PHYSIOLOGY AND ENDOCRINOLOGY II

1400 (T210) Fertility of lactating dairy cows treated with gonadotropin-releasing hormone at estrus, 5 d after AI, or both, during summer heat stress. L. G. D. Mendonça*[1], F. M. Mantelo[1], and J. S. Stevenson[2], [1]*Dep. of Animal Sciences and Industry, Kansas State University, Manhattan,* [2]*Kansas State University, Manhattan.*

Objective was to evaluate fertility of lactating dairy cows after treatment with GnRH on the day of estrus, 5 d after AI, or both, under heat stress conditions in two Kansas dairies. Cows (n = 2855) detected in estrus based on tail paint removal were assigned to four treatments in a 2 × 2 factorial arrangement: 1) control (CON = 722); 2) GnRH treatment at AI (G0 = 739); 3) GnRH treatment 5 d post-AI (G5 = 697); or 4) GnRH treatment at AI and 5 d post-AI (G0G5 = 697). Daily temperature and humidity data were collected during study enrollment and temperature humidity index (THI) was calculated. Blood samples were collected from a subgroup of cows at AI (d 0), d 5 and d 12 to determine progesterone concentrations (CON = 58, G0 = 57, G5 = 58, G0G5 = 65). Pregnancy diagnosis was conducted by transrectal ultrasonography on d 36 and 94. Treatment with GnRH at AI did not affect pregnancy per AI (P/AI) on d 36 (P = 0.89) or d 94 (P = 0.53). Treatment with GnRH 5 d after AI did not affect P/AI on d 36 (P = 0.49) or d 94 (P = 0.36). Furthermore, the interaction between GnRH treatments on d 0 and 5 did not affect P/AI on d 36 (P = 0.90) or d 94 (P = 0.75). In contrast, the interaction between lactation number and treatment with GnRH on d 5 affected P/AI on d 36 (P = 0.01) and d 94 (P = 0.03) because GnRH treatment increased P/AI of ≥ 3-lactation cows (27.0 vs. 19.3%, 23.1 vs. 16.1%, respectively). Average THI at AI was 83.8 ± 0.1 and tended (P = 0.08) to be associated with P/AI at d 36, but was not (P = 0.34) associated with P/AI on d 94. Overall, treatment with GnRH on d 0 (P = 0.82), d 5 (P = 0.61), and the interaction between treatments on d 0 and 5 (P = 0.28) did not affect progesterone concentration on d 5 and 12 (1.8 ± 0.1 and 6.7 ± 0.2 ng/mL, respectively). Treating cows under heat stress conditions with GnRH at AI did not increase P/AI, but treatment with GnRH 5 d post-insemination increased fertility of ≥ 3-lactation cows.

Key Words: fertility, summer heat stress, dairy cow

1401 (T211) Luteolysis and pregnancy outcome in 5-d Resynch dairy cows after 1 or 2 injections of prostaglandin $F_{2\alpha}$. J. S. Stevenson*, S. L. Pulley, and S. L. Hill, *Kansas State University, Manhattan.*

Our objective was to determine pregnancy outcome after 50 mg PG administered on d 6 or 25 mg PG delivered on d 5 and 6, respectively, in a 5-d Ovsynch-Resynch (GnRH 5 d before [d 0] and 56 [p.m. on d 7] or 72 h [d 8] after 25-mg doses of PG [d 5 and 6 after GnRH]; timed artificial insemination [AI] on d 8). Lactating Holsteins in herd 1 diagnosed not pregnant between 30 and 36 d since last AI were enrolled randomly to receive either 50 mg PG on d 6 (1 × 50; n = 134) or 25 mg PG on d 5 and 6 (2 × 25; n = 139) after GnRH-1 (d 0), with GnRH-2 at 56 h after PG (d 5) and timed AI 16 h after GnRH-2. In herd 2, even-tagged cows received the 2 × 25 (n = 422) treatment and odd-tagged cows received the 1 × 50 (n = 450) treatment after a not pregnant diagnosis between 34 and 40 d since last AI. Blood collected from all cows in herd 1 at d 0, 5, 6, and 8 was assayed for progesterone. Defined luteolysis occurred when progesterone was ≥ 1 ng/mL on d 5 and 72 h later was < 0.5 ng/mL or < 1 ng/mL on d 8. Progesterone was similar between treatments on pretreatment d 0 and 5, but was greater (P < 0.01) in 1 × 50 than 2 × 25 cows on d 6 (4.7 ± 0.2 vs. 1.1 ± 0.2 ng/mL) and d 8 (0.43 ± 0.04 vs. 0.19 ± 0.04 ng/mL), respectively. Luteolysis was greater (P < 0.01; 93.3 vs. 78.5%) in the 2 × 25 vs. 1 × 50 treatment when the cutpoint was 0.5 ng/mL on d 8, whereas no difference was detected when the cutpoint was < 1 ng/mL (100 vs. 96.3%), respectively. Luteolytic failure was greater in cows classified as early cycle on d 0 or having a new corpus luteum after d 0 than for cows classified as late cycle on d 0 or having low progesterone on d 0 and 5. Luteolytic failure also was greater (P < 0.01) in 1 × 50 than 2 × 25 cows with a cutpoint of 0.5 ng/mL at AI and pregnancy per AI in combined herds was slightly reduced (30.4 vs. 25.1%), respectively.

Key Words: luteolysis, pregnancy per AI, progesterone

1402 (T212) Physiological characteristics of cows with divergent genetic merit for fertility traits during the transition period. S. Moore*[1,2], P. Lonergan[2], T. Fair[2], and S. Butler[3], [1]*Teagasc Moorepark, Fermoy, Ireland,* [2]*University College Dublin, Ireland,* [3]*Animal & Grassland Research and Innovation Centre, Teagasc, Moorepark, Fermoy, County Cork, Ireland.*

Cows with similar genetic merit for milk production, but with extremes of good (Fert+; n = 15) or poor (Fert-; n = 10) genetic merit for fertility traits were monitored. DMI was recorded daily from wk -2 to 5 relative to calving. Blood metabolites and metabolic hormones were measured from wk -2 to 8 relative to calving. Vaginal mucus (VM) was scored weekly on a scale 0 (no pus) to 3 (≥ 50% pus) from parturition to wk 6. Uterine polymorphonuclear neutrophil count was measured at

wk 3 and 6. Continuous data were analyzed using mixed model procedures. PROC NPAR1WAY was used to analyse VM score data. Logistic regression was performed to analyse the proportion of animals classified as having endometritis or to have resumed cyclicity by wk 6 postpartum. Prepartum DMI was similar between genotypes, but during the postpartum period, Fert+ cows had greater DMI than Fert- cows (19.7 vs. 16.8 kg DM/d, $P = 0.02$). Energy balance at wk 1 was greater in Fert+ cows than Fert- cows (2.3 vs. -1.12 UFL/d, $P = 0.02$). Fert+ cows had greater milk solids production (1.89 vs. 1.74 kg/d, $P = 0.05$). Fert+ cows had greater mean circulating insulin-like growth factor-I (102.62 vs. 56.85 ng/mL, $P = 0.001$) and tended to have greater mean circulating insulin (3.25 vs. 2.62 µIU/mL, $P = 0.08$) compared with Fert- cows from wk -2 to 8 relative to parturition. Mean circulating glucose (3.40 vs. 3.01 mmol/L, $P = 0.04$) concentrations were greater in Fert+ cows compared with Fert- cows from wk -2 to 3 relative to parturition. Fert+ cows maintained greater mean BCS throughout lactation compared with Fert- cows (2.98 vs. 2.74 units, $P < 0.0001$). Fert+ cows had better uterine health compared with Fert- cows as evidenced by lower weekly VM scores during wk 2 to 6 postpartum, and based on uterine cytology a smaller proportion were classified as having endometritis at wk 3 (0.42 vs. 0.78, $P = 0.09$) and 6 (0.25 vs. 0.75, $P = 0.04$). A greater proportion of Fert+ cows had resumed cyclicity by wk 6 postpartum (0.86 vs. 0.20, $P = 0.009$) compared with Fert- cows. These results indicate that good genetic merit for fertility traits is associated with a more favourable bioenergetic and uterine health status, earlier resumption of cyclicity and greater BCS, without antagonizing milk production.

Key Words: genetic merit for fertility, transition period, endometritis

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1403 (T213) Characterization of luteal dynamics in lactating dairy cows for 32 d after synchronization of ovulation and timed artificial insemination.

