

ABSTRACTS
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* Author Presenting Paper

Physiology: Nutrition-reproduction, gametes and uterus

T1 Effects of supplemental high-linoleate safflower seeds on ovarian follicular development and hypophyseal gonadotropins and GnRH receptors. E. J. Scholljegerdes*, B. W. Hess, E. A. Van Kirk, and G. E. Moss, *University of Wyoming*.

Experimental objectives were to evaluate effects of supplemental high-linoleate safflower seeds on ovarian follicular development and hypophyseal LH, FSH, and GnRH-receptors. Beginning 1 d postpartum, 18 primiparous, crossbred beef cows (BW = 410 ± 24.2 kg) were fed foxtail millet hay at 2.13% of BW and either a low-fat control supplement (61.2% corn, 32.1% safflower seed meal, 3.7% liquid molasses; Control) or a supplement containing 94% cracked high-linoleate (67% 18:2) safflower seeds and 6.0% liquid molasses (Linoleate). Supplements were formulated to be isonitrogenous and isocaloric and the Linoleate diet was formulated to contain 5% fat. Cattle were slaughtered for collection of tissues after 35 d of experimental treatment. Average total number of follicles, number of follicles with in each size classification (1 to 4), and diameter of follicles were not influenced ($P = 0.17$ to 1.0) by dietary treatment. Treatment did not influence hypophyseal GnRH receptors ($P = 0.42$; 2.7 # 10⁻¹⁴ and 2.2 # 10⁻¹⁴ M/mg of protein for Control and Linoleate, respectively) or concentrations of LH ($P = 0.14$; 700 96.9 ng/mg) in the anterior pituitary gland. Conversely, concentrations of FSH in the anterior pituitary gland were greater ($P = 0.02$) for Linoleate (1100.6 ng/mg) than Control (805.1 ng/mg) cows. Fat supplementation enhanced stores of FSH in the anterior pituitary gland; however, increased hypophyseal FSH storage was not accompanied by increased ovarian follicular development. Overall, dietary fats high in linoleic acid do not improve development of ovarian follicles of primiparous cows early in the postpartum period either because of insufficient hypophyseal FSH secretion or decreased ovarian responsiveness to FSH.

Key Words: Beef cattle, Fat supplementation, Gonadotropin

T2 Reproductive effects of feeding lambs high-oleate or high-linoleate safflower seeds. Z. Kiyama*, B. W. Hess, M. R. Bolte, E. A. Van Kirk, and G. E. Moss, *University of Wyoming, Laramie*.

The objective of this project was to evaluate potential reproductive effects of feeding high-oleate or high-linoleate safflower seeds to ewe lambs. White-faced ewe lambs (BW = 34 ± 0.4 kg; n = 36) were fed a beet

pulp, oat hay and soybean meal basal diet (CON). Safflower seeds were fed as isocaloric and isonitrogenous replacement in the basal diet so that Oleate (OLE) and Lineolate (LIN) diets contained 5% additional fat. Lambs were slaughtered when they reached a final BW of 61.3 ± 0.9 kg. Based on serum progesterone or the presence of a corpus luteum (CL), 6, 5 and 2 lambs reached puberty prior to slaughter in LIN, OLE and CON groups, respectively. Lambs fed LIN had more ($P = 0.02$) and OLE tended ($P = 0.10$) to have more CLs than CON lambs. No treatment effects were detected ($P \geq 0.18$) for any other characteristics. Subsequently, data from only prepubertal lambs were re-analyzed. Within the prepubertal population, treatment did not influence ($P \geq 0.38$) hypothalamic contents of GnRH, hypophyseal GnRH-receptors, ovarian weights, or number of large follicles. Hypophyseal concentrations of LH ($P = 0.04$) were, and FSH tended ($P = 0.10$) to be influenced by treatment. Concentrations of LH ($P = 0.01$) and FSH ($P = 0.04$) were the greater in OLE than CON, and intermediate ($P \geq 0.24$) in LIN lambs. Uterine weight ($P = 0.09$) and number of small follicles ($P = 0.10$) tended to be influenced by treatment. Uteri from CON were heavier ($P = 0.03$) than those of OLE and were intermediate ($P = 0.13$) in LIN lambs. Similarly, numbers of small ovarian follicles were greater ($P = 0.05$) in CON than OLE and LIN lambs were intermediate ($P = 0.11$). Dietary fat supplied by safflower seeds may potentiate the onset of puberty in ewe lambs. It appears the response may be unique to the fatty acid composition of the supplement, but actions of other potential constituents of safflower seeds remains to be determined.

