Physiology and Endocrinology I

M183 Cortisol levels during roping acclimation in rodeo calves. K. Comeaux, B. Pousson, A. Greathouse, D. Terro, J. Browning, and C. E. Ferguson,* *McNeese State University, Lake Charles, LA.*

Roping calves (n = 10) were evaluated for chute behavior and cortisol concentration for the first 9 weeks of being roped. These calves were between 90 to 120 d of age and weighed between 93 to 120 kg at the beginning of the study. Each Monday (haul), calves were penned via horseback, placed in a chute, and given a chute score from 1 (calm) to 5 (frantic). Calves were then weighed, a jugular blood sample was collected, and they were loaded onto a stock trailer and transported ~10 min to the rodeo area. At the rodeo arena, calves were left in a pen for 3 h. They then received a chute score, second blood sample (collected at arena), and returned to the pen. On Tuesday, calves were roped twice and on Wednesday (rope) received a chute score, and a blood sample was collected twice, once before roping. Within 30 min of second roping, a second chute score was given and blood sample was collected. Following this, calves were returned to the farm for the remainder of the week. This routine was repeated for 9 weeks and at the end of the 9-week period blood samples were analyzed for plasma cortisol via radioimmunoassay. Statistical differences among chute scores and plasma cortisol concentrations (μg/dL) pre-event, post-event, the change in cortisol (post-event – pre-event), and weight gain were analyzed using SAS Proc GLM with repeated measures. The change in cortisol decreased following roping ($P < 0.05$) compared with hauling during wk 3, −1.04 ± 0.50 (rope) vs. 2.40 ± 0.46 (haul) and wk 4, −3.08 ± 0.56 (rope) vs. 0.03 ± 0.77 (haul). There was no difference in the change of cortisol for wk 5 and 6. However, roping during wk 7 differed ($P < 0.05$) from hauling; −4.62 ± 0.92 (rope) vs. −1.54 ± 89 (haul). There was no difference in the chute score for pre-event, but chute score decreased ($P < 0.05$) post-hauling after wk 3 and remained lower compared with wk 1 and 2. The chute score was lower ($P < 0.05$) after roping compared with after hauling during first 3 weeks but did not differ afterward. Additionally, weight gain in calves significantly increased after the third week. From these results it can be concluded that although after 3 weeks calves begin to acclimate to hauling and roping they acclimate more quickly to roping than hauling ~10 min.

Key Words: rodeo, cortisol, roping calf

M184 Improving reproductive performance of Ossimi ewes using hormonal and enzymatic treatments. E. B. Abdalla*[1], A. Q. Al-Momani[2], F. A. Khalil[1], H. M. Gado[1], and F. S. Al-Barakeh[3], *[1]Ain Shams University, Cairo,Egypt, [2]Ministry of Agriculture, Amman, Jordan, [3]National Center for Agricultural Research and Extension, Al-Baq'a, Al-Balqa, Jordan.*

The current experiment was conducted to evaluate the effect of dietary supplementation of melatonin, Zado (patent product contains cellulases, xylanases, protease and α amylase) and their combination on the reproductive performance of Ossimi ewes. Fifty-seven, multiparous, adult Ossimi ewes were randomly assigned to 4 groups, each group was fed ration supplemented with one of the following components: melatonin (G1; n = 14), Zado (G2; n = 14), melatonin and Zado (G3; n = 15) and ration with no additives (G4; control, n = 14). All ewes were fed maintenance ration for one month (during June) followed by flushing ration for 5 weeks (2 weeks before and 3 weeks after ram introduction). Melatonin (3mg/h/d) and Zado (15g/h/d) started to be added with maintenance and flushing rations, respectively. Fertile rams were introduced to ewes for 2 successive estrous cycles. General Linear Model (GLM) procedure of SAS, ANOVA and Duncan's multiple range test were used for statistical analysis. Percentage of ewes expressed estrus was significantly different ($P < 0.05$) among the treated groups. All ewes expressed estrus in G1, while the lowest percentage (71%) was detected in G4. The interval from ram introduction to the onset of estrus differed ($P < 0.01$) among the treated groups. Ewes received melatonin in G1 and G3 expressed estrus earlier than ewes didnâ€t receive melatonin in G2 and G4. Neither body weight nor body condition score was different at the first estrus following ram introduction, while average daily gain was slightly promoted by treatments particularly with melatonin. Melatonin treated groups (G1 and G3) had significantly higher ($P < 0.01$) progesterone concentration than that in G2 and G4. Lambed ewes as percentage of total ewes were similar in G3 and G4 (50%), while they increased in G1 and G2 (57 and 67%, respectively). Prolificacy was greater in G1 and G3 than that in G2 and G4. Maximum prolificacy was detected in G3 while the minimum was observed in G4 ($P < 0.01$). Fecundity was lower ($P < 0.01$) in G4 (control group) than all the treated groups (G1, G2 and G3).Results of the present study indicated that melatonin could increase the estrus expression of ewes and decrease the interval to estrus after ram introduction. Thus, treatment with melatonin alone, or in combination with Zado, may provide a viable means to improve the fecundity and prolificacy of Ossimi ewes.

Key Words: Zado, melatonin, estrus

M185 Prostaglandin-$F_{2\alpha}$ may not be necessary in short-term progesterone-based estrous synchronization protocols in cyclic ewes. K. N. D'Souza,* S. L. Rastle-Simpson, Q. S. Baptiste, and M. Knights, *West Virginia University, Morgantown.*

Two experiments were conducted to determine the requirement for prostaglandin F2α to achieve ample synchrony of fertile estrus in short-term progesterone-based (STPB) estrous synchronization protocols in cyclic ewes. In Exp. 1, ewes (n = 175) were randomly assigned to receive 0, 1 or 2 CIDR inserts for 7 d before introduction of rams. At insert removal, approximately half of the ewes in each group were injected with 20mg lutalyse (dinoprost; PGF) or received no further treatment. In Exp. 2, crossbred Katahdin ewes (n = 83) were randomly assigned to receive a CIDR for 5 or 7 d before the introduction of rams, with or without PGF at insert removal. The effects of treatments on reproductive performance were analyzed using logistic regression and ANOVA for categorical and continuous variables, respectively. In Exp. 1, the use of CIDR inserts tended to increase the proportion of ewes lambing to the first service period ($P = 0.08$) and reduced the mean lambing day ($P = 0.1$) by 13 percentage points and 1.7 d, respectively. Ewes treated with 1 CIDR device were more prolific ($P < 0.001$; 1.7 ± 0.07 vs 1.4 ± 0.07), lambed earlier ($P < 0.05$; 6.4 ± 0.8 vs 9.2 ± 0.8), and a greater portion tended to lamb to the first service period ($P = 0.1$; 88 vs 74%) than ewes treated with 2 CIDR devices. There was no significant effect of PGF on any of the reproductive performance variables. In Exp. 2, neither the length of treatment with CIDR device nor the use of PGF affected estrous response. Ewes treated with CIDR for 5-d tended to have higher conception rates ($P = 0.07$; 81.3%) and pregnancy rates ($P = 0.1$; 66.7%) compared with ewes treated for 7-d (61.8 and 51.2%, respectively). Injection with PGF decreased ($P < 0.01$) both conception and pregnancy rates (53.6 and 42.9% and 84.2 and 71.1%, for PGF and non- PGF treated ewes, respectively). The effect of PGF was not altered by the duration of CIDR treatment. The results of the current study

indicate that there are no beneficial effects of increasing the duration of treatment from 5 to 7 d, or using an additional CIDR device when using a STPB estrous synchronization protocol in cyclic ewes. Treatment with a CIDR device for 5-d alone is sufficient to induce fertile estrus and synchronize lambing.

Key Words: fertility, prostaglandin, synchronization

M186 Is a CIDR as effective as a sponge in a novel follicle wave emergence and estrus synchronization protocol in anestrous ewes? M. B. Gordon[1], M. Bidarimath[1], M. Moggy[1], M. Camara[1], J. A. Small[3], P. M. Bartlewski[2], and D. M. W. Barrett*[1], *[1]Department of Plant & Animal Science, Nova Scotia Agricultural College, Truro, NS, Canada, [2]Ontario Veterinary College, University of Guelph, Guelph, ON, Canada, [3]Atlantic Food & Horticulture Research Centre, Agriculture & Agri-Food Canada, Truro, NS, Canada.*

The characteristics of ovulating follicles and the timing of ovulation can be very inconsistent when estrus is synchronized in anestrous ewes. Moreover, the well-utilized MAP sponge, containing synthetic progesterone (P_4), was discontinued and replaced by the controlled internal drug releasing device (CIDR), containing natural P_4. The objectives of this study were to compare the patterns of follicular and luteal development of seasonally anestrous ewes treated with CIDR-estradiol-eCG or MAP-estradiol-eCG. During seasonal anestrus, Texel ewes were randomly assigned to receive MAP sponges (60 mg MAP; Veramix; n = 7) or CIDRs (0.33 g P_4; Eazi-Breed CIDR; n = 7) for 12 d. An intramuscular injection of estradiol-17β (350 μg) was given 6 d before intravaginal device removal and an intramuscular injection of eCG (500 IU; Folligon) at intravaginal device removal. Daily ovarian ultrasonography was performed on the day of intravaginal device insertion and continued until ovulation and corpora lutea were confirmed. Blood for P_4 analysis was collected from 5 to 15 d after device removal. To determine the exact timing of ovulation, ovarian ultrasonography was done twice daily from eCG treatment until ovulation was detected. There were no differences in the interval from eCG treatment to ovulation (4.1 ± 0.2 d; $P = 0.67$), number of follicles that ovulated (1.4 ± 0.2; $P = 0.69$), or number of corpora lutea that developed (0.9 ± 0.2; $P = 0.44$) between the sponge and CIDR treatments. There were also no differences between the sponge and CIDR treatments for P_4 concentrations from 5 (0.2 ± 0.06 ng/mL) to 15 (1.2 ± 0.3 ng/mL) d after eCG treatment ($P = 0.92$). This study demonstrates that the CIDR-estradiol-eCG treatment may be as efficacious as the MAP-estradiol-eCG treatment. Further research is required to examine the affects of the CIDR-estradiol-eCG treatment on other critical criteria of ewe reproductive performance, such as pregnancy and lambing rates.

