZ-UNESP, Botucatu, SP, Brazil, [2]Pioneiros Veterinary Clinic, Carambei, PR, Brazil.*

Two experiments were performed in lactating Holstein cows. Experiment 1 (n = 565) evaluated the influence of progesterone (P4) concentrations at removal of the P4 intravaginal device (CIDR) on pregnancy rates of cows assigned to the following estrous synchronization protocol + fixed-time AI: d 0 – 2 mg of estradiol benzoate and insertion of a new or used CIDR (previously used once or twice, originally containing 1.9 g of P4); d 7 – 25 mg PGF2α injection; d 8 - removal of the CIDR and administration of 1 mg of estradiol cypionate; d 10 – fixed-time AI. Blood samples were collected concurrently with CIDR removal for P4 analysis. Experiment 2 (n = 610) evaluated if administration of PGF2α at d 7 (PG7) or d 8 (PG8) of the same protocol utilized in Exp. 1 affects pregnancy rates in cows submitted to fixed-time AI or ET. Data from Exp. 1 and 2 were analyzed with the PROC LOGISTIC of SAS. In Exp. 1, the number of the CIDR use did not affect ($P > 0.05$) P4 concentrations at CIDR removal (1.47, 1.29 and 1.16 ng/mL of P4 for new, used once, or used twice CIDR) or pregnancy rates (27, 26, and 32% of pregnant cows/total cows for new, used once, or used twice CIDR). There was no effect ($P > 0.05$) of P4 concentration on d 8, independently of CIDR usage, on subsequent pregnancy rates (P4 < 1.0 ng/mL = 27% pregnant cows/total cows; P4 between 1.0 and 2.0 ng/mL = 28% pregnant cows/total cows; and P4 > 2.0 ng/mL = 32% pregnant cows/total cows). In Exp. 2, PG7 cows had greater ($P < 0.05$) pregnancy and conception rates to fixed-time AI and ET, respectively, compared with PG8 cows (35 vs.. 25% pregnant cows/total cows at fixed-time AI, respectively; 54 vs.. 46% pregnant cows/transplanted cows at fixed-time ET, respectively). In conclusion, in synchronization protocols where age of follicles are similar, the interval between beginning of circulating P4 decrease and ovulation may affect pregnancy rates in lactating dairy cows.

Key words: progesterone, prostaglandin, dairy cows

M244 Period of dominance of the ovulatory follicle influences conception rates in Nelore pubertal heifers detected in estrus. T. Martins[1], A. Rodrigues[1], F. Aono[1], M. Pereira[1], R. Peres[2], H. Graff[2], E. Carvalho[2], and J. L.M. Vasconcelos*[1], *[1]FMVZ-UNESP, Botucatu, SP, Brazil, [2]Agropecuaria Fazenda Brasil, Nova Xavantina, MT, Brazil.*

Length of dominance of the ovulatory follicle and exposure to estradiol during proestrus can affect fertility in beef females. The aim of this study was to compare intravaginal progesterone devices (CIDR; containing 1.9 g of progesterone) non-previously (CIDR1) or previously used for 18 d (CIDR3) during estrous synchronization, as well as effects of timing of CIDR removal on conception rates of pubertal Nelore heifers. Cycling Nelore heifers (n = 705) were randomly assigned to receive on d 0 either CIDR1 or CIDR3 and 2 mg of estradiol benzoate. On d 7 all heifers received 12.5 mg of PGF2α and were assigned within CIDR1 and CIDR3 to CIDR removal on d 7 (D7; n = 335) or d 9 (D9; n = 370). Estrus detection was performed twice daily after CIDR removal and heifers were inseminated 12 h after being detected in estrus. At insemination, the largest follicle was measured by transrectal ultrasonography. Pregnancy diagnosis was performed on d 61 also via transrectal ultrasonography. Data were analyzed with PROC LOGISTIC and PROC MIXED of SAS. Conception rates were not affected by CIDR use ($P > 0.05$) but were affected ($P < 0.05$) by timing of CIDR removal (57.8 vs.. 66.5% of pregnant heifers/inseminated heifers for D7 and D9, respectively). Estrus detection rate was 60.9% for D7 and 67.0% for D9 ($P < 0.10$). Follicle diameter at AI was affected ($P < 0.01$) by CIDR use (11.4 vs.. 12.0 mm for CIDR 1 and CIDR3, respectively). Further, follicle diameter was greater for cows assigned to CIDR3 and removal on d 9 ($P < 0.05$) compared with all other treatment combinations. Interval between CIDR removal and heat detection was also affected ($P < 0.01$) by CIDR use (3.62 vs.. 2.92 d for CIDR1 and CIDR3, respectively) and timing of CIDR removal (3.71 vs.. 2.83 d for D7 and D9, respectively). According to these results, variation in the period of dominance of the ovulatory follicle influences fertility of heifers inseminated after estrus detection.

Key words: conception rates, Nelore heifers, progesterone

M245 Impacts of L-arginine on ovarian function and reproductive performance at the time of maternal recognition of pregnancy in ewes. C. Schauer*[1], C. Saevre[1,2], A. Meyer[2], M. VanEmon[1,2], J. Kirsch[2], M. Kapphahn[2], J. Luther[3], J. Caton[2], and D. Redmer[2], *[1]Hettinger Research Extension Center, North Dakota State University, Hettinger, [2]Department of Animal Sciences, North Dakota State University, Fargo, [3]Department of Animal and Food Science, University of Wisconsin-River Falls, River Falls.*