Key Words: Dietary fat, Lambs, Puberty

T3 Feed intake, serum leptin, and puberty in Brangus heifers sired by bulls with differing EPDs for growth and scrotal circumference. K. L. Shirley*¹, M. G. Thomas¹, D. H. Keisler², D. M. Hallford¹, D. M. Montrose¹, G. A. Silver¹, M. D. Garcia¹, and L. A. Narro¹, ¹New Mexico State University, Las Cruces, ²University of Missouri, Columbia.

Spring-born Brangus heifers sired by bulls with differing EPDs for growth and scrotal circumference were evaluated for growth, level of feed intake, serum concentrations of leptin, and puberty from 12.5 to 15.5 months of age. Sire EPD and accuracy for weaning weight, yearling weight, and scrotal circumference were 36.2 (0.61), 61.0 (0.54), and 0.1 (0.42) for a large growth-moderate scrotal circumference sire (LG-MS);

n = 7 heifers), 29.5 (0.66), 36.3 (0.59), and 0.9 (0.47) for a moderate growth-large scrotal circumference sire (MG-LSC; n = 8 heifers), and 25.9 (0.61), 39.3 (0.54), and 0.4 (0.37) for a sire with Balanced EPD values (n = 8 heifers). Heifers were weaned, trained to a Calan[®] gate system, and fed a diet of 11.6% CP and 79.4% TDN. Individual feed intake was measured daily and body weight was measured every two wk. Blood samples were collected twice weekly to evaluate serum concentrations of leptin and progesterone with RIA, and two consecutive samples of progesterone > 1 ng/mL was considered as day of puberty. Heifers were exposed to breeding for 90-d beginning at 14.3 months of age. Adjusted 205- and 365-d weight and ADG were similar ($P > 0.10$) in heifers from these sires, but feed intake was greater ($P < 0.01$) in heifers from the LG-MSc and MG-LSC sire groups relative to heifers from the Balanced EPD sire group (1.07 and 1.05 > 0.89 0.09 kg of feed consumed/100 kg of BW). Serum concentrations of leptin increased linearly ($P < 0.01$, slope = 0.02 ng/mL/d) in all heifers, but were similar ($P > 0.47$) among sire groups. Age of puberty was earlier ($P < 0.02$) in heifers from the Balanced EPD sire relative to heifers from the LG-MSc and MG-LSC EPD sires (13.7 < 15.1 = 15.5 0.4, d), however, pregnancy percentage was similar among sire groups (87.5% = 71.4% = 75.0%, $\chi^2 = 0.72$). Data suggest that Brangus heifers from a sire with balanced EPDs for growth and scrotal circumference achieve reproductive competency earlier than heifers from a sire with EPDs for either large growth-moderate scrotal circumference or moderate growth-large scrotal circumference. This relationship may be related to differences in mechanisms which influence feed intake, but appears to be independent of serum concentrations of leptin among the sires groups.

Key Words: Brangus, Puberty, Leptin

T4 Intracerebroventricular infusion of Neuropeptide Y and leptin differentially influence the episodic secretion patterns of GH in well-fed ovariectomized cows. L. A. Narro^{*1}, M. G. Thomas¹, M. D. Garcia¹, D. H. Keiser², M. Amstalden³, G. L. Williams³, and D. M. Hallford¹, ¹New Mexico State University, Las Cruces, ²University of Missouri, Columbia, ³Texas A&M University Agricultural Research Station, Beeville.