Key Words: anestrous ewes, CIDR, synchronization

M187 Effects of parity and litter size on body reserves dynamics across a complete physiological year in Romane ewes reared under extensive grazing conditions. E. González-García*[1], V. Gozzo de Figuereido[2], D. Foulquie[3], E. Jousserand[3], A. Tessniere[1], F. Bocquier[1], and M. Jouven[1], *[1]INRA UMR868 Systèmes d'Èlevage Méditerranéens et Tropicaux (SELMET), 34060 Montpellier, France, [2]Escola Superior de Agricultura, São Paulo, Brazil, [3]INRA UE0321, Domaine de La Fage, 12250 Roquefort-sur-Soulzon, France.*

How adaptation mechanisms interact across functional levels to control the adaptability of an individual during its own lifetime and also across generations is a core complex question of contemporary research. This work belongs to a series of first efforts in our team looking for gaining insights in identifying and understanding the adaptation mechanisms of ruminants through decrypting functional levels effects in a series of environmental and physiological conditions changes. We consider the evolution of energy body reserves (BR) mobilization or accretion process as an indicator of adaptability while evidencing individual differences responsive of internally or environmentally driven changes in grazing ruminants. Forty-one Romane ewes reared in a natural extensive rangeland of Center France were allocated in homogeneous groups according to body weight (BW) and body condition score (BCS), and distributed by parity [multiparous (MULT), n = 20; primiparous (PRIM), n = 21] and litter size (LSi) [having singletons (SING), n = 21 or TWIN, n = 20]. Feeding was restricted to grazing and other fibrous sources (hay), thus avoiding luxury energy intake and enhancing BR mobilization in function of requirements. Individual BW, BCS (1 to 5 scale), plasma NEFA and glucose (GLU) were monitored across a complete physiological year at –44, 0, 24, 61, 88, 119, 168, 207, 227, 257 and 312 d relatives to lambing (DIM). Blood sampling were performed early morning before the first meal. Data were analyzed by mixed procedure of SAS (2003), considering parity, litter size and its interactions as fixed effects. MULT (59.8 ± 1.21 kg) were systematically heavier ($P < 0.0001$) than PRIM (51.7 ± 1.18 kg). Beginning of lambing, LSi affected ($P < 0.05$) BW in MULT [where ewes with SING (46.5 ± 0.95 kg) were heavier than TWIN (43.3 ± 0.92 kg)] but not in PRIM. BCS was not affected by parity but, from lambing and throughout the experiment, MULT with SING (2.8 ± 0.06) expressed consistent higher ($P < 0.0001$) BCS than those MULT with TWIN (2.5 ± 0.06). NEFA was higher for ewes having more than 1 fetus (TWIN; 0.61 ± 0.040 vs. 0.49 ± 0.041 mmol. L^{-1}) just 1.5 mo before lambing, afterward this difference disappeared probably because of a lack of sensitiveness of this parameter for the fluctuating feeding regimens in extensive grazing conditions. This was coherent with the higher concentration of GLU at lambing in MULT with one fetus (SING; 0.83 ± 0.047 vs. 0.68 ± 0.043 g.L^{-1}, $P < 0.0385$) but not in PRIM. Results indicates that MULT are able to mobilize BR easier than PRIM in situations of negative energy balance (e.g., TWINS vs. SING; peri-lambing days), confirming that flexibility in BR utilization comes with experience, as evidence of an environmentally driven character. Trying to unravel how interactions between heritable and environment-dependent differences between individuals lead to interindividual differences in BR dynamics is our main research goal in the next future.

Key Words: body reserves, extensive grazing, adaptive capacity

M188 Pregnancy per AI (P/AI) after presynchronizing estrous cycles with Presynch-10 or PG-3-G before Ovsynch-56 in four dairy herds. J. S. Stevenson* and S. L. Pulley, *Kansas State University, Manhattan.*

Our objective was to monitor P/AI at first service in 3,005 lactating dairy cows located in 4 herds in northeast Kansas. Cows with eartags ending with odd digits at calving were enrolled in Presynch-10 (Pre-10): 2 25-mg injections of $PGF_{2\alpha}$ (i.e., PG-1 and PG-2) 14 d apart. Cows with eartags ending with even digits were enrolled in PG-3-G: one 25-mg injection of PG (PrePG) 3 d before 100 μg GnRH (Pre-GnRH), with the PrePG injection administered at the same time as PG-2 in the Pre-10 treatment. Cows were enrolled in a timed AI protocol (Ovsynch-56; injection of GnRH 7 d before [GnRH-1] and 56 h after [GnRH-2] PG with AI 16 to 18 h after GnRH-2) 10 d after PG-2 or PrePG injections. Median DIM at scheduled timed AI was 74 d. The study began in September 2009 and was completed in September 2011. Cows detected in estrus before the scheduled timed AI were inseminated (early bred; EB). Pregnancy was diagnosed at d 32–39 and at d 60–67 after timed AI by

transrectal ultrasound or palpation. Data were analyzed using procedure GLIMMIX, with herd as a random effect and with fixed effects of treatment (EB, Pre-10, PG-3-G), lactation number (1 vs. 2+), season (hot [June through September] vs. cool-cold [October through May]), DIM, estrus at timed AI (0 vs. 1), and all 2-way interactions with treatment. The P/AI at d 32–39 for EB (n = 472), Pre-10 (n = 1,247), and PG-3-G (n = 1,286) was: 31.4, 35.0, and 41.2% ($P = 0.071$); P/AI at d 60–67 was: 29.8, 32.2, and 37.3% ($P = 0.107$); and pregnancy loss: 5.1, 7.0, and 9.2% ($P = 0.523$), respectively. Season ($P < 0.001$) influenced P/AI at d 32–39 and d 60–67, but a treatment x season interaction was not detected (d 32–39: $P = 0.468$; d 60–67: $P = 0.195$). The P/AI for PG-3-G and Pre-10 treatments did not differ during cool weather (d 32–39: 46.8 vs. 44.3%; d 60–67: 41.6 vs. 41.1%), respectively, but during hot weather, P/AI in PG-3-G was greater than in Pre-10 (d 32–39: 35.9 vs. 26.7% [$P = 0.001$] or d 60–67: 33.2 vs. 24.4% [$P = 0.009$]), respectively. We concluded that presynchronizing estrous cycles with PG-3-G produced more P/AI than Pre-10 during hot weather.

Key Words: Presynch-10, PG-3-G, pregnancy per AI

M189 Effect of bovine somatotropin (bST) injected at fixed-timed insemination of Holstein cows exposed to an ovsynch protocol. A. Reyes-Gomez, C. F. Arechiga,* M. A. Lopez-Carlos, J. I. Aguilera, R. R. Lozano, R. M. Rincon, F. De la Colina, and F. J. Escobar, *Autonomous University of Zacatecas, Zacatecas, Mexico.*

Objective of present work was to determine the effect of growth hormone (GH), or bovine somatotropin (BST; Lactotropin, Elanco, Mexico), injected at fixed-timed insemination in Holstein cows exposed to an Ovsynch protocol. Holstein cows (n = 849) from Las Palomas Dairy at Aguascalientes, Mexico, were included in the study and randomly alloted into 4 experimental groups: Control (n = 244), Ovsynch (n = 225), Ovsynch+CIDR (n = 241) and Ovsynch+BST (n = 139). Cows received 2 prostaglandin injections (i.e., 26–32 d PP and 40–46 d PP, respectively). By 52–58 d PP cows were included into treatment: 1) Control (d 0, PGF2α; d 3–7 estrus detection and insemination). 2) Ovsynch (d 0, GnRH; d 7, PGFα; d 9, GnRH; d 10, Timed A.I.). 3) Ovsynch/CIDR (d 0, GnRH + CIDR; d 7, CIDR removal + PGF2α; d 9, GnRH; d 10, timed A.I.). 4) Ovsynch/BST (d 0, GnRH; d 7, PGF2α; d 9, GnRH; d 10, timed A.I. + BST). Data was analyzed by PROC FREQ of SAS using X-square and Fisher test. Ovsynch with or without CIDR or BST, increased estrus response and estrus detection (i.e., 97% vs. 68%; total average of 89%). BST at fixed-timed insemination (i.e., d 10, of Ovsynch protocol), increased pregnancy rates determined at 62 + 3 d postpartum by rectal palpation). (30.9% Ovsynch + BST vs. 24.4%, without BST), vs. 21.7% with conventional management or 19.1% using Ovsynch + CIDR. There were differences on estrus response ($P < 0.001$), pregnancy rates to first service and/or treatment and by 160 d PP ($P < 0.02$) and differences in PR disappear by 360 d PP ($P > 0.05$). BST administration at fixed- timed insemination during Ovsynch decreased the number of services per conception (NSC = 2.12 vs. 2.44 SC) compared with Ovsynch without BST; or to Control group (2.45). Contrarily, CIDR insertion increased the number of SC (2.58 SC; $P < 0.05$). In conclusion, a BST-injection at the time of insemination increased pregnancy rates and reduced services per conception in cows exposed to ovsynch.

Key Words: dairy cows, somatotropin, fixed-insemination

M190 Effect of adding a GnRH or $PGF_{2\alpha}$ between the Presynch and Ovsynch program for first AI in lactating dairy cows. R. G. S. Bruno*[1,2], A. M. Farias[1], J. A. Hernández-Rivera[1], A. E. Navarrette[1], D. E. Hawkins[2], and T. R. Bilby[1], *[1]Texas A&M University, College Station, [2]West Texas A&M University, Canyon.*

The objective was to evaluate the effect of 3 reproductive programs for first AI on fertility in lactating dairy cows. Lactating cows (n = 1521) from a dairy in Texas were presynchronized (PS) with 2 injections of prostaglandin (PG) given at 36 and 50 ± 3 DIM. At 50 ± 3 DIM, 915 multiparous and 606 primiparous cows were blocked by parity and assigned to 1 of 3 programs: GPG (n = 552) which initiated the Ovsynch protocol 14 d after PS, GGPG (n = 402) in which a GnRH injection was given 7 d after PS followed by the Ovsynch protocol 7 d later and PG7-GPG (n = 567) in which a PG injection was given 7 d after PS followed by the Ovsynch protocol 7 d later. Cows were AI based on signs of estrus (ED) beginning after the PS and, if AI, cows were removed from subsequent injections. Ovaries were examined and blood was sampled for progesterone levels on day of first GnRH and PG of Ovsynch. Pregnancy per AI (P/AI) was diagnosed at 36 and 66 d after AI. Overall 52.3% of cows were AI based on ED with the GGPG program having the least ($P < 0.01$) number of cows being identified in estrus (GGPG = 46.8 vs. GPG = 50.7 and PG7-GPG = 57.7%). Reproductive program did not affect ($P > 0.33$) overall P/AI at 33 and 66 d after AI (36d GPG = 34.1, PG7-GPG = 34.6 and GGPG = 31.3%; 66d GPG = 32.3, PG7-GPG = 31.9 and GGPG = 28.1%) or pregnancy loss ($P = 0.68$) between 36 and 66 d after AI. Cows AI upon ED had higher P/AI than TAI (ED = 37.9 vs. TAI = 28.8%, $P < 0.01$). However, treatment did not affect ($P > 0.61$) P/AI for cows AI upon ED or TAI (36d, GGPG = 34.1, PG7-GPG = 34.6 and GGPG = 31.3%; 66d GPG = 32.3, PG7-GPG = 31.9 and GGPG = 28.1%). Reproductive program affected ($P < 0.01$) the median DIM at first service (PG7-GPG = 59 vs. GPG = 68 and GGPG = 68 d). At beginning of Ovsynch, more GGPG cows had CL (GGPG = 72.7 vs. GPG = 54.4 and PG7-GPG = 46.4%, $P < 0.01$). However, treatment did not affect ($P = 0.26$) ovulation to first GnRH of Ovsynch. In conclusion, adding a GnRH injection 7 d after presynchronization reduced number of cows AI upon ED. Pregnancy per AI was not affected by reproductive program but adding a PG injection 7 d after PS decreased the median DIM at first AI.