A. Ricci*[1], P. D. Carvalho[2], M. C. Amundson[1], and P. M. Fricke[1], *[1]Dep. of Dairy Science, University of Wisconsin–Madison, Madison, [2]University of Wisconsin, Madison.*

Approximately 20% of cows diagnosed not pregnant 32 d after TAI do not have a CL, and cows that begin a resynchronization protocol in the absence of a CL have about 10% fewer pregnancies per AI compared to cows with a CL. An understanding of luteal dynamics after synchronization of ovulation and timed AI (TAI) may help to refine strategies for resynchronizing cows failing to conceive. Lactating Holstein cows ($n = 141$) were synchronized for first TAI (80 to 86 DIM) using a Double Ovsynch protocol. Thrice weekly (MWF) from 4 to 32 d after TAI, luteal diameter was measured using ultrasonography and blood samples were collected for evaluation of progesterone (P4) concentrations. Pregnancy status was determined using ultrasound 32 d after TAI. Cows ($n = 13$) were removed if they had twins ($n = 2$), if they did not synchronize ($n = 4$), or if they had pregnancy loss ($n = 7$). For cows diagnosed pregnant ($n = 48$), luteal volume increased from 4 to 13 d after TAI then remained constant until 32 d, whereas P4 increased from 4 to 15 d after TAI then remained constant until 32 d. For cows diagnosed not pregnant 32 d after TAI ($n = 80$), P4 profiles were evaluated using statistical cluster analysis (PROC CLUSTER of SAS) based on the day after TAI that P4 decreased to < 1 ng/mL. Cows diagnosed not pregnant were segregated into 5 clusters: 1) luteal regression 15 d after TAI (1.3%, 1/80); 2) luteal regression 18 to 22 d after TAI (55.0%, 44/80); 3) luteal regression 25 to 27 d after TAI (18.8%, 15/80); 4) luteal regression 29 to 32 d after TAI (3.8%, 3/80); and 5) original CL present 32 d after TAI (21.3%, 17/80). Pregnancy-associated glycoproteins (PAG) were measured in serum samples collected 25 and 32 d after TAI using a commercial assay (IDEXX Laboratories, Inc., Westbrook, ME). Relative serum PAG levels (mean ± SEM S-N values) differed among clusters at both 25 ($P = 0.03$) and 32 ($P < 0.01$) d after TAI and were similar for cows in clusters 2, 3, and 4 but were greater for cows in cluster 5. We conclude that cows maintaining their original CL for 32 d after TAI were initially pregnant but underwent pregnancy loss based on residual serum PAG levels at 24 and 32 d after TAI. *Supported by Hatch project WIS01171.*

Key Words: progesterone, luteal dynamics, pregnancy loss

1404 (T214) Influence of fat supplementation on LH pulses and FSH concentration in Nellore heifers.

R. S. Cipriano*[1], M. C. V. Miguel[2], H. F. Costa[2], J. S. Souza[2], L. M. Pavanello[2], M. A. Maioli[2], D. Giraldo-Arana[2], D. M. Pinheiro[2], F. M. Abreu[3], L. H. Cruppe[3], M. L. Day[3], and G. Nogueira[2], *[1]UniSalesiano, Araçatuba, Brazil, [2]Unesp, Araçatuba, Brazil, [3]Ohio State University, Columbus.*

The aim of this study was to verify whether protected fat supplementation, after weaning increases LH pulses and FSH concentration in Nellore heifers (*Bos taurus indicus*). Contemporary heifers ($n = 30$; 167 ± 13 kg; 9 mo) were sorted into three experimental groups: Control Group (CG, $n = 10$), sugarcane bagasse plus 4.2 kg concentrate and 500 g of ground corn; Fat Group (FG, $n = 10$), sugarcane bagasse, plus 4.2 kg of concentrate and plus 200 g of rumen protected fat (Ca salts of soybean oil); and Excess Group (EG, $n = 10$), sugarcane bagasse plus 4.2 kg of concentrate, 500 g of ground corn plus 200 g of rumen protected fat per animal per day (13.85% of palmitic acid, 17.92% of oleic acid and 49.09% of linoleic acid). After an adaptation period, animals remained under nutritional treatments for 92 d (13 to 16 mo of age). Blood samples were collected every 24 h during 17 d, in 10, 12, 14, and 16 mo of age, and every 20 min per 12 h on 11, 13, 14, and 16 mo of age for FSH and LH quantification. The

results were evaluated by repeated measures ANOVA and the Duncan´s test was the post-test of SAS. During the treatment, the FG presented a higher number LH pulses (3.12 ± 1.64; P = 0.05) in comparison with EG (1.86 ± 0.90) and CG (2.63 ± 0.74), from samples collected every 20 min per 12 h. The CG showed higher FSH concentration area (15.71 ± 3.72 ng/ml/day), P = 0.06) than GG (11.23 ± 2.51 ng/ml/day) and EG (14.17 ± 4.22 ng/ml/day) at the 14 mo of age. There was no difference on FSH concentration area between groups in 10 (P = 0.80), 12 (P = 0.55) and 16 (P = 0.35) mo of age. The CG showed higher FSH amplitude (1.86 ± 0.72 ng/ml, P = 0.08) than GG (1.12 ± 0.51 ng/ml) and EG (1.54 ± 0.22 ng/ml) also at the 14 mo of age. There was no difference on FSH amplitude between groups at the 10 (P = 0.89), 12 (P = 0.78) and 16 (P = 0.36) mo of age. We concluded that fat treatment increased frequency LH pulses, decreased FSH amplitude and FSH concentration area during supplement period.

Key Words: *Bos indicus*, LH, pulses, FSH, fat, supplementation

1405 (T215) Pregnancy outcomes based on pregnancy-associated glycoproteins in milk and serum during the first trimester of gestation in Holstein dairy cows.

A. Ricci[*1,2], P. D. Carvalho[3], M. C. Amundson[1], S. Koller[4], R. H. Fourdraine[5], L. Vincenti[2], and P. M. Fricke[1], *[1]Dep. of Dairy Science, University of Wisconsin-Madison, Madison, [2]University of Turino, Italy, [3]University of Wisconsin, Madison, [4]IDEXX Laboratories, Inc, Westbrook, ME, [5]AgSource Laboratories, Verona, WI.*

Our objective was to compare pregnancy outcomes based on pregnancy-associated glycoproteins (PAGs) in milk and serum samples from cows of known pregnancy status during the first trimester of gestation. Lactating Holstein cows (n = 141) were synchronized using a Double-Ovsynch protocol for first timed AI (TAI). Blood and milk samples were collected from all cows 25 and 32 d after TAI, and pregnancy status was determined 32 d after TAI using ultrasound. Pregnant cows with singletons (n = 48) continued the experiment in which blood and milk samples were collected and pregnancy status was assessed weekly from 39 to 102 d after TAI. Milk samples were assayed for PAGs by AgSource Laboratories (Menomonie, WI) and serum samples were assayed for PAGs by IDEXX Laboratories (Westbrook, ME). Milk and serum assay outcomes included relative PAG levels (S-N values), and cows were classified as pregnant (PG), nonpregnant (NP), or recheck (RC) based on threshold S-N values. Sensitivity, specificity, negative predictive value, positive predictive value, accuracy for milk PAG outcomes were 88, 87, 92, 83, and 88%, respectively 25 d after TAI, and 98, 83, 98, 79, and 88%, respectively 32 d after TAI. These values for serum PAG outcomes were 94, 92, 96, 88, and 93%, respectively, 25 d after TAI, and 100, 88, 100, 83, and 92%, respectively, 32 d after TAI. Overall, 87% (48/57) of cows maintained their pregnancy until 102 d after TAI. For the milk assay, NP and RC outcomes occurred for pregnant cows 25 (11 and 36%), 46 (4 and 17%), 53 (4 and 20%), 60 (5 and 18%), and 67 (7 and 20%) d after TAI when relative PAG levels were low. For the serum assay, NP and RC outcomes occurred for pregnant cows 25 d after TAI (6 and 35%), whereas RC outcomes occurred for pregnant cows 39 (30%), 46 (46%), 53 (59%), 60 (70%), 67 (52%), 74 (28%), 81 (20%) and 88 (11%) d after TAI. Relative PAG levels in both milk and serum were negatively correlated (P < 0.01) with milk production in multiparous but not primiparous cows at 53 and 60 d after TAI when relative PAG levels were at their nadir. We conclude that low relative PAG levels in both milk and serum resulted in NP and RC outcomes in pregnant cows using these assays and that both parity and milk production affected relative PAG levels in milk and serum. *Supported by Hatch project WIS01171.*

Key Words: pregnancy diagnosis, pregnancy associated glycoprotein, milk

1406 (T216) Comparison of two gonadorelin formulations and two luteolytic agents on pregnancy rates in beef cattle synchronized with a 5-d CO-Synch + CIDR program.

S. Bas[*1], T. A. Brick[1], G. Starkey[1], G. Messerschmidt[1], A. A. Barragan[1], G. M. Schuenemann[1], and M. L. Day[2], *[1]Dep. of Veterinary Preventive Medicine, Ohio State University, Columbus, [2]Ohio State University, Columbus.*

The objective of the present study was to compare the effect of two gonadorelin formulations and two luteolytic agents (PGF) injected as part of a 5 d CO-Synch + CIDR program on fixed timed AI (FTAI) pregnancy rates (PR) in beef cattle. Postpartum beef cows (n = 473) and heifers (n = 78) from two herds received GnRH and a CIDR insert on d 0; 5 d later, at CIDR removal, animals received two doses of PGF. On d 8, cows and heifers received a second dose of GnRH and were FTAI. At the initiation of the breeding program, cows were blocked by age and d postpartum (DPP) and randomly assigned into one of two treatment groups. For animals in the control group (CON = 280), the hormones used for the synchronization program were gonadorelin diacetate tetrahydrate (100 μg; Cystorelin) and dinoprost tromethamine [50 mg (two 25 mg doses); Lutalyse]; while animals in the Parnell group (PAR = 271) received gonadorelin acetate (100 μg; GONAbreed) and Cloprostenol sodium [1000 μg (two 500 μg doses); estroPLAN]. Determination of pregnancy status was performed by transrectal ultrasonography at 35 to 45 d after FTAI and after the conclusion of the breeding season. Age (CON = 4.8 ± 0.2; PAR = 4.6 ± 0.2), DPP (CON = 73.8 ± 1.6; PAR = 75.9 ± 1.5), and body condition score (CON = 6.6 ± 0.9; PAR = 6.6 ± 0.1) were not different (P > 0.05) between treatments. No difference (P > 0.05) in PR at FTAI was observed for the

CON (54.9%) and PAR (55.9%) treatment groups. Similarly, no difference ($P > 0.05$) in PR was observed between treatments for cows [CON ($n = 236$) = 55.1%; PAR ($n = 243$) = 56.9%] and heifers [CON ($n = 37$) = 54%; PAR ($n = 35$) = 51.4%]. Breeding season PR (89.8%) did not differ ($P > 0.05$) between treatments. In conclusion, the use of gonadorelin diacetate tetrahydrate plus dinoprost tromethamine (CON) resulted in similar FTAI PR when compared to gonadorelin acetate plus cloprostenol sodium (PAR).