Anterior pituitary secretion of GH is regulated by hypothalamic secretion of somatostatin and GHRH. There are also appetite-regulating signals that modulate this axis at the level of the hypothalamus; however, these data are limited in cattle. Objectives of this study were to further evaluate the central effects of the orexigenic and anti-orexigenic peptides, neuropeptide Y (NPY) and leptin, on pituitary secretion of GH in cattle. Six *Bos taurus* x *Bos indicus* crossed cows were ovariectomized by paralumbar laparotomy and then surgically fitted with a third cerebroventricle cannula at least 3 wk before the start of the sampling period. Cows were fed a diet of hay and concentrate to maintain BW and condition. Body condition scores were 5.6 0.3 (i.e., scale of 1 = emaciated to 9 = obese) throughout the experiment. Cows were randomly assigned to receive infusions of Control (200 l of 0.3% BSA, 0.9% NaCl), NPY (500 μ g of pNPY dissolved in 200 l of 0.3% BSA, 0.9% NaCl) or Leptin (600 μ g leptin dissolved in 200 l of 0.3% BSA, 0.9% NaCl) solutions into the third cerebroventricle in a replicated 3 x 3 latin square arrangement. At least 1 d of rest occurred between sampling periods. Ten milliliters of blood were collected immediately before infusion and every 10 min thereafter for 4 h. Blood plasma was analyzed for concentrations of GH with RIA. Mean concentrations of GH, frequency and amplitude of GH pulses, and area under the response curve were estimated using CLUSTER[®]. No differences were detected among Control, Leptin, and NPY treatments in mean concentrations of GH (19.0 = 19.6 = 23.4 3 ng/mL, $P > 0.18$), area under the response curve (4545 = 4733 = 5648 747, $P > 0.17$), or frequency of GH pulses (1.6 = 1.2 = 1.7 0.4 per 4 h, $P > 0.17$). However, amplitude of GH pulses were greater (42.5 > 25.8 4.4 ng/mL, $P = 0.05$) in cows treated with NPY than Control, while leptin-treated cows did not respond differently ($P > 0.10$) from Control or NPY-treated cows. Results suggest that in well-fed mature cows, acute intracerebroventricular administration of NPY modulates pituitary secretion of GH by increasing the amplitude of GH pulses, but pituitary secretion of GH does not appear to be influenced by central administration of leptin.

Key Words: Neuropeptide Y, Leptin, Growth hormone

T5 Concentrations of antigonadotropic decapeptide in ovine tissues. S. N. Sandstede*, M. E. Wise, and D. M. Hallford, New Mexico State University, Las Cruces, NM/USA.

Antigonadotropic decapeptide (AGD) has been shown to inhibit secretion of GnRH and LH in several species. The mechanism of action of AGD is unknown but appears to be integral to the GnRH secretory process. The objectives of this study were to 1) develop a RIA to measure tissue concentrations of AGD and 2) determine which ovine tissues contained AGD. The AGD double antibody RIA utilized rabbit antiserum against AGD conjugated to KLH (Covance Res. Prod.) and synthetic AGD (Princeton Biomolecules) as the standard. The 125I-AGD was produced using chloramine T and purified using sephadex G-10 chromatography. No cross reactivity was detected for the following peptides: oxytocin, CRH, GHRH, TRH, ADH, SRIF, GnRH, and NPY. Detection limit was 0.3 ng, recovery of added AGD to tissue extracts was 108%, and the assay CV was 3%. Liver, kidney, skeletal muscle, and brain tissues were collected from six ewes, immediately frozen at -80°C, and subsequently homogenized in PBS. Tissues (0.02 to 1.0 g) were vortexed for 2 min using 3 mL of acid methanol (12% 2N HCl: 88% methanol; vol: vol) followed by centrifugation at 4100 x g for 10 min and dried at 40°C for 30 min. Using this procedure, an average of 82 \pm 1.2% of added 125I-AGD was recovered. The dried extract was resuspended in 0.5 mL of PBS containing 1% BSA which was then assayed in duplicate for AGD. No AGD was detected in control tissues (liver, kidney, and skeletal muscle). Likewise, the decapeptide was not detected in brainstem, cortex, pituitary, anterior or posterior hypothalamus. The pineal gland averaged 8.0 \pm 3.0 ng AGD/g tissue while the greatest concentration of AGD was detected in the median eminence (69.0 \pm 19.0 ng/g). These data demonstrate development of a sensitive AGD RIA. This RIA reveals the presence of substantial concentrations of AGD in the ovine median eminence and pineal gland.

