Physiology and Endocrinology II

T206 Quantitative bioluminescence imaging of functional estrogen receptor activity within intact porcine ovarian follicles in vitro. S. Jung* and S. T. Willard, *Mississippi State University, Mississippi State.*

Activated estrogen receptors (ER) in response to estrogen bind to specific sequences (estrogen response elements; ERE) to induce transcription. The objective of this study was to evaluate whether the estrogen induced ER binding activity in granulosa cells of antral ovarian follicles can be detected by bioluminescence imaging in vitro, and correlated to estrogen concentrations in follicular fluid. In this study, we used lipid–mediated gene transfer and an ERE–luc reporter gene (which consisted of 3 tandem repeats of EREs upstream from the luciferase gene) to transfect granulosa cells within intact follicles. When the endogenous functional and activated ERs bind to the ERE–luc sequences within the transfected granulosa cells, the expression of luciferase is enacted for detection. A total of n = 58 follicles between 4.2 to 9.4 mm in diameter were dissected from the ovaries. DNA–lipid complexes were formed at a DNA (μg): lipid (μl) ratio of 1:3, by adding FuGene 6 in PBS to 3 μg of ERE–luc DNA and injected into each follicle using a microinjector. The follicles were cultured individually with αMEM and 45% O_2; 50% N_2; 5% CO_2 at 39°C. After 20 h post–transfection, the luminescence from each follicle was detected using an IVIS 100 imaging system. Each follicle was imaged with 10 min exposure and signal intensity was reported (and normalized) as mean ± SEM of photons per second (p/s). Estradiol concentrations of follicular fluid were measured by radioimmunoassay in each follicle. Regression coefficients were determined, and a *P*–value of <0.05 was considered significant. Concentrations of estradiol in follicular fluid significantly increased as follicle size increased (r = 0.607; $P < 0.05$). Estradiol total content in each follicle was positively correlated with ERE-driven luciferase expression level of follicles (r = 0.39; $P < 0.05$). These results demonstrate the initial development of a new methodology for measuring functional and ligand activated estrogen receptor activity within intact porcine ovarian follicles in vitro using bioluminescence imaging methodologies.

Key words: bioluminescence imaging, estrogen receptor, porcine ovarian follicles

T207 Propionate increases mitochondrial phosphoenolpyruvate carboxykinase mRNA in Madin-Darby bovine kidney epithelial cells. S. I. Tindell*, S. L. Koser, and S. S. Donkin, *Purdue University, West Lafayette, IN.*

Phosphoenolpyruvate carboxykinase (PEPCK) is a rate determining enzyme for gluconeogenesis that is present in both a cytosolic (PEPCK-C) and a mitochondrial (PEPCK-M) form in bovine liver. Considerable research has documented the importance and control of PEPCK-C however there is little information available on hormonal and nutritional control of bovine PEPCK-M. The objectives of this study were to clone the promoter region of bovine PEPCK-M, to determine the transcription factor binding sites within the proximal promoter region, and determine the response of bovine PEPCK–M to nutrients and hormones. Genomic DNA isolated from liver of lactating dairy cows was used to clone a 906 nucleotide (nt) sequence that includes promoter specific elements and the first few bases of the coding sequence for bovine PEPCK–M promoter. Computer assisted analysis of this sequence revealed that all the elements necessary for promoter activity are contained within 896 nt relative to the transcription start site and there is no sequence similarity between bovine PEPCK-M and PEPCK-C promoters. The direct individual effects of 1 μ*M* dexamethasone, 10 μ*M* Wy14643, 100 n*M* insulin, 4.5 n*M* somatotropin, 1 μ*M* cAMP, 2 m*M* propionate, 2 m*M* acetate, 2 m*M* butyrate, or 2 m*M* lactate for 24 h on bovine PEPCK-M mRNA were determined in Madin-Darby bovine kidney epithelial (MDBK) cells. The data indicate that expression of PEPCK-M mRNA is increased ($P < 0.05$) by propionate (1.62 vs. 2.94 ± 0.19; control vs. propionate, respectively) but there were no effects ($P > 0.05$) of the other hormones and metabolites tested. The data would suggest that PEPCK-M may be regulated by propionate supply which may serve to enhance the capacity for mitochondrial phosphoenolpyruvate flux and gluconeogenesis.

Key words: PEPCK, gluconeogenesis, gene

T208 Staining bovine sperm for sex-sorting: Concentration effects of seminal plasma, sperm and Hoechst 33342. C. A. Burroughs*[1], J. K. Graham[1], R. W. Lenz[2], and G. E. Seidel[1], *[1]Colorado State University, Fort Collins, [2]Sexing Technologies Inc., Navasota, TX.*

We investigated various combinations of sperm, seminal plasma, and Hoechst 33342 (H33342) concentrations during staining of bull sperm to improve sex-sorting of sperm. Ejaculates from 11 bulls with at least 60% motile and 70% morphologically normal sperm were collected by artificial vagina on 2 different days. Semen was centrifuged at 1,000 × *g* for 15 min to separate sperm from seminal plasma, which was then clarified by additional centrifugation (2,000 × g for 10 min). Sperm were resuspended in TALP (pH 7.4) at 160 × 10^6 or 240 × 10^6 sperm per ml with 0 or 10% seminal plasma. H33342 was added (final concentrations of 49, 65 or 81 μ*M*) followed by incubation for 45 min at 34.5°C. An equal volume of TALP (pH 5.5) containing red food dye was added; sperm were sorted using a MoFlo SX (Dako, Denmark) flow cytometer and analyzed for % live-oriented cells, X sort rate, % dead (sperm membrane permeable to red dye), and splitability (peaks to valley ratio - degree of separation of X and Y populations). Overall, staining with 0% seminal plasma resulted in higher % live-oriented cells (57.4% vs. 53.7%) and a faster sort rate (3.60 × 10^3sperm per s vs. 3.28 × 10^3sperm per s) compared with 10% seminal plasma (both $P < 0.01$). There was an interaction between sperm concentration and H33342 concentration for ability to separate X and Y populations and for sort rate (Table 1). Using 65 μ*M* H33342 was sufficient to optimally stain 160 × 10^6 sperm per ml, while 240 × 10^6 sperm required 81 μ*M* H33342 to reach similar splitability and sort rates. The optimal combination for staining bull sperm was 0% seminal plasma, 160 × 10^6 sperm per ml, and 65 μ*M* H33342.

Table 1. Sperm and H33342 concentration responses averaged over 0 and 10% seminal plasma

Sorting Parameter	Sperm Conc (10^6)	49μM H33342	65μM H33342	81μM H33342
% live-oriented cells	160	55.0^{ab}	57.1^{b}	56.8^{b}
% live-oriented cells	240	52.2^{a}	55.5^{ab}	56.9^{b}
X Sort Rate (10^3 sperm/sec)	160	3.41^{bc}	3.82^{d}	3.76^{cd}
X Sort Rate (10^3 sperm/sec	240	2.67^{a}	3.32^{b}	3.66^{bcd}
Splitability %	160	29.2^{c}	39.6^{d}	37.7^{cd}
Splitability %	240	5.0^{a}	16.7^{b}	37.2^{cd}

Means without common superscripts differ ($P < 0.05$).

Key words: sex-sorting, sperm, bull

T209 Effect of feed restriction on reproductive and metabolic hormones in dairy cows. H. Gencoglu[1,2], A. Nascimento[1], K. Hackbart[1], L. F. Ferraretto*[1], F. Dalla Costa[1], J. Guenther[1], R. Meyer[1], R. D. Shaver[1], and M. C. Wiltbank[1], *[1]Department of Dairy Science, University of Wisconsin-Madison, Madison, [2]Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, University of Uludag, Bursa, Turkey.*

The objective of this trial was to evaluate the effects of feed restriction (FR) on serum concentrations of glucose, nonesterified fatty acids (NEFA), progesterone (P4), insulin and follicle-stimulating hormone (FSH), and milk production in dairy cows. Eight pregnant multiparous Holstein cows, 114 ± 14 d in milk and 685 ± 39 kg body weight (BW) at trial initiation were randomly assigned to a replicated 4 × 4 Latin Square design with 14-d periods. During the first 8 d of each period, all cows were fed for ad libitum feed intake. On d 9 through d 12 of each period, the feed restricted groups were fed 25 and 50% of the average daily dry matter intake (DMI) based on previous 8-d ad libitum feeding. The 4 dietary treatments were: ad libitum (AL), 25% feed restriction (25FR), 50% feed restriction (50FR), and 50% feed restriction produced by adding 50% wheat straw to diet (50FRS). Blood samples were collected before feeding from jugular vein at 0700, 1500, and 2300h on d 8 and continuing through d 14 of each period. On d 12 of each period, blood samples were collected before and at 60, 120, 180, 240, 300, 360, 420, and 480 min after morning feeding. The conventional TMR compared with the TMR with straw was higher in crude protein (15.1 vs. 10.8%) and starch (26.8 vs. 17.0%) and lower in NDF (32.1 vs. 50.5%) concentrations. Cows fed AL had 6.7, 12.7, and 11.3 kg/d greater DMI than cows fed 25FR, 50FR, and 50FRS, respectively ($P < 0.0001$). Likewise milk production and glucose concentration followed the same linear decrease ($P < 0.0001$). Serum concentrations of insulin (μIU/mL) were lower ($P < 0.0001$) for cows fed 50FR (8.27) and 50FRS (6.24) than cows fed AL (16.65) and 25FR (11.16). Furthermore, plasma NEFA concentrations increased linearly ($P < 0.0001$) followed by linear BW ($P < 0.0003$) and BCS ($P < 0.02$) loss. In addition, serum P4 concentrations were lower for cows fed AL than cows fed 50FRS and 25FR ($P < 0.01$). FSH concentrations did not differ among treatments ($P > 0.10$). The current trial suggests that, FR results in lower glucose and insulin levels, fat mobilization, BW and BCS loss, and increased circulating P4 concentration.