Objectives were to determine if arginine supplementation surrounding the time of maternal recognition of pregnancy enhances ovarian function and minimizes early reproductive losses. Ewes received L-arginine HCL (equivalent to 27 mg of L-arginine/kg of BW; ARG; n = 47) or saline (CON; n = 47) i.v. once daily from d 9 to d 14 following

estrus (d 0). Daily blood samples were obtained from a subset of 10 ewes/group to assess progesterone (P4) concentrations and at −0.5, 0, 0.5, 1, 2, 4, 6, and 8 h following treatment on d 10 to determine serum amino acid concentrations. Reproductive losses were determined with B–mode ultrasonography on d 25, 45, and 65 of gestation. On d 10, serum concentrations of arginine (nmol/mL) were elevated in ARG vs.. CON ewes at 0, 0.5, 1, 2, and 4 h ($P < 0.001$), but were similar ($P \geq 0.70$) at −0.5, 6, and 8 h. Despite similarities in the number of corpora lutea (CL) per ewe (ARG, 1.69 ± 0.12 and CON, 1.67 ± 0.16; $P > 0.05$), serum progesterone concentration (ng/mL) was greater in this subset of CON compared with ARG ewes on d 9 ($P < 0.02$) and 10 ($P < 0.005$), but similar for the remaining treatment period ($P \geq 0.06$). On d 12, there were no differences in pulsatility index and resistance index in those ewes treated with arginine in the ovarian hilus or the CL ($P > 0.05$). Treatment with arginine increased overall pregnancy rate at d 25 (ARG, 55% and CON, 30%). Pregnant ewes were similar in CL number per ewe (ARG, 1.69 ± 0.12 vs.. CON, 1.67 ± 0.13; $P > 0.05$) and embryo number (ARG, 1.62 ± 0.12 vs.. CON, 1.53 ± 0.13; $P > 0.05$) at d 25 of gestation. As pregnancy progressed to d 45, similar ($P > 0.05$) number of embryos per ewe were observed in pregnant ARG ewes (1.45 ± 0.14) vs.. pregnant CON (1.50 ± 0.15) with overall pregnancy rate remaining greater ($P \leq 0.02$) in ARG (47%) compared with CON (26%). In summary, treatment with arginine surrounding the time of maternal recognition of pregnancy may have prevented pregnancy loss, but did not enhance ovarian hemodynamics or progesterone concentration.

Key words: L-arginine, ovarian hemodynamics, sheep

M246 Failure of differences in prepubertal dietary intake to affect ovarian development in pubertal beef heifers. 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 to a lighter body weight at first breeding can reduce input costs but may impede follicular development and onset of puberty. The objective was to determine whether lower dietary intake prepubertally impedes ovarian development in purebred or crossbred heifers. In 2009 and 2010, 8-mo-old Angus and composite MARC II heifers were assigned equally by body weight and genetic line (n = 120 / line) to receive either a low- (LE) or high- (HE) energy diet to achieve an ADG of 0.45 or 0.9 kg/d for 180 d or 55 vs.. 65% of mature BW at 14 mo of age. At 14 mo, heifers were monitored twice daily for estrus behavior for 21 d. Total number of antral follicles (AFC), ovarian length and height, and preovulatory follicle diam. were measured by transrectal ultrasonography at about 12 h after estrus; corpus luteum (CL) diam. was measured 7 to 14 d later. Uterine horn diam. was only measured in 2010. Data were analyzed by ANOVA with diet, line, and year as independent variables and their 2-way interactions. At 14 mo, HE heifers were heavier (419.4 vs.. 364.9 ± 7.1 kg) and fatter (6.8 vs.. 5.5 ± 0.1 BCS) than LE heifers ($P < 0.01$); LE heifers were 11% heavier in 2010 than 2009 (diet x year; $P < 0.01$). Puberty occurred in 94.6% of heifers by 14 mo of age. Size of preovulatory follicle (13.8 ± 0.2 mm), AFC (22.2 ± 0.6), ovary (length = 26.5 ± 0.3 mm; height = 15.1 ± 0.2 mm), CL (19.3 ± 0. Three mm), and uterine horn diam. (11.6 ± 0.2 mm) did not differ between HE and LE, but follicle diam. (14.3 vs.. 13.2 ± 0. Two mm; $P < 0.01$) and ovarian length (26.4 vs.. 25.3 ± 0.4 mm; $P = 0.07$) were greater for MARC II vs.. Angus heifers. AFC was correlated with ovarian length ($r = 0.56$; $P < 0.01$), and CL diam. was correlated with preovulatory follicle diam. ($r = 0.34$; $P < 0.01$). Results indicate that AFC and ovarian size in pubertal heifers are not influenced by differences in prepubertal growth and body condition associated with diet. USDA is an equal opportunity provider and employer.

Key words: beef heifers, diet, ovarian follicles

M247 Follicular fluid composition of the preovulatory follicle in beef cows grazing different forage allowances of native pastures. M. Carriquiry*[1], P. Soca[1], A. C. Espasandín[1], A. Meikle[2], and C. Viñoles[3], *[1]School of Agronomy, UdelaR, Montevideo, Uruguay, [2]School of Veterinary Sciences, UdelaR, Montevideo, Uruguay, [3]National Research Institute for Agriculture, Tacuarembó, Uruguay.*