Key Words: Antigonadotropic decapeptide, Radioimmunoassay, Neuropeptide

T6 Pituitary expression of ghrelin mRNA during the luteal phase of the bovine estrous cycle. H. C. Moore*, P. C. Gentry, R. J. Collier, and A. M. Turzillo, University of Arizona, Tucson, AZ.

Ghrelin, an endogenous growth hormone (GH) secretagogue, has been implicated as a regulator of metabolism and growth. Although predominantly secreted by the stomach, ghrelin expression was recently reported in somatotropes, lactotropes, gonadotropes, and corticotropes of the human pituitary gland. The presence of ghrelin mRNA in various pituitary cell types indicates that ghrelin may modulate the release of pituitary hormones other than GH. The potential influence of ghrelin on synthesis and secretion of gonadotropins as well as potential feedback effects of steroid hormones on ghrelin actions are yet to be determined. The objectives of this study were to determine 1) if the ghrelin gene is expressed in the bovine pituitary gland, and 2) if pituitary ghrelin mRNA expression is modulated by progesterone (P4) during the luteal phase of the estrous cycle. Following estrous synchronization, ovarian follicular dynamics in beef heifers were monitored with ultrasound and pituitary glands were harvested on days 2, 4, 6, 8, and 10 (n=6) after initiation of the first follicular wave following ovulation. Blood samples were collected daily and serum concentrations of P4 were determined by RIA. As expected, concentrations of P4 increased from day 2 to day 10 (0.7, 1.9, 3.3, 5.7, and 7.3 ng/ml on days 2, 4, 6, 8 and 10, respectively) and were correlated with luteal development. Total cellular RNA was reverse transcribed and primers spanning bases 40-481 of bovine ghrelin were used to amplify ghrelin cDNA by PCR. Representative cDNA products were sequenced to verify identity. Ghrelin gene expression was detected in pituitary glands on days 2-10. However, preliminary analysis indicated that pituitary ghrelin expression does not change during the luteal phase of the estrous cycle. We conclude that pituitary expression of ghrelin is not regulated in an endocrine fashion by P4. Studies are underway to determine cellular sites of ghrelin expression in the bovine pituitary gland.

Key Words: Ghrelin, Pituitary, Bovine

T7 Effects of short-term fasting on reproductive function in beef cows. A. Ramos III¹, K. K. Kane¹, D. E. Hawkins¹, W. D. Bryant¹, D. M. Hallford¹, G. E. Moss², and R. S. Kelling¹, ¹New Mexico State University, ²University of Wyoming.

To determine the influence of short-term fasting on reproductive function, normally cycling, crossbred cows were randomly assigned to one of three treatments: control (CONT; n = 8), CIDR (n = 9), or fasted (FAST; n = 9). CONT and CIDR cows were fed ad libitum while the FAST cows were not fed from d 10 through 14 (d 0 = estrus). CIDR cows received an intravaginal CIDR (InterAg, Hamilton, NZ; 1.38 g progesterone (P4)) on d 11. On d 15, cows received PGF2 α (Lutalyse[®], 25 mg, i.m.) at 0600 h and CIDRs were removed at 1800 h. Body weights were obtained on d 10 and 15 at 0600 h. Blood samples were collected d 10 through 16 for determination of P4. To evaluate serum LH, samples were collected every 15 min for 4 h on d 10 and 14 and every 4 h on d 17 through ovulation. Ultrasonography was performed daily from d 0 through d of ovulation. On d 15, BW loss of FAST cows was greater (-29 kg) than CONT (-1 kg) and CIDR cows (0 \pm 4 kg; P \leq 0.001). Mean P4 from d 10 through 14 did not differ among treatments (7.1, 7.1, 6.3 \pm 0.5 ng/mL, CONT, CIDR, and FAST, respectively; P \geq 0.05). Concentration of LH within treatments was similar between d 10 and 14 (CONT: 4.9 vs 4.2 \pm 1.3 ng/mL; CIDR: 1.3 vs 1.8 \pm 0.5 ng/mL; FAST: 3.5 vs 2.9 \pm 0.6 ng/mL, d 10 and d 14, respectively; P \geq 0.05). Time from PGF2 α to P4 \leq 1 ng/mL did not differ among treatments (30.0, 33.3, 26.2 \pm 3.3 h, CONT, CIDR, and FAST, respectively; P \geq 0.05). No differences were observed in times from PGF2 α to peak LH (79.7, 84.6, 127.7 \pm 26.7 h, CONT, CIDR, and FAST, respectively; P \geq 0.05) and peak LH to ovulation (24.0, 16.0, 41.1 \pm 9.8 h, CONT, CIDR, and FAST, respectively; P \geq 0.05). Time from PGF2 α to ovulation was greater in FAST (255 h) than CONT (96 h) and CIDR (101 \pm 55.3 h, P \leq 0.05). Short-term fasting did not affect serum P4 or LH during the luteal phase, P4 disappearance following PGF2 α , or timing of the LH surge. However, short-term fasting increased BW loss and time from PGF2 α to ovulation, suggesting an influence of short-term fasting on time of ovulation.