Key words: feed restriction, reproductive hormones, dairy cows

T210 Fetal growth and maternal body condition following melatonin supplementation in adequately fed or nutrient restricted ewes. C. O. Lemley*, A. M. Meyer, L. E. Camacho, T. L. Neville, D. J. Newman, J. S. Caton, and K. A. Vonnahme, *North Dakota State University, Fargo.*

Low birth weight offspring often exhibit poor growth performance and lower daily rates of gross energy accretion. Using a maternal nutrient restriction model, we examined fetal growth following melatonin supplementation as a 2 × 2 factorial design. At d 50 of gestation 16 primiparous ewes were allocated to receive 100% (adequate; ADQ) or 60% (restricted; RES) of nutrient requirements and were supplemented daily with 5 mg of melatonin (MEL) or no melatonin (CON) until d 90. All ewes were exposed to a 12:12 light dark cycle with lights on at 0700 h and off at 1900 h. Ewes were fed a pelleted ration with or without melatonin 5 h before the end of the photophase (1400 h). Serum melatonin was determined over a 24 h period using an ELISA kit. Maternal BCS, back fat, and loin muscle area were examined at 50 and 90 d of gestation, while fetal growth was measured at d 48, 50, 60, 70, 80 and 90 of gestation using ultrasonography. The melatonin feeding schedule resulted in a melatonin treatment by h interaction ($P < 0.01$), where serum melatonin concentrations peaked at 1500 h in MEL. Melatonin concentrations remained elevated in MEL versus CON until the scotophase (1900 h). A gestational d by nutritional plane interaction ($P < 0.01$) was observed for maternal BCS, which was similar at d 50 and decreased in RES vs. ADQ at d 90. Moreover, a nutritional plane by melatonin interaction ($P < 0.05$) was observed for maternal BCS, where MEL-ADQ had a greater BCS vs. CON-ADQ and MEL-RES had a lower BCS vs. CON-RES. Maternal back fat tended ($P < 0.1$) to decrease while loin muscle area increased ($P < 0.05$) from d 50 to 90. All fetal growth parameters listed below increased ($P < 0.01$) with gestational day. Biparietal distance was similar between treatments ($P > 0.3$), while abdominal diameter tended ($P < 0.1$) to be larger in MEL vs. CON fetuses. Fetal kidney length and width were increased ($P < 0.05$) in MEL vs. CON. In conclusion, dietary melatonin appears to increase fetal kidney development, which may have direct implications in improving offspring performance.

Key words: fetal growth, melatonin, pregnancy

T211 Effects of realimentation after nutrient restriction during early to mid-gestation on uterine blood flow in pregnant beef cows. L. E. Camacho*[1,2], C. O. Lemley[1,2], B. W. Neville[1,2], C. R. Dahlen[1,2], G. P. Lardy[1,2], and K. A. Vonnahme[1,2], *[1]Center for Nutrition and Pregnancy; Department of Animal Sciences, Fargo, ND, [2]North Dakota State University, Fargo.*

During pregnancy, dramatic changes occur in the maternal cardiovascular system alongside prominent growth and development of the uteroplacental vascular bed. Pregnancy is associated with increases in cardiac output and uterine blood flow and a fall in systemic vascular resistance. We hypothesized the duration of nutrient restriction would impact uterine blood flow and vascular resistance. Moreover, we further hypothesized that upon realimentation, blood flow would ultimately surpass blood flow in control animals. Our objectives were to examine the effects of maternal realimentation after nutrient restriction during early to mid-gestation on uterine blood flow. Multiparous beef cows (n = 17) were assigned randomly to one of 3 treatments: 1) 100% NRC requirements from d 30 to 226 of gestation (CCC; n = 6); 2) 60% NRC from d 30 to 85, thereafter being re-alimented to 100% NRC to d 226 (RCC; n = 5); 3) or receive 60% NRC from d 30 to 140, thereafter being re-alimented to 100% NRC to d 226 (RRC; n = 6). Cows were individually fed once daily in a Calan gate system at 1500 h. Baseline measurements were obtained via Doppler ultrasonography at 0700 h on d 30 and every 14 d thereafter until d 226. Measurements include maternal heart rate (HR), uterine blood flow (BF), pulsatility index (PI), and resistance index (RI). Percentage change of each measurement from the initial measurement on d 30 was calculated. There was a treatment by day interaction ($P = 0.01$) for HR percentage change, where RCC reached a greater HR at d 156 compared with CCC and RRC was intermediate. Uterine BF percentage change was not affected by treatment ($P > 0.72$). However, there were treatment by day interactions ($P \leq 0.02$) for PI and RI where RRC cows had a greater reduction in resistance than CCC cows after d 140. In summary, although maternal realimentation after nutrient restriction did not affect uterine BF percentage change, maternal diet affected PI and RI percentage change. Further investigations in uterine and placental vascular reactivity may help explain the differences observed in resistance indices.

Key words: nutrient restriction, pregnancy, uterine blood flow

T212 Effects of propiogenic supplements on serum concentration of insulin and progesterone in nonlactating cows: I. Monensin. T. Leiva[1], M. Barbosa[1], R. O. Rodrigues[1], R. F. Cooke[2], and J. L. M. Vasconcelos*[1], *[1]UNESP – Faculdade de Medicina Veterinária e Zootecnia, Botucatu, SP, Brazil, [2]Oregon State University – Eastern Oregon Agricultural Research Center, Burns.*

Insulin has been shown to increase circulating progesterone (P4) concentrations by stimulating ovarian steroid synthesis and alleviating hepatic steroid catabolism. Therefore, the objective was to determine if monensin is a nutritional alternative to increase circulating concentrations of insulin and consequently P4 in forage-fed cows. Fifteen nonlactating ovariectomized Gir × Holstein cows were ranked by BW and BCS and randomly assigned to receive, in a crossover design, 0.1 kg/d of corn in addition to 2 g/d of kaolin (control) or 0.2 g/d of monensin Na (MO). During the study, cows were maintained in *Brachiaria brizantha* pastures, and received treatments individually in a feed bunk every morning. Each period contained 21 d, where the initial 5 d served as adaptation when MO and control cows received 0.1 kg/d of corn in addition to, respectively, 0.1 g of monensin or 1 g of kaolin. Within each period, cows received a previously used intravaginal P4 device (CIDR, originally containing 1.9 g of P4) on d 0, which was replaced by a new CIDR at the end of the adaptation period (d 5). Blood samples were collected on d 12, 13, 19, and 20 immediately before (0 h) and 6, 12, 18, and 24 h relative to treatment feeding. On d 12 and 19 cows had access to pastures between samplings, whereas on d 13 and 20 cows were maintained in the working facility without access to forage. Blood samples were analyzed for serum concentrations of insulin and P4. Within samples collected when cows had no access to pastures, a treatment × time interaction was detected ($P = 0.05$) for P4 and insulin. Cows receiving MON had greater ($P < 0.01$) P4 concentrations compared with control at h 18 (2.6 vs. 2.1 ng/mL, respectively) but reduced insulin concentrations at h 0 (4.9 vs. 6.9 μIU/mL, respectively; $P < 0.01$) and h 6 (3.3 vs. 4.7 μIU/mL, respectively; $P = 0.07$). No treatment differences were detected for insulin and P4 concentrations when cows had access to pastures. In conclusion, supplemental monensin reduced serum insulin concentration but increased, by alternative physiological mechanisms, serum P4 concentrations in feed restricted cows.

Key words: monensin, progesterone, insulin

T213 Effects of propiogenic supplements on serum concentration of insulin and progesterone in nonlactating cows: II. Propylene glycol. A. M. L. Madureira[1], M. A. S. Borges[1], R. O. Rodrigues[1], R. F. Cooke[2], and J. L. M. Vasconcelos*[1], *[1]UNESP – Faculdade de Medicina Veterinária e Zootecnia, Botucatu, SP, Brazil, [2]Oregon State University – Eastern Oregon Agricultural Research Center, Burns, OR, USA.*

Insulin can increase circulating progesterone (P4) concentrations by stimulating ovarian synthesis and alleviating hepatic catabolism. Therefore, the objective was to determine if propylene glycol (PPG) supplementation increases circulating concentrations of insulin and thus P4 in forage-fed cows under negative or positive nutritional balance. From d −7 to d 14, 15 nonlactating ovariectomized Gir × Holstein cows were maintained in a *Brachiaria brizantha* pasture with proper forage availability and received 2 kg of concentrate/d. From d 15 to d 42, cows were move to a *B. brizantha* pasture with reduced forage availability without supplementation. Cows were inserted with an intravaginal device containing 1.9 g of P4 on d −7, which was replaced every 14 d during the study. Mean cow ADG was 0.57 ± 0.1 kg/d from d −7 to d 14, and −0.37 ± 0.1 kg/d from d 15 to 28. On d 6 of the study, cows were ranked by BW and BCS and assigned to receive a drench with PPG or water (control) at 2.5 mL/kg of $BW^{0.75}$. Blood was collected, relative to treatment application (h 0), at −0.5, 0, 0.5, 1, 2, 3, 4, 5, 6, and 7 h for determination of serum insulin and P4 concentrations. Cows received concentrate and returned to pastures in-between samplings. On d 7, cows received the converse treatment in a crossover design and were sampled similarly as in d 6. On d 13 and 14, 20 and 21, and 27 and 28, cows were again assigned to the same crossover and sampling schedule, but were not fed during the collection period. When cows were on adequate nutritional status, independently if feed restricted or not, PPG cows had greater ($P < 0.01$) insulin but reduced ($P < 0.01$) P4 concentrations compared with control cows (8.5 vs. 4.3 μIU/mL of insulin and 1.89 vs. 2.04 ng/mL of P4, respectively). However, when cows were on negative nutritional balance, PPG cows had greater ($P < 0.01$) insulin and P4 concentrations compared with control cows (7.0 vs. 3.4 μIU/mL of insulin and 2.16 vs. 2.03 ng/mL of P4, respectively). In conclusion, PPG supplementation increased circulating insulin and P4 concentrations only in cows under negative nutritional balance.