Key Words: beef, GnRH, prostaglandin

1407 (T217) Rams treated with testosterone induce sexual activity in anovulatory dorper adult sheep.

L. M. Tejada*, *Universidad Autónoma Agraria Antonio Narro, Torreón, México.*

The aim of this study was to determine whether rams treated with testosterone induce estrus in multiparous anovulatory ewes in northern México. On April 15, Dorper multiparous ewes ($n = 60$) were randomly distributed to two experimental groups; all females received daily 30 mg i.m. of progesterone from d −8 and d −4 before contact with males. On April 20, while one group (GMT, $n = 31$) was exposed to three males treated with testosterone (25 mg i.m., every 3 d × 2 wk before mating), the other group (GMC, $n = 29$) was exposed to three non-testosterone treated males. Estrous response was evaluated in two periods. The first period from d 0 to 14 while the second period from 15 to 25 d after male introduction (twice daily, during 1 h). On d 10 and 25 of the experimental period, ovulation rate was assessed throughout ultrasonographic scanning. Estrus activity was compared using chi2 (SYSTAT program 12). During the first 14 d after male introduction, 87% of the GMT-ewes (27/31) ovulated but only 9.6% (3/31) showed estrus activity, whereas 68% of the GMC-females (20/29) ovulated and 3.4% (1/29) showed estrous activity. After 15 d post-male introduction, 83% of the GMT-females(26/31) ovulated, with 80% of the ewes showing signs of estrus (25/31). Regarding the GMC-females, 51% (15/29) ovulated, and 68% (13/29) showed signs of estrus activity. Results of this study confirm that males treated with testosterone are more effective to induce ovulation and estrus activity during the second phase of the experimental period (15 to 25 d after male introduction) in Dorper adult ewes, regarding the untreated-males.

Key Words: testosterone, sheep, anestrus, estrus activity

Table 1407. Sexual response in sheep exposed to male control group (GC) or treated with testosterone (GT)

	Response 0 to 14 d after introduction of males		Response 15 to 25 d after introduction of males	
	Ovulation	Estrous	Ovulation	Estrous
GT	87% (27/31)a	9.6% (3/31)a	83%(26/31)a	80%(25/31)a
GC	68%(20/29)a	3.4%(1/29)a	51%(15/29)b	68%(13/29)b

Different letters in columns indicate different statistical differences $P > 0.05$.

1408 (T218) Regulation in vivo and in vitro of G protein-coupled receptor 34 (GPR34) mRNA in ovarian granulosa cells of cattle and its role in steroidogenesis.

L. J. Spicer*[1], J. A. Williams[1], L. F. Schutz[1], M. L. Totty[1], N. B. Schreiber[1], and J. Gilliam[2], *[1]Oklahoma State University, Stillwater, [2]Oklahoma State University Center for Veterinary Health Sciences, Stillwater.*

Abundance of *G* protein-coupled receptor 34 (GPR34) mRNA is greater in granulosa cells (GC) of cystic follicles *vs*. normal dominant follicles of cattle. The present experiments were designed to determine if: 1) GPR34 expression in GC changes during normal follicular development in estrogen-active (EA) and estrogen-inactive (EI) follicles of cattle, 2) hormones that have been shown to influence steroidogenesis such as IGF-I and FSH regulate expression of GPR34 mRNA, and 3) GPR34 ligands function to regulate GC function. In Exp 1, estrous cycles of non-lactating Holstein cows were synchronized and ovariectomized on either Day 3 or 6 after ovulation; a 2 × 2 factorial ANOVA (Day 3 vs. 6; EA vs. EI) indicated that GPR34 mRNA abundance in GC increased ($P < 0.05$) from 6.1 to 14.1 ± 4.3 relative mRNA units between Day 3 ($n = 5$ cows) and 6 ($n = 5$ cows) post-ovulation but did not differ ($P > 0.10$) between EA ($n = 15$) and EI ($n = 23$) follicles. In Exp 2, ovaries were collected at a local slaughterhouse and GC were isolated and treatments applied in vitro for 24 h; a 2 × 2 factorial ANOVA (± IGF-I with ± tumor necrosis factor (TNF)-α) indicated that IGF-I increased ($P < 0.05$) GPR34 expression from 3.5 to 7.8 ± 0.3 relative mRNA units and TNFα decreased ($P < 0.05$) the IGF-I-induced GPR34 mRNA abundance to 6.0 ± 0.4 relative mRNA units in small-follicle (1–5 mm) GC ($n = 3$ replicates and GC pools/treatment). Also in Exp 2, IGF-I and TNFα decreased ($P < 0.05$) GPR34 expression from 17.1 to 9.4 and 2.2 relative mRNA units, respectively, in large-follicle (8–22 mm) GC, indicating a change in GPR34 responsiveness occurs during follicle development. Other in vitro experiments (Exp 3–7; $n = 3$ replicates) revealed that treatment with IL-2, prostaglandin E2 and angiogenin decreased ($P < 0.05$) GPR34 expression by 62, 19, and 21%, respectively, whereas FSH, IL-6 and cortisol did not affect ($P > 0.10$) *GPR34* expression in small-follicle GC. In Exp 8, the presumed ligand of GPR34, L-a-lysophosphatidyserine (LPPS), increased GC numbers by 1.74-fold and estradiol production by 5.4-fold (0.19 vs. 1.03 ng/10^5 cells/24 h) in small-follicle GC ($n = 3$ replicates). For the first time, we have identified the lysophosphatidylserine receptor GPR34 as a developmentally and hormonally regulated gene in GC, the ligand of which enhances GC proliferation and estradiol production.

Key Words: G protein coupled receptor, granulosa cell, cattle

1409 (T219) Interaction between a mammary immune response to lipopolysaccharide and luteal function in lactating dairy cows. J. Luettgenau[1], O. Wellnitz*[1,2], R. M. Bruckmaier[2], and H. Bollwein[1], *[1]Clinic of Reproductive Medicine, Vetsuisse Faculty University of Zurich, Switzerland, [2]Veterinary Physiology, Vetsuisse Faculty, University of Bern, Switzerland.*

In a previous study we observed negative effects of an intravenous injection of *Escherichia coli* lipopolysaccharide (LPS) on luteal size and blood flow (LBF) as well as on plasma P4 concentrations. Because there are several reports about negative effects of mastitis on fertility of dairy cows, the objective of the present study was to investigate if LPS applied into the mammary gland could also suppress luteal function. Each of 8 lactating dairy cows received once 200µg LPS into one quarter of the mammary gland on d 9 of the estrous cycle (d 1 = ovulation). Plasma cortisol (stress hormone) and haptoglobin (acute phase protein), both indicating a systemic immune response, as well as P4 were determined immediately before (0h), hourly until 9, 12, and 24 h after administration of LPS. Luteal size and LBF were measured 0, 3, 6, 9, 12, and 24 h after LPS-injection. Cows showed local and systemic symptoms (swelling of the udder, pyrexia, increased cardiac and respiratory frequencies), increased ($P \leq 0.02$) cortisol concentrations between 2 and 8 h, and a fivefold increase ($P = 0.02$) of haptoglobin between 0 and 24 h after treatment. Plasma P4 increased between h 2 and 4, and decreased between h 4 and 6 after LPS exposure. There was no effect ($P > 0.05$) of treatment on luteal size, but LBF increased ($P = 0.05$) during the first 3 h after LPS-injection, remained constant ($P > 0.05$) between h 3 and 6, and decreased ($P < 0.0001$) between h 6 and 12. Results indicate that in contrast to an intravenous injection the application of LPS into the mammary gland does not show an obvious suppression of luteal function, although inducing systemic effects.

Key Words: mastitis, corpus luteum

1410 (T220) Influence of maternal nutrient restriction and realimentation on vascularity of bovine placentomes. B. R. Mordhorst*[1], L. E. Camacho[2], C. O. Lemley[3], P. P. Borowicz[1], D. A. Redmer[1], K. C. Swanson[1], and K. A. Vonnahme[1], *[1]North Dakota State University, Fargo, [2]University of Arizona, Tucson, [3]Mississippi State University, Starkville.*

To investigate maternal global nutrient restriction and realimentation impacts on placentome vascularity and mRNA expression for angiogenic and vasoactive factors in cotyledonary (COT) and caruncular (CAR) tissues, multiparous beef cows were randomly assigned to either 100% (CON; $n = 18$) or 60% NRC requirements for all nutrients (RES; $n = 28$) on d 30 of gestation. On d 85, tissues were collected, or cows remained on CON or RES diets, or were realimented to CON. On d 140, tissues were collected and all remaining cows were placed on CON diets until d 254 where all remaining cows were slaughtered to collect tissues. At tissue collection, placentomes were separated and portions snap frozen until qPCR analyses for mRNA expression of platelet endothelial cell adhesion molecule-1 (PECAM-1), soluble guanylate cyclase-β, endothelial nitric oxide synthase, vascular endothelial growth factor, fms-like tyrosine kinase, and kinase insert domain containing receptor were performed with all normalized to 18S. Vascularity measurements in CAR and COT were stained for PECAM-1, Rhodamine labeled lectin, and DAPI, and micrographs analyzed with Image-Pro Premiere. Treatment did not affect ($P \geq 0.06$) mRNA expression in any tissue on any day. Data from d 85 was presented previously where COT capillary size was smaller in RES vs. CON (465 vs. 764 ± 91 μm^2). Treatment did not affect any CAR or COT measurements d 140 or 254. In CON cows, CAR tissue area decreased ($P = 0.02$) and capillary number density increased ($P < 0.01$) from d 85 to 254 (0.76 vs. 0.59 ± 0.05 mm^2; 114 vs. 301 ± 26 number/μm^2). In COT, tissue area increased ($P = 0.02$) from d 85 to 254 (2.66 vs. 2.84 ± 0.05 mm^2). Capillary area and surface densities were similar ($P \geq 0.19$) on d 85 and 140 and increased ($P \leq 0.04$) by d 254. Capillary size decreased ($P < 0.01$) from d 85 to 140 and were similar ($P = 0.14$) from d 140 to 254. Capillary number density increased ($P < 0.03$) throughout gestation (45.6, 66.6, vs. 162.6 ± 6.5 number/μm^2). Capillary changes throughout gestation are more prevalent in COT vs. CAR. While we have previously reported that realimentation can augment uterine blood flow and placental arteriole vasoreactivity, the histologic and mRNA expression for angiogenic/vasoactive factors do not appear to be altered by maternal dietary intake. *Supported partly by AFRI Competitive Grant no. 2009–65203–05812 from the USDA-NIFA.*