The follicular microenvironment has been shown to play a critical role in determining follicular fate. To evaluate the effect of long-term nutrition on metabolite follicular fluid composition in beef cows on grazing conditions, multiparous cows (Angus, Hereford and F1 crossbred, n = 32) at 2 forage allowances of native pastures throughout the year (6 vs.. 10 kgDM/100kgBW/d; LO vs.. HI) were used in a complete randomized block design. At the end of the third year, at 178 ± 15 d postpartum, cows were synchronized with 2 prostaglandin (PG) injections 11 d apart and slaughtered 32 ± 1 h after the last PG injection. Cows were classified as cyclic or in anestrus based on the presence of a corpus luteum on the ovaries. Ovaries of cyclic cows were collected (n = 16 and n = 12 for HI and LO, respectively) and largest follicle present on the ovarian surface was dissected, and follicular fluid was aspirated for metabolite analyses. At slaughter, cow BCS did not differ ($P = 0.32$) between groups and averaged 3.9 ± 0.08. The preovulatory follicle was larger for HI than LO cows (13.1 ± 0.8 vs.. 10.3 ± 1.3 mm; $P < 0.05$). Glucose (23.1 vs.. 25.9 ± 5.4 mg/dL), NEFA (0.79 vs.. 0.57 ± 0.11 mmol/L) and urea (19.6 vs..19.1 ± 2.4 mg/dL) concentrations in follicular fluid did not differ ($P > 0.74$) due to forage allowance. However, follicular fluid cholesterol concentrations were greater in LO than HI cows (112 vs.. 79 ± 8 mg/dL, $P = 0.01$). Glucose and cholesterol concentrations increased ($P < 0.02$) with size of the preovulatory follicle (3.3 ± 1.3 mg/dL of glucose and 6.4 ± 1.8 mg/dL of cholesterol for each mm of increase in follicle size). Results showed minor effects of long-term nutrition in follicle fluid composition of the preovulatory follicle of beef cows grazing different forage allowances of native pastures.

Key words: cattle, grazing, ovary

M248 Longitudinal assessment of the somatotropic axis in free-ranging, juvenile Steller sea lions. K. D. Hebert*[1], J. P. Richmond[1,2], L. D. Rea[3], and S. A. Zinn[1], *[1]University of Connecticut, Storrs, CT, USA, [2]University of North Florida, Jacksonville, FL, USA, [3]Alaska Department of Fish and Game, Fairbanks, AK, USA.*

The decline of the Western population (144 degrees west longitude) of Steller sea lions is hypothesized to be the result of impaired nutritional status and decline of growth rate, especially in juveniles, and subsequent natality. Because changes in components of the somatotropic axis can be predictive of nutritional status and growth rate in this species, 2 groups of free-ranging juvenile Steller sea lions were captured in Prince William Sound, AK. Group 1 (n = 30) was initially captured at 5 mo and recaptured at 10 mo of age, whereas group 2 (n = 9) was captured at 7 and 8 mo of age. At capture, animals were anesthetized, age estimated, and blood and BW collected. Concentrations of GH and IGF-I were quantified (ng/mL) using RIA and IGFBP-2 and −3 were quantified [Arbitrary Units, (AU)] using Western ligand blots. Data were analyzed using the Mixed Procedure in SAS. Mass of Steller sea lions increased ($P < 0.01$) with age from 69 ± 1.3 kg at 5 mo to 100

± 2.8 kg at 10 mo (group 1) and 93 ± 5.8 kg at 7 mo to 101 ± 5.7 kg at 8 mo (group 2). Concentrations of IGFBP-2 decreased with age from first to second capture (group 1; 37.8 ± 2.5 vs.. 36.0 ± 2.5; group 2; 43.7 ± 4.8 vs.. 39.8 ± 4.4 AU; $P < 0.01$) and across all animals GH, IGF-I and IGFBP-3 averaged 1.6 ± 0.1 ng/mL, 165.7 ± 10.4 ng/mL, 304.0 ± 13.4 AU respectively, but there was no effect ($P > 0.1$) of age on concentrations of these hormones. Greater concentrations of IGFBP-3 were positively associated with greater growth rate ($P = 0.06$) across all animals. In group 2, the increase in IGF-I concentrations between captures was positively correlated with growth rate ($P < 0.05$), indicating that changes in IGF-I and IGFBP-3 may be useful indicators of growth rate in juvenile Steller sea lions. These data provide a more detailed description of the changes in the components of the somatotropic axis and their relationship with growth rate in juvenile Steller sea lions, and may provide insight into survival and the continued decline of the Western population.

Key words: somatotropic axis, Steller sea lions, insulin-like growth factor binding proteins

M249 Analysis of bovine liver transcriptomics data due to level of prepartal dietary energy using two bioinformatics approaches. K. Shahzad*, M. Bionaz, and J. J. Loor, *University of Illinois, Urbana.*

We used a newly-developed approach (dynamic impact analysis, DIA) that allows visualizing the dynamic adaptations of pathways, and the well-established enrichment analysis using DAVID to evaluate at the transcriptomic level the impact of prepartal plane of energy intake [overfed (OF) or restricted (RE)] on biological pathways in liver. Both approaches rely on freely-available pathway databases from the KEGG database. Analysis of variance with a false discovery rate (FDR) correction resulted in 4,111 genes with a time × diet interaction (FDR <0.05, DEG). For the DIA analysis the whole data set with Entrez gene IDs, FDR, fold-change, and post-hoc *P*-value between the 2 treatments at each time point were uploaded. For the DAVID analysis a list of up-and downregulated genes with Entrez Gene ID was uploaded. A cut-off of FDR = 0.05 and *P* -value = 0.05 was applied in both approaches. Among DEG between OF vs. RE, DAVID analysis uncovered oxidative phosphorylation as the most significantly-enriched (FDR < 0.05) pathway followed by ribosome and proteasome. Without multiple correction (i.e., simple *P*-value < 0.05) other enriched pathways included fatty acid metabolism, glycan biosynthesis, lysosome, and complement, which were more induced in RE vs. OF; whereas, base excision repair, ubiquitination, and ECM receptor were more induced in OF vs. RE. The DIA approach revealed that RE vs. OF led to a higher utilization of glucose, amino acids (AA), and fatty acids (FA) to produce energy (e.g., more impacted/induced TCA cycle, oxidative phosphorylation, and FA metabolism together with degradation of most of AA). Dietary OF vs. RE resulted in large induction of cell cycle, protein turnover, glycan metabolism, with larger ECM receptor activity (e.g., glycan degradation, ubiquitin, Wnt and Notch signaling pathways). Overall, results from the 2 bioinformatics approaches indicated that OF vs. RE prepartum increased liver proliferation and ECM components while reducing utilization of energy and protein synthesis. This adaptation might partly explain the greater liver lipid accumulation due to OF vs. RE postpartum.