Key words: propylene glycol, insulin, progesterone

T214 Follicular fluid composition in cyclic Hereford cows supplemented with rice bran in grazing conditions. L. Veloz[1,2], M. E. Trobo[1,2], C. García Pintos[1,2], C. Viñoles[2], and M. Carriquiry*[1], *[1]School of Agronomy, UdelaR, Montevideo, Uruguay, [2]National Research Institute for Agriculture, Tracuarembó, Uruguay.*

The aim of this study was to evaluate the effect of short-term supplementation with rice bran before initiation of the breeding period on follicular fluid composition of beef cows grazing native pastures. Fifteen non-pregnant nonlactating Hereford cows (492 ± 6 kg BW and 5.6 ± 0.1 BCS, scale 1–8) were randomly allocated to 2 groups: control, non-supplemented (CON, n = 7) and supplemented (SUP, n = 8). The supplement (2.5 kg/cow of whole rice bran; 90.3%DM, 10%CP, 9%EE, 14%NDF) was fed daily for 23 d. All cows grazed on native pasture. Cows were synchronized with 3 prostaglandin (PG) injections 11 d apart. Thirty-six hours after the last PG injection, cows were castrated and all follicles ≥5mm were dissected and follicular fluid was aspirated for metabolite and hormone analyses. Means from a mixed analyses were considered to differ when $P < 0.05$. Follicular size did not differ between cow groups and averaged 10.2 ± 1.0 mm. Estrogen (19750 vs. 10009 ± 16570 pmol/L) and progesterone (135.7 vs. 108.5 ± 29.9 ng/mL) concentrations as well as estrogen/progesterone ratio (229.9 vs 273.2 ± 188.1) in follicular fluid were not different between SUP and CON cows. Similarly, glucose, nonesterified fatty acids, and cholesterol concentrations in follicular fluid (74.3 vs. 81.1 ± 8.5 mg/dL, 0.87 vs. 0.82 ± 0.05 mmol/L, 111.0 vs. 95.3 ± 10.1 mg/dL, SUP vs. CON, respectively) were not affected by nutritional treatment. Glucose concentrations increased and cholesterol concentrations tended ($P = 0.09$) to increase with follicle size (3.8 ± 0.9 mmol/L and 2.9 ± 1.6 mmol/L for each mm of increase in follicle size, respectively). Results susggest that short-term supplementation did not affect follicular fluid composition in cyclic beef cows in good BCS on grazing conditions

Key words: cattle, nutrition, ovary

T215 Capability of a new or once-used CIDR to develop persistent follicles and the capability of additional progesterone for persistent follicle turnover in replacement beef heifers. G. H. L.

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Two experiments evaluated the capability of new or once-used (for 7 d) CIDR to develop a persistent follicle and we hypothesized that high concentrations of progesterone with an additional CIDR would induce turnover of the persistent follicle. In Exp. 1, 59 crossbred heifers received a new (n = 29) or once-used (n = 30) CIDR from d 0 to 11. On d 8 heifers assigned randomly to receive either an injection of saline (n = 19), a second new CIDR from d 8 to 11 (n = 20), or follicular aspiration of all follicles ≥ 5mm (n = 20) resulting in a 2 × 3 arrangement of treatments. Transrectal ultrasonography was used daily to monitor follicular development and follicle turnover. When the dominant follicle had failed to turnover by d 8 the follicle was deemed to be persistent. Follicle turnover was defined as a dominant follicle that was present on d 8 that had disappeared by d 11. Heifers receiving the once-used CIDR (83%) tended ($P = 0.10$) to develop more persistent follicles than those receiving the new CIDR (64%). For treatments on d 8, 100% of heifers receiving follicular aspiration had follicle disappearance by d 11 which was greater ($P < 0.05$) than those receiving a second new CIDR (69%) which was greater ($P < 0.05$) than those receiving saline (32%). In Exp. 2, 41 heifers received a once-used CIDR from d 0 to 13 and all follicles ≥ 5mm were aspirated on d 0. On d 10 heifers were assigned randomly to one of 2 treatments: 1) injection of saline (Sal; n = 21); or 2) new additional CIDR insert for 3 d (CIDR; n = 20). Blood samples and ultrasonography were performed daily from d −10 to 16 to evaluate concentrations of progesterone (P4) and monitor follicle development and turnover. Concentrations of P4 were greater ($P < 0.01$) for CIDR than Sal at 4, 8, and 72 h after treatment on d 10. However, the ability of the CIDR (58%) and Sal (64%) treatments were similar. We conclude that a once-used CIDR develops more persistent follicles than new CIDR, whereas follicular aspiration was more effective at follicle turnover than an additional CIDR or saline treatments.

Key words: persistent follicle, beef heifer, progesterone

T216 Influence of CIDR-based protocols associated with supplementation of calcium soap on reproductive performance of Nellore cows. M. V. Biehl*[1], A. V. Pires[1,2], I. Susin[2], D. D. Nepomuceno[2], J. R. S. Gonçalves[4], L. H. Cruppe[3], F. M. Da Rocha[1], and M. L. Day[3], *[1]University of Sao Paulo, Pirassununga, SP, Brazil, [2]University of Sao Paulo, Piracicaba, SP, Brazil, [3]Ohio State University, Columbus, [4]Experimental Station Georgina Hildegard von Pritzelwitz, Londrina, PR, Brazil.*

The aim of this study was to compare reproductive performance of lactating Nellore cows (n = 264) submitted to estrus synchronization using either 7 or 9 d CIDR+Estradiol Benzoate (EB) program and 3 mineral supplements. Cows were blocked according to BW (428.5 ± 50.4 kg) and BCS (2.84 ± 0.23, 1 to 5) in a 2x3 (2 protocols and 3 supplements) factorial arrangement. The supplement treatments were mineral mixture (MM); MM+Megalac E+Citrus Pulp (CP) (MEG); MM+Kaolim+CP (KAO). At the beginning of the experiment, cows were 55 ± 0.36 d postpartum. Supplementation treatments began 30 d before the initiation of the synchronization program and were terminated 30 d after timed AI. Blood samples for progesterone analysis were collected 10 d before and at CIDR insertion to classify cows as cyclic. The CIDR was inserted with a 2 mg injection of EB and it was removed either 7 or 9 d later. All cows received 25 mg PGF2α (Lutalyse) 48 h before CIDR withdrawal and 300 IU eCG (Novormon) and 0.6 mg estradiol cipionate (ECP) at CIDR removal. Treatments were defined as follow: 7dMM (n = 42; e.g., 7d CIDR and MM supplementation), 7dKAO (n = 46), 7dMEG (n = 47), 9dMM (n = 40), 9dKAO (n = 46), and 9dMEG (n = 43). Estrus was detected for 5 d after CIDR removal and timed AI was performed 50 h after CIDR withdrawal. Second estrus detection was performed 16 d after timed AI for 6 d and AI submitted. Pregnancy diagnosis was performed by US 60 d after timed AI and at the end of the breeding season. At CIDR insertion, 85% (224/263) of cows were in anestrus. Estrus was detected in 52.8% (139/263) of the cows and time to estrus (43.5 ± 5.7 h after CIDR removal) did not differ among treatments. Timed AI pregnancy rates did not differ between supplements MM, 51; KAO, 69.5; MEG, 54.7% or with 7 d, 57.1, 58.7 and 52.5%, 9 d of CIDR treatment. Pregnancy rates were not different at the end of the breeding season. In conclusion, supplementation with Megalac E or Kaolin, did not improve reproductive performance in this study. In addition, timed AI pregnancy rates did not differ between CIDR treatments of 7 or 9 d in the CIDR-EB program used in the present study.

Key words: Megalac E, estrus, cows

T217 Effect of dietary conjugated linoleic acid on reproduction and tissue responses in dairy cows. G. Esposito*[1,2], A. Schneider[3], V. A. Absalón Medina[2], S. H. Pelton[2], and W. R. Butler[2], *[1]University of Naples Federico II, Naples, Italy, [2]Cornell University, Ithaca, NY, [3]Universidade Federal de Pelotas, Pelotas, RS, Brazil.*

Feeding rumen-protected isomers of conjugated linoleic acid (CLA) to early lactation dairy cows reportedly improves fertility by reducing the postpartum interval to first ovulation and enhancing circulating IGF-I levels. CLA supplementation increases high density lipoprotein (HDL) cholesterol in mice. Also, in vitro studies with bovine granulosa and luteal cells have shown that HDL and LDL promoted granulosa cell viability and stimulated IGF-I production. The objectives of this study were to examine ovarian follicles, corpora lutea, and liver tissues for effects induced by dietary CLA supplementation (top-dressed once daily from 15 d before expected calving to 65 DIM). Twenty-four lactating Holstein cows were assigned to 2 treatments: control and CLA diet. Milk production and DMI were recorded daily and milk components every 10 d. At 26 DIM ovulation was synchronized with a vaginal controlled internal drug-releasing device and injection of GnRH followed by an injection of PGF2α after one week. Blood samples were collected every 4 d. Follicular fluids, from follicles larger than 9mm, were collected every 8 d from 34 DIM. Plasma and follicular fluid samples were analyzed for estradiol (E2), progesterone, IGF-I, cholesterol, LDL, and HDL. At 56 DIM ovulations were synchronized in all cows. The resulting CL and the liver were biopsied at 64 and 65 DIM, respectively. Tissues were analyzed for gene expression of IGF-I, GHR, VEGFA and ANGPT2 or for PPARα, IGF-I, GHR, PC and PECK, respectively. In the CLA treated cows milk fat production was lower ($P < 0.05$) and energy balance was improved ($P < 0.05$). No differences between the 2 groups were observed for milk production. CLA-supplemented cows tended to have higher plasma concentrations of E2 and LDL ($P < 0.1$) and plasma concentrations of IGF-I were higher ($P < 0.001$). No differences were observed for the mRNA expression in tissues. This study confirms the improvement of plasma IGF-I levels, but dietary CLA did not alter plasma lipoprotein concentrations in cows as had been shown in mice. Moreover, CLA supplementation failed to alter gene expression in the tissues examined.