Key Words: cow, placenta, vascularity

1411 (T221) Lysophosphatidic acid (LPA) activates ERK1/2-P90RSK signaling in porcine trophoblast cells. J. Kim*, J. Lee, S. Jung, H. Bang, Y. Sung, Y. Choi, and J. Kim, *Dankook University, Cheonan, South Korea.*

LPA (lysophosphatidic acid) is a phospholipid having diverse biological effects on various types of tissues. Recently, we indicated that LPA and their specific G protein-coupled receptors appear to play a lipid regulator during implantation and establishment of pregnancy in a human, mice and pig. In pig, LPA with various fatty acyl groups and receptors (LPA_{1-3}) were expressed in the uterine endometrium and conceptus during pregnancy. The extracellular-signal-regulated kinase (ERK1/2) pathway has emerged as one of the critical components in LPA signaling cascades. However, little is known of the biological role of LPA in the porcine conceptus during implantation. Therefore, this study examined LPA and the ERK1/2 signal transduction pathway in porcine conceptuses

during early pregnancy. The effects of LPA on the ERK1/2 signaling pathway were studied using established porcine trophectoderm cells (PTr) isolated from Day 12 pig conceptuses. The pTr cells were serum starved for 24 h and then treated with LPA (0–20 uM) for 30 min. LPA dose dependently increased ERK1/2 phosphorylation. Western blot analyses of whole cell extracts with antibodies to target proteins also found that LPA increased levels of pERK1/2 and pP90RSK (ribosomal protein S6 kinase, 90 kDa) by 2.3- and 1.6-fold, respectively, within 15 min which was maintained for up to 90 min. MEK inhibitor U0126 and LPA_3 receptor blocker significantly decreased LPA-induced ERK and P90RSK activity. Collectively, these results indicate that LPA coordinately activates ERK1/2, P90RSK in pTr cells and supports the hypothesis that LPA is a critical regulator of trophoblast survival, growth and differentiation during early pregnancy.

Key Words: LPA, pig trophoblast, ERK1/2

1412 (T222) Relationship between dry-matter intake and subclinical endometritis in healthy postpartum dairy cows.

A. H. Souza*[1], P. D. Carvalho[2], A. R. Dresch[3], L. M. Vieira[4], K. S. Hackbart[3], R. D. Shaver[2], and M. C. Wiltbank[2], *[1]University of California Cooperative Extension, Tulare, [2]University of Wisconsin, Madison, [3]Dep. of Dairy Science, University of Wisconsin—Madison, Madison, [4]University of Sao Paulo-VRA, Brazil.*

The main objective was to study the relationship between dry matter intake (DMI) and subclinical endometritis of postpartum dairy cows. A secondary objective was to evaluate whether colostrum quality at calving was associated with subclinical endometritis. Postpartum Holstein cows (n = 70 total; primiparous = 28; multiparous = 42), were milked twice daily and housed and fed individually in tie-stalls. Dry matter intake was measured daily from individual cows from calving to 10 wk postpartum. Four cows that were evidently sick in the first wk after calving with depressed DMI for over 3 d and/or detected with fever were removed from further analysis. Colostrum was collected from the first milking and frozen for later quality analysis (digital Brix refractometer, 0 to 53% scale). To measure level of subclinical endometritis, uterine swabs were performed at 40 ± 3 d postpartum and a single treatment-blind technician evaluated all the slides by counting a minimum of 100 cells at 400x magnification and determined the number and percentage of polymorphonuclear cells (PMN) in the endometrial smear. The statistical analyses were performed with PROC CORR and PROC GLIMMIX of SAS. Dry matter intake averaged 18.5 ± 0.3 kg/d and 23.6 ± 0.4 kg/d, for primiparous and multiparous, respectively. There was no significant association between proportion of uterine PMN cells and average DMI (r = 0.16; P = 0.20), with no significant interactions with parity. In a further retrospective analysis, cows were divided in three classes of subclinical endometritis [0% PMN (n = 22); 1 to 20% PMN (n = 32); or > 20% PMN (n = 16)]. Similarly, the repeated measures comparison indicated no effects (P = 0.42) of subclinical endometritis on DMI. Interestingly, greater colostrum quality at calving was associated with greater DMI in multiparous (r = 0.40; P = 0.01), but not in primiparous cows (r = -0.08; P = 0.69). Further, colostrum quality was not associated with subclinical endometritis in older cows (r = 0.19; P = 0.25); but surprisingly, greater colostrum quality was associated with lower subclinical endometritis in primiparous cows (r = -0.37; P = 0.05). In conclusion, healthy postpartum cows with lower DMI had similar incidence of subclinical endometritis as compared to cows with greater intake levels. Associations between colostrum quality at calving and DMI and/or proportion of subclinical endometritis need further examination, but could represent an interesting tool to predict postpartum performance. *Support: USDA Grant 2010–85122–20612.*

Key Words: dairy cows, postpartum, dry matter intake, subclinical endometritis

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1413 (T223) The effect of the initial GnRH and dose of PGF2α on pregnancy rate to TAI in beef heifers submitted to the 5-d CO-Synch + CIDR program.

L. H. Cruppe*[1], S. L. Lake[2], F. M. Abreu[1], S. G. Kruse[3], S. L. Bird[3], K. Heaton[4], B. R. Harstine[1], M. L. Day[1], and G. A. Bridges[3], *[1]Ohio State University, Columbus, [2]University of Wyoming, Laramie, [3]University of Minnesota, Grand Rapids, [4]Utah State University, Logan.*

The objective was to determine the effects of GnRH (Factrel) at CIDR insertion and number of doses of $PGF_{2\alpha}$ (PGF; Lutalyse) at CIDR removal in a 2 × 2 factorial on pregnancy rates to timed AI (TAI) in beef heifers enrolled in a 5-d CO-Synch + CIDR program. Yearling beef heifers (n = 1105) in seven locations (Ohio [2 locations; n = 149 and n = 116; Angus x Simmental], Utah [n = 274; Angus x Hereford], Wyoming [n = 156; Angus] and Minnesota [3 locations; n = 150, n = 150 and n = 110; Crossbred]) were enrolled in the 5-d CO-Synch + CIDR protocol and randomly assigned to either receive 100 µg GnRH (G+, n = 547) or not to receive GnRH (G-, n = 558) at CIDR insertion (d 0 of the experiment). At CIDR removal (d 5), heifers within G+ and G- groups were randomly assigned to receive either a single 25 mg PGF (PGF1) or two 25 mg PGF 6 ± 2 h apart (PGF2), resulting in four distinct treatments (G+PGF1, n = 272; G+PGF2, n = 275; G-PGF1, n = 277; G-PGF2, n = 281). All heifers received either tail paint or Estrotectpatches at CIDR removal to determine estrus response and were inseminated by TAI concomitant with 100 µg GnRH at 60 h after CIDR removal. Pregnancy diagnosis was performed between 32 and 36 d after TAI. Estrus response, as determined by estrous detection aids, did not differ among treatments. Pregnancy rate to TAI averaged 55.5% and was similar among treatments (G+PGF1, 53.3%; G+PGF2,

57.4%; G-PGF1, 55.2%; G-PGF2, 55.9%). Heifers classified as having been in estrus before TAI had a greater ($P < 0.05$) pregnancy rate to TAI (64.6%; 277/429) than either heifers with minimal (49.8%; 150/301) or no (48.7%; 172/353) evidence of estrus before TAI. In conclusion, omission of the initial GnRH treatment in the 5-d CO-synch + CIDR program did not influence TAI pregnancy rate in yearling beef heifers. Moreover, an additional dose of PGF at CIDR removal did not improved fertility in these yearling beef heifers, regardless of whether or not the initial GnRH treatment was given.

Key Words: GnRH, PGF, yearling beef heifers

1414 (T224) Use of a CIDR in the 5-d CO-synch estrous synchronization protocol improves pregnancy rates to timed artificial insemination. G. A. Bridges[1], R. P. Lemenager[2], E. Taylor[3], and P. J. Gunn[*4], *[1]University of Minnesota, Grand Rapids, [2]Purdue University, West Lafayette, IN, [3]Purdue University, Lafayette, IN, [4]Iowa State University, Ames.*

The objective of this experiment was to compare timed-AI (TAI) pregnancy rates in suckled beef cows synchronized with the 5-d CO-Synch protocol with (5CIDR) or without (5NoCIDR) the inclusion of an EAZI-BREED CIDR insert (CIDR). The experiment was conducted in 879 cows over 2 yr, and at three locations with a total of five replications. Cows were assigned to either the 5CIDR ($n = 438$) or 5NoCIDR ($n = 436$) protocol by breed, age, and days postpartum. Blood samples were collected to determine estrous cyclicity status for four of five replications. On d 0 all cows received GnRH (100 μg) and cows in the 5CIDR treatment received a CIDR. On d 5 CIDR were removed (5CIDR) and all cows received two separate doses of $PGF_{2\alpha}$ (25 mg/dose) between 2 and 10 h apart. Cows were TAI 72 h after CIDR removal (d 8), concurrent with GnRH (100 μg). Timed-AI and breeding season pregnancy rates were determined via ultrasonography 32 to 38 d after TAI and end of the breeding season, respectively. Data were analyzed with the GLMIIX procedure of SAS. There were no significant treatment-based interactions with year, age, or cyclic status; therefore data were pooled across year and cyclic status. In reps that had cyclicity determined, the proportion of cyclic cows was 89.3% (583/653). Timed-AI pregnancy rates were greater ($P = 0.0002$) in 5CIDR (62.3%, $n = 438$) than 5NoCIDR (50.7%, $n = 436$) treatment. Age classification, year, and cyclicity did not affect TAI pregnancy rates ($P \geq 0.33$). In conclusion, to optimize TAI pregnancy rates in beef cows synchronized with the 5 d CO-Synch protocol, the inclusion of a CIDR is recommended.