Key Words: Fasting, Ovulation, Reproduction

T8 Reproductive Performance of Dairy cows under the Pasture Production System of New Zealand. Z. Z. Xu, L. J. Burton, and D. L. Johnson, *Livestock Improvement Corporation Ltd, Hamilton, New Zealand.*

Reproductive performance is a key determinant of the efficiency of dairy production, especially for seasonal dairy production systems based on pasture. Studies in several countries have shown a gradual decline in reproductive performance. The objectives of this study were to benchmark current reproductive performance of dairy cows in New Zealand and to quantify the effects of various factors on reproductive performance. Data were collected from 101,185 cows over a 3-year period (414 herd-year groups). Pregnancy status was determined either by palpation per rectum and/or from calving information in the following calving season. The overall conception rate to first AI (CR1) for all cows was 53.0% (n=89,367) with a large variation among herds. The average CR1 for herds in the top and bottom quartiles were 62.3% and 43.4%, respectively. The conception rate to AI after a natural estrus was 55.3% compared with 38.6% to AI after an estrus induced with hormonal treatment.

Data were analyzed using the MIXED procedure of SAS and herd-year was included as a random factor. All following results are least squares means produced by the model. CR1 increased with age from 2 (53.5%) to 4 years (59.2%) and then decreased with age to 46.7% in cows aged \geq 10 years. Jersey cows had a lower CR1 (53.3%, P<0.0001) than Holstein-Friesian (56.1%) or crossbred cows (56.4%). Cows giving birth to twins had lower CR1 (47.0%, P<0.0001) than cows giving birth to a single (55.8%). Cows that had calving difficulty had a lower conception rate (50.3%, P<0.001) than cows that had no calving difficulty (55.8%). The relationship between the interval from calving to first AI and CR1 was curvilinear, with the linear (1.51), quadratic (-0.011) and cubic (0.000027) terms all being significant (P<0.01). The day of AI relative to the start of the breeding season also had a curvilinear relationship with CR1, with significant (P<0.01) linear (0.24), quadratic (-0.015) and cubic (0.000106) terms. These results show that the conception rate of dairy cows in New Zealand is higher than that reported in many other countries. The large variation among herds in conception

rate suggests tremendous scope for improving reproductive performance through management. (Supported by the Dairy Global Program)

Key Words: Reproductive performance, Conception rate, Risk factors

T9 Effect of genetic potential for milk yield on the onset of reproductive activity and corpus luteum function in Holstein cows. W. J. Weber¹, S. J. Kolath², M. C. Lucy², H. Chester-Jones¹, L. B. Hansen¹, and B. A. Crooker¹, ¹University of Minnesota, St. Paul, ²University of Missouri, Columbia. USA.