Key words: CLA, ovarian follicles, corpus luteum, liver

T219 Endocrine and ovarian parameters associated with increased fertility after resynchronized timed artificial inseminations in lactating dairy cows. J. O. Giordano*, M.C. Wiltbank, and P. M. Fricke, *Department of Dairy Science, University of Wisconsin, Madison.*

Lactating dairy cows failing to conceive to a previous timed AI (TAI) were resynchronized to receive TAI either using Double-Ovsynch (DO, Pre-Resynch, GnRH–7 d–PGF–3 d–GnRH, 7 d later Breeding-Resynch, GnRH–7 d–PGF–56 h–GnRH–16 h–TAI) or Ovsynch initiated 32 d after TAI (D32, GnRH–7 d–PGF–56 h–GnRH–16 h–TAI). All DO cows received the first GnRH injection of Pre–Resynch 22 d after TAI, and cows (n = 981) diagnosed not pregnant using ultrasonography (US) 29 d after TAI continued the protocol. All D32 cows received GnRH 32 d after TAI, and cows (n = 956) diagnosed not pregnant using transrectal palpation 39 d after TAI continued the protocol. In subgroups of DO and D32 cows, the proportion of cows with a functional corpus luteum (CL) at the first GnRH of Breeding-Resynch and D32 protocols (G1), CL regression after PGF, and ovulation to the last GnRH (G2) of both protocols was determined using US and serum progesterone (P4). Pregnancy diagnosis was performed 29 d after TAI using US. Overall, P/AI was greater ($P < 0.01$) for DO vs. D32 cows (38.7 vs. 30.0%). The proportion of cows with high (HP4) vs. low P4 (LP4; cutoff 0.5 ng/mL) at G1 was greater ($P < 0.01$) for DO vs. D32 cows [86.7 (368) vs. 62.5% (375)]. At 29 d after TAI, cows with HP4 at G1 had greater ($P < 0.01$) P/AI than LP4 cows [33.9 (554) vs. 19.5% (190)], and cows that ovulated to G1 had greater ($P < 0.01$) P/AI than cows that did not [32.6 (285) vs. 28.3% (466)]. Synchronization rate (HP4 at PGF, LP4 at G2, and ovulation to G2) was greater ($P < 0.01$) for DO vs. D32 cows [71.8 (223) vs. 50.5% (210)]. At 29 d, P/AI were similar between treatments for synchronized [D32 = 43.4 (106) vs. DO = 43.1% (160)] and non-synchronized [D32 = 9.6 (104) vs. DO = 4.8% (63)] cows. Similarly, synchronization rate was greater ($P < 0.01$) for cows with HP4 at G1 [69.2 (321) vs. 37.1% (105)] and for cows that ovulated to G1 [65.6 (151) vs. 58.9% (275)]. We conclude that resynchronized lactating cows that had high P4 at G1 and that ovulated after G1 had an increased synchronization rate resulting in increased fertility to TAI. Supported by Hatch project WIS01171

Key words: double-Ovsynch, resynchronization

T220 Use of the CIDR+EB synchronization program in prepubertal Nellore heifers. M. V. Biehl*[1], A. V. Pires[1,2], I. Susin[2], L. H. Cruppe[3], D. D. Nepomuceno[2], J. R. S. Gonçalves[4], F. M. Da Rocha[1], and M. L. Day[3], *[1]University of Sao Paulo, Pirassununga, SP, Brazil, [2]University of Sao Paulo, Piracicaba, SP, Brazil, [3]Ohio State University, Columbus, [4]Experimental Station Georgina Hildegard von Pritzelwitz, Londrina, PR, Brazil.*

The objective of this study was to compare reproductive performance of prepuberal Nellore heifers (n = 407) using either a 5, 7, 9 or 11 d CIDR treatment with an estradiol benzoate (EB)-based synchronization program. Heifers were blocked to treatments based on body weight (282 ± 18 Kg) and body condition score (2.63 ± 0.21, scale of 1 to 5) in a 4 × 2 factorial arrangement. All animals received 2 mg EB at time of CIDR insertion, and after either 5, 7, 9 or 11d the CIDR was removed and all heifers received 25mg PGF2α (Lutalyse) and 300 IU of eCG (Novormon). Approximately half of the heifers in each treatment were administered 1mg EB at 48 h after CIDR withdrawal. Therefore, experimental treatments were designated as 5d-EB (n = 61; e.g., 5 d of CIDR with 1mg EB 48 after CIDR removal), 5d (n = 51; e.g., 5 d of CIDR without EB after CIDR removal), 7d-EB (n = 51), 7d (n = 47), 9d-EB (n = 49), 9d (n = 50), 11d-EB (n = 52) and 11d (n = 46). Estrus detection was performed for 5 d after CIDR removal and artificial insemination (AI) was performed according to the AM/PM protocol. Detection for return to estrus and AI, in heifers not conceiving to the initial AI, began 16 d after CIDR withdrawal and continued for 6 d. Pregnancy was diagnosed by ultrasonography 60 d after the first AI. Binominal data were analyzed using GLIMMIX procedures of SAS. During the synchronization period (5 d after CIDR withdrawal), a greater ($P < 0.05$) proportion (87.3%) of heifers that received EB presented estrus as compared with the proportion (54.6%) of heifers with no second EB treatment in estrus. Timing (68.1 ± 17.3 h after CIDR removal) and distribution of estrus did not differ among treatments. The additional EB did not increase conception rate, but tended ($P = 0.06$) to improve pregnancy at first AI (with EB, 21.6; without EB, 14.9%). Final pregnancy rates did not differ among treatments. Effects of the duration of CIDR treatment were not detected. In prepubertal Nellore heifers, inclusion of EB treatment at 48h after CIDR removal improved estrus response, did not influence conception rate, but tended to improve pregnancy rate following treatment with CIDR.

Key words: prepuberal, heifers, estradiol benzoate

T221 Effects of ethanol and acetic acid fed to high-producing dairy cows on blood parameters. J. L. P. Daniel*, L. G. Nussio, R. C. Amaral, E. H. C. Garcia, A. W. Bispo, F. C. L. Oliveira, I. F. Silva, and M. Zopollatto, *University of Sao Paulo, College of Agriculture "Luiz de Queiroz", Piracicaba, SP, Brazil.*

Ethanol and acetic acid are common end products from silages, especially from tropical forages. The objective of this study was to determine whether ethanol and acetic acid affect plasmatic glucose, insulin, ethanol, and gamma-glutamyl transferase (GGT) at peripheral blood. Hypothetically, ethanol present in the diet could reach portal blood, damage liver and increase GGT activity. Thirty lactating Holstein cows averaging 40 kg/d of milk at beginning of trial were grouped in 10 blocks and fed either: Control (33% Bermuda hay + 67% concentrates); Ethanol (control diet + 5% ethanol on DM basis); or Acetic acid (control diet + 5% acetic acid on DM basis) diets. Ethanol and acetic acid were diluted in water (1:2) and sprayed onto total mixed ration twice daily before feeding. The same amount of solution was replaced with water in the control diet. During the 1st week of trial the cows received half-dose of these chemical compounds. Blood was collected from coccyges vessels into evacuated tubes containing sodium heparin, 6 h after morning feeding on d 7, 14 and 42. Tubes were immediately centrifuged and plasma was frozen for a 28d period. Plasmatic concentration of ethanol was lower than 0.1 g/L in all cows. Diets did not affect ($P = 0.95$) plasmatic GGT (26.2, 27.0, and 26.6 U/L, respectively for control, ethanol and acetic acid diets). Insulin concentration was unaffected ($P = 0.96$) across diets (0.28, 0.27, and 0.29 mU/L, respectively). However, blood from cows supplemented with acetic acid showed lower ($P = 0.04$) glucose concentration (58.3 mg/dL) than those fed ethanol containing diets (62.7 mg/dL). It might be due to the lower dry matter intake observed, during 2nd and 3rd experimental weeks, from cows fed acetic acid (not showed). Control diet presented an intermediary blood sugar level (61.0 mg/dL). In conclusion, blood parameters were not altered by feeding ethanol to high producing dairy cows, up to studied level. The conversion of ethanol to acetate into the rumen might be a plausible explanation to understand the ordinary blood parameters to the alcohol containing diet.

Key words: GGT, insulin, glucose

T222 Estrous response in yearling and multiparous ewes during reduction on the synchronized luteal phase and eCG injection. J. L. Cordero[1], T. Sánchez[1], P. Molina[2], R. Nieto[1], J. Peralta[2], O. Mejía[3], L. Olivares[4], E. García*[5], and J. L. Figueroa[1], *[1]Colegio de Postgraduados, Texcoco, Estado de México, [2]Universidad Autónoma del Estado de Hidalgo, Tulancingo, Hidalgo, México, [3]FMVZ, Universidad Autónoma de México, Tres Marías, México, [4]Universidad Autónoma del Estado de México, Toluca, Estado de México, [5]CUCSUR, Universidad Autónoma de Guadalajara, Jalisco, México.*

The aim of the experiment was to evaluate the effect of reducing the synchronized luteal phase and eCG injection in yearling and multiparous ewes and their response on estrous and pregnancy rate. Seventy-nine ewes were divided according to their reproductive status in yearling (n = 36) and multiparous (n = 43), which were then randomly subdivided into groups for the assignment of hormonal treatments. Ewes were pre-synchronized with 2 doses of prostaglandin F2α (cloprostenol, 65 mg) 8 d apart, before sponge insertion. Yearling ewes (P) were synchronized with cronolone sponges (20 mg) for a 12 d period (P12+0, n = 8) without and with 100 IU of eCG injection (P12+eCG, n = 9); and for a 6 d period without (P6+0, n = 9) and with 100 IU of eCG injection (P6+eCG, n = 10), both eCG injections were given at sponge removal. Multiparous ewes (M) were synchronized by the same protocol (M12+0, n = 12; M12+eCG, n = 11; M6+0, n = 9 and M6+eCG, n = 11, respectively). All ewes (P and M) showed estrous response (100%) after sponge removal. There were no differences ($P \geq 0.05$) for reduction of the synchronized luteal phase and eCG injection in beginning and estrous duration in yearling ewes. However in multiparous ewes estrous duration was affected ($P \leq 0.05$) by reduction of the luteal phase (M12, 37.04 ± 1.9 vs M6, 45.2 ± 2.1) and eCG injection (M+0, 36.2 ± 1.5 vs M+eCG, 32.8 ± 1.1). There was no difference ($P \geq 0.05$) in gestation rate between groups (P = 86 and M = 81%). It is concluded that reducing the period of synchronization or eCG injection does not alter the response, onset and duration of estrous in yearling ewes, but it does alter estrous duration in multiparous ewes, so it should be considered when performing AI at fixed time or when yearling and multiparous are synchronized together.