Key Words: 5-d CO-synch, beef cow, CIDR

1415 (T225) Incidence of ovulation to GnRH at onset of 5-d CO-synch + CIDR and impact on reproductive responses. H. P. Dias[*1], S. G. Kruse[2], S. L. Bird[2], B. J. Funnell[2], T. C. Geppert[3], E. L. Lundy[3], P. J. Gunn[3], and G. A. Bridges[2], *[1]Aluno do Programa de Pós Graduação em Zootecnia, FMVZ-UNESP-Botucatu, Brazil, [2]University of Minnesota, Grand Rapids, [3]Iowa State University, Ames.*

The objective of this study was to determine how response to GnRH at the onset of the 5-d CO-Synch + CIDR protocol (5dCO) affected estrous response, follicular dynamics, and pregnancy success to timed-AI (TAI) in beef cows. Suckled primiparous ($n = 95$) and multiparous ($n = 264$) beef cows at four locations (1; $n = 126$, 2; $n = 121$, 3; $n = 73$, 4; $n = 39$) were enrolled in the 5dCO that consisted of GnRH (GnRH-1) and CIDR insertion on d −8, CIDR removal and two 25-mg doses of $PGF_{2\alpha}$ given concurrently on d -3, and TAI on d 0 concurrent with GnRH (GnRH-2). Estrus was detected twice daily from d -3 to 0. Estrous cyclicity (70.6%) was determined at locations 1 and 2 via assessment of circulating progesterone concentrations. Ovarian ultrasound was conducted on d −8, -3, 0, and 2. Ovulation to GnRH-1 was defined by the disappearance of a dominant follicle observed on d −8 and development of a new corpus luteum on d -3. Follicle diameter at GnRH-2 (d 0) was assessed and ovulation confirmed on d 2 via ultrasonography. Pregnancy to TAI was determined approximately 30 d after TAI via ultrasonography. Cows were classified as having ovulated (OV; $n = 196$) or not ovulated (NoOV; $n = 163$) to GnRH-1. Statistical analyses were conducted using the MIXED and GLIMMIX procedures of SAS with location included as a random variable. Response to GnRH-1 (54.6%) was not influenced by parity (multiparous versus primiparous) or estrous cyclicity status. Estrus before TAI was greater ($P < 0.05$) in NoOV (47.6%) than OV (40.8%) cows. In cows that displayed estrus, interval from CIDR removal to estrus tended to be greater ($P = 0.07$) in OV (64.4 ± 0.9 h) than NoOV (60.6 ± 1.0 h), and was greater ($P < 0.01$) in multiparous (64.4 ± 0.8 h) than primiparous (58.3 ± 1.4 h) cows. Ovulation to GnRH-1 did not impact follicle diameter at GnRH-2. Pregnancy rate to TAI was greater ($P < 0.05$) in NoOV (65.0%) than OV (51.5%), primiparous (68.4%) than multiparous (53.8%) cows, and those cows that did (63.9%) than did not (52.7%) exhibit estrous. In summary, ovulation in response to GnRH-1 at the onset of the 5dCO protocol reduced estrous response and TAI pregnancy rates in suckled beef cows.

Key Words: timed-AI, beef cow, 5-d CO-synch + CIDR, GnRH

1416 (T226) The use of 5-d CO-synch+CIDR and 7-d EB+CIDR synchronization programs in Nellore females. M. V. C. Ferraz Jr.[*1], A. V. Pires[2], M. V. Biehl[2], R. Sartori[2], J. R. S. Gonçalves[3], E. M. Moreira[1], M. H. Dos Santos[1], L. H. Cruppe[4], and M. L. Day[4], *[1]University of São Paulo– FMVZ/USP, Pirassununga, Brazil, [2]University of São Paulo–ESALQ/USP, Piracicaba, Brazil, [3]Experimental Station Hildegard Georgina Von Pritzelwitz, Londrina, Brazil, [4] Ohio State University, Columbus.*

Reproductive performance of heifers and cows submitted to either the 5-d CO-Synch+CIDR or 7-d EB+CIDR program was evaluated. Nellore females (n = 411) were used (nulliparous, n = 198; primiparous, n = 80; multiparous, n = 133). The 5-d CO-Synch+CIDR program consisted of insertion of a CIDR and 100 μg of GnRH (Fertagyl) on d 0. On d 5, CIDR was removed and two doses of 25 mg $PGF_{2\alpha}$ (PGF; Lutalyse) were administered 6 h apart. Timed-AI was performed on d 8 (72 h after CIDR removal). The 7-d EB+CIDR program consisted of insertion of a CIDR and 2 mg estradiol benzoate (EB) on d 0. On d 7, CIDR was removed and 25 mg of PGF, 0.6 mg of estradiol cypionate (ECP) and 150 IU of eCG (Novormon) administered. Timed-AI was performed 55 h after CIDR removal. Estrotect patches were applied at CIDR removal and visual estrus detection performed on a 12 h interval for the following 96 h. Blood samples for progesterone (P4) analysis were collected 10 d after AI to confirm ovulation. Concentration of P4 was assessed by chemiluminescent immunoassay. Data were analyzed using the GLIMMIX and PROC MIXEDs of SAS. Estrus response was greater (P < 0.05) in 7-d EB+CIDR than the 5-d CO- Synch+CIDR program (nulliparous, 95.8 vs. 66.0%; primiparous, 48.7 vs. 0.0%; and multiparous, 76.9 vs. 13.4%, respectively). In contrast, ovulation rate (89.8%) was similar between programs. Concentration of P4 10 d post AI was greater (P < 0.05) in primiparous and multiparous cows in the 7-d EB+CIDR than 5-d CO-Synch+CIDR program (6.8 vs. 4.9 ng/mL; and 6.9 vs.5.8 ng/mL, respectively); but did not differ between treatments in nulliparous females (4.4 ± 0.14 ng/mL). Timed-AI pregnancy rate was greater (P < 0.05) in multiparous cows (58.4 vs. 32.8%), but did not differ for nulliparous (51.0 vs. 41.0%) and primiparous, (25.6 vs. 31.7%) for the 7-d and 5-d program, respectively). The 7-d EB+CIDR program resulted in a greater number of females in estrus, and either greater or similar P4 on the subsequent estrous cycle and timed-AI pregnancy rates. In conclusion, reproductive performance seems to be enhanced with the 7-d EB + CIDR in comparison to the 5-d CO-Synch+CIDR program in Nellore females.

Key Words: Nellore, 7-d EB-P4, 5-d CO-synch

1417 (T227) The efficacy of different PGF2α treatments to promote luteolysis on D 7 or D 9 of the estrous cycle in nonlactating Nellore cows. M. V. Biehl[*1], A. V. Pires[1], L. H. Cruppe[2], M. V. C. Ferraz Jr.[3], R. Sartori[1], A. D. B. Ribeiro[3], J. A. Faleiro Neto[3], J. R. S. Gonçalves[4], and M. L. Day[2], *[1]University of São Paulo– ESALQ/USP, Piracicaba, Brazil, [2]Ohio State University, Columbus, [3]University of São Paulo–FMVZ/USP, Pirassununga, Brazil, [4]Experimental Station Hildegard Georgina Von Pritzelwitz, Londrina, Brazil.*

The objective was to evaluate the luteolytic competence of different PGF2α (PGF, Lutalyse) treatments on d 7 and 9 of the estrous cycle. Nonlactating Nellore cows (n = 270) were synchronized with the 7-d EB + CIDR program. Cows received Estrotect patches at CIDR removal to determine estrus response. Presence of an ovulatory follicle and its disappearance were confirmed 48 and 72 h after CIDR removal, respectively. Cows detected in estrus within 48 h and with confirmed ovulation 72 h after CIDR removal remained in the study (n = 225). Cows were assigned to treatments according to BW (389 ± 3.1) and BCS (2.7 ± 0.01, scale 1 to 5). One of four PGF treatments were administered either 7 or 9 d after confirmed ovulation (a single 12.5-, 25- or 50-mg dose or two 25-mg doses 8 h apart), in a 2 × 4 factorial. Presence of a corpus luteum was determined by ultrasound and progesterone (P4) analyses (P4 ≥ 1 ng/ml) on either d 7 (h 0) or d 9 (h 0) of the estrous cycle. Blood samples were collected at h 24, 48, and 72 after PGF treatment to assess the incidence of luteal regression (defined as concentrations of P4 < 1 ng/ml at 48 and 72 h after PGF). Serum P4 concentrations were quantified using a chemiluminescent immunoassay. Cows received a new Estrotect patch at PGF and were observed for estrus twice daily for 5 d. The incidence of luteal regression was greater (P < 0.05) in cows receiving either a single 50-mg (89.3%; 50/56), or two 25-mg doses of PGF (89.5%; 51/57) compared to cows receiving 12.5-mg (67.9%; 38/56) or one 25-mg PGF dose (66.1%; 37/56). Moreover, estrus response was greater (P < 0.05) for cows receiving the single 50-mg or two 25-mg doses of PGF (80.4 and 78.9%, respectively) compared to cows receiving 12 mg (55.4%); cows that received a single 25-mg dose were intermediate (66.1%) and did not differ from other treatments. Neither day of estrous cycle (7 or 9) nor its interaction with PGF treatment influenced luteal regression and estrus response. In conclusion, luteal regression and estrus response were greater in nonlactating cycling Nellore cows treated with 50 mg of PGF either in a single or split dose injection on d 7 or 9 of a synchronized estrous cycle.