Key words: systems biology, pathway analysis, transition cow

M250 Follicle-stimulating hormone induces the canonical WNT/beta-catenin pathway in bovine granulosa cells. B. I. Castañon*, A. D. Stapp, L. J. Spicer, C. A. Gifford, and J. A. Hernandez Gifford, *Oklahoma State University, Stillwater.*

The WNTs are a family of secreted glycoproteins that evoke a response by interacting with specific 7 transmembrane frizzled (FZD) receptors. In the canonical WNT/β-catenin pathway, WNT binding to a FZD receptor leads to inactivation of the β-catenin (CTNNB1) degradation complex. Disruption of the destruction complex allows CTNNB1 to accumulate in the cytoplasm and translocate to the nucleus where it activates transcription by contact with T-cell factor and lymphoid enhancer-binding factor. Several WNT and FZD transcripts are expressed at defined stages of follicular development in the adult ovary. However, the role of the WNT/CTNNB1 pathway in folliculogenesis remains to be elucidated. This study evaluates FSH regulation of the WNT signaling pathway components that contribute to steroid production in bovine granulosa cells. Granulosa cells were isolated from small ovarian follicles (1–5 mm) and plated ($2.3–4 \times 10^5$ cells/35 mm dish) in DMEM/F12 medium. At 48 h after plating, cells were incubated in the presence or absence of 100 ng/ml FSH for 24 or 48 h (n = 6). Expression of *WNT2* mRNA was induced 3.75 (±0.68) fold after 24 h of FSH stimulation compared with controls (0.12 ± 1.09; $P < 0.05$). Likewise, at 48 h *WNT2* tended to be induced (3.14 vs. 1.00 ± 1.03; $P < 0.06$) with FSH treatment. Analysis of other members of the WNT/CTNNB1 signaling pathways did not demonstrate hormone-regulated expression. Granulosa cells and follicular fluid were collected from large mid-luteal antral follicles (8–22 mm) and classified as estrogen active (n = 8) (>25 pg/mL) or estrogen inactive (n = 5). Nuclear and cytoplasmic protein fractions were enriched and CTNNB1 was analyzed using Western blot. Preliminary evidence indicates estrogen active follicles have greater amounts of nuclear CTNNB1 compared with estrogen inactive follicles. Together, these data demonstrate FSH regulates WNT signaling pathway components which are important in granulosa cell steroidogenesis.

Key words: WNT, beta-catenin, follicle-stimulating hormone

M251 Effects of organic versus inorganic trace mineral supplementation on bull semen quality before and after freezing. M. P. Rowe*, C. L. Williams, R. J. Page, T. D. Lester, C. F. Rosenkrans, E. B. Kegley, J. G. Powell, and R. W. Rorie, *University of Arkansas, Fayetteville.*

Limited information is available on the effects of organic trace mineral supplementation on bull fertility. The objective of this study was to evaluate the effect of trace mineral supplementation on bull semen quality before and after freezing, as measured by computer-assisted sperm analysis (CASA). Angus and Balancer bulls were assigned to inorganic (n = 9) and organic (n = 10) trace mineral treatments, based on semen quality, breed, body weight, and age. The bulls were maintained in a dry lot pen and fed mixed grass hay. Three times a week bulls were individually fed a grain supplement that served as the carrier for treatments containing trace mineral for 123 d (May to September). Treatments were supplemental Zn (450 mg/d), Cu (150 mg/d), Co (12 mg/d), Mn (300 mg/d), Se (3 mg/d), and I (5 mg/d) as either inorganic or as a portion of the same levels as organic sources. Starting on d 60, semen was collected by electro-ejaculation on wk 1, 4, and 8. Semen was evaluated by CASA for percent motile and progressive sperm within 5 min of each collection. Sperm was extended, slowly cooled to 4°C, loaded into 0.5 mL straws, and frozen in liquid nitrogen. After thawing, semen was washed to remove extender and then re-suspended in TALP media. Semen was then evaluated using CASA at 0 and 2 h post-thaw. Data was analyzed by treatment, week and their

interaction, using PROC GLM. Week and treatment by week were not significant ($P > 0.05$), so they were dropped from the analysis. At collection, motile (69.1 vs. 55.2%) and progressive (50.3 vs. 38.5%) sperm were greater ($P < 0.05$) for bulls in the organic than the inorganic groups. After thawing, motile (16.3 vs. 7.9%) and progressive (8.9 vs. 4.1%) sperm were also higher ($P < 0.05$) for semen from bulls in the organic vs. inorganic treatments, respectively. At 2 h post-thaw, motile sperm remained higher (8.5 vs. 3.7% $P < 0.05$) but progressive sperm (4.2 vs. 1.7%) was similar ($P > 0.05$) for the organic and inorganic groups, respectively. Although post-thaw motility was low for both treatments, results suggest organic trace mineral supplementation may improve bull semen quality.