Key words: *Ovis aries*, estrus synchronization, progestogen

T223 Fertility following fixed-time AI in infertile CIDR-treated dairy cows given rbST throughout extended (>500 d) lactations. A. Zúñiga-Serrano*, F. G. Véliz-Deras, J. Méndez-Lara, L. M. Tejada-Ugarte, and M. Mellado-Bosque, *Universidad Autónoma Agraria Antonio Narro, Torreón, Coahuila, México.*

With extended lactations (beyond 17 mo) due to the use of rbST throughout lactation, attempts to fecundate subfertile cows around 300 d in milk (DIM) can be commercially viable. Thus, the purpose of this study was to determine, using multiple logistic models, factors affecting pregnancy rates (PR) following fixed-time AI (FTAI) in subfertile (up to 12 services) cows treated with rbST throughout lactation. Four hundred ninety-eight Holstein cows of all parities, unable to become pregnant when approaching 300 d in milk received a CIDR device and 100 mg of GnRH on Day 0. CIDR removal and PGF2α (25 mg) treatment were done concurrently on Days 7. Estradiol benzoate (2 mg) was injected on d 8 and GnRH on d 9; cows were inseminated 16–20 h later. Cows that produced <15000 kg of milk in their previous lactation had only half the chance ($P < 0.05$) to become pregnant compared with cows with total lactations of > 15000 kg. Cows with an average milk fat <3% in their previous lactation were 43% more likely ($P < 0.05$) to become pregnant at FTAI than cows with milk fat >3%. Cows with <5 services had significantly increased chances of becoming pregnant than cows with >5 services at FTAI (PR 36 vs. 27%; $P < 0.05$). Cows with less than 2 lactations were 1.7 times more likely ($P < 0.05$) to become pregnant than older cows. Cows with >350 DIM were less likely to become pregnant (PR 27 vs. 35%; $P < 0.05$) than cows subjected to FTAI with <350 DIM. Cows with peak milk yields lower than 55 kg were 1.5 times more likely to conceived than cows with peak milk yields greater than 55 kg (PR 28 vs. 37%; $P < 0.05$). Cows subjected to FTAI with a temperature-humidity index (THI) <73 were 45% more likely ($P < 0.05$) to become pregnant than cows inseminated with a THA >73. It was concluded that an acceptable percentage of subfertile cows can become pregnant with the protocol used in the present study, and this practice seems to be biological feasibility and economically justifiable in dairy operations with 3X milking and the use of rbST throughout lactations, which would assure lactations >500 d.

Key words: reproductive performance, estrus synchronization, Holstein cows

T224 Adiponectin system and peroxisome proliferator-activated receptor gamma2 (PPARγ2) mRNA abundance in different bovine fat depots considering conjugated linoleic acids (CLA) or lactation stage related changes. B. Saremi*[1], H. Sauerwein[1], D. von Soosten[2], S. Dänicke[2], and M. Mielenz[1], *[1]Institute of Animal Science, Physiology and Hygiene Unit, University of Bonn, Bonn, North Rhine-Westphalia, Germany, [2]Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI), Federal Research Institute for Animal Health, Braunschweig, Lower Saxony, Germany.*

Adiponectin (Ad) is secreted from adipose tissue (AT) and exerts insulin sensitizing effects via Ad receptor 1 and 2 (AdR1/2) in humans. PPARγ2 increases plasma Ad concentrations and thus improves insulin sensitivity in monogastrics. We hypothesized that the CLA-induced reduction of milk fat might improve energy balance and insulin sensitivity in dairy cows. From 25 heifers, 5 were slaughtered on d 1 postpartum. Remaining heifers were randomly allocated to CLA (Lutrell pure, BASF, Germany, n = 10) or control fat supplementation (Silafat, BASF, n = 10) each at 100 g/d. Five animals per group were slaughtered at d 42 or 105. Subcutaneous (Sc) (chest, wither and tail head) and visceral (Vc) AT (mesenterial, omental and retroperitoneal) samples were collected. Ad, AdR1/2 and PPARγ2 mRNA abundance (Ab) was quantified by qPCR. Pearson correlation, GLM or non parametric tests were used for statistical analysis (SPSS 17; $P < 0.05$). Ad, AdR1 and PPARγ2 Ab increased from d 1 and 42 to d 105 in most VcATs. AdR1 Ab was highest at d 105 in all ATs except omental fat. In the merged data from VcATs, Ad and AdR2 were reduced in CLA-treated heifers at d 105. Comparing individual AT depots, Ad and AdR2 Ab was reduced in omental and retroperitoneal AT from CLA-treated animals. PPARγ2 was increased by CLA in Vc depots regardless of time. In general, the different depots had different Ab values, e.g., retroperitoneal AT displayed higher Ad, AdR1 and AdR2 Ab than mesenterial AT. Ad Ab was correlated to AdR1/2 and PPARγ2 (r = 0.5, 0.8, and 0.5, respectively). In conclusion, the observed CLA effects on Ad and AdR2 as well as the timely changes of Ad, AdR1 and PPARγ2 Ab were fat depot dependent. The increase in Ad and AdR1 Ab at d 105 (post peak lactation) is possibly regulated by PPARγ2, and might improve insulin sensitivity as compared with pre-peak lactation (d 42). As to whether the effects of CLA on the Ad system and PPAR expression will affect insulin sensitivity in the different depots and in the entire organism remains to be clarified.

Key words: adipose tissue, adiponectin, PPARγ2

T225 Relationship between follicular and ovulatory responses with embryo production during superovulatory treatment in cattle. H. Kohram[1,2] and M. Poorhamdollah*[1], [1]*Department of Animal Science, Faculty College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran,* [2]*Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University, Ahvaz, Iran.*

The efficiency of embryo transfer technology is still limited because of high variability and unpredictability of superovulatory responses of the donor cows. The aim of this study was to investigate relationship between follicular and ovulatory responses with embryo production during superovulatory treatment in cattle. The experiment was carried out in one to 4 random cycles of superovulation in 47 Holstein cows, for a total of 88 superovulatory cycles. Animal were superstimulated between d 8 and 12 of estrous cycle with 400 mg Folltropin-v given in decreasing doses: 3.5; 3, 3; 2.5, 2.5; 2, 2; 1.5 mg (a.m. and p.m.) over 4 d and luteolysis was induced with 2 mg intramuscularly injection of Cloprostenol with the 7 injection of folltropin-v. The ovaries of all cows were examined by ultrasonography on the day of estrus following superovulatory treatment and on the day of embryonic collection (7 d after estrus). Ova and embryos were collected by a nonsurgical procedure, evaluated and classified as quality I (freezable), quality II (transferable) and degenerated embryos. Criteria used to classify each type of superovulatory responses as low, medium or high. For each type of the superovulatory responses, data were analyzed by means of the GLM procedure of the SAS. The results showed in Table 1. In conclusion the high follicular or ovulatory responses are not necessarily coupled with a high yield of embryos.

Table 1. Percent of superovulation cycles with various types of responses

	Class		
Type of response	Low (%)	Medium (%)	High (%)
Follicle ≥ 7 mm at estrus	10	21	69
Ovulations	23	24	53
Quality I embryos	59	22	19
Quality II embryos	77	18	5
Quality I+ II embryos	52	24	24
Degenerated embryos	80.6	17.1	2.3

Key words: embryo, superovulation, Folltropin-v

T226 Differentiation of estrus versus nonestrus cow cervix morphology: Verification of a cost-effective methodology. A. Nikkhah*, M. A. Sirjani, A. A. Assadzadeh, and H. Amanloo, *University of Zanjan, Zanjan, Iran.*

The increasing trend in milk solids secretion over the last few decades has noticeably depressed cow fertility. Accurate estrus detection has been a major challenge to achieve. Our objective was to quantify dairy cow cervix morphology during standing-estrus (SE) and nonestrus (NE) days of the estrus cycle. Four multiparous Holstein cows (50 ± 14 d in milk, 31 ± 3.6 kg milk yield, 643 ± 66 kg BW, 3.0 ± 0.18 BCS) were monitored daily for cervical region morphologies for an entire 21-d estrus cycle. The cervix was videotaped using a recording on-farm apparatus to score tissue morphology as affected by estrus. The apparatus had 45 cm length and 2.7 cm diameter, internal electrical settings, external polyvinyl coat, lights on the front, wires at its terminal, and a connection to a laptop computer with an image processing software. Cervix was scored for distinctness from surrounding vaginal tissues, central positioning, motility, and secretions on a 5-scale basis. The score of 1 described cervices with quite distinct, central, stable, and mucosal manifestation, and the score of 5 represented quite nonseparate, noncentral, moving, and dry appearance. Data were analyzed as a mixed model with fixed day effect and random effects of cow within day plus residuals. Findings demonstrated that cervix area was markedly ($P < 0.01$) more distinct (1.0 vs. 3.0), more central (1.1 vs. 3.6), more stable (1.5 vs. 2.8), and more mucosal (1.1 vs. 3.7) during SE than NE days. As such, on SE days, the cervix was rigidly observable in the central end of vaginal tract, whereas NE cervices were hardly separable from the surrounding tissues. The data verify our earlier results and establish the on-farm feasibility of using the new inexpensive technique (e.g., <US$200) to differentiate SE and NE cervices.

Key words: cervix, morphology, estrus

T227 Metabolic characteristics of pregnant gilts fed low and excess protein diets associated to intrauterine growth retardation (IUGR). C. C. Metges*[1], I. S. Lang[1], U. Hennig[1], M. Peters[1], K.-P. Brüssow[1], E. Kanitz[1], M. Tuchscherer[1], F. Schneider[1], J. Weitzel[1], A. Ooster[2], H. Sauerwein[2], G. Nürnberg[1], C. Rehfeldt[1], and W. Otten[1], [1]*Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany,* [2]*Institute of Animal Science, Rheinische Friedrich-Wilhelms-Universität, Bonn, Germany.*

Limited and excess dietary protein in pregnant gilts lead to IUGR (Rehfeldt et al. 2011, J. Anim. Sci.). To explore metabolic reasons, gilts' plasma metabolite and hormone concentrations were analyzed. Low (6.5%, LP), adequate (12%, AP), and high (30%, HP) protein diets were fed to 16, 17, and 15 pregnant gilts, respectively. At d −5, 24, 66, and 108 of pregnancy fasted blood was collected. Concentrations of glucose (Glc), triglyceride (TG), nonesterified fatty acids (NEFA), cholesterol (C), low density lipoprotein cholesterol (LDLC), high density lipoprotein cholesterol (HDLC), insulin, glucagon (Gg), leptin, insulin-like growth factor-1 (IGF1), cortisol and progesterone (P_4) were analyzed in plasma; urea (U) and protein were analyzed in serum. At d 92, in 9 gilts/group diurnal blood samples were analyzed for Glc, NEFA, TG, C, and U metabolic profiles. Diet effects were evaluated with repeated measure ANOVA. Concentrations of HDLC were reduced in HP compared with AP gilts ($P < 0.01$). In LP gilts LDLC was lowest ($P < 0.01$). Highest and lowest U were observed in HP and LP ($P < 0.001$). Serum protein was lowest in LP gilts ($P < 0.05$). In the HP group Gg was higher than in LP and AP, whereas IGF1 was lower in LP than in AP gilts at d 24 and 66 ($P < 0.05$). Plasma P_4 was higher in HP than in LP and intermediate in AP ($P < 0.05$). In HP gilts cortisol levels were lower than in AP gilts ($P < 0.05$). Metabolic profiles indicated that plasma Glc was lower in HP ($P = 0.04$) and LP ($P = 0.09$) gilts. In HP U was 3 times the values observed in AP whereas in LP U reached only 60% of AP ($P < 0.001$). Plasma TG levels tended to be lower in LP than in AP ($P = 0.09$) and HP ($P = 0.07$). In HP diurnal NEFA levels were higher compared with AP and LP ($P < 0.01$). In LP gilts C was higher than in AP and HP ($P < 0.01$). In conclusion, HP gilts have a low glucose and energy status as reflected in higher NEFA and lower body fat. In LP gilts, deficiency of essential amino acids altered lipoprotein and C metabolism and favored lipid disposal. Both conditions are related to IUGR. Supported by Deutsche Forschungsgemeinschaft ME1420/8-1 and OT 137/3-1 (PAK 24)