Key Words: dairy cows, fertility, synchronization

M191 Application of progesterone insert for the induction of lactation in nonpregnant dairy cows or heifers. F. Rivera-Acuña*[1], R. C. Fierros[1], E. M. Prado[1], P. Luna-Nevarez[1], J. G. Aceves[1], L. R. Avendaño[2], and A. C. Correa[2], *[1]Instituto Tecnológico de Sonora, Ciudad Obregón, México, [2]Universida Autónoma de Baja California, Mexicali, México.*

The induced lactation of cows or heifers can be an alternative to reduce the rate of culling and to increase the economic benefits in a dairy operation. The objective of this study consisted of evaluating the effectiveness of replacing the traditional progesterone (P4) source with injections in programs of induced lactation by means of 2 P4 vaginal inserts containing 0.186 g each. During the study 15 animals, dairy cows and heifers were blocked by days dry and parity and were assigned at random to one of 3 treatments. Natural lactation (LN, n = 5) 2 P4 inserts (CRONI2, n = 5) injections only (INY, n = 5). The LN group initiated their lactation at the time of the calving date and was handled like dairy fresh cows. Treated groups during the first 7 d received P4 of different forms, one by means of daily i.m. injections 0.25 mg/kg/d and another one with 2 P4 vaginal inserts. Additionally blood was sampled during the same period to determine P4 levels. Furthermore during the first 14

d both groups received estradiol-17Î[2] 0.1 mg/kg/d i.m. In addition 3 bST applications at intervals of 10 or 14 d during the study and lactation respectively and dexamethasone 10 mg/cow i.m. during the d 17, 18 and 19 and the lactation beginning to the 21 d. All the cows were submitted to the Co-Synch timed artificial insemination protocol (TAI) at 60 d in milk (DM) and were inseminated at 70 DM. The data was analyzed in random blocks with replications through the commands Repeated and Random Mixed procedure of SAS. The P4 levels during the first 7 d of treatment had a similar behavior ($P = 0.9$), the milk yield at 112 DM was highly significant ($P = 0.0001$) for LN and INY groups, the milk yield to the tip result ($P = 0.001$) in LN and INY groups. On the other hand there were no differences ($P = 0.59$) between the interval of beginning of the lactation to the tip of lactation either on days of persistence period. Milk yield during the persistence period result significant ($P = 0.0004$) for LN and INY groups. Finally the 305 d adjusted milk yield also were significant ($P = 0.0001$) for LN and INY groups. The BCS at TAI tended ($P = 0.08$) to be better for CRONI2 in relation to the other groups. The pregnancy per AI was better ($P = 0.71$) in the LN group (40%) compared other 2 (20%) each. Change P4 source by means of vaginal inserts during induced lactation programs did not improved lactation performance nevertheless the application of P4 by i.m. injections can show very similar results to natural lactation cows.

Key Words: induced lactation, progesterone, milk yield

M192 Enhancing endogenous progesterone during growth of the ovulatory follicle is positively associated with fertility in dairy cows treated with Presynch-11/Ovsynch, Double Ovsynch, and G6G/Ovsynch. F. Jiminez-Krassel,* J. P. Martins, B. S. Raghavendra, M. Kron, and J. R. Pursley, *Michigan State University, East Lansing.*

Presynchronization strategies that enhance ovulation to first GnRH of Ovsynch have a greater chance of enhancing progesterone during ovulatory follicle growth. The objectives of this study were to 1) determine the effect of 3 presynchronization strategies on pregnancy/AI (PAI) of lactating dairy cows, 2) determine the relationship between P4 at PG of Ovsynch on PAI, and 3) determine if decreasing time from follicular wave induction to luteolysis during Ovsynch enhances fertility. Cows (n = 2453) were assigned to 4 treatments by parity to receive Presynch-11 (P11), G6G/Ovsynch (G6G), and Double Ovsynch 7 or 5 d between G and PG of Ovsynch (DO7, DO5) beginning 39 to 51 DIM. Cows received final GnRH of Ovsynch 72 h after PG and AI 16 h later. Ovulation was diagnosed with ultrasound. Serum was collected at strategic intervals to assay P4. Pregnancy was diagnosed with ultrasound 32 d post-AI. Percent cows with P4 > 1 ng/mL were different between treatments at final PG of the Presynch program (65, 56, 78, 77%; $P < 0.01$, n = 2451), at first GnRH of Ovsynch (67, 76, 87, 87%; $P < 0.001$, n = 2453), and at PG of Ovsynch (84, 86, 93, 93%; $P < 0.001$, n = 2451) for P11, G6G, DO7 and DO5. Ovulation rate in response to the first GnRH of Ovsynch (80, 85, 89, and 86%; $P < 0.05$, n = 1878), percent cows with luteolysis after final PG of Ovsynch (96, 92, 93, and 43%; $P < 0.001$, n = 2223) were different for P11, G6G, DO7 and DO5. PAI in cows (n = 1802) that ovulated to first GnRH of Ovsynch and had P4 > 1ng/mL at PG and decreased to <0.3 ng/mL P4 72h later were 52, 50, 49, and 48% ($P = 0.7$) for P11, G6G, DO7 and DO5. Concentrations of P4 at time of PG injection was positively associated with pregnancy outcome: 33, 43, 44, 50, 51 and 57% PAI for P4 ranges of 1–2 (n = 171), 2–3 (n = 136), 3–4 (n = 201), 4–5 (n = 308), 5–6 (n = 327) and > 6 (n = 659) ng/mL P4 ($P < 0.05$ for P4 < 4ng/mL vs. P4 > 4 ng/mL). Enhancing progesterone during growth of the ovulatory follicle is associated with enhanced PAI of lactating dairy cows. Decreasing time from wave induction to luteolysis did not increase fertility.

Key Words: Ovsynch, progesterone, fertility

M193 Effect of progesterone (P4) supplementation after AI on circulating P4 and development of the corpus luteum (CL) in dairy cattle. P. L. J. Monteiro Jr.*[1], F. L. M. Silva[1], M. Borsato[1], G. P. Nogueira[2], G. B. Mourão[1], L. D. Silva[1], M. C. Wiltbank[1], and R. Sartori[1], *[1]University of São Paulo, Piracicaba, SP, Brazil, [2]São Paulo State University, Araçatuba, SP, Brazil, [3]University of Wisconsin-Madison, Madison, WI.*

Supplementation with P4 at 3 d after AI may improve fertility of lactating dairy cows, however, concerns have been expressed that early P4 supplementation may inhibit normal CL development or lead to early CL regression. The objective of this study was to evaluate CL development, by transrectal ultrasonography, and circulating P4, as measured by radioimmunoassay. Lactating dairy cows were synchronized (2 mg estradiol benzoate + CIDR on d 0 with prostaglandin F2α on d 7 and CIDR removal and 1 mg estradiol cypionate on d 8) and received timed AI (TAI). All cows that demonstrated synchronized ovulation (ovulated 1.5 to 3.5 d after CIDR removal; n = 42) were randomized to 1 of 2 treatments: No treatment (Control; n = 21) or CIDR supplementation starting on d 3 until d 20 after TAI (CIDR-treated; n = 21). Ultrasound was performed 2, 3, and 4 d after CIDR removal to confirm ovulation. Luteal volume was measured on d 4, 7, 11, 14, and 20 after TAI and circulating P4 concentrations on day of TAI and d 3, 4, 7, 11, 14, 17, 20, and 21. The procedure GLIMMIX of SAS was utilized for statistical analysis. The circulating P4 concentration demonstrated a tendency for an effect of P4 supplementation ($P = 0.07$) and an effect of day ($P = 0.001$) and an interaction of day by P4 supplementation ($P = 0.01$). This interaction was related to the largest difference due to CIDR-treatment during the earliest stages of supplementation (d 4: 0.9 ± 0.2 vs 2.2 ± 0.2 ng/mL and d 7: 2.7 ± 0.2 vs 3.6 ± 0.2 ng/mL). In contrast, the CL volume demonstrated an effect of day ($P < 0.001$) but there was no effect of P4 supplementation ($P = 0.921$) and no interaction of P4 supplementation by day ($P = 0.352$). For example, CL volumes on d 7 were 9,030.1 ± 126.4 vs 9,021.4 ± 129.1 mm^3 for Control and CIDR-supplemented cows, respectively. Thus, P4 supplementation 3 d after TAI induced an early increase in circulating P4, but did not compromise CL development in lactating dairy cows. Acknowledgments: Pfizer, FAPESP and CNPq of Brazil.

Key Words: progesterone supplementation, corpus luteum, dairy cow

M194 Reproductive performance of lactating dairy cows managed for first service using timed artificial insemination with or without detection of estrus using an accelerometer system. P. M. Fricke,* A. Valenza, J. O. Giordano, M. C. Amundson, and G. Lopes Jr., *University of Wisconsin, Madison.*

Lactating dairy cows (n = 564) on a commercial dairy farm were fitted with accelerometers (Heatime, SCR Engineers Ltd., Netanya, Israel) at 21 DIM and were randomly assigned to one of 3 treatments: 1) Heatime + Ovsynch (HOv): cows received AI to detected estrus from the end of the VWP (50 DIM) until initiation of Ovsynch (GnRH-7d-PGF-56h-GnRH; cows initiated Ovsynch at 65 ± 3 DIM if not detected in estrus from 21 to 65 DIM, whereas cows initiated Ovsynch at 79 ± 3 DIM if detected in estrus from 21 to 50 DIM but not from 51 to 79 DIM); 2) Presynch-Ovsynch (PGF-14d-PGF-12d-GnRH-7d-PGF-56h-GnRH) + Heatime (PSOvH): cows received AI to estrus detected after the

second PGF injection of Presynch at 50 DIM, and cows not detected in estrus initiated Ovsynch at 65 ± 3 DIM; and 3) Presynch-Ovsynch (PSOv): cows were monitored for estrus after the second PGF injection of Presynch, but all cows received TAI at 75 ± 3 DIM. Pregnancy outcomes were determined by the herd veterinarian using transrectal ultrasonography 39 ± 3 d after AI. Average days to first service (DFS) differed ($P < 0.0001$) among treatments with PSOvH cows having the fewest ($P < 0.0001$) DFS (62.4 ± 8.3 d), followed by HOv cows (67.4 ± 10.7 d) and finally PSOv cows (74.7 ± 2.1 d). Overall, pregnancies per AI (P/AI) did not differ ($P = 0.58$) among treatments for HOv cows (33%; 64/194), PSOvH cows (29%; 57/194), or PSOv cows (34%; 60/176). For HOv cows, 57% were detected in estrus (P/AI = 33%), whereas 43% received TAI (P/AI = 33%). For PSOvH cows, 71% were detected in estrus (P/AI = 26%), whereas 29% received TAI and had P/AI of 36.8% (21/57). For PSOv cows, 76% showed estrus after PGF but completed the protocol and received TAI (P/AI = 35%), whereas 24% did not show estrus and received TAI (P/AI = 30%). Based on these preliminary results, the fewest DFS occurred when cows were submitted to a Presynch-Ovsynch protocol combined with detection of estrus using the Heatime system, whereas no overall differences in P/AI were observed among the 3 management strategies for submitting cows to first service.