Key words: fertility, bulls, trace minerals

M252 Exposure of beef females to the biostimulatory effects of bulls prior to AI. K. E. Pfeiffer*[1], J. A. Binversie[1], J. D. Rhinehart[2], and J. E. Larson[1], *[1]Mississippi State University, Mississippi State, [2]University of Tennessee, Nashville.*

The objective of this study was to evaluate the biostimulatory effect of bull exposure on the expression of estrus and pregnancy rate to AI in cattle. Beef heifers (n = 86) and cows (n = 193) during 2 consecutive yr were allocated to one of 3 treatments: 1) no bull exposure (CON; n = 95), 2) exposure to a bull with a surgically deviated penis for 21 d before AI (SB; n = 88), or 3) exposure to a vasectomized bull for 21 d before AI (VB; n = 96). The SB treatment provided the physical presence of the bull but prevented intromission whereas the VB treatment allowed for intromission and deposition of seminal plasma but not spermatozoa. The estrous cycles of all females were synchronized using the Hybrid-Synch+CIDR protocol ($GnRH^{+}$CIDR insertion-7 d-CIDR removal+$PGF_{2\alpha}$, visual detection of estrus 3 × daily with AI 12 h later for 82 h, and clean-up TAI+GnRH at 82 h). Blood samples were collected on d −17 and −7 relative to the initial injection of GnRH and analyzed for concentrations of progesterone to determine cyclicity status at the initiation of the experiment (at least one sample ≥1 ng/mL). Pregnancy was detected by transrectal ultrasonography on d 35 post-AI. At the onset of the experiment, 75.7% of heifers and 86.1% of cows were cycling. The percentages of females that displayed estrus after CIDR removal were increased ($P < 0.001$) in Year 1 (52.3%) compared with Year 2 (23.1%) as well as increased ($P < 0.05$) in nulliparous (52.3%) compared with primiparous and multiparous females (26.0 and 31.5%, respectively). The percentages of females that displayed estrus were similar ($P = 0.15$) among treatments (31.6, 39.8, and 39.6% for CON, SB, and VB, respectively). Pregnancy rates were increased ($P < 0.01$) in Year 2 (55.8%) compared with Year 1 (42.4%) and were increased ($P < 0.05$) in females treated with CON and SB (49.5 and 59.1%, respectively) compared with females treated with VB (40.6%). In conclusion, a similar percentage of females among treatments displayed estrus during the 82 h detection period but pregnancy rates were decreased in females exposed to a vasectomized bull compared with those exposed to either no bull or a bull presence only.

Key words: beef cattle, biostimulatory effects, bull exposure

M253 Effect of selenium and a glucogenic precursor on fertility in Creole Rodeo cows synchronized with CIDR, PGF2α, eCG, and GnRH. C. Sanchez-Arcineiga*, J. A. Ramirez-Godinez, D. Dominguez-Diaz, A. Flores-Mariñelarena, E. Santellano-Estrada, J. A. Grado-Ahuir, G. Corral-Flores, and L. A. Borunda-Pacot, *Universidad Autonoma de Chihuahua, Chihuahua, Chihuahua, Mexico.*

The objective was to evaluate the effect of calcium propionate (CaP) and sodium selenium (Se) on average daily gain (ADG), backfat thickness (BF), body condition (BCS) and pregnancy rate (PR) in Creole Rodeo cows (CC) supplemented for 60 d with 2 Kg of a concentrate (29 ± 1.6% CP) every other day. Forty-five dry CC were randomly assigned to T1 (n = 11), concentrate only; T2 (n = 11), concentrate + 10.95 mg of Se/50 Kg BW; T3 (n = 11), concentrate + 100 g of CaP and T4 (n = 12), 10.95 mg of Se/50 Kg of BW + concentrate + 100 g of CaP. Cows selected across treatments based on the presence of a palpable corpus luteum (n = 34, 9 from T1, 7 from T2, 9 from T3, and 9 from T4) received a 8 d CIDR. Later, 25 mg of Lutalyse were injected at CIDR removal, and 18 cows (5 from T1, 3 from T2, 6 from T3, and 4 from T4) were treated with 400 IU of eCG. All CC received 100 mg of GnRH 56h after CIDR removal, and fixed-time AI (TAI). Data were analyzed under a 2x2 factorial design with repeated measures. For PR a chi-squared test was used to analyze the effect of eCG. BCS was similar ($P > 0.05$) between treatments, CaP supplementation had a negative effect ($P < 0.0001$) on ADG over time (T) and the interaction CaP*T was significant ($P = 0.0192$). BF was similar between treatments ($P > 0.05$). The use of Se had no effect on BCS, ADG and BFT. Supplementing Se or CaP had no effect ($P > 0.05$) on PR; similarly, the use of eCG at CIDR removal did not improved fertility (44.1% and 55.8%, $P > 0.05$, respectively) in GnRH treated CC at TAI.

Key words: Creole cattle cow, calcium propionate, eCG

M254 Effects of heat stress on skeletal muscle insulin responsiveness in lactating Holstein cows. L. C. Cole[1], M. V. Skrzypek[1], S. R. Sanders[1], M. R. Waldron[3], L. H. Baumgard[2], and R. P. Rhoads*[1], *[1]University of Arizona, Tucson, [2]Iowa State University, Ames, [3]University of Missouri, Columbia.*

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 (TN) conditions (18°C, 20% humidity) with ad libitum intake 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. To evaluate skeletal muscle insulin responsiveness, biopsies were obtained immediately before and after an insulin tolerance test (ITT). Insulin receptor (IR), insulin receptor substrate (IRS), Akt/protein kinase B (AKT) and phosphorylated AKT (P-AKT) were measured by Western blot analyses. During P2, HS cows had ($P < 0.01$) a 1.48°C increase in Tre and a 2.4-fold increase in RR compared with PF cows. 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 greater glucose disposal in P1 compared with P2 ($P < 0.05$), but did not differ between environments in P2. Protein abundance of the IR, IRS and AKT remained stable between periods and environments. Insulin increased P-AKT in each period ($P < 0.05$), but this response tended to decline in P2 for PF animals ($P = 0.10$), but not during HS. These results indicate that mild insulin resistance during HS may be related to reduced nutrient intake. Moreover, a reduction in skeletal muscle insulin responsiveness may stem from a post-receptor signaling defect.