Holsteins from a breeding project initiated in 1964 to develop a stable control line (CL) that represents US breed average in 1964 and a select line (SL) that represents contemporary US Holsteins were used to determine effects of selection on corpus luteum function. Management of heifers (25 CL, 30 SL) and cows (13 CL primiparous (CL-P), 28 CL multiparous (CL-M), 25 SL-P, 38 SL-M) was identical for both lines. Blood samples were obtained weekly from heifers from 240 d of age until first observed estrus and 3X weekly for 6 wk from the later of first estrus or 12 mo of age and weekly from cows during the first 10 wk postpartum (PP). Blood from heifers and cows was collected 10, 28 \pm 3 and 42 \pm 3 d post-insemination (AI), unless a subsequent estrus was observed. Post-AI sampling was repeated prior to 730 d of age (heifers) or 200 d PP (cows) until conception. Growth and production variables were determined. Growth rate, production data, day of first plasma progesterone (P4) greater than 1.0 ng/ml, estrous cycle length, and area under the estrous cycle P4 curve (AUC) were analyzed using PROC MIXED and body measures and post-AI P4 as repeated measures using PROC MIXED of SAS. Means differed when P < 0.05. From 8 to 18 mo of age, growth rate was greater (800 vs. 730 g/d) but BCS consistently less (3.8 vs. 4.0 at 18 mo) in SL heifers. Daily 3.5% FCM yield through 17 wk PP was greater in SL-P (31.1 vs. 20.6 kg) and SL-M (43.8 vs. 29.7 kg) cows. Age at first P4 rise (291 vs. 302 8.0 d), cycle length (19.0 vs. 19.6 0.5 d), or AUC (85.3 vs. 82.6 4.4 ng/ml/d) were not affected by line. First P4 rise PP was delayed for SL cows (38.5 vs. 28.5 \pm 2.3 d) and not altered by parity. Plasma P4 increased from 10 to 42 d post-AI (5.6, 8.8, 9.3 \pm 0.2 ng/ml) but did not differ between lines. Corpus luteum function was not affected by greater growth rate of SL calves but PP alterations contribute to an increased interval of anestrus in SL cows.

Key Words: Selection, Progesterone

T10 Comparison of artificial insemination (AI) versus embryo transfer (ET) in lactating dairy cows. R. Sartori^{*}, A. Gümen, J. N. Guenther, A. H. Souza, and M. C. Wiltbank, *University of Wisconsin-Madison.*

Based on the idea that oocyte integrity and early embryonic development are compromised in dairy cows, we tested the hypothesis that conception rate (CR) can be improved by ET compared with AI. During 365 d, 550 potential breedings were used from 243 lactating Holstein cows with average milk production of 34.9 kg/d. Cows were synchronized with a modified Ovsynch protocol (GnRH-7d-PGF2 α -3d-GnRH) and were randomly assigned to receive AI immediately after the second GnRH injection (d 0) or to receive transfer of one embryo 7 d later (21.5 and 78.5% fresh and frozen embryos, respectively). Circulating progesterone and follicular and luteal size (by ultrasound) were determined on d 0 and 7. Cows with circulating progesterone \geq 0.5 ng/ml on d 0 (n = 66; 12.0%), < 0.5 ng/ml on d 7 (n = 9; 1.6%), or without a responsive follicle to GnRH on d 0 (n = 76; 13.8%) were considered not synchronized. Pregnancy diagnosis was performed by ultrasonography on d 25 or 32, and pregnant cows were reevaluated on d 60-66. Synchronized cows with single ovulation had similar (P > 0.30) CR on d 25-32 with ET (n = 176; 40.3%) and AI (n = 160; 35.6%). Pregnancy loss between d 25-32 and 60-66 also did not differ (P > 0.20) between ET (26.2%) and AI (18.6%) cows. When single (n = 336) and multiple (n = 57) ovulators were compared, independent of treatment, multiple ovulators had greater (P < 0.001) circulating progesterone on d 7 than single ovulators (2.7 vs. 1.9 ng/ml) and there was a tendency (P = 0.07) for a greater CR for multiple ovulators (50.9% vs. 38.1%). However, there was no difference in CR between AI and ET cows with multiple ovulation (50.0% vs. 51.7%). The CR tended to be lower for AI than ET in single-ovulatory cows ovulating smaller (\leq 15mm; 23.7 vs. 42.3%; P = 0.11) or larger (\geq 20mm; 34.3 vs. 51.0%; P = 0.13) follicles but not average ovulatory sized follicles (16-19 mm; 41.2 vs. 37.3%; P = 0.69). Thus, ET did not

improve overall CR in lactating cows but size and number of ovulating follicles may determine success with these procedures.