Key Words: Heatime, Presynch-Ovsynch, timed AI

M195 Accuracy of pregnancy diagnosis outcomes using transrectal ultrasonography 29 days after artificial insemination in lactating dairy cows. J. O. Giordano* and P. M. Fricke, *University of Wisconsin-Madison, Madison.*

Our objective was to assess the effect of different criteria to determine pregnancy status on the accuracy of transrectal ultrasound (TU) 29 d after a timed AI in lactating dairy cows in a commercial setting. Pregnancy status was determined 29 d after timed AI (TAI) using transrectal ultrasonography (TU; Easi-scan, BCF Technology Ltd.) based on the following criteria: presence or absence of a corpus luteum (CL), presence, absence, volume and appearance of uterine fluid (UF) typical for a 29 d conceptus, presence or absence of an embryo with a heartbeat. Cows were classified as 1) non-pregnant (NP): presence or absence of a CL, absence of UF, or insufficient UF, and absence of an embryo; 2) pregnant (P): CL present, normal UF, and no embryo; 3) pregnant embryo (PE): CL present, normal UF, and at least one embryo visualized; and 4) questionable pregnant (QP): CL present, and one or more of the following: UF, insufficient UF, and either no embryo or a non-viable embryo. At 39 and 74 d after TAI, pregnancy status was determined using transrectal palpation (TP). Overall, 802 cows were classified as NP 29 d after TAI, whereas 799 cows were classified as NP 39 d after TAI resulting in misdiagnosis rate of 0.5% (4/802) for TU 29 d after TAI. At 29 d after TAI, 1,116 cows were classified as either P (28.9%; n = 322), PE (67.9%; n = 758), or QP (3.2%; n = 36). Among QP cows, 69.4% (25/36) were classified as NP 39 d after TAI, and an additional 45.5% (5/11) of the cows were classified as NP 74 d after TAI. For cows classified as P and PE using TU 29 d after TAI, more ($P < 0.0001$) P (17.7%) than PE (4.0%) cows were classified as NP using TP 39 d after TAI. Similarly, more ($P = 0.0004$) P (12.1%) than PE (5.4%) cows diagnosed at 39 d after TAI using TP were classified as NP 74 d after TAI. From the initial pregnancy exam at 29 d to the last exam at 74 d after TAI, more ($P < 0.0001$) P (27.6%) than PE (9.1%) cows were diagnosed as NP. Cows classified as P using TU 29 d after TAI were 3.8 (95% CI = 2.7 to 5.4) times more likely to be classified as NP than PE cows 74 d after TAI. We conclude that the accuracy of TU outcomes are increased when an embryo is visualized compared with TU outcomes based only on the presence of a CL and the volume of uterine fluid. Supported by Hatch project WIS01171.

Key Words: transrectal ultrasonography, pregnancy diagnosis, dairy cattle

M196 Early detection of pregnancy-specific protein B (PSPB) following conception in Holstein heifers. J. Howard*[1,2], C. Autran[1], J. Branen[2], G. Sasser[2], and A. Ahmdzadeh[1], *[1]University of Idaho, Moscow, [2]BioTracking LLC, Moscow, ID.*

Pregnancy-specific protein B (PSPB) is produced by ruminant placentae during pregnancy, and can be detected in maternal blood by a specific ELISA. The objective of this study was to determine the earliest appearance of PSPB after AI and when it can reliably be utilized to determine pregnancy status in Holstein Heifers as compared with the standard ultrasonography method. One hundred twenty-one Holstein replacement heifers were synchronized and subsequently submitted to timed-AI. Blood samples from all heifers were obtained at initiation of the experiment (at the time of AI, d 0), and again on d 18, d 25 and d 32 after AI for PSPB concentration. Pregnancy status was then determined by ultrasonography on d 32 after AI. The results of the PSPB data were then categorized into non-pregnant and pregnant animals based on ultrasonography results. Data were analyzed using a mixed model ANOVA for repeated measures. There was an effect of pregnancy status and pregnancy status × time ($P < 0.01$) on PSPB. Mean PSPB concentrations, expressed as optical density (OD), did not differ between pregnant and non-pregnant heifers on d 0 or d 18. However, PSPB concentrations increased by 1.7 fold, and were greater ($P < 0.01$) in pregnant compared with non-pregnant heifers on d 25 (0.083 ± 0.002 vs. 0.138 ± 0.003; $P < 0.01$) and d 32 (0.079 ± 0.002 vs. 0.296 ± 0.003; $P < 0.01$). Similarly, PSPB levels expressed as ng/mL did not differ on d 0 or d 18, but were greater ($P < 0.01$) in pregnant compared with non-pregnant heifers on d 25 (0.006 ± 0.031 vs. 0.290 ± 0.038; $P < 0.01$) and d 32 (0.026 ± 0.030 vs. 1.124 ± 0.037; $P < 0.01$). These results indicate that PSPB increases overtime and can be detected as early as d 25 after breeding and potentially used to identify non-pregnant heifers.

Key Words: PSPB, pregancy detection, heifers

M197 Possible associations between ova-embryos characteristics in early lactating cows and subsequent reproductive performance. R. L. A. Cerri*[1], W. W. Thatcher[2], and J. E. P. Santos[2], *[1]University of British Columbia, Vancouver, BC, Canada, [2]University of Florida, Gainesville.*

Ova-embryos (348) from 321 single ovulating Holstein cows were recovered 6 d after AI. Objectives were to determine if previously collected ova-embryo influences future reproductive outcome. Cows were enrolled in a presynchronized Ovsynch program starting at 30 ± 3 DIM and only cows that responded to the first GnRH of the Ovsynch were included. The BCS was measured at enrollment; cyclicity was monitored by ultrasonography. After ova-embryos collection, cows were subjected to a timed AI protocol. Cows were then re-inseminated if in estrus or resynchronized after diagnosed non-pregnant. Pregnancy at first AI, pregnancy loss at first AI, proportion of pregnant cows at 300 DIM, number of AI and interval to pregnancy were analyzed according to grade of ova-embryos collected from the same cows. Ova-embryos were classified based on fertilization and grade quality (IETS). The following combinations of fertilization and embryo grade quality were used to predict fertility responses of dairy cows: fertilized vs. unfertilized; grade 1 vs. remaining; grades 1 and 2 vs. remaining; grades 1 to 3 vs.

remaining. Data were analyzed using GLM, LOGISTIC, and LIFETEST procedure of SAS. Cows that yielded grades 1–3 embryos had a greater number of AI to become pregnant (3.2 ± 0.2 vs. 2.3 ± 0.3), had more median days open (117 vs. 90 d) compared with other structures, but proportion of cows pregnant at 300 DIM was unaltered (90.4 vs 97.9%). Conversely, cows that yielded fertilized ova compared with unfertilized ones required fewer AI (3.0 ± 0.1 vs. 3.8 ± 0.3) and had fewer median days open (109 vs. 122 d) and greater proportion of pregnant cows at 300 DIM (91.8 vs 81.2%). No other combinations of fertilization and embryo quality were associated with number of AI, days open or conception rate at first AI or at 300 DIM. In conclusion, embryo quality in early lactation does not seem to be related to subsequent reproductive performance, but fertilization was associated with reduced subsequent days open and greater conception rate. The genetic potential to produce fertilized ova, but not necessarily good quality embryos, seems to influence reproductive performance.

Key Words: embryo, fertilization, genetic potential

M198 Effects of induced clinical and subclinical mastitis on oocyte developmental competence in bovine. S. Asaf[1], O. Furman[1], G. Leitner[2], D. Wolfenson[1], and Z. Roth*[1], *[1]The Robert H. Smith Faculty of Agriculture, Food and Environment, the Hebrew University, Rehovot, Israel, [2]The Veterinary Institute, Bet Dagan, Israel.*

Clinical and subclinical mastitis can lead to decreased fertility in cows. We examined the effects of gram-positive exosecretions (G+; *S. aureus* ex.) or gram-negative (G-; *E. coli* LPS) toxin induced mastitis on oocyte developmental competence. Two models were established to simulate short-term, acute, clinical mastitis and long-term, subclinical mastitis. Holstein cows were synchronized with GnRH and $PGF_{2\alpha}$ to induce development of a preovulatory follicle. Follicular fluid (FF) was aspirated by transvaginal probe from (1) uninfected cows, (2) cows with clinical mastitis induced by G+ or G-, and (3) cows with subclinical mastitis induced by G+ or G-. FFs were used as maturation medium for IVF procedures. Oocytes were matured (FF, 22 h, 5% CO_2, 38.5°C), fertilized (18 h, 38.5°C, 5% CO_2), and cultured for 8 d (KSOM, 5% CO_2, 5% O_2, 38.5°C). Cleavage rate and proportion of oocytes developed to the blastocyst stage were recorded 44 h and 7 to 8 d post-fertilization. Apoptotic index for blastocysts was determined by TUNEL assay. Total RNA and poly(A) mRNA were isolated from mature oocytes and 4-cell-stage embryos and subjected to real-time PCR for *COX2*, *POU5F1*, *GLUT1*, *HSF1* and *GDF9* genes. Maturation in FF aspirated from clinical- or subclinical-mastitic cows reduced ($P < 0.05$) the cleavage rate and the proportion of developing embryos and increased ($P < 0.05$) the proportion of apoptotic cells in blastocysts. Alterations in gene expression were evident in embryos developed from oocytes matured in FF obtained from mastitic cows, with the most prominent impairments being in *COX2* expression. While clinical G- mastitis decreased *COX2* expression in MII-stage oocytes ($P < 0.05$), both clinical G- and G+ mastitis and subclinical G- and G+ mastitis increased *COX2* expression in 4-cell-stage embryos ($P < 0.05$). In addition, both clinical G+ and subclinical G+ mastitis reduced *OCT4* expression in 4-cell-stage embryos ($P < 0.05$). The findings indicate a deleterious effect of FF obtained from both clinical- and subclinical-mastitic cows on oocyte developmental competence. The disruptive mechanism seems to be associated with alteration in embryonic gene expression.