Key words: high protein, metabolites, pregnancy

T228 Induction of luteal tissue in PGF_{2a}-treated sows. D. Gandy*, A. L. Greathouse, H. Klienman, F. M. LeMieux, and C. E. Ferguson, *McNeese State University, Lake Charles, LA.*

In the sow, corpora lutea must be present the entire length of gestation in order for the pregnancy to result in normal parturition at ~114 d post-mating. The objective of this experiment was to determine if induced luteal tissue following PGF_{2a} treatment could support pregnancy in the sow. A total of 12 cross-bred mature sows between the ages of 2 and 5 years were mated with a boar and evaluated for pregnancy at 30 to 60 d post-mating via ultrasonography. Pregnant sows were then allotted to 1 of 2 treatments, control or induction of luteal tissue. Sows randomly selected for the control treatment (n = 5) received 15 mg of PGF_{2a} 12 h apart and were then administered 30.8 mg of matrix at time of PGF_{2a} treatment. Then the matrix dose (daily) was reduced in a declining manner from to 22 mg, 15.4 mg, 6.6 mg and 0 mg matrix in 7 d intervals. Treatment sows (randomly selected for ovulation induction) were maintained on the same matrix schedule however, they received 750 IU of eCG 7 d post-PGF_{2a} and 500 IU hCG 48 h post-eCG. Blood samples were collected on all sows at 0, 4, 8, 12 and 24 h post-PGF_{2a} and 48 h post-hCG. There were no differences in the number of sows aborting in the control group (5/5, 100%) and the treatment group (7/7, 100%). The time from the start of 0 mg matrix to abortion was not different between the control group (1.8 ± 0.3 d) and the treatment group (5.0 ± 2.8 d). Among treatment sows, 2/7 (29%) developed luteal tissue (≥2 ng/mL P_4 48 h post-hCG) in response to eCG and hCG treatment while 5/7 (71%) did not (≤2 ng/mL P_4 48 h post-hCG) and success of induced luteal tissue may have affected the length of pregnancy maintenance. The length of time from 0 mg of matrix to abortion for sows with induced luteal tissue was 13 ± 7.5 d compared with 5.0 ± 2.8 d for sows with no induced luteal tissue. There was a high positive correlation $r^2 = 0.90$ between P_4 levels 48 h post-hCG and days to abortion post-hCG. These results indicate that pregnancy can be maintained in a PGF_{2a}-treated sow and induced luteal tissue can extend gestation in the absence of supplemental P_4.

Key words: abortion, pregnancy, luteal

T229 Effects of increased GnRH dose post–TAI in Brahman influenced cattle. B. Pousson*[1], D. J. Kesler[2], M. Poole[1], W. Storer[1], and C. E. Ferguson[1], *[1]McNeese State University, Lake Charles, LA, [2]University of Illinois, Urbana-Champaign.*

Brahman cattle have a history of lower pregnancy rates following artificial insemination (AI) compared with European cattle. The decrease in pregnancy rates among Brahmans has been linked to the higher excitability in stressful situations in which an increase in cortisol can result in delaying or blocking ovulation. This experiment was designed to determine if an increased GnRH dose at AI would improve pregnancy rate in Brahman and Brahman–type cattle. From 6 different location in Texas and Louisiana a total of n = 50 heifers, n = 123, cross–bred Angus heifers (no Brahman influence), n = 83 lactating cross-bred Brahman cows were bred using conventional semen following a CO-Synch+CIDR schedule with timed artificial insemination (TAI) at 48 to 58 h. Additionally, n = 32 Brahman dry cows were bred using sex–sorted Brahman semen (sorted for Y–chromosome) and the same synchronization schedule and TAI. All females were randomly selected to receive either 100 μg (n = 84) or 200 μg (n = 81) of GnRH at TAI and ultrasound for pregnancy ~30 d post-TAI. The administration of 200 μg GnRH at TAI resulted in a significantly higher ($P < 0.004$) pregnancy rate (0.43 ± 0.05) compared with 100 μg GnRH (0.21 ± 0.04). This pattern existed in heifers receiving 200 μg of GnRH (0.63 ± 0.10) vs. 100 μg GnRH, (0.29 ± 0.09), cows receiving conventional semen and 200 μg (0.40 ± 0.08) vs. 100 μg (0.23 ± 0.67) and cows receiving sex–sorted semen and 200 μg (0.21 ± 0.11) vs. 100 μg (0.06 ± 0.06). Among non-Brahman heifers increasing the dose of GnRH at TAI did not affect pregnancy rates 200 μg (0.49 ± 0.06) compared with 100 μg (0.55 ± 0.06). These results indicate that increasing the dose of GnRH at time of AI can result in an increase in the pregnancy rate in Brahman and cross-bred Brahman cattle, however there was no effect on pregnancy rate among non-Brahman heifers.

Key words: Brahman, ovulation, stress

T230 Dynamics of fat cell turnover in visceral and subcutaneous fat tissue in dairy cows. S. Häussler*[1], S. Dänicke[2], K. Friedauer[1], D. Germeroth[1], D. von Soosten[2], and H. Sauerwein[1], *[1]University of Bonn, Germany, [2]Federal Research Institute, Braunschweig, Germany.*

Adipose tissue can expand either by cell proliferation, cell enlargement or both. The number of adipocytes seems constant in mature humans; in cattle, in particular dairy cows, dynamics of fat cell turnover were unknown. To characterize lactation-induced changes in fat cell number, we targeted cell proliferation (marker Ki67) and preadipocyte differentiation (marker Pref-1) using immunohistochemistry, and apoptosis via the TUNEL method in both a visceral (retroperitoneal (RP)) and a subcutaneous (tailhead (SC)) depot obtained from 25 Holstein heifers. The heifers were divided in a control (CTR) and a CLA group; from d 1 of lactation until sample collection, animals from the CLA group were fed with 100 g CLA (Lutrell Pure, BASF, Germany) per day. On d 1, 42 and 105 postpartum, 5 animals of CTR were slaughtered; from CLA, 5 cows were slaughtered each on d 42 and 105. For the detection of apoptosis and Pref-1, deparaffinized sections (12 μm), for Ki67 frozen sections (14 μm) were used. Bovine lymph nodes (apoptosis), placenta (Pref-1) and liver (Ki67) served as controls. The portion (%) of positive cell was defined as mean number of positive stained cells/mean number of total cells x 100 and analyzed using the general linear model and the Student's *t*-test (SPSS). An apoptosis-proliferation index (A:P index) was calculated from the portions of apoptotic and proliferating cells. The A:P index was lower in RP than in SC due to a higher apoptotic rate in SC ($P \leq 0.001$) and concomitantly very low cell proliferation rates, irrespective of time and treatment. The same applied for preadipocyte portions being similar in both RP and SC, with mean values of 1.17 ± 0.33% for RP and 1.76 ± 0.65% for SC, respectively. In addition to decreasing fat cell size demonstrated in our group recently, the reduction of fat mass in early lactation seems to be dominated by apoptosis. Further dynamics of fat cell turnover are more likely defined by activation of preadipocytes than by cell proliferation.

Key words: bovine adipocyte, cell turnover, CLA

T231 Insulin sensitivity in obese (Iberian) and lean (Landrace) 50-kg barrows. I. Fernandez-Figares*, L. Gonzalez-Valero, J. M. Rodriguez-Lopez, and M. Lachica, *EEZ-CSIC, Granada, Spain.*

The Iberian pig is a slow growing obese breed with a distinct serum hormone and metabolite profile compared with lean (Landrace) pigs (Fernandez-Figares et al., 2007 Livest. Sci.110:73-81). The objective of the present work was to explore the possibility that Iberian pigs show peripheral insulin resistance. An intravenous glucose tolerance test was performed using Iberian (n=4) and Landrace barrows (n=5), 50 kg average BW, fitted with permanent carotid artery catheters. After surgery recovery, they were injected intravenously 500 mg glucose/kg

BW and blood samples collected at −10, 0, 5, 10, 15, 20, 25, 30, 45, 60, 90, 120, 150 and 180 minutes. Plasma samples were frozen in aliquots until analysis for glucose and insulin. Glucose was determined using Precision PCx Blood Glucose Test Strips. Insulin was determined using a Linco porcine insulin RIA kit and human insulin was used as standard. Responses of plasma glucose and insulin to the intravenous glucose challenge test was evaluated by computing areas under the response curves (AUC) for the 3-hour period following glucose administration determined using trapezoidal geometry. Insulin/glucose ratios were used as an index of insulin resistance. Area under the curves were analyzed using the GLM procedure of SAS. Insulin/Glucose ratios were evaluated using a mixed ANOVA with repeated measures with breed, time of sampling and their interaction in the model statement. Concentration at time zero of the analyte was included as a covariate in the statistical analysis. Significant differences among treatments were assessed using Bonferroni's multiple-range test. Iberian pigs had increased plasma insulin AUC (67.9%, $P < 0.01$) and insulin/glucose ratio (78.8%, $P < 0.001$) although no statistical difference in plasma glucose AUC was found ($P = 0.18$) compared to Landrace pigs. In conclusion, Iberian pigs showed lower insulin sensitivity of peripheral tissues evaluated using an intravenous glucose tolerance test.