Key Words: beef cows, luteolysis, PGF

1418 (T228) Effect of timing of artificial insemination and estrus expression using sexed semen on pregnancy rates in Holstein dairy cows.
S. E. Crego*, E. L. Larimore, and G. A. Perry, *South Dakota State University, Brookings.*

The use of sexed semen has become important in dairy herds across the United States, but reported lower conception rates have limited the adaptation in some herds. The objective of this study was to determine if timing of AI and expression of estrus impacted fixed-time AI pregnancy success with sexed semen. Primiparous and multiparous lactating Holstein cows (n = 130) were synchronized with a Presynch-Ovsynch protocol (PGF d -29; PGF d -19; GnRH and CIDR insertion d −9; PGF and CIDR removal d -2; GnRH d 0) starting at 35 DIM. The cows were allotted into a 2x2 factorial randomized block (lactation) design with 1) sexed (n = 68) versus conventional (n = 62) semen, and 2) insemination at second GnRH (n = 54) versus 16 h later (n = 76). Follicle size was determined in all cows by transrectal ultrasonography at GnRH and ovulation was confirmed on d −5 and 4. Only those cows that ovulated after AI were utilized in the analysis (n = 130). Estrus detection was determined by visual observation with the aid of tail chalk. Blood samples were collected on d -16, −9, -2, 0, and 4 to determine circulating concentrations of progesterone and estradiol by RIA. Data were analyzed using the GLIMMIX procedures of SAS. There was a significant effect of time of insemination (P = 0.04) and estrus expression (P = 0.02) on pregnancy success. Cows inseminated 16 h after GnRH had greater pregnancy success compared to cows bred at time of second GnRH (53 vs. 35%, respectively), and cows expressing estrus had greater pregnancy success compared to cows not expressing estrus (54 vs. 34%, respectively). However, there was no effect of semen (P = 0.20; 50 vs. 38% for conventional and sexed, respectively) or any interaction of semen by estrus (P = 0.55); semen by time (P = 0.47); or time by estrus (P = 0.23) on pregnancy success. There was no difference between treatments (P = 0.62) or between cows that became pregnant and cows that did not (P = 0.45) for follicle size at the second GnRH injection, but cows that expressed estrus had larger (P < 0.01) follicles than cows that did not express estrus. In conclusion, pregnancy success was significantly influenced by time of insemination and estrus expression, but was not influenced by semen, or any interactions.

Key Words: fixed-time AI, sexed semen, estrus

1419 (T229) Evaluation of the hypothalamic kisspeptin system throughout the estrous cycle in gilts.
E. S. Jolitz*, and J. A. Clapper, *South Dakota State University, Brookings.*

Kisspeptin has been demonstrated to increase peripheral concentrations of LH in pigs, presumably by its actions on GnRH, as has been demonstrated in other species. To determine if hypothalamic expression of kisspeptin (Kiss1), kisspeptin receptor (Kiss1R), estrogen receptor α (ER-α), and estrogen receptor β (ER-β) varies throughout the estrous cycle, the following experiment was performed. Forty crossbred prepubertal gilts of similar age (191 d) and weight (121 kg) were administered an intramuscular injection of PG600 (200 IU human chorionic gonadotropin and 400 IU equine chorionic gonadotropin). Twelve days after the administration of PG600, gilts were fed 15 mg of altrenogest (Matrix) daily for 15 d to synchronize estrus. Estrus detection was performed by exposing gilts to a mature boar beginning 4 d after the cessation of altrenogest and continuing for 4 d. The first day gilts stood immobile was denoted d 1 of the estrous cycle. Blood samples were collected via jugular venipuncture on d 1, 4, 7, 10, 13, 16, 19, and 21 of the estrous cycle. Ten animals were slaughtered on d 1, 9, 14, and 21 of the estrous cycle, when hypothalami, anterior pituitary glands, and blood were collected. Relative expression of hypothalamic Kiss1, Kiss1R, ER-α, ER-β, and β-actin was determined using real-time reverse transcriptase PCR. Fold changes in relative expression were determined using the Relative Expression Software Tool. Relative expression is based on the expression ratio of a target gene versus a reference gene. The expression ratio results of the investigated transcripts were tested for significance by a Pair Wise Fixed Reallocation Randomized Test with day compared as independent time effects. Relative expression of Kiss1 was increased (P < 0.05) 3.2-fold on d 1 vs. d 21 and 2.3-fold on d 9 versus d 21 of the estrous cycle. Relative expression of Kiss1 was not different (P > 0.05) among the remaining days. Relative expression of ER-β was decreased (P < 0.05) 0.8-fold on d 9 versus d 21 and 0.7-fold on d 14 vs. d 21. Relative expression of ER-β was not different (P > 0.05) when comparing the remaining slaughter days. Relative expression of Kiss1R and ER-α were each not different (P > 0.05) among days. These data provide preliminary evidence that hypothalamic expression of kisspeptin varies throughout the porcine estrous cycle, which may modulate the subsequent release of GnRH.

Key Words: kisspeptin; hypothalamus; pig

1420 (T230) Levels of IGF-1, thyroxine, triiodothyronine and cortisol in yearling bulls in feedlot or silvopastoral system.
M. E. Romero*[1], J. A. Ramírez-Godínez[1], G. Corral-Flores[1], A. Flores-Mariñelarena[1], C. Rodríguez-Muela[1], P. F. Mancillas[1], J. A. Gutierrez[1], and A. Ayala[2], *[1]Universidad Autónoma de Chihuahua, México, [2]Universidad Autónoma de Yucatán, Mérida, México.*

The aim of this research was to compare serum levels of Insulin-like growth factor (IGF-1), triiodothyronine (T3), thyroxine (T4) and Cortisol (C) in 80 yearling bulls from two genetic groups, Brahman x Charolais (40 BhXCH) and Brahman x Brown Swiss (40 BhXBS), which were randomly assigned to the feeding system in the dry tropic region in Michoacan,

México; feedlot (F) or intensive silvopastoral (ISPS). Animals were bled from the jugular vein at 0, 71, 132, and 195 d to quantify serum hormones by radioimmunoassay. Effects of feeding system and genetic group across time were analyzed using mixed models from SAS with time repeated measures. Differences were found for IGF-1, T3 and C by feeding system ($P < 0.05$). Means were higher in IGF-1 (201.36 ± 8.25; 117.74 ± 7.74 ng mL^{-1}).T3 of F was higher ($P < 0.05$) than ISPS (1.15 ± 0.03; 0.99 ± 0.03 ng mL^{-1}). C of F was lower ($P < 0.05$) than ISPS (19.13 ± 1.80; 25.30 ± 1.71 ng mL^{-1}), respectively. Genetic group had no effect ($P > 0.05$) on T3, T4 and C concentration; however, effect on IGF-1 was found ($P < 0.05$). T4 was similar ($P > 0.05$) between systems. It was conclude that IGF-1 and T3 were higher in F than in ISPS. Genetic group only had effect on IGF-1. Handling of ISPS animals were more susceptible to stress than F, since the values for C were higher but decreased with time.

Key Words: silvopastoral, IGF-1, thyroid hormones, bovine.

1421 (T231) Meta-analysis of the effect of estrus expression before fixed-time AI on conception rates in beef cattle. B. N. Richardson*[1], S. L. Hill[2], J. S. Stevenson[2], G. D. Djira[1], and G. A. Perry[1], *[1]South Dakota State University, Brookings, [2]Kansas State University, Manhattan.*

Expression of estrus after PGF and before fixed-time AI has been reported to change the uterine environment, increase fertilization rates, accessory sperm numbers, and overall embryo survival. Thus, expression of estrus can strongly impact overall pregnancy success. Because of variation in percentage of beef animals exhibiting estrus and number of animals per study, it can be difficult to detect a significant effect of estrus on pregnancy success. Thus, a meta-analysis was conducted using data from 6981 animals in 20 studies that utilized variations of the 5 most common fixed-time AI protocols (CO-Synch, CO-Synch+CIDR, 5-d CIDR, PG 6-d CIDR, and the 14-d CIDR protocols) to examine the effect estrus had on conception rates. A random-effects model was used to combine the studies. The overall model indicated a positive effect of estrus on conception rates with cows expressing estrus before fixed-time AI having a 27% greater conception rate compared with those not exhibiting estrus ($P < 0.05$; 95% CI = 22% to 32%). Next we determined factors that influenced expression of estrus. Data were available on 547 cows synchronized with one of the CIDR based fixed-time AI protocols and observed for estrus for 2 to 4 yr. Analysis of these cows indicated that days postpartum ($P = 0.22$) did not impact estrus expression. In contrast, BCS influenced estrus expression ($P = 0.04$) with cows in a BCS of ≤ 4 (51 ± 5%) having decreased expression of estrus compared to those with a BCS > 4 ($\geq$ 70 ± 4%). Initiation of estrous cycles before the breeding season also influenced estrus expression ($P = 0.03$), with anestrous cows having greater expression of estrus compared with estrus-cycling cows (78 ± 5% versus 70 ± 5%, respectively). In conclusion, among all currently recommended fixed-time AI protocols, cows expressing estrus before fixed-time AI improved conception rates, and BCS and estrus-cycling status had the greatest influence on expression of estrus.