Key Words: mastitis, oocyte, developmental competence

M199 Assessing the relationships of prostaglandin E2 in uterine flush fluid, peripheral blood prostaglandin E2 and progesterone with pregnancy outcome in dairy cattle. J. L. Fain*[1], M. W. Overton[2], D. J. Hurley[2], and G. P. Birrenkott[1], *[1]Clemson University, Clemson, SC, [2]University of Georgia, Athens.*

Uterine inflammation can negatively affect animal welfare, milk production and rebreeding capacity in dairy cattle. The objectives of the current work were to investigate the relationship between prostaglandin E2 (PGE) found in uterine flush fluid to that found in peripheral blood and their potential effect on reproductive success in postpartum dairy cows. Animals (n = 34) were enrolled in a presynch program beginning at 35 ± 3 d postpartum with a $PGF_{2\alpha}$ injection followed by a second at d 49. Cows then received the Ovsynch protocol (100µg GnRH d 60, 25mg $PGF_{2\alpha}$ d 67, 100µg GnRH d 69 with TAI 16–18 h later on d 70). Uterine flush samples of both horns were collected from animals before the $PGF_{2\alpha}$ injection of the Ovsynch protocol. All animals received 100µg of GnRH on d 28 post TAI for the resynchronization program. Any animals open to the first TAI as determined by transrectal ultrasonography on d 35 had a second flush sample collected from the uterine body while receiving 25mg $PGF_{2\alpha}$ with an injection 100 µg GnRH following on d 37 and TAI 16–18 h later on d 38. Cows were then monitored for and bred off standing estrus with any animals not seen in estrus having pregnancy confirmed. If open to a third breeding, a third and final flush sample was collected. Blood samples were collected at d 35, 49, 60 and 67 post-parturition and d 14, 18, 22, 25, 28, 32 and 35 post TAI for P4 and PGE analysis. Additional blood samples were collected before each flush for testing by BioPRYN and for PGE. Blood and uterine PGE values shared no relationship with P4 concentrations. Significantly higher P4 values were found on d 18 post TAI in animals that were pregnant ($P < 0.05$). Though numerically there was evidence that reduced PGE value increased the likelihood of pregnancy, neither the PGE values in uterine flush fluid nor blood were significant predictors of pregnancy success at the 1st, 2nd or 3rd breeding or the likelihood of securing pregnancy after 3 breedings ($P > 0.05$). There was a high correlation between uterine PGE values and BioPRYN optical density readings ($P < 0.05$). Though the second flush showed a strong correlation between blood PGE and uterine flush PGE when SCC was added as a covariate ($P < 0.05$), this was not consistent across flushes ($P > 0.05$). Correlation potentials with BioPRYN as well as blood PGE would lend a less complicated method for detection of uterine inflammation as assessed by PGE.

Key Words: prostaglandin E2, dairy, uterine inflammation

M200 Effect of oral or subcutaneous administration of vitamin E and selenium on milk quality and reproductive function of Holstein cows. C. Garcia-Barrios, M. Rodriguez-Loera, C. F. Arechiga, M. A. Lopez-Carlos, J. I. Aguilera, R. M. Rincon, H. Rodriguez-Frausto, D. Rodriguez-Tenorio, and Z. Cortes,* *Universidad Autonoma de Zacatecas, Zacatecas, Mexico.*

Aim of this study was to determine factors affecting milk quality and reproductive efficiency of Holstein dairy cows in response to subcutaneous or oral administration of vitamin E and selenium. Cows (n = 159) were randomly allotted into 3 groups: 1) Control cows (n = 53; non-injected or supplemented cows); 2) Cows (n = 53), injected subcutaneously with 10 mL of vitamin E and selenium at days −45, −22, 0, before parturition and d 15 and 30 after parturition, (each injection contained 600 mg of vitamin E and 109.5 mg of selenium (BeefSe, Aranda

Labs., Queretaro,Mexico) 3) Cows (n = 53), were supplemented orally with 20 g of Selenio E (Ripoll, Mexico, D.F.), containing 2,500 mg of vitamin E/cow/d, and 3 mg of selenium/cow/d) from days −45 before parturition through d 30 after parturition. Milk quality and reproductive efficiency of cows was evaluated. Data obtained include determination of fat, protein, lactose, total solids (TS), non-fat solids (NFS) using a Milkoscan equipment (Foss, Hillerød, Denmark). Reproductive data were obtained and analyzed using PROC MIXED of SAS. The model includes the effects of season, lactation, treatment and treatment by season, a REML and PPDIFF methods were utilized considering days as repeated measurements. In conclusion, milk components were reduced during autumn ($P < 0.05$). However, antioxidant administration during the peripartum period, by subcutaneous injection of vitamin E and selenium were effective preventing milk protein reduction during autumn. Moreover, oral supplemented administration of vitamin E and selenium did not prevented seasonal reductions of milk components. Whereas, reproductive function was improved during autumn

Key Words: dairy cow, milk components, reproduction

M201 Effects of supplementation with different PUFA during the postpartum periods of early lactating dairy cows, estradiol concentration and luteal function. E. Dirandeh[1], A. Towhidi*[1], S. Zeinoaldini[1], M. Ganjkhanlou[1], Z. Ansari Pirsaraei[2], and T. Saberifar[1], *[1]Department of Animal Science, Faculty of Agricultural Science and Engineering, University College of Agriculture and Natural Resources, University of Tehran, Karaj, Tehran, Iran, [2]Department of Animal Science, Faculty of Animal Science and Fishery, Sari University of Agricultural and Natural Resources, Sari, Mazandaran, Iran.*

Ninety high-yielding multiparous Holstein dairy cows with no over clinical illnesses were blocked according to calving date and parity. Cows were assigned randomly to be fed either 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 d 60 postpartum (dpp). There was no difference between groups (mean ± SEM) in parity (3.0 ± 1.90) or BCS at calving (3.2 ± 0.07). Beginning at 30 DIM, cows were induced into a synchronized ovulatory cycle with 2 injections of PGF2α 14 d apart. Blood was collected daily; from the day that second PGF2α inject until the day of next estrous. Ovarian follicular development was monitored daily by transrectal ultrasonography with a real-time linear scanning ultrasound diagnostic system (B mode; Piemedical, Falco 100; 8 MHz transducer). Ultrasonography was performed once daily from the day that second PGF2α inject until the day of next estrous. Data were analyzed with PROC MIXED of SAS. Cows offered diet S and L had a greater size of corpus luteum than cows offered Diet C. There were no significant differences between groups L and S in size of corpus luteum (17.70 ± 0.86 and 18.30 ± 0.86 mm respectively; $P < 0.05$). Although mean serum progesterone concentrations on d 17 of synchronized cycle were higher in cows fed S (8.40 ± 0.10 ng/ml) or L (9.10 ± 0.20 ng/ml) than cows fed palm oil (6.30 ± 0.15 ng/ml, $P < 0.01$), there were no significant differences between groups L and S groups. Mean estradiol concentration was higher at the time of estrus in cows fed S or L compared with those fed palm respectively (12.60 ± 0.016, 13.00 ± 0.08 vs. 10.10 ± 0.09 pg/mL; $P < 0.05$) in the cows.

Key Words: dairy cows, n-3 and n-6 fatty acid, luteal function

M202 Hepatic patatin-like phospholipase domain-containing protein 3 mRNA expression is increased during feed restriction and in transition dairy cows. M. E. Viner*[1], S. S. Donkin[2], and H. M. White[1], *[1]Department of Animal Science, University of Connecticut, Storrs, [2]Department of Animal Sciences, Purdue University, West Lafayette, IN.*

Patatin-like phospholipase domain-containing protein 3 (PNPLA3), commonly known as adiponutrin, is part of a novel subfamily of triglyceride lipase enzymes with potential effects on triglyceride metabolism in adipose and hepatic tissues. In rodents and humans, PNPLA3 mRNA expression is suppressed during fasting and is increased by subsequent refeeding. The bovine predicted PNPLA3 sequence has been identified; however, expression of this gene has not yet been examined. The objectives of this study were to verify expression of the bovine predicted PNPLA3 gene and to determine response to whole-animal energy balance. Genomic DNA was isolated from liver biopsy samples collected from cows at +28 d relative to calving (DRTC) and the predicted PNPLA3 region was amplified via PCR and visualized to confirm expression. To determine if energy balance alters expression of PNPLA3, RNA was isolated and quantified in liver samples from mid-lactation cows (n = 5) after a 5-d ad libitum period and after a subsequent 5-d 50% feed restriction period, to determine if energy balance altered expression. Expression of hepatic PNPLA3 was decreased ($P < 0.05$) after a period of feed restriction (8.14 vs. 1.08 ± 2.17, arbitrary units, ad libitum vs. fasted). Dairy cows commonly experience negative energy balance during the transition to lactation; therefore, PNPLA3 expression was also examined in liver biopsy samples cows (n = 16) at −14, +1, +14, and +28 DRTC. Expression was decreased ($P < 0.05$) at +1 and +14 DRTC compared with −14 DRTC (23.35, 7.28, 10.17, and 14.5 ± 4.9, arbitrary units, −14, +1, +14, and +28 DRTC, respectively). The data indicate increased hepatic PNPLA3 expression that is part of the adaptive response to the transition to lactation and suggests a link to negative energy status associated with this interval.

Key Words: patatin-like phospholipase domain-containing 3, adiponutrin, energy balance

M203 Changes of the serum and milk proteome in lactating dairy cows duodenal infused with α-linolenic acid. J. H. Yang, J. Q. Wang,* T. J. Yuan, D. P. Bu, Y. X. Yang, P. Sun, and L. Y. Zhou, *State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.*

This study investigated the effect of a duodenal infusion of a C18:3 free fatty acid on the serum and milk proteome of lactating dairy cows. Four primiparous Holstein cows were fitted with duodenal cannulas and received 0, 100, 200, 300, and 400 g/d of α-linolenic acid (LNA) in a 2-treatment crossover design. Blood and milk were collected for determination of protein composition by 2-dimensional gel electrophoresis. Quantitative analysis of protein dyeing density were prosessed by Quantity One 4.6. Alteration of protein spots were detected and identified using matrix-assisted laser desorption/ionization time-of-flight/time-of-flight tandem mass spectrometry (MALDI-TOF-TOF MS). Data were analyzed by SPSS 16.0. Serum haptoglobin levels, and milk β-casein A2, αs1-casein variant, and albumin, did not differ in cows after infusion of 0, 100, 200 and 300 g/d of LNA, but were increased after the cows received duodenal infusion of 400 g/d of LNA. Western blot analysis of haptoglobin expression in the serum confirmed the alterations in protein

expression seen using mass spectrometry. This study demonstrated that infusion of high doses of LNA by duodenal cannula can result in metabolic stress to the cows and changes in milk composition.