Key words: heat stress, lactation, insulin

M255 Withdrawn

M256 Effects of heat-stress and fresh or frozen semen on reproductive efficiency in dairy cows treated with rbST throughout lactation. E. Sepúlveda*[1], O. Ange-García[1], CA Meza-Herrera[2], FG Veliz[1], and M. Mellado[1], *[1]Universidad Autonoma Agraria Antonio Narro, Torreón, Coahuila, México, [2]Universidad Autonoma Chapingo, Bermejillo, Durango, México.*

The objective of this study was to assess the effect of high ambient temperature and the use of fresh or frozen semen on reproduction performance of dairy cows in a hot arid environment. Reproductive variables (n = 18,037 services) of a large Holstein dairy herd in northern Mexico were evaluated with respect to the average temperature-humidity index [(ITH = (0.8 x temperature + (relative humidity/100) x (temperature - 14.4) + 46)] of the 1 and 3 d before breeding, the day of breeding, and 1 and 3 d following breeding. Increased ITH from < 70 to > 95 was associated with a decrease in pregnancy rate from 47% to 26%. Pregnancy rates for cows serviced on days with an ITH 85–90 but cooler temperatures before breeding were 6 percent points higher than cows exposed to higher ITH before breeding. Pregnancy rates for cows serviced on days with an ITH 80-85 but cooler temperatures before breeding were 3-4 percent points higher than cows exposed to higher ITH before insemination. Pregnancy rates for cows serviced on days with an ITH 75-80 but cooler temperatures before breeding did not differ compared to cows exposed to higher ITH before insemination. With ITH cooler the days after insemination pregnancy rates were also higher for all ITH classes the day of breeding. Pregnancy rates were higher ($P < 0.05$) from January to March compared with all other months of the year. On the other hand, the average number of inseminations per pregnancy was higher from May to July (3.0-3.4) than from all other months of the year (2.1 to 3.0). Pregnancy rate was higher (36 vs. 28%) with insemination with fresh semen (natural mating) than frozen semen, although this difference was noted only during the warmest period of the year. It was concluded that the climatic conditions of the site where this dairy operation is located, drastically hampers the reproductive performance of Holstein cows subjected to three milking and treated with somatotropin throughout lactation. This data also show that natural service markedly increases pregnancy rate during the warm months of the year, compared to AI with frozen semen.

Key words: heat stress, pregnancy rate, natural service

M257 Expression patterns of eNOS in 13 different tissues shows a new isoform in bovine brain stem. M. De Donato*[1], M. A. Adefenwa[1,2], and I. G. Imumorin[1], *[1]Dept of Animal Science, Cornell University, Ithaca, NY, [2]Dept of Cell Biology and Genetics, University of Lagos, Lagos, Nigeria.*

Endothelial nitric oxide synthase (eNOS), along with inducible nitric oxide synthase (iNOS) and neuronal nitric oxide synthase (nNOS), catalyze the generation of nitric oxide, a reactive free radical which acts as a biologic mediator in several processes, including neurotransmission and antimicrobial and antitumoral activities. Expression of eNOS mRNA is restricted to the endothelial cell layer of arterial blood vessels, and is a critical mediator of cardiovascular homeostasis through regulation of the diameter of blood vessels and maintenance of an antiproliferative and antiapoptotic environment in the vasculature. Here we report the expression pattern of eNOS in 13 different bovine tissues as a first step to study possible association of different isoforms with animal performance and health. We used semiquantitative PCR to assess expression with specific primers that amplified exons 5 and 6, with GAPDH as control. Similar expression was detected in cerebellum, cerebral cortex, heart, skeletal muscle, lung, kidney, spleen, liver, pancreas, stomach, placenta and ovary. However, in brain stem tissue, a larger fragment, which represents the unspliced section of exon 5, intron 5 and exon 6, was the major expressed isoform, with low expression of the smaller isoform. Small amounts of this larger isoform were also seen in the cerebral cortex, skeletal muscle, kidney, placenta and ovary. The presence of this isoform as a major protein product in the brain stem could indicate a more specialized function of the gene in this tissue. Further studies will be needed to confirm this observation and compare differences in the function of this protein.

Key words: eNOS, bovine, brain stem

M258 Analysis of bovine adipose transcriptomics data during the transition from pregnancy to early lactation using two bioinformatics approaches. K. Shahzad*[1], J. Sumner-Thomson[2], J. P. McNamara[2], and J. J. Loor[1], *[1]University of Illinois, Urbana, [2]Washington State University, Pullman.*

We used a newly-developed approach (dynamic impact analysis, DIA) that allows visualizing the dynamic adaptations of pathways, and the well-established enrichment analysis using DAVID to evaluate the impact of change in physiological state on biological pathways in adipose tissue of Holstein dairy cattle. Both approaches rely on freely available pathway information from the KEGG database. RNA was hybridized to Affymetrix Bovine Gene Array containing 14,200 elements. Analysis of variance with a false discovery rate (FDR) correction resulted in 1,692 genes with a time effect (FDR <0.10, DEG). For the DIA analysis the whole data set (encompassing −21, −7, 7, and 28 d relative to parturition) with Entrez gene IDs, FDR, fold-change, and post-hoc P value between time points was uploaded. For the DAVID analysis a list of up-and downregulated genes with Entrez Gene ID was uploaded. A cut-off of FDR = 0.05 and P-value = 0.05 was applied in both approaches. Among DEG between time points, DAVID analysis uncovered that fatty acid biosynthesis, linoleic acid metabolism, biotin metabolism, and glycerolipid metabolism were markedly inhibited postpartum than prepartum; whereas, complement and coagulation cascades and riboflavin metabolism were the only pathways with sustained induction postpartum than prepartum. The DIA approach revealed that the onset of lactation resulted in a gradual decrease in the utilization (metabolism) of glucose, lactate, and acetate to produce energy (e.g., most impacted pathways included TCA cycle, Pyruvate metabolism). Furthermore, fatty acid biosynthesis, desaturation, elongation, and PPAR signaling were markedly inhibited during lactation. Overall, the combined results from both bioinformatics approaches indicated that the adipogenic capacity of adipose tissue is quite robust during late pregnancy while the innate immune response of the tissue becomes predominant during early lactation. The latter may be a response of the tissue to stressors including cytokines/hepatokines, NEFA, and/or pathogens. Alternatively, it may represent a mechanism associated with tissue remodeling.