Key words: insulin sensitivity, Iberian pig, glucose tolerance

T232 Reproductive performance of replacement beef heifers when estrus was synchronized with progesterone (CIDR) for 5 or 7 d, GnRH, and $PGF_{2\alpha}$. K. M. Bischoff*[1], T. E. Black[1], R. D. Estermann[2], G. A. Bridges[3], G. C. Lamb[1], and J. V. Yelich[2], *[1]North Florida Research and Education Center, University of Florida, Marianna, [2]Depertment of Animal Sciences, University of Florida, Gainesville, [3]North Central Research and Outreach Center, University of Minnesota, Grand Rapids.*

We determined if reproductive performance differed in replacement *Bos indicus* × *Bos taurus* beef heifers when estrus was synchronized with one of 3 protocols that included CIDR, GnRH, and $PGF_{2\alpha}$ (PG). Reproductive tract scores (RTS; scale 1 to 5 with 1 = immature and 5 = estrous cycling) were determined on d −7 and used to stratify heifers to one of 3 treatments: 1) GnRH (100 μg) and a CIDR insert d −7 and PG (25 mg) and CIDR removal on d 0 (7dCIDR; n = 113); 2) GnRH and CIDR insert on d −5 and CIDR removal and PG on d 0 (5dCIDR; n = 113); 3) PG and CIDR on d −7, GnRH on d −5, and CIDR removal and PG on d 0 (7dMOD; n = 117). All heifers received a second PG (25 mg) 8 h after CIDR removal. Estrus was detected for 60 h after CIDR removal, with heifers detected in estrus inseminated using the AM/PM rule. Heifers not detected in estrus received TAI 72 h after CIDR removal coincident with GnRH (100 μg). Bulls were inserted 10 d following TAI for a 103 d breeding season. Transrectal ultrasonography was used to diagnose pregnancy 55 d after TAI and 30 d following bull removal. Estrous response was reduced ($P < 0.05$) in the 5dCIDR (21%) treatment compared to the 7dMOD (43%) and 7dCIDR (35%) treatments. A. greater ($P < 0.05$) percentage of heifers with RTS of 3 (40%), 4 (49%), and 5 (46%) exhibited estrus than those with a RTS of 1 (14%) or 2 (11%). Conception rates of heifers exhibiting estrus were greater ($P < 0.05$) for the 7dMOD (62%; 31/50) than the 5dCIDR (33%; 8/24) and 7dCIDR (39%; 15/39) treatments. For heifers failing to exhibit estrus, TAI pregnancy rates (16%) did not differ between treatments. Synchronized AI pregnancy rates were greater ($P < 0.05$) in the 7dMOD (38%) treatment than the 5dCIDR (20%), and 7dCIDR (23%) treatments. Synchronized pregnancy rates for heifers with RTS of 1 (10%) and 2 (16%) were less ($P < 0.05$) than those with RTS 3 (34%), 4 (36%), and 5 (32%). Breeding season pregnancy rates did not differ between treatments (81%). In summary, synchronized AI pregnancy rates were greatest with the 7dMOD protocol and RTS affected reproductive performance.

Key words: estrus synchronization, beef heifer, CIDR

T233 Fat mobilization during early lactation: Effects on milk performance, feed intake, body condition and metabolic changes in dairy cows. C. Weber*[1], F. Becker[1], C. Hametner[1], B. Losand[2], R. M. Bruckmaier[3], W. Kanitz[1], and H. M. Hammon[1], *[1]Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany, [2]State Institute for Agriculture and Fishery, Dummerstorf, Germany, [3]Veterinary Physiology, Vetsuisse Faculty, Bern, Switzerland.*

Dairy cows mobilize body fat during early lactation to provide energy for milk production due to insufficient feed intake. However, there is huge individual variation in postnatal fat mobilization among cows indicated by a broad range of postnatal NEFA changes in blood plasma and liver fat concentration (LFC). The objective of the present study was to investigate feed intake, milk performance, and metabolic and endocrine changes in German Holstein cows (>11,000 kg milk/305 d in 2nd lactation) grouped according to different mean LFC on d 1, 14, and 28 after calving: L (<200 mg total fat/g DM; n = 10), M (200 – 300 mg total fat/g DM; n = 10), and H (>300 mg total fat/g DM; n = 7). Cows were studied from dry off up to 63 DIM in their 3rd lactation and were fed TMR ad libitum. DMI and milk yield were recorded daily, BW, BCS, and milk composition were measured weekly. Plasma concentrations of NEFA, BHBA, glucose, and insulin were measured in blood taken at 56, 28, 15, 5 d before expected calving and once weekly up to 63 DIM. Liver biopsies were taken at 1, 14, 28 DIM to measure total fat content. Data were analyzed by the Mixed Model of SAS with LFC and time as fixed effects. Mean hepatic fat concentration for H, M, and L were different ($P < 0.05$) among groups: 351 ± 14, 250 ± 10 and 159 ± 9 mg/g liver DM, for H, M, and L, respectively. DMI was lowest ($P < 0.05$) before calving in H, increased ($P < 0.01$) after calving in all groups, but was highest ($P < 0.05$) in L. Milk yield was not affected by LFC, but energy balance was least negative ($P < 0.01$) in L. BCS were highest ($P < 0.05$) before calving in H and the postnatal decrease was higher ($P < 0.05$) in H and M than in L. Plasma concentrations of NEFA and BHB increased more around calving ($P < 0.05$) in H than M and L, but plasma glucose was lowest in H. Plasma insulin concentrations after calving were highest in L. Greater fat mobilization in cows with elevated BCS before calving was associated with reduced DMI and a more severe negative energy balance, did not affect milk production, but influenced postnatal glucose and insulin status in cows.

Key words: dairy cow, fat mobilization, energy metabolism

T234 Fat mobilization around calving in high-yielding dairy cows affects hepatic gene expression of gluconeogenic enzymes but not enzymes involved in fatty acid oxidation. H. M. Hammon*[1], C. Weber[1], F. Becker[1], C. Hametner[1], B. Losand[2], and W. Kanitz[1], *[1]Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany, [2]State Institute for Agriculture and Fishery, Dummerstorf, Germany.*

Energy demands largely increase after calving in dairy cows and hepatic energy metabolism is affected by elevated glucose output due to milk production. As cows differ in fat mobilization around calving the objective of the study was to investigate hepatic gene expression of key-enzymes involved in gluconeogenesis, fatty acid oxidation, and ketone body formation in cows with variable liver fat concentration

(LFC) after calving. German Holstein cows were grouped according to mean LFC (as indicator of fat mobilization) on d 1, 14 and 28 after calving in low (L) (<200 mg total fat/g DM; n = 10), middle (M) (200 – 300 mg total fat/g DM; n = 10), and high (H) (>300 mg total fat/g DM; n = 7). Cows were fed TMR ad libitum. Liver biopsies were taken at 56 and 15 d before calving and at 1, 14, 28, and 49 DIM to measure LFC, glycogen, and mRNA concentrations of pyruvate carboxylase (PC), cytosolic phosphoenolpyruvate carboxykinase, glucose-6-phosphatase, propionyl-CoA-carboxylase (PCCA), carnitine palmitoyl-transferase 1A, acyl-CoA-synthetase long chain, acyl-CoA-dehydrogenase very long chain, hydroxy methyl glutaryl-CoA-synthetase 2, and peroxisome proliferator-activated factor (PPAR)α. Data were analyzed by the Mixed Model of SAS with LFC and time as fixed effects. Mean hepatic fat concentration for H, M, and L differed ($P < 0.05$) among groups: 351 ± 14, 250 ± 10 and 159 ± 9 mg/g DM, for H, M, and L, respectively. Glycogen concentrations decreased after calving in all groups, but were lowest in H ($P < 0.05$). All measured enzymes changed with time during the experimental period. PC mRNA concentrations increased immediately after calving highest ($P < 0.05$) in H and M. PCCA and PPARα tended to be lowest in H. Elevated fat mobilization indicated by LFC during early lactation affected hepatic glycogen and gene expression involved in glucose production and nuclear energy sensing, but not gene expression referred to hepatic fatty acid oxidation and ketone body formation.

Key words: dairy cow, fat mobilization, hepatic energy metabolism

T235 Ovarian characteristics, serum estradiol and progesterone concentrations, and fertility in lactating dairy cows in response to equine chorionic gonadotropin (eCG). S. L. Pulley*, L. D. Wallace, H. I. Mellieon, and J. S. Stevenson, *Kansas State University, Manhattan.*

Numbers of FSH receptors are greatest in maturing follicles on d 4 of the first follicular wave of the estrous cycle when LH receptors are first detected in granulosa cells of the dominant follicle. After having acquired LH receptors dominant follicles respond to both LH and FSH or eCG. Because the potential for eCG to influence follicle size and estradiol secretion, objectives were to evaluate the effects of eCG on serum ovarian steroids, corpus luteum (CL) diameter, estrual activity, and timed AI pregnancy rates. Cows (n = 121) in a single herd were enrolled in a Presynch-Ovsynch program. Cows received 2 injections of prostaglandin F2α (PG) 14 d apart (Presynch), with the second injection given 11 d before the onset of the Ovsynch protocol (GnRH injection 7 d before [GnRH-1] and 56 h after PG [GnRH-2], with AI administered 14 to 18 h after GnRH-2). Cows randomly received either saline or 400 IU eCG concurrent with the PG injection of the Ovsynch protocol (d 0). Blood samples were collected to monitor serum progesterone (d −7, 0, 2, 4, 9, 16, and 33) and serum estradiol (d 0, 1, 2, and 3). Serum estradiol did not differ between treatments from d 0 to 3. Estrual activity also was not affected by treatment. Overall expression of estrus was poor (eCG: 15.4%; 10/65 vs. saline: 16.4%; 9/55). Treatment with eCG improved neither ovulation response (96.9% vs. 100%; $P = 0.15$) nor multiple ovulation rates (20.3% vs. 18.2%) after GnRH-2 injection. Administration of eCG tended to increase the number of CL and on d 9 ($P = 0.08$) and d 16 ($P = 0.09$) after PG. Volume of luteal tissue was increased ($P = 0.04$) only on d 16 in response to eCG. Pregnancy diagnosis was determined by transrectal ultrasonography on d 33. Timed AI pregnancy rates did not differ between eCG (36.9%) and saline-treated cows (41.8%) cows. We concluded that administration of eCG at the time of PG provided no profertility advantages to dairy cattle when programmed for a timed insemination at first service.