Key Words: fixed-time AI, estrus, pregnancy success

1422 (T232) Comparison of estrus parameters in nulliparous heifers by two automated activity monitoring systems. B. F. Silper*, A. M. L. Madureira, T. A. Burnett, M. Kaur, E. L. Drago Filho, A. M. de Passillé, J. Rushen, and R. L. A. Cerri, *Faculty of Land and Food Systems, University of British Columbia, Vancouver, Canada.*

The aim of this study was to compare a commercial (Heatime, SCR Engineers, Israel) and a research based (IceTag, IceRobotics, Scotland) activity monitoring system on their ability to measure estrus episodes in nulliparous heifers (n = 57; 119 estrus episodes) starting at 12 mo old. Only heifers detected by Heatime system were evaluated, therefore only the sensitivity of the systems could be measured for accuracy. Ultrasound scanning to describe ovarian structures and blood sampling for estradiol were performed at each estrus episode and later used to determine heat detection precision. Secondary signs of estrus (mucus, uterine tonus, and visual mounting and standing) were also recorded. Heifers were housed in dynamic groups of 24 animals in a free stall barn from May 2012 to August 2013. Data was analyzed using ANOVA and Pearson correlations using proc GLM, CORR and REG of SAS. Sensitivity of Heatime system was 84.7% (94/111), whereas IceTag had 98.7% (74/75) sensitivity. Estrus episodes lasted 12.7 ± 5.6 h on Heatime and 15.0 ± 3.9 h on IceTag and were highly correlated between them ($r = 0.60$; $P < 0.01$). Mean time difference of estrus initiation and end was 3.5 ± 4.3 h and 2.9 ± 4.9 h (IceTag as reference). Peak activity was also positively correlated ($r = 0.62$; $P < 0.01$) between systems and was 76.6 ± 19.9 index value and 4.6 ± 1.7 times increase related to baseline on Heatime and IceTag, respectively. Duration and peak activity were highly correlated for Heatime ($r = 0.63$; $P < 0.01$), but not significant when using the IceTags ($r = 0.13$; $P = 0.26$). Diameter of preovulatory follicle (15.7 ± 2.6 mm; mean ± SD) had no correlation with duration or peak activity as measured by both sensors. However, concentrations of estradiol in plasma were correlated with both duration ($r = 0.47$) and peak activity ($r = 0.36$; $P < 0.01$) measured only by the Heatime system. Number of baseline steps/h measured by the IceTag system had a strong negative correlation ($P < 0.01$) with peak activity in both sensors (r = -0.37 and -0.70). Heifers with more than one secondary sign of estrus had great peak activity and duration ($P < 0.05$) on the Heatime system, but not on the IceTag sensor. Secondary signs were not affected by follicle diameter and concentration of estradiol. Re-

sults indicate that both activity monitoring systems identified estrus with high sensitivity and that major measurements (i.e., episodes, duration, peak/intensity, initiation of estrus) were mostly correlated. However, some variables such as concentration of estradiol and secondary signs of estrus should be analyzed independently for research purposes.

Key Words: automated monitoring system, dairy cows, estrus detection

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1423 (T233) Cryopreserved sperm quality in young Brangus bulls raised on pasture and supplemented with vitamin E.

P. P. Tsuneda*, L. K. Hatamoto-Zervoudakis, R. D. Almeida, W. A. D. S. Marinho, J. T. Zervoudakis, B. H. Tsuneda, F. A. D. P. D. B. Arguello, M. F. Duarte, Jr., and T. B. Castaldeli, *Federal University of Mato Grosso, Cuiaba, Brazil.*

Vitamin E (or tochopherol) is a fat-soluble antioxidant that inhibits propagation of chain reactions induced by reactive oxygen (ROS) species in biological membranes, being an important defense mechanism against oxidative damage caused to sperm membrane, and reducing lipid peroxidation of membranes. The objective of this study was to evaluate semen characteristics and semen quality after freezing of young bulls raised on pasture and supplemented with vitamin E (α-tochopherol acetate). Sixteen Brangus bulls with 24 mo of average age and 462.2 kg of body weight mean, were randomly assigned to two treatments. Treatments were control group (CG, without supplementation) and group supplemented with vitamin E (GE- 400 IU of vitamin E/day added into concentrate). Each group was kept in separate paddocks formed by Panicum maximum cv. Mombaça and received 4.5 kg concentrate/animal/day. Animals received vitamin E supplementation for 60 d. Semen was collected by electroejaculation and diluted in TRIS–egg-yolk citrate extender with 4% of glycerol and were manually frozen. Cryopreserved semen was thawed in a water bath at 360°C for 30 sec. Immediately after thawing were evaluated sperm motility (percentage of mobile sperm), sperm vigor (intensity of motility, 1–5), sperm viability (percentage of live sperm), acrosome integrity (percentage of acrosome membrane integrity), sperm integrity (percentage of sperm with membrane integrity), and occurrence of acrosome reaction (percentage). The experiment was a completely randomized design with repeated measures and data were analyzed by ANOVA with a significance level of 5%. Supplementation with vitamin E improved the sperm viability ($P = 0.0225$) post-thaw (76.83 ± 2.07 vs. 81.91 ± 2.56). None effects of supplementation ($P > 0.05$) was observed in other traits. Based on parameters evaluated and results obtained from supplemented animals, it is concluded that this level of supplementation was beneficial for semen subjected to cryopreservation process indicating a better protection of the sperm membrane to membrane damage caused by freezing semen, justified by increased percentage of viable cells found in the supplemented group compared with control group.

Key Words: cryopreservation, oxidative stress, sperm viability

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1424 (T234) Addition of vitamin C extender and post-cryopreservation semen quality in bulls.

A. L. Cândida de Resende Fraga, L. K. Hatamoto-Zervoudakis, T. B. Castaldeli*, R. A. Minozzo, J. T. Zervoudakis, P. P. Tsuneda, F. M. Wingert, and R. D. Almeida, *Federal University of Mato Grosso, Cuiaba, Brazil.*

The process of spermatozoa freezing provides a resting state of cell while preserving cellular structure and fertilizing capacity of sperm. However, after thawing, semen quality is reduced compared to fresh semen. Sperm cell is able to generate and degrade reactive oxygen species (ROS) necessary for cell functioning, and oxidative stress is a cellular damage caused by imbalance between increased ROS and decreased antioxidant mechanisms. During cryopreservation can occur an increase in oxidative stress due deficiency of intra and extra cellular antioxidant defense system. Vitamin C is considered a great antioxidant of extracellular fluid, working mainly preventing formation of lipid hydroperoxide in plasma lipoproteins and protecting phospholipids in cell membranes. Thus, this study aimed to evaluate the use of vitamin C in cryopreservation extender medium of bull semen to reduce damage caused by cryopreservation process. Sixteen Brangus bulls in reproductive age were used. Ejaculate was collected by electrostimulation. After analyzed, samples were diluted in a extender (TRIS-citrate-egg-yolk with 4% of glycerol), divided into two treatments: the control group (without additive, CG) and other group supplemented with vitamin C (0.45 mg/mL, GS). Thawing was performed in water bath at 37°C for 30 sec. After that aliquots were evaluated to: sperm motility, sperm vigor, sperm viability and sperm membrane integrity. Data were analyzed by analysis of variance with a significance level of 5%. Vitamin C did not improved sperm motility after cryopreservation ($P > 0.005$, CG 45.06 ± 5.51 vs. SG 43.18 ± 4.04). Sperm vigor on GC (0.93 ± 0.85) not differ ($P > 0.05$) of SG (1.00 ± 0.89). Sperm viability in GC (51.71 ± 9.70) was not different ($P > 0.05$) to SG (49.99 ± 6.46). Sperm membrane integrity was not affected by supplementation ($P > 0.05$). Medium supplementation with vitamin C did not affect seminal parameters evaluated, demonstrating that supplementation was not effective in reducing damage caused by cryopreservation in bovine semen.

Key Words: antioxidant, sperm viability, oxidative stress

1425 (T235) Concentrations of progesterone during early follicular development and pregnancy rate to AI in beef cows. F. M. Abreu*[1], M. L. Day[1], M. A. Coutinho da Silva[1], C. A. Madsen[2], T. Martins[2], L. H. Cruppe[1], B. R. Harstine[1], G. A. Bridges[3], and T. W. Geary[2], *[1]Ohio State University, Columbus, [2]USDA ARS Fort Keogh, Miles City, MT, [3]University of Minnesota, Grand Rapids.*

The objective was to investigate if decreased progesterone (P4) concentrations earlier during follicular growth would impact fertility in beef cows. Crossbred (Angus x Hereford) cows (n = 261) received estradiol benzoate (EB; 1 mg, i.m.) and a previously used CIDR on d −7, to induce emergence of a new follicular wave approximately 3 d later (d −4). On d 0, all cows received 100 µg GnRH and were randomly assigned to one of the two treatments. In the high P4 (H; n = 131) treatment, the previously inserted CIDR was replaced with a new CIDR on d 0. In the low P4 (L; n = 130) treatment, 25 mg PGF was administered on d 0, and the CIDR previously inserted on d −7 remained. On d 5, blood samples to determine P4 concentrations were collected, all cows received two 25 mg PGF doses and CIDRs were removed. Estrous detection coupled with artificial insemination (AI) 12 h later (Estrus-AI) was performed for 60 h after PGF. Cows not detected in estrus within this period were bred by timed-AI (TAI) and received 100µg GnRH at 72 h. Pregnancy diagnosis was performed approximately 40 d after AI. P4 concentrations at CIDR withdrawal (d 5) were greater ($P < 0.01$) in the H (2.81 ± 0.10 ng/ml) than in the L (1.73 ± 0.05 ng/ml) treatment. Within the first 60 h after PGF, estrus response (82% vs. 85%) and estrus distribution (56.1 ± 0.7 h vs. 54.0 ± 0.7 h) did not differ between H and L treatments, respectively. Synchronized pregnancy rate was similar between H (77.1%) and L (82.3%) treatments. Across treatments, pregnancy rates were greater ($P < 0.01$) with Estrus-AI (82.9%) than TAI (63.6%). Concentrations of P4 on d 5 were negatively related ($P < 0.01$) with estrus response and time to estrus; across treatments. In conclusion, P4 concentrations during early follicular development did not influence synchronized pregnancy rate in beef cows.