Key words: systems biology, transition cow

M259 Reproduction of dairy cows receiving 1 vs. 3 timed AI (TAI) when not observed for estrus and subjected to natural service (NS). F. S. Lima*[1], R. S. Bisinotto[1], E. S. Ribeiro[1], H. Ayres[1], L. F. Greco[1], C. A. Risco[2], W. W. Thatcher[1], and J. E. P. Santos[1], *[1]Animal Sciences Department, University of Florida, Gainesville, [2]Large Animal Clinical Sciences, University of Florida, Gainesville.*

Objectives were to determine the effects 1 vs. 3 TAI followed by NS on time to pregnancy of lactating dairy cows not observed for estrus. Holstein cows, 1,050 received the double Ovsynch TAI program (d −27 GnRH, d −20 PGF2a, d −17 GnRH, d −10 GnRH, d −3 PGF2a, d −1 GnRH, and d 0 AI) for first AI. On the day of first AI, cows were blocked by parity and randomly assigned to receive one (1TAI, n = 533) or 3 TAI (3TAI, n = 517) before subjected to NS. Cows were moved to NS 7 d after the first or third AI according to treatment. Pregnancy was evaluated 32 d after TAI and each 28 d during NS. Nonpregnant cows in 3TAI were resynchronized with the Ovsynch program starting on d 32 after the previous insemination, such that the re-insemination interval was 42 d. Pregnant cows were re-evaluated for pregnancy 28 d after the initial diagnosis. Cows were scored for body condition 32 d after the first AI. All cows had a period of 231 d after the first AI to become pregnant, and non- pregnant cows were censored. Data were analyzed with the Cox's proportional hazard model or by Logistic regression using SAS. Models included the effects of treatment, parity, body condition and season. As expected, pregnancy at the first TAI did not differ between 1TAI and 3TAI on d 60 after insemination (30.9 vs. 33.4%). Cows receiving 3TAI had greater ($P = 0.04$) rate of pregnancy than those in 1TAI (AHR = 1.15; 95% CI = 1.01–1.31). This resulted in median d open of 142 (95% CI = 130–150) and 123 (95% CI = 121–144) for 1TAI and 3TAI, respectively. Primiparous cows had greater ($P < 0.01$) pregnancy rate than multiparous cows (AHR = 1.44; 95% CI = 1.16–178). Cows receiving the first TAI in the cool season had greater ($P < 0.01$) pregnancy rate than cows exposed to heat stress (AHR = 1.77; 95% CI = 1.53–2.05). Finally, cows with BCS > 2.75 had greater ($P < 0.01$) pregnancy rate than those with BCS < 3.0 (AHR = 1.59; 95% CI = 1.38–1.84). In conclusion, in spite of the long re-insemination interval, cows receiving 3TAI had improved reproductive performance than those receiving 1TAI.

Key words: dairy cow, natural service, timed AI

M260 Effect of intravaginal progesterone insert on GnRH-induced GnRH-induced LH release, follicle growth, and plasma progesterone, estradiol, and inhibin concentrations. L. G. D. Mendonça*[1], M. Amstalden[2], and R. C. Chebel[1], *[1]Department of Veterinary Population Medicine, University of Minnesota, St. Paul, [2]Department of Animal Science, Texas A&M, College Station.*

The objectives of this experiment were to evaluate the effect of treatment with a controlled internal drug release (CIDR) insert containing 1.38 g of progesterone (P4) at the time of GnRH injection on GnRH-induced GnRH-induced LH release, follicular growth and plasma concentrations of P4, estradiol and inhibin. Non-pregnant lactating Holstein cows were randomly assigned to one of 3 treatments after balancing for parity, body condition score and 305-d projected milk yield. The treatments were control (CON, n = 7), 1GP4 (n = 10) and 2GP4 (n = 10). All cows were presynchronized with a CIDR insert for 5 d, one day before and upon CIDR removal cows received a 25mg PGF injection, and 2 d later a 100µg GnRH injection. The day of the GnRH injection was considered d0 of the estrous cycle. On d6, CON cows received 100µg of GnRH, 1GP4 cows received 100µg GnRH injection and a CIDR insert, and 2GP4 cows received 200µg of GnRH and a CIDR insert. Ovaries were scanned 0, 10, and 20h after the GnRH given on d6. Blood was sampled at 0, 15, 30, 60, 120, 240, 345, 600, and 1200 min after the GnRH given on d6. Data were analyzed by ANOVA for repeated measures. Although LH concentration from 0 to 345 min was greater ($P < 0.01$) for 2GP4 cows (3.1 ± 0.2ng/ml) than CON (2.1 ± 0.3ng/ml) and 1GP4 cows (2.2 ± 0.2ng/ml), that was mainly because at 60 (CON = 2.6 ± 0.4, 1GP4 = 2.7 ± 0.3, 2GP4 = 3.7 ± 0.3ng/ml) and 120 (CON = 4.6 ± 0.8, 1GP4 = 5.1 ± 0.6, 2GP4 = 7.6 ± 0.6ng/ml) min LH concentrations were ($P < 0.01$) greatest for 2GP4 cows. Progesterone concentrations were smaller ($P < 0.01$) for CON cows (1.9 ± 0.3ng/ml) than 1GP4 (3.3 ± 0.2ng/ml) and 2GP4 (3.4 ± 0.2ng/ml) cows, but there were no ($P = 0.82$) differences between 1GP4 and 2GP4 cows. There were no differences ($P = 0.75$) among treatments in size of the dominant follicle at 10 and 20 h after the GnRH injection given on d6. Treating cows with intra-vaginal P4 concurrently with GnRH does not decrease LH concentration or peak, but treatment with 200µg of GnRH results in earlier rise in LH and greater LH peak concentration than treatment with 100 µg of GnRH.