Key words: eCG, fertility, Ovsynch

T236 A mechanistic metabolic model of regulation of reproductive processes in dairy cattle. J. P. McNamara[1], S. L. Shields*[1], and I. Lean[2], *[1]Washington State University, Pullman, [2]University of Sydney, Camden, NSW, Australia.*

The objective was to expand and continue evaluation of a deterministic, mechanistic, dynamic model of reproductive processes in the dairy cow. This research model will be suitable for evaluation of data, concepts and hypotheses regarding underlying genetic, nutritional and physiological control of reproduction. We began with an existing model of metabolism in the cow, published and validated in the literature (Molly, UC Davis); which describes utilization of glucose, amino acids and fatty acids by muscle, adipose, visceral and mammary tissues at an aggregated metabolic pathway level. Elements of genetic background, response to nutritional environment and metabolic hormones are explicit. The physiological processes are integrated at the pathway level into one system to link genetic elements, nutrient use and reproductive processes. For example, equations link glucose, IGF-I and growth hormone to rates of follicle stimulating hormone, luteinizing hormone, and follicular growth. The days in milk at which cycling commences is directly related to amount of body fat (days at start cycling = 37.41 DIM − 0.1489 × body fat kg; $r^2 = 0.979$), which is an integral function of the sum of nutritional pathways. Degradation of estrogen and progesterone by the liver is a function of metabolic rate, as described by total ATP demand, so that for example, peak progesterone and estrogen in the first cycle are reduced by 10.9% and 6.3% as milk yield increases from 34 to 50 kg/d for the first 90 DIM. Related to feed intake, progesterone and estrogen first peak are reduced 15.2 and 8.1% as DMI increases from 19.6 to 27.0 kg/d. Progesterone directly affects early embryonic growth (energy used in embryonic growth (kcal/d) = 13.59 + 1.923 × (progesterone, ng/ml); $r^2 = 0.946$) and must be maintained at a pre-set level to allow embryonic growth to continue after 21 to 45 d after conception. The model behavior (pattern and direction of response) is consistent with literature values. This research model should be useful to frame specific hypotheses on control of reproductive processes by genetic and nutritional driven mechanisms.

Key words: reproduction model, nutrition, genetics

T237 Effect of prostaglandin $F_{2\alpha}$ on growth of *Escherichia coli* and *Streptococcus uberis* associated with bovine mastitis. C. Autran*[1], B. Shafii[2], M. McGuire[1], J. Dalton[3], and A. Ahmadzadeh[1], *[1]University of Idaho, Moscow, [2]Statistical Programs, College of Ag & Life Sci, Moscow, ID, [3]Caldwell R & E Center, Caldwell, ID.*

Certain fatty acids have been shown to inhibit the growth of mastitis pathogens. Moreover, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$: dinoprost tromethamine) inhibits the growth of *Staph. aureus* in vitro. The objective was to determine the bacteriostatic effects of $PGF_{2\alpha}$ on growth of *E. coli* and *Strep. uberis* in vitro. In Exp. 1, flasks containing tryptic soy broth were inoculated with *E. coli*, and subsequently treated with $PGF_{2\alpha}$ at concentrations of 0, 1.2, 2.4, 4.8 and 9.6 mg/mL. Cultures were sampled every 4 h over 24 h to determine bacterial growth (log cfu). The experiment was repeated 3 times. In Exp. 2, *Strep. uberis* was treated with $PGF_{2\alpha}$ (0, 0.6, 1.2, 2.4 and 4.8 mg/mL). The procedures were identical to Exp. 1. Data were analyzed using ANOVA, and regression procedures. In Exp. 1 there was an effect of treatment by time interaction on mean log cfu ($P < 0.05$) for *E. coli*. Only the mean log cfu values of the 9.6 mg/mL $PGF_{2\alpha}$ were different ($P < 0.05$) at 12 h from

the control. The regression models showed that the pattern of bacterial growth over time for 4.8 mg/mL ($P = 0.05$) and 9.6 mg/mL $PGF_{2\alpha}$ ($P < 0.05$) were different from the control. Moreover, 9.6 mg/mL of $PGF_{2\alpha}$ was the most effective dose inhibiting *E. coli* growth. In Exp. 2, there was an effect of treatment and treatment by time interaction on mean log cfu for *Strep. uberis* ($P < 0.05$). At 12 and 24 h of growth, mean log cfu for all $PGF_{2\alpha}$ concentrations differed ($P < 0.05$) from the control, in a dose-dependent manner. Regression results revealed that the growth curve pattern of *Strep. uberis* over 24 h for each treatment was different compared with the control ($P < 0.05$), and the increase in growth rate over time for treatments 2.4 and 4.8 mg/mL was different from the control ($P < 0.05$). These results provide evidence for the first time that $PGF_{2\alpha}$ has inhibitory effects on growth of *Strep. uberis* in vitro; however, growth inhibition of *E. coli* was only achieved with the greatest concentration of $PGF_{2\alpha}$. Gram-positive mastitis causing bacteria (*Strep. uberis*) appear to be more susceptible to $PGF_{2\alpha}$ than gram-negative bacteria (*E. coli*).

Key words: prostaglandin $F_{2\alpha}$, *E. coli*, *Strep. uberis*

T238 Effects of sequential injections of GnRH at 17 and 24 d after AI on progesterone concentration and pregnancy losses. A. L. A. Scanavez*[1], J. G. N. Moraes[1], R. G. Bruno[2,3], K. J. Lager[2,3], J. A. H. Rivera[2], P. R. B. Silva[1], L. G. D. Mendonça[1], T. R. Bilby[2], and R. C. Chebel[1], *[1]Department of Veterinary Population Medicine, University of Minnesota, St. Paul, [2]Texas AgriLife Research and Extension Service, Texas A&M System, Stephenville, [3]Department of Agricultural Science, West Texas A&M University, Canyon.*

Objectives of the current study were to determine whether sequential injections of GnRH at 17 ± 3 and 24 ± 3 d after pre-enrollment artificial insemination (AI), would reduce pregnancy losses between 31 ± 3 and 66 ± 3 d after AI by increasing progesterone concentrations (P4) at 24 ± 3 and 31 ± 3 d after AI. Lactating cows from 2 dairies (MN-Jersey cows and TX-Holstein cows) were enrolled in the study at 17 ± 3 d after pre-enrollment AI. At enrollment cows were grouped by parity and number of AI and assigned to 1 of 3 treatments in a ratio of 1:2:1. Cows assigned to the 2GnRH treatment received 100 µg of GnRH at 17 ± 3 and 24 ± 3 d after pre-enrollment AI; cows assigned to the 1GnRH treatment received 100 µg of GnRH at 24 ± 3 d after pre-enrollment AI; and, control cows received no GnRH. All cows were examined by ultrasound at 31 ± 3 d after pre-enrollment AI and those diagnosed pregnant were re-examined at 66 ± 3 d after pre-enrollment AI. Blood samples were collected from a subgroup of cows at 24 ± 3 (MN-123 cows and TX-160 cows) and 31 ± 3 (MN-142 cows) d after pre-enrollment AI for determination of P4. There were 514 2GnRH, 1099 1GnRH, and 648 control cows pregnant at 31 d after AI and re-examined at 66 d. At 24 d after pre-enrollment AI P4 was not ($P = 0.70$) affected by treatment (7.5 ± 0.2ng/mL). At 31 d after pre-enrollment AI P4 was greatest ($P < 0.01$) for 2GnRH cows (8.5 ± 0.4ng/mL), but was not ($P = 0.16$) different between 1GnRH (6.2 ± 0.4 ng/mL) and control (7.0 ± 0.5ng/mL) cows. Treatment ($P = 0.99$) and site ($P = 0.81$) did not affect pregnancy loss, but the interaction between treatment and site affected ($P = 0.04$) pregnancy loss. In the MN-dairy 2GnRH (7.5%) cows had fewer pregnancy losses than 1GnRH (11.8%) and control (10.4%) cows and in the TX-dairy 2GnRH (11.9%) had more pregnancy losses than 1GnRH (7.9%) and control (8.8%) cows. There was a quadratic correlation between P4 at 31 d after AI and pregnancy loss [pregnancy loss = 87.4 − (26.5xP4)+(2.1xP42); r2 = 99.7%]. Jersey cows treated with GnRH at 17 and 24 d after AI had greater P4 at 31 d after AI and reduced pregnancy losses, but Holstein cows did not benefit from GnRH treatment after AI.

Key words: dairy cow, pregnancy loss, GnRH

T239 Effect of GnRH treatment at critical stages of estrous cycle following artificial insemination on pregnancy rate in lactating Holstein dairy cows. Z. Hakimi, A. Z. Shahne, H. M. Yegane, and R. Masoumi*, *University of Tehran, Karaj, Karaj, Iran.*

Embryonic mortality is regarded as one of the major causes of reproductive failure in cattle resulting in reduced pregnancy rates, slower genetic improvement and substantial financial losses to dairy operations. Progesterone hormone deficiency after insemination has an important place among the causes of early embryonic death. Even though average fertilization rates of heifer and cows are between 88 and 90, 20% or more of the embryos are lost after insemination before 21th days. GnRH injections are applied from 4 to 15 d after insemination for the prevention of early embryonic death which is attached to inadequate progesterone hormone in cattle. The aim of present field study was to determine the effect of GnRH treatment on pregnancy rate of lactating Holstein dairy cows in critical stages of estrous cycle following AI. A total of 174 Holstein cows were randomly assigned into one of 5 following treatment groups: Cows in group A (n = 35) were not treated and served as control group. Cows in group B (n = 35) were treated intramuscularly with a GnRH analog (Gonadorelin acetate; 25 µg) on d 0 (at the time of AI). Cows in group C (n = 39) were treated with GnRH on d 0 and 5. Cows in group D (n = 36) were treated with GnRH on d 0 and 11. Cows in group E (n = 29) were treated with GnRH on d 0, 5 and 11. Pregnancy was diagnosed by rectal palpation 45–50 d after insemination. Dichotomous data were analyzed using PROC LOGISTIC of SAS. Pregnancy rate for treatment groups were 42.2, 60, 51.8, 63.9, 55.1%, respectively. Although, there was a notable difference between control and treatment groups in PR, but the differences were not statistically significant ($P < 0.05$). Therefore, GnRH administration to stimulate CL function using single or multiple doses of GnRH during the luteal phase could not increase pregnancy rate in present study.

Key words: GnRH, pregnancy rate, dairy cattle