Key Words: linolenic acid, proteome, dairy cow

M204 Investigation of the relationship between resumption of ovarian cyclicity and plasma nutritional markers in lactating dairy cows. A. Ahmadzadeh[1], J. Spencer*[1], B. Shafii[1], C. Johnson[1], J. Dalton[2], K. Carnahan[1], and S. Reeds[1], *[1]University of Idaho, Moscow, [2]University of Idaho R & E Center, Caldwell.*

Previous reports have shown the association between metabolic hormones, ovulation intervals and reproductive performance. The objective of this study was to investigate the relationship between the resumption of ovarian activity and plasma nutritional markers including glucose, NEFA, plasma urea nitrogen (PUN), cholesterol and β-hydroxybutyrate (BHBA). Forty-three lactating Holstein cows, housed in a free stall barn, were randomly selected from a commercial herd. From wk 2 to wk 7 postpartum, weekly ultrasonography and blood sampling was performed to characterize ovarian status, plasma metabolites, and blood progesterone. The occurrence of the first postpartum ovulation was determined by the analysis of plasma progesterone and confirmation of ovulation using visualization of a corpus luteum by ultrasonography. Based on plasma progesterone concentration and ovarian status, cows were divided into 2 treatment groups, cows that ovulated ≤25 DIM were designated early ovulators (EO) and cows that ovulated >25 DIM were considered late ovulators (LO). Data were analyzed using mixed model ANOVA for repeated measures. The mean interval to 1st ovulation for EO was 16.7 d, and for LO was 34.7d. Mean BCS was greater ($P < 0.05$) for EO than LO and (3.3 ± 0.1 vs 2.9 ± 0.1). There were effects of week ($P < 0.01$) and week by group interaction ($P < 0.05$) on blood cholesterol. Mean blood cholesterol increased over time for both EO and LO, however, mean cholesterol concentrations between wk 2 and 3 were less ($P < 0.05$) for EO compared with LO. Across all weeks, the mean glucose concentrations tended to be greater ($P = 0.06$) in EO than LO. There was no effect of group, week, or their interaction on blood BHBA. Blood NEFA did not differ between EO and LO, however, the mean concentration of NEFA decreased over time ($P < 0.01$) for both groups. Mean PUN was not different between EO and LO throughout the experiment. These results provide evidence that the 1st postpartum ovulation may be associated with blood glucose and cholesterol during the early postpartum period and may be indicative of the resumption of reproductive function in lactating dairy cows.

Key Words: blood metabolites, ovulation, dairy cows

M205 Insulin responses in dairy cows with different fat mobilization during early lactation. U. Kautzsch[1], B. Kuhla[1], M. Röntgen[1], S. Görs[1], R. M. Bruckmaier[2], C. C. Metges[1], and H. M. Hammon*[1], *[1]Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany, [2]Veterinary Physiology, Vetsuisse Faculty, Bern, Switzerland.*

Dairy cows differ in fat mobilization around calving. Insulin has antilipolytic effects and may antagonize body fat mobilization, but insulin action is probably impaired in cows early postpartum (pp). The objective of the present study was to compare insulin responses ante partum (ap) and pp in cows differing in fat mobilization. German Holstein cows (>10,000 kg milk/305 d; ≥ 2nd lactation) were classified by liver fat concentration (LFC) pp in low (L; < 240 mg total fat/g DM; n = 9) and high (H; > 240 mg total fat/g DM; n = 10). Cows were studied from dry off up to 30 DIM and were fed TMR ad libitum. DMI and milk yield were recorded daily. Liver biopsies were taken on d 3, 18, and 30 pp to measure LFC. Hyperglycemic (HGC) and euglycemic-hyperinsulinemic clamps (EGHIC) were performed in wk 5 ap and wk 3 pp to measure pancreatic and peripheral insulin responses. In HGC glucose was infused to reach plasma concentrations 50% higher than basal glucose concentrations. In EGHIC insulin (6 mU/kg BW × min) was infused for 6 h and plasma glucose was kept constant according to pre-clamp concentrations. Blood samples were taken to measure plasma glucose and insulin and glucose infusion rates (GIR) were determined during steady-state conditions. Before and during the EGHIC pp [U-^{13}C]-glucose (prime: 5.4 μmol/kg BW; infusion: 7.5 μmol/[kg BW × h] for 9 h) was infused to measure of endogenous glucose production (eGP), glucose turnover (GT), and oxidation (GOx). Data were analyzed by Mixed Model of SAS with LFC and time as fixed effects. LFC differed between groups ($P < 0.01$) (H: 306 ± 0.2 mg/g; L: 195 ± 0.1 mg/g). Basal glucose and insulin concentrations were higher ($P < 0.05$) ap than pp. In HGC GIR was higher ($P < 0.001$) and insulin release was lower ($P < 0.01$) pp than ap. In EGHIC GIR tended to be higher ($P < 0.1$) in H than L. During insulin infusion pp eGP decreased to 19% of pre-clamp eGP. GT was higher ($P < 0.05$), but GOx relative to glucose turnover tended to be lower ($P < 0.1$) in H than L. Insulin action was not affected by elevated fat mobilization pp but stimulation of insulin secretion was impaired pp. Supported by DFG, Germany.

Key Words: dairy cow, glucose, insulin response

M206 Effects of heat stress and plane of nutrition on adipose tissue metabolism-related gene expression in lactating Holstein cows. G. Xie*[1], L. W. Hall[2], M. Nearing[2], L. C. Cole[2], J. Allen[2], L. H. Baumgard[3], D. M. Spurlock[3], and R. P. Rhoads[1], *[1]Virginia Polytechnic Institute and State University, Blacksburg, [2]University of Arizona, Tucson, [3]Iowa State University, Ames.*

During heat-stress (HS) in lactating dairy cows, adipose tissue appears to become refractory to lipolytic signals whereas pair-fed cows employ mechanisms allowing lipid mobilization to spare glucose utilization in peripheral tissues. Despite this, little is known regarding the effects of HS on metabolic gene expression in adipose tissue. Multiparous cows (n = 12; parity = 2, 305 ± 33 DIM; 665 ± 18 kg BW) housed in climate chambers were fed a TMR consisting primarily of alfalfa hay and steam-flaked corn and subjected to 2 experimental periods (P): 1) thermoneutral (TN) conditions (18°C, 20% humidity) with ad libitum intake for 9d and 2) either HS conditions (cyclical temperature 31–40°C, 20% humidity: min THI = 73, max THI = 86) fed for ad libitum intake (n = 6), or TN conditions, pair-fed (PF in TN conditions, n = 6) for 9d. Rectal temperature (Tre) and respiration rate (RR) were measured thrice daily at 0600, 1400 and 1800h. To evaluate adipose tissue gene expression, biopsies were obtained from the tail-head region at the end of each period and total RNA isolated for real-time PCR analyses. Data was normalized using β-actin as a control gene and analyzed by the Proc Mixed procedure in SAS. During P2, HS cows had a 1.8°C increase in Tre and a 3-fold increase in RR compared with TN cows ($P < 0.01$). Pair feeding did not alter Tre or RR. Heat stress reduced ($P < 0.01$) DMI by 20% and by design PF cows had similar intake reductions. Milk yield was decreased 12% during HS and 8% in PF cows. Adipose triglyceride lipase (ATGL), lipoprotein lipase (LPL), and pyruvate carboxylase (PC) significantly

decreased ($P < 0.05$) during HS compared with TN, but was unaffected by PF. Fatty acid synthase (FAS) mRNA abundance decreased during PF ($P < 0.05$) but was unchanged by HS. In contrast, gene expression of β1 adrenergic receptor (AR) and perilipin (PLIN) tended to increase ($P = 0.10$) while β2 AR and the truncated leptin receptor (ObRa) increased significantly during PF ($P < 0.05$) but not HS. These results indicate that HS directly alters adipose tissue metabolism-related gene expression independently of reduced plane of nutrition. This project was supported by NRI Competitive Grant no. 2008-35206-18817 and AFRI Competitive Grant no. 2010-65206-20644 from the USDA NIFA.

Key Words: heat stress, adipose, metabolism

M207 Relevance of mineralocorticoid receptors in different fat depots of dairy cows supplemented with CLA. K. Friedauer*[1], S. Dänicke[2], D. von Soosten[2], H. Sauerwein[1], and S. Häussler[1], *[1]University of Bonn, Bonn, NRW, Germany, [2]Federal Research Institute, Braunschweig, Lower Saxony, Germany.*

Adipose tissue (AT) consists of different cell types, among those preadipocytes, marked by preadipocyte factor-1 (Pref-1), are crucial for differentiation into mature adipocytes. This process is induced by glucocorticoids mediating their effects through the glucocorticoid (GR) and mineralocorticoid receptor (MR). Whether bovine adipocytes differentiate through GR or MR activation is not known. To investigate the main pathway of adipocyte differentiation, 25 primiparous Holstein cows were divided into control (CON, n = 15) and CLA group (n = 10), slaughtered on 1, 42 and 105 DIM. CLA cows received 100 g/d of a CLA-mixture (Lutrell BASF) from 1 DIM until slaughter. CON cows were fed with 100 g/d of a fatty acid mixture (Silafat BASF) without CLA. Samples from 3 visceral (vc) and 3 subcutaneous (sc) AT depots were obtained. MR and Pref-1 were localized on paraffin embedded sections by immunohistochemistry. Data are given as percentage of positive cells per total cell number (mean ± SEM) and were correlated by Pearson correlation (SPSS 19). Both MR and Pref-1 were detected exclusively in the stromal vascular cell fraction (SVF) of all fat depots, while GR was detected solely in adipocytes. The mean values were 1.05 ± 0.1% and 2.99 ± 0.2% for Pref-1 and MR, respectively. Significant correlation coefficients between MR and Pref-1 are given in the table ($P \leq 0.05$). We assume that bovine adipocyte differentiation is mediated through MR, for MR and Pref-1 are allocated to the SVF. In vc AT lipolysis in early lactation might be compensated by preadipocyte differentiation and seems to vary between different AT depots.

Table 1. Correlation coefficients (r) between mineralocorticoid receptor (MR) and Pref-1 (only significant data are shown; $P \leq 0.05$)

	CON	CON	CLA	CON	CLA
DIM:	1	42	42	105	105
vc mesenteric	0.916	—	—	0.914	—
vc retroperitoneal	0.905	—	—	—	—
vc omental	—	0.883	—	—	0.885
sc tailhead	—	0.917	—	—	—
sc withers	—	0.897	—	—	—
sc sternum	—	0.896	—	—	—

Key Words: preadipocyte, mineralocorticoid receptor, dairy cow

M208 The effects of a soybean and canola diet during pre-pubertal growth on dairy heifer fertility. M. B. Gordon*[1], E. Thompson[1], T. Gowan[2], D. Mosely[3], J. A. Small[2], and D. M. W. Barrett[1], *[1]Department of Plant & Animal Science, Nova Scotia Agricultural College, Truro, NS, Canada, [2]Atlantic Food & Horticulture Research Centre, Agriculture & Agri-Food Canada, Truro, NS, Canada, [3]AgraPoint, Bible Hill, NS, Canada.*

Dietary phytoestrogens (such as those in soybean) can have detrimental influences on the rate of sexual maturation and reproductive physiology of animals; however, the precise effect of phytoestrogens on reproductive function in dairy cattle has not been fully elucidated. This study examined the effects of feeding a soybean (SOY) and canola (CAN) calf starter diet during pre-pubertal development on the fertility of Holstein dairy heifers. At 8 wk of age calves (n = 24) were randomly assigned to receive CAN or SOY until 24 wk of age. Calves were fed approximately 1.5 kg/calf/day and ad libitum hay and water. At 24 wk of age heifers were group housed and fed a TMR of grass and corn silage and hay. At approximately 60 wk of age heifers were synchronized for AI using Double Ovsynch (GnRH–$PGF_{2\alpha}$, 7 d–GnRH, 3 d followed 7 d later by GnRH–$PGF_{2\alpha}$, 7 d–GnRH 48 h–AI 16 to 20 h). Blood samples were collected on d −3, 0 (AI), 7, and 14 for progesterone (P_4) analysis. Transrectal ultrasonography was performed twice daily for 3 d, starting 24 h before AI, to determine timing of ovulation and ovulatory follicle size and on d 42 for pregnancy diagnosis. Ovarian and hormone data were analyzed using ANOVA; pregnancy rate (PR) was analyzed using logistic regression. The timing of ovulation following the last GnRH injection was not different (35.9 ± 2.6 h; $P = 0.39$). However, 1 CAN and 2 SOY heifers did not ovulate. The maximum ovulatory follicle diameter was similar (15.3 ± 0.5 mm; $P = 0.58$). The CAN treatment had greater P_4 concentrations than the SOY on d −3 (4.9 ± 0.5 ng/mL vs. 2.7 ± 0.5 ng/mL; $P = 0.003$). There were no differences in P_4 concentrations on d 7 (2.3 ± 0.2 ng/mL $P = 0.42$) or 14 (2.6 ± 0.3 ng/mL; $P = 0.20$). Pregnancy rates were 66.7% vs. 41.7% ($P = 0.62$) for CAN and SOY, respectively. Concentrations of P_4 on d −3 ($P = 0.10$) and 7 ($P = 0.09$) tended to influence PR. In summary, there were no differences between treatments for the timing of ovulation, ovulatory follicle size, and post-AI P_4 concentrations. However, P_4 concentrations on the day of $PGF_{2\alpha}$ treatment of Ovsynch TAI tended to influence PR and on this day CAN treatment had higher P_4 concentrations.