Key words: dairy cow, CIDR, LH

M261 Environmental effects on semen quality of beef bulls used for artificial insemination. D. O. Stepp*, K. J. Stutts, M. M. Beverly, and S. F. Kelley, *Sam Houston State University, Huntsville, TX.*

Semen quality, like other phenotypic expressions, consists of a genetic component, an environmental component, and a variety of interactions between the 2. The objective of this study was to evaluate environmental effects on semen quality of beef bulls used for artificial insemination. Angus and Brangus bulls (n = 76) that were housed at a commercial collection facility in southeast Texas were used in this study. Bulls were collected twice per week with 2 collections attempted on each collection day. Following collection, volume, concentration, and motility of the sample were evaluated. The sample was then cooled, extended, and frozen in liquid nitrogen following standard protocol of the collection facility. A post-thaw analysis of the sample was performed the following day. Data collected on each sample included: motility immediately after thawing (MOT0), motility 3 h post-thaw (MOT3), and the number of primary, secondary, and tertiary morphological abnormalities. Mean ambient temperature and relative humidity were recorded for the time of collection and the preceding 60 d. ANOVA was performed using Minitab15.1. All main effects and all 2-way interactions were included in the model. There was a significant effect of season on MOT0, MOT3, and number of primary, secondary, tertiary, and total abnormalities. MOT0 was higher ($P < 0.05$) in the winter (34.2%) and spring (33.6%) than in the fall (32.2%). MOT3 was higher ($P < 0.01$) in the spring (31.60%) than in the winter (29.77%) and fall (29.61%). Total abnormalities were highest ($P < 0.03$) in the summer (28.05%), followed by the fall (25.47%), spring (23.74%) and winter (23.35%). Season also had significant effects on ejaculate volume and concentration. Ejaculate volume was higher ($P < 0.01$) in the fall than in the winter, and sperm cell concentration was highest ($P < 0.01$) in the spring. These results indicate that environmental factors have a negative effect on semen characteristics of beef bulls. Semen quality is most degraded in the summer and fall seasons after exposure to a combination of high ambient temperatures and high relative humidity in southeast Texas.

Key words: beef bulls, semen quality, environment

M262 Plasma progesterone concentration and follicle dynamics of lactating Jersey cows treated with 1 or 2 intra-vaginal progesterone insert. J. G. N. Moraes*, P. R. B. Silva, N. Bortoletto, A. L. A. Scanavez, and R. C. Chebel, *Department of Veterinary Population Medicine, University of Minnesota, St. Paul.*

The objectives of the current study were to determine the progesterone (P4) concentration and the follicle dynamics of lactating Jersey cows treated with 1 or 2 intra-vaginal P4 insert. Cows were enrolled in the study at 34 ± 3 DIM and were paired by parity, BCS (3.1 ± 0.1), body weight (421.7 ± 5.2kg), and milk yield (28.8 ± 0.6kg/d). All cows were presynchronized with an injection of GnRH concurrent with controlled internal drug release (CIDR) insert containing 1.38 g of P4 and 5 and 6 d later all cows received a PGF2α injection. The day of the first PGF2α injection was determined d −2 of the study. Cows assigned to the 1CIDR treatment received a CIDR insert from d 0 to 8, cows assigned to the 2CIDR treatment received 2 CIDR inserts from d 0 to 8, and control cows did not receive further treatment. Cows were examined by ultrasound and ovarian structures were measured and mapped on d −2 and daily from d 0 to 8. Blood samples were collected for determination of P4 on d −2 and daily from d 0 to 8 and blood samples were collected for determination of estradiol concentration from d 0 to 8. Average P4 concentration from d 0 to 8 was ($P < 0.01$) smallest for control cows (0.73 ± 0.17 ng/ml) followed by 1CIDR (1.37 ± 0.10ng/ml) and 2CIDR (2.21 ± 0.09ng/ml) cows, respectively. Diameter of the largest follicle on d 0 (16.2 ± 0.6mm) was not different ($P = 0.14$) among treatments, but percentage of cows that developed codominant follicles was smallest for 1CIDR cows (1CIDR = 8.0, 2CIDR = 30.8, control = 50%; $P = 0.02$). Percentage of cows ovulating the dominant follicle identified on d 0 was greatest for control cows (1CIDR = 0, 2CIDR = 3.9, control = 80%; $P < 0.01$) and the interval to ovulation was 96 h from d −2 for the 2CIDR cow and averaged 123.0 ± 12.4 h from d −2 for control cows. Control cows were more likely to develop a new dominant follicle from study d 0 to 8 (1CIDR = 12, 2CIDR = 7.7, control = 60%; $P < 0.01$), but there was no ($P = 0.65$) difference in interval to identification of the new dominant follicle (106.9 ± 9.9h from d −2). Treatment with CIDR insert results in increase in P4 similar to those described for Holstein cows.

Key words: progesterone, Jersey cow, follicle