Key Words: beef cattle, progesterone, pregnancy rate

1426 (T236) Tocopherol in bovine semen cryopreservation extender: Fertility and oxidative stress. L. K. Hatamoto-Zervoudakis*, L. Soares, J. T. Zervoudakis, F. M. Wingert, P. P. Tsuneda, M. F. Duarte Junior, and L. E. S. Silva*, Federal University of Mato Grosso, Cuiaba, Brazil.*

The study was conducted to evaluate effects of supplementation with tocoferol (α-tocopherol acetate) in bovine semen extender for cryopreservation on sperm quality and oxidative stress. Thirty-eight Nellore bulls with average age of 36 mo and average body weight of 490 kg were used. Ejaculate was obtained by electroejaculation and semen was diluted in lactose–egg yolk extender with 4% of glycerol. After semen + extender were divided in four fractions and subjected to four concentrations of vitamin E: TC, Control treatment, without supplementation medium; T10, 10 mmol mL of tocopherol supplementation/mL; T30, 30 mmol of tocopherol supplementation/mL; T50, 50 mmol of tocopherol/mL. Semen were frozen with a concentration of 45 million sperm per straw. Straws were thawed at 36°C for 30 sec, and evaluated for sperm motility, sperm viability, and oxidative stress. The experiment was a completely randomized design and data were analyzed using ANOVA and SNK test (Student- Newman-Keuls) with a significance level of 5%. Treatment control afforded less decrease in progressive motility rectilinear (P = 0.0135) after thawing (13.31 ± 2.34%) compared to the other experimental groups (T10: 5.36 ± 0.92%; T30: 8.15 ± 1.80%; and T50: 27.90 ± 3.89%). Oxidative stress on sperm in T10, T30 and T50 treatments (50.91 ± 7.22, 65.88 ± 2.58, 84.22 ± 11.68 ng/mL respectively) were higher (P = 0.0001) shown in the TC control treatment (13.07 ± 1.87 ng/ml). However, the treatment T10 provided a higher sperm viability (P = 0.0001, 73.91% ± 3.72) compared to supplementation levels (54.29% ± 4.20 T30, T50 46.41% ± 5.49), but was not superior to CT (68.95% ± 3.88). It can be concluded that use of antioxidants in bovine semen extender for cryopreservation did not protect sperm plasma membrane of lipid peroxidation, or against damage caused by cryopreservation, and the levels used in this work were detrimental to sperm fertility.

Key Words: TBARS, fertility, antioxidant

1427 (T237) Embryonic growth between d 33 and 45 of pregnancy in lactating dairy cows differing in hormone and metabolite concentrations. T. J. Stratman*[1], S. E. Poock[2], S. L. Murphy[1], A. Thomas[1], D. Bouhan[1], D. H. Keisler[1], and M. C. Lucy[1], *[1]University of Missouri, Division of Animal Sciences, Columbia, [2]University of Missouri, College of Veterinary Medicine, Columbia.*

Embryonic loss in dairy cows continues to occur into the 5 and 6 wk of pregnancy, and there is an association between slower embryonic growth and pregnancy loss during this period. Individual differences in hormone and metabolite concentrations may affect the growth of the embryo via endocrine mechanisms or by affecting nutrient flux across the placenta. The objective was to examine the relationship between postpartum hormones/metabolites and embryonic growth from d 33 to 45 of pregnancy. Holstein cows (n = 56; 86 ± 17 d postpartum at AI) were examined by transrectal ultrasonography on d 33, 35, 38, 40, 42, and 45 of pregnancy. Length (l) and width (w) of the embryo and amnionic vesicle were measured and the volumes for the embryo (e_vol) and amnionic vesicle (a_vol) were calculated [volume = 4/3*π*(0.5*l)*(0.5*w)*(0.5*w)]. There was an effect of day of pregnancy ($P < 0.001$) because

e_vol and a_vol increased from d 33 to d 45 (0.14 ± 0.01 cm^3 vs. 0.60 ± 0.06 cm^3; and 1.52 ± 0.05 cm^3 vs. 10.57 ± 0.59 cm^3, respectively). Across all days, the a_vol of male embryos was larger than female (4.25 ± 0.20 vs. 3.70 ± 0.20 cm^3) but e_vol was similar for male vs. female. Blood was collected on the d of ultrasound and plasma analyzed for glucose, progesterone (*P*4), growth hormone (GH), IGF1, and insulin (INS). Plasma hormone and metabolite concentrations were not affected by day of pregnancy ($P > 0.1$) but differed for individual cows ($P < 0.001$; range = 51 to 82 mg/dL for glucose, 4.7 to 13.5 ng/mL for P4, 2.3 to 13.4 ng/mL for GH, 50 to 131 ng/mL for IGF1, and 0.2 to 0.7 ng/mL for INS). Individual cows were categorized as being above or below the median for each blood hormone/metabolite concentration. Cows that were above or below the median for glucose, P4, GH, or INS were similar for e_vol and a_vol ($P > 0.1$). There was a category by day interaction ($P < 0.05$) for IGF1, however, because cows with IGF1 above the median (mean = 102.4 ± 16.4 ng/ml) had greater e_vol on d 42 compared with low IGF1 cows (mean = 69.9 ± 13.0 ng/mL) (1.11 ± 0.04 vs. 0.98 ± 0.04 cm^3; above vs. below). A_vol was not affected by IGF1 category. Conclusions were that male embryos have greater amnion vesicle volume from d 33 to 45 of pregnancy. Plasma concentrations of IGF1 were positively associated with a larger embryo on d 42. Chronically low IGF1 concentrations in lactating cows may lead to embryonic loss via slower embryo growth.

Key Words: bovine, embryo, metabolites

1428 (T238) Altered ovarian dynamics in lactating dairy cows undergoing embryonic mortality. *R*. Wijma*[1], M. L. Stangaferro[1], J. R. Branen[2], J. M. Howard[2], and J. O. Giordano[1], *[1]Dep. of Animal Science, Cornell University, Ithaca, NY, [2]Biotracking LLC, Moscow, GA.*

Our objective was to characterize ovarian dynamics in lactating dairy cows undergoing embryonic mortality. Cows ($n = 62$) received timed AI at 60 to 79 DIM after Presynch-Ovsynch. At AI, cows were blocked by parity (primiparous vs. multiparous) and randomly assigned to AI with regular semen ($n = 52$) or extender only ($n = 10$; Cycling; CY). Blood was collected every 48 h from 14 to 42 d after AI to determine concentrations of progesterone (P4) and pregnancy specific protein B (PSPB). Transrectal ultrasound was performed daily to assess ovarian dynamics. Cows were considered: 1) pregnant (PG; $n = 18$) if a viable embryo with a heartbeat was observed, 2) embryonic mortality (EM; $n = 6$) when a viable embryo or its heartbeat was no longer observed, and/or PSPB concentrations were initially above and then below 0.8 ng/mL, and 3) Non-pregnant (NP; $n = 28$) when no viable embryo was observed and PSPB concentrations remained below 0.8 ng/mL. Pregnant and EM cows had greater ($P < 0.01$) PSPB concentrations than NP and CY cows beginning 24 d after AI. Percentage of cows with complete luteal regression (CLR) was affected ($P = 0.02$) by group (CY and NP = 100 vs. 66.7% for EM) and occurred later ($P < 0.01$) in EM (39.5 ± 2.1) than in CY and NP cows (20.2 ± 1.3 and 22.9 ± 0.8). At 18 d after AI, NP cows had greater ($P < 0.05$) P4 than CY cows, whereas EM and PG cows had greater ($P < 0.05$) P4 concentrations than CY and NP cows. Among cows with CLR, the percentage ovulating was similar ($P = 0.21$) for CY, NP, and EM (90.0, 85.7, and 50.0%) cows. The interovulatory interval (IOI) was affected by group ($P < 0.01$). Cycling cows had the fewest days to ovulation (22.9 ± 0.96) followed by NP cows (25.8 ± 0.6) whereas EM cows had the longest interval to ovulation (40.0 ± 2.0 d). Days from CLR to ovulation was similar ($P = 0.52$) for CY (5.0 ± 0.9), NP (6.0 ± 0.6), and EM (7.0 ± 1.9) cows. Ovulatory follicle growth rate for the 5 d preceding ovulation was similar ($P = 0.28$) for all groups (CY = 1.6 ± 0.2, NP = 1.5 ± 0.1 and EM = 0.8 ± 0.5 mm/day). Likewise, diameter at ovulation was similar ($P = 0.77$) for all groups (CY = 22.7 ± 1.1, NP = 23.9 ± 1.8 and EM = 24.8 ± 3.8 mm). Thus, cows with EM were less likely to undergo complete luteal regression, and had extended IOI. The observed differences in IOI were due to delayed luteal regression rather than alterations in follicular wave dynamics. The longer IOI for NP than CY cows may have been caused by undetected EM. *Supported by Hatch project NYC127813.*

Key Words: ovarian dynamics, embryonic mortality, dairy cow