Key Words: phytoestrogens, dairy heifers, fertility

M209 Reproduction in grazing dairy cows treated with 14-d CIDR for presynchronization before a timed AI (TAI) compared with AI after observed estrus. R. C. Escalante,* S. E. Poock, D. J. Mathew, W. R. Martin, E. M. Newsom, S. A. Hamilton, K. G. Pohler, and M. C. Lucy, *University of Missouri, Columbia.*

Progesterone-releasing devices (CIDR; Pfizer, New York, NY) inserted for 14 d are used to presynchronize the estrous cycle for TAI in beef heifers (14-d CIDR-PG program). The objective was to test a similar program in dairy cows by measuring first service conception rates (FSCR), pregnancy rates, and time to pregnancy compared with a control (AI after observed estrus). Postpartum cows (Holstein, Jersey or crossbred; n = 1363) from 4 grazing dairy farms were assigned to one of 2 programs: 14dCIDR_TAI [TRT; CIDR in, 14 d, CIDR out, 19 d, $PGF_{2\alpha}$ (5 mL Lutalyse, Pfizer), 56 h, GnRH (2 mL Factrel, Pfizer), and then 16 h, TAI; n = 737] or control [AI after observed estrus; reproductive

program with $PGF_{2\alpha}$ (cycling cows) and CIDR (non-cycling cows) to synchronize estrus with the start of the breeding season; n = 626]. Body condition (BCS; 1 to 5; thin to fat) was scored at trial start. The interval from the start of breeding to first AI was shorter for TRT vs control (3.0 ± 0.2 vs 5.3 ± 0.2 d; $P < 0.001$) but TRT cows had lesser FSCR than control (48 vs 61%; $P < 0.05$). Farm affected ($P < 0.002$) FSCR (69, 50, 58, and 51% for farms 1 to 4) but there was no treatment by farm interaction ($P > 0.10$). BCS affected FSCR (50, 55, and 62%; 2, 2.5, and 3; $P < 0.05$). Cows that either calved the year before (carry-over) or that calved early in the calving season had greater FSCR than cows that calved later in the calving season (55, 61, and 42%, respectively; $P < 0.001$). The percentage of cows pregnant to AI (6 wk breeding season) was similar for TRT and control (65 vs 70%; $P > 0.10$) but farm (81, 66, 69, and 62; $P < 0.01$) and time of calving (70, 76, and 56%; carry-over, early, and late; $P < 0.001$) affected the percentage. Survival analyses showed an initial advantage for TRT [more cows inseminated ($P < 0.001$) and more pregnancies achieved ($P < 0.07$) early in the breeding season] that was not maintained over time. Conclusions were that the 14dCIDR_TAI program achieved acceptable FSCR (48%) and overall 6 wk pregnancy rates (65%) for a TAI but did not surpass a control program that employed AI after observed estrus (61 and 70%; respectively).

Key Words: dairy, cow, timed AI

M210 Hormonal therapies on repeat breeder cows of a dairy production unit of central Mexico (Aguascalientes State). F. Lugo-Garcia, C. F. Arechiga,* A. Reyes-Gomez, R. R. Lozano, F. J. Escobar, R. M. Rincon, J. I. Aguilera, and M. A. Lopez-Carlos, *Universidad Autonoma de Zacatecas, Zacatecas, Mexico.*

The aim of this study was to determine the factors that influence the reproductive efficiency of Holstein repeat-breeder cows (n = 320) in response to 5 different protocols or treatments used in an intensive dairy production unit at Aguascalientes State. Hormonal therapies were: 1) control; 2) GnRH at d 5 post-AI; 3) hCG at d 5 post-AI; 4) GnRH at d 5 post AI + CIDR; 5) GnRH at d 11 post-AI. Data was recorded from February to September 2011. Information obtained was analyzed by Chi-squared using the SAS statistical package (SAS, 2006). Subsequently, orthogonal contrasts were performed (control vs. hCG vs. GnRH). A high incidence of repeat-breeder cows showed reduced conception rates, which, in turn, compromise general reproductive performance of the herd and it has economic and managemental implications such as the genetc progress and replacement programs of the herd. Only 17% of repeat breeder cows were diagnosed pregnant whereas 83% were non-pregnant. Responses to all 5 hormonal therapies implemented were considerably low. However, a GnRH injection 11 d post-insemination presented higher pregnancy rates compared with the control group (31 vs. 21%), or to other GnRH (15.7%) or hCG treatments (0%). In conclusion, a GnRH injection 11 d post-insemination could be effective increasing fertility of repeat breeder cows.

Key Words: dairy cows, reproduction, repeat breeder

M211 Effects of month of breeding on reproductive efficiency of dairy cows inseminated with sexed or nonsexed semen in a hot arid environment. E. Sepulveda*[1], O. Angel-Garcia[1], J. M. Guillen[1], C. A. Meza-Herrrera[2], F. G. Veliz[1], and M. Mellado[1], *[1]Universidad Autonoma Agraria Antonio Narro, Torreon, Coahuila, Mexico, [2]Universidad Autonoma Chapingo-Unidad Regional Universitaria de Zonas Aridas, Bermejillo, Durango, Mexico.*

The objective of this study was to assess the effect of month of breeding on reproduction performance of Holstein cows treated with rbST throughout lactation and inseminated with sexed or nonsexed semen in a hot arid environment. Pregnancy per artificial insemination (P/AI; 62666 services over a 5-year period) both in nulliparous heifers (n = 20313) and cows (42353) from a large dairy herd in northern Mexico (26° N) were evaluated with respect to month of AI. The GENMOD procedure of SAS was implemented to assess the effect of month of breeding on P/AI. Overall, P/AI with sex-sorted semen was greater ($P < 0.01$) in heifers (41.6%) than cows (17.3%). P/AI for cows serviced with conventional semen was 10 percent points higher ($P < 0.01$) in January and December (31 vs 21) than cows serviced with sex-sorted semen. While there was no difference in P/AI between the sex-sorted sperm, and conventional semen in cows inseminated in June, P/AI plummeted for both groups of cows at this time of the year (16 and 18%, respectively). What was observed in cows, P/AI was not different between heifers serviced with sex-sorted or conventional semen during the hottest months of the year (July to October). However, during the coldest months of the year (January and February), P/AI was 10 percentage points greater ($P < 0.01$) in heifers serviced with conventional semen than heifers serviced with sex-sorted semen. It was concluded that in this hot-arid climate of northern Mexico breeding cows and heifers during summer compromise the breeding success. This data also show that the use of nonsexed semen resulted in meaningful advantages in terms P/AI compared with sexed semen, however, this advantage was reduced during extreme heat loads.

Key Words: nulliparous, sex-sorted, conventional

M212 Effects of follicular wave and progesterone concentration during follicle growth on conceptus global gene expression in dairy cows. R. S. Bisinotto*[1], E. S. Ribeiro[1], L. F. Greco[1], N. Martinez[1], R. L. A. Cerri[2], W. W. Thatcher[1], and J. E. P. Santos[1], *[1]University of Florida, Gainesville, [2]University of British Columbia, Vancouver, BC, Canada.*

Effects of wave of the ovulatory follicle and progesterone (P4) concentration during follicle growth on subsequent conceptus gene expression were evaluated. Nonlactating Holstein cows received a timed artificial insemination (AI) protocol (d-9 GnRH, d-2 and d-1 $PGF_{2\alpha}$, d0 GnRH and AI, d1 AI) initiating either during proestrus or early diestrus. The rationale was to induce ovulation of either a first (FW, n = 13) or second wave follicle (SW, n = 12) at AI, respectively. To evaluate the effects of P4 during follicle development, a third group of cows induced to ovulate first wave follicles received 3 intra-vaginal inserts containing P4 inserted sequentially at 12, 24 and 48 h after the initial GnRH (FWP4, n = 8). All inserts were removed on d-2. Cows were killed on d17 after d0 AI and uteri were flushed with PBS solution. Recovered concepti mRNA was extracted and global gene expression was evaluated using Affymetrix GeneChip Bovine Genome arrays. Data analyzed using the MIXED procedure of JMP-Genomics/SAS. Orthogonal contrasts were performed to determine the effects of P4 (FW vs. FWP4) and follicle wave (FW+FWP4 vs. SW). Differentially expressed genes were selected if $P < 0.05$ and fold-difference >1.5. Analyses identified 155 upregulated and 478 downregulated genes in response to the ovulation of a FW compared with a SW follicle. Upregulated genes were associated with 18 pathways from the Kyoto Encyclopedia of Genes and Genomes (KEGG). Downregulated genes are involved with a greater variety of biological processes (50 pathways), including several of those encompassing the upregulated transcripts. Progesterone supplementation during growth of the FW follicle induced upregulation of 73 genes from 13 pathways, including MAPK (hsa04010) and Wnt signaling pathways (hsa04310), focal adhesion (hsa04510) and regulation of cytoskeleton (hsa04810). Interestingly,

P4 induced upregulation of TCF7 and EGFR in concepti. These genes have been associated with endometrial cell proliferation and cancer in humans; therefore, are potential candidates to mediate P4 maternal-conceptus communication.

Key Words: embryo gene expression, progesterone, dairy cow

M213 Expression of CYP11A1, CYP17, and CYP19A1 in granulosa cells, and determination of hormone levels in follicular fluid from dominant follicles and follicular cysts in Holstein cows. P. Hernández-Briano, J. A. Grado-Ahuir,* E. Burrola-Barraza, R. M. Villaseñor González, L. E. Escobedo-Morales, and S. A. Quintana-Quintana, *Facultad de Zootecnia y Ecología, Universidad Autónoma de Chihuahua, Chihuahua, Chihuahua, México.*

Follicular cysts are a major cause of infertility in dairy cattle. The objective was to assess the expression of genes coding for CYP11A1, CYP17, and CYP19A1 in granulosa cells, and to measure the level of IGF-1, 17-β estradiol (E_2), and progesterone (P_4) in follicular fluid of dominant follicles and follicular cysts. We analyzed 17 dominant follicles and 16 follicular cysts obtained from dairy cows slaughtered in Chihuahua, México. Gene expression relative to GAPDH was measured by real-time PCR using the comparative threshold cycle method. The concentration of IGF-1, E_2 and P_4 was measured by enzyme immunoassay. The experiment was conducted using a completely randomized design, with the fixed effect of follicle (cystic or dominant). We also studied the correlation among gene expression, follicle diameter and hormone levels. There were no differences ($P > 0.05$) between follicular cysts and dominant follicles for the expression of CYP11A1 (3.51 ± 0.25 vs 3.42 ± 0.26), CYP17 (1.88 ± 0.26 vs 1.84 ± 0.29), CYP19A1 (4.07 ± 0.36 vs 3.37 ± 0.42), and the concentration of E_2 and P_4 (1142.00 ± 1.82 vs 1384.65 ± 1.82, and 23.40 ± 1.45 vs 13.10 ± 1.45 ng/mL, respectively). The concentration of IGF-1 was higher ($P < 0.05$) in follicular cysts compared with dominant follicles (132.78 ± 1.88 vs 78.21 ± 1.88 ng/mL). We found significant correlations ($P < 0.05$) between the expression level of CYP11A1 with CYP17 and CYP19A1 (0.57 and 0.73, respectively) in dominant follicles. There was a negative correlation ($P < 0.05$) between IGF-1 and CYP11A1 expression (−0.69), and CYP11A1 and follicle diameter (−0.60) in follicular cysts. Level of expression for CYP19A1 was positively correlated to E_2 concentration in both follicular cysts and dominant follicles (0.93 and 0.88 respectively). Cystic follicles show greater concentration of IGF-1, which is negatively related to CYP11A1.

Key Words: follicular cyst, gene expression, IGF-1

M214 Comparison of dry matter intake and somatotropic axis components of Holstein and crossbred dairy cows. L. G. D. Mendonça*[1], N. B. Litherland[2], M. C. Lucy[3], D. H. Keisler[3], and R. C. Chebel[1], *[1]Department of Veterinary Population Medicine, University of Minnesota, Saint Paul, [2]Department of Animal Science, University of Minnesota, Saint Paul, [3]Division of Animal Sciences, University of Missouri, Columbia.*

Objectives of the study were to compare dry matter intake (DMI), somatotropic axis components, plasma cortisol (COR), and leptin (LEP) concentrations of Holstein (HO) and Montbéliarde sired crossbred (MS) cows. Cows were enrolled in the study 45 d before calving (d −45) and followed until d 90. Daily DMI was measured from d −45 to 45. Liver was biopsied on d −14, 7, 14, and 28 to determine mRNA expression of growth hormone receptor (GHR) 1A, insulin-like growth factor-I (L-IGF1), and insulin receptor b (IRB). Plasma concentrations of growth hormone (GH), insulin-like growth factor-I (IGF1), insulin (INS), and LEP were determined on d −7, 1, 7, 14, 21, 42, and 56 and that of COR was determined on d −14, −7, 1, 7, 14, 21, and 42. Data were analyzed by ANOVA for repeated measures. Breed tended ($P = 0.08$) to be associated with DMI from d −45 to 45 (HO = 16.5 ± 0.7kg/d vs MS = 14.9 ± 0.6kg/d) and DMI expressed as percentage of body weight (DMIBW) tended ($P = 0.10$) to be affected by the interaction between breed and day because among HO cows DMIBW on d −15 and −1 were 1.89 ± 0.12% and 1.43 ± 0.14%, respectively, and among MS cows DMIBW on d −15 and −1 were 1.51 ± 0.10% and 1.41 ± 0.11%, respectively. There were no associations between breed and expression of GHR1A ($P = 0.83$), L-IGF1 ($P = 0.70$), and IRB ($P = 0.68$) mRNA. The interaction between breed and day was ($P = 0.02$) associated with expression of IRB mRNA because among HO cows IRB expression was ($P < 0.05$) greater on d 7 compared with d 28 and on d 14 compared with d −14 and 28 but no changes in IRB expression were observed among MS cows. There were no associations between breed and concentrations of IGF1 (55.4 ± 1.7ng/mL; $P = 0.81$), INS (65.2 ± 0.7ng/mL; $P = 0.69$) and LEP (2.8 ± 0.2ng/mL; $P = 0.30$), but HO cows had ($P < 0.01$) greater concentrations of GH (7.4 ± 0.4 vs 5.1 ± 0.4 ng/mL) and cortisol (9.4 ± 0.8 vs 7.1 ± 0.8ng/mL) than MS cows. The greater decrease in prepartum DMI for HO cows may have caused the increased levels of GH and COR. The similar IGF1 concentration among HO and MS cows, despite HO cows having greater GH concentration and similar expression of GH1RA mRNA to MS cows, may suggest a more pronounced decoupling of the somatotropic axis in purebred HO cows.

Key Words: transition cow, somatotropic axis, crossbreeding

M215 Effect of subclinical mastitis and postpartum uterine disease on expression of estrous behavior in cows. Y. Lavon[1], M. Kaim[2], G. Leitner[3], H. Voet[4], and D. Wolfenson*[4], *[1]Israel Cattle Breeders Association, Caesarea, Israel, [2]Agricultural Research Organization, Bet-Dagan, Israel, [3]The Veterinary Institute, Bet Dagan, Israel, [4]Faculty of Agriculture, Food and Environment, The Hebrew University, Rehovot, Israel.*

The objective of this study was to determine the effect of mammary and uterine diseases on manifestation of estrus in cows. Cows (n = 1247) from 50 to 130 d of lactation, in the summer and winter, were synchronized with 2 $PGF_{2\alpha}$ injections administered 14 d apart (before 1st AI); 36 h after the 2nd injection, cows were checked for estrus 4 times daily for 6 d. Uterine diseases were recorded during the first week postpartum. Cows were sorted to uninfected control or chronic (most likely subclinical) mastitic cows by somatic cell count in the monthly milk samples taken before and after 2nd $PGF_{2\alpha}$ injection. Body condition score difference (BCS-diff) between calving and 2nd $PGF_{2\alpha}$ injection, and milk yield were recorded. Factors relevant to manifestation of estrus were selected by stepwise logistic regression. As some interactions were detected, separate logistic regressions were performed by season and parity. In the winter, the percentage of cows not expressing estrus increased, compared with controls (26.6 vs. 12.6%; odds ratio [OR] 2.4, $P < 0.002$), in multiparous cows with uterine diseases. Similarly, a high BCS-diff tended to increase the rate of cows not expressing estrus (21.3 vs. 12.1%; OR 1.93, $P < 0.06$). In primiparous cows, subclinical mastitis as well as high milk yield increased the percentage of cows not expressing estrus (34.7 vs.14.3%; OR 3.4, $P < 0.02$, and 24.1 vs. 9.4%; OR 3.2, $P < 0.03$, respectively). In the summer, an increased percentage of cows not expressing estrus (24.2 vs. 16.9%; OR 1.7, $P < 0.05$) was recorded in multiparous cows with high BCS-diff. In primiparous cows, a subclinical mastitis by

uterine disease interaction was detected, showing a higher rate of cows not expressing estrus (41.4 vs. 17.2%; OR 3.9, $P < 0.05$). Results indicate a higher risk for not expressing estrus, which is parity- and season-dependent, in cows with subclinical mastitis and postpartum uterine diseases. The above findings could result in increased days open in dairy herds, and could be associated with disease induction of anestrus, or with disruption of the endocrine process associated with expression of estrous behavior.

Key Words: estrus, mastitis, cows

M216 Dietary protein:carbohydrate ratio affects glucose tolerance and oxidation in pregnant gilts. C. C. Metges,* S. Görs, I. Lang, K.-P. Brüssow, C. Rehfeldt, and W. Otten, *Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany.*

Low as well as high dietary protein:carbohydrate ratios during pregnancy cause changes of maternal metabolism and body composition, and intrauterine growth restriction (IUGR) in the pig (Rehfeldt et al., J Anim Sci 2011,189:329–341; Metges et al., PLoS ONE 2012, in press). We performed intravenous glucose tolerance tests (IVGTT) with labeled glucose to further characterize maternal constraints of glucose metabolism in late pregnant gilts. Twenty-seven German Landrace gilts were fed diets with low (6.5%, L), adequate (12%, A), or high (30%, H) protein levels (n = 9/group) made isoenergetic by adjusted carbohydrate content throughout pregnancy. Gilts were fitted with jugular catheters and an IVGTT was performed on d 90 of pregnancy. After overnight fast 0.5 g/kg BW glucose and 0.5 mg/kg U^{13}C-glucose were administered i.v. Blood samples were analyzed for plasma glucose (GLC), insulin (INS), glucagon (GCG) concentrations, and U^{13}C-glucose and blood $^{13}CO_2$ enrichments by mass spectrometry. Areas under curve (AUC) and cumulative $^{13}CO_2$ appearance in blood ($^{13}CO_2$cum) were calculated. Diet effects were evaluated by PROC MIXED of SAS. L gilts had a lower BW as well as a lesser basal INS concentration and INS-AUC than controls, whereas H gilts showed a higher basal GCG level and GLC-AUC (Table 1). In H gilts lower $^{13}CO_2$cum suggested a lower GLC oxidation (Table 1). Apparently higher and lower INS sensitivity in L and H, respectively, might be explained by lower pancreatic INS secretion caused by protein deficiency, and reduced GLC oxidation due to GLC sparing, respectively. Thus, L and H diets might cause altered feto-maternal glucose interaction possibly associated to the observed offspring IUGR in these groups. Supported by Deutsche Forschungsgemeinschaft ME1420/8–1.

Table 1. Body weight and glucose metabolism analyzed by IVGTT in pregnant gilts fed L, A, or H diet throughout pregnancy

	Diet			
Item	L	A	H	*P*-value
BW, kg	174^{a}	196^{b}	197^{b}	0.017
GLC_{basal}, mmol/L	5	5	5	0.970
INS_{basal}, μU/mL	7^{a}	11^{b}	9^{a,b}	0.077
GCG_{basal}, ng/L	44^{a}	46^{a}	65^{b}	0.013
GLC-AUC, mmol/L × min	134^{a}	136^{a}	167^{b}	0.001
INS-AUC, μU/mL × min	724^{a}	1336^{b}	1035^{a,b}	0.021
$^{13}CO_2$cum, APE × min	0.176^{a}	0.165^{a}	0.128^{a}	0.012

Key Words: high protein, glucose, pregnancy