Key Words: Embryo transfer, Dairy cattle, Pregnancy

T11 Effects of varying dry period length and prepartum diet on reproduction in dairy cattle. A. Gümen, R. R. Rastani, R. R. Grummer, and M. C. Wiltbank, *University of Wisconsin-Madison*.

The effect of dry period length on reproductive performance of lactating dairy cows has not been previously evaluated. Sixty Holstein cows were assigned in a randomized block design to one of three treatments: 1) Traditional (T) dry period (56 d) with dry cows fed 28 d on low energy followed by 28 d on a moderate energy diet, 2) Shortened (S) dry period (28 d) with cows continuously fed a high energy diet, or 3) Zero (Z) dry period with cows continuously fed a high energy diet. Cows had ovaries evaluated by ultrasound and blood samples collected 3 times per week beginning from d 6 or 7 postpartum (PP) until 7 d after second ovulation. Average d from calving until the first 10 mm follicle were fewer ($P < 0.05$) in Z (8.1 d) and S (9.0 d) than T (10.5 d). Time from calving to first ovulation was earlier ($P < 0.01$) in Z (14.5 d) than T (28.9 d) with S (21.5 d) intermediate. A follicle of the first follicular wave ovulated in more ($P < 0.01$) Z (84%; 16/19) than T (43%; 9/21) with S (65%; 13/20) intermediate. Double ovulation rate at the first ovulation was greater ($P < 0.02$) in T (62%) than Z (21%) with S (35%) intermediate. However, there was no difference in double ovulation rate at second ovulation (15/60). There were no differences among treatments in size and volume of the ovulatory follicle or in luteal volume and serum progesterone concentrations on day 7 after ovulation for cows with single or double ovulation. Number of cows with persistent CL (>30 d; 18/60) was not different among groups; however, short luteal phases were greater ($P < 0.05$) in Z (21%; 4/19) than S (0%; 0/20). Days to first AI were shorter ($P < 0.06$) in Z (68.7 d) and S (68.0 d) than T (75.4 d). First service conception rate was greater ($P < 0.06$) in Z (58%; 11/19) than T (25%; 5/20) with S (29%; 6/21) being intermediate. Days open in pregnant cows were fewer ($P < 0.05$) in Z (80.7 d) than S (121.1 d) or T (114.4 d). Thus, shortening or eliminating the dry period leads to earlier PP ovulation and may improve reproduction in lactating dairy cows.

Key Words: Dry Period, Reproduction

T12 Relationship between milk production and estrous behavior of lactating dairy cows. H. Lopez^{*1}, L. D. Satter^{1,2}, and M. C. Wiltbank¹, ¹Dairy Science Department, *University of Wisconsin*, ²US Dairy Forage Research Center, *USDA-ARS, Madison, WI*.

The objective of this study was to evaluate the association between level of milk production and duration of estrus as determined by mounting activity recorded by a radiotelemetry system. Holstein cows ($n=267$; 50 DIM) were fitted with a transmitter that allowed 24h/d recording of mounting activity. Cows were housed in a free-stall barn with concrete flooring and milked twice daily with milk weights recorded. Ovulation was confirmed for all estruses ($n=323$) by ultrasonography. Average milk production for 10d before the day of estrus was used to classify cows as low (<39.5 kg/d) or high (≥ 39.5 kg/d) producers at the time of estrous expression. Follicle diameter and serum estradiol (E2) concentrations were determined in a subset of single-ovulating cows ($n=71$) on the day of estrus. Duration (6.2 ± 0.5 vs. 10.9 ± 0.7 h; $P < 0.0001$), mounts (6.3 ± 0.4 vs. 8.8 ± 0.6 ; $P = 0.001$), and mounting time (21.7 ± 1.3 vs. 28.2 ± 1.9 s; $P = 0.007$) were shorter for estruses from high (46.4 ± 0.4 kg/d; 91±3DIM; $n=146$) than low producers (33.5 ± 0.3 kg/d; 96±3DIM; $n=177$). The effect of milk production on estrous duration was similar in primiparous (5.7 ± 0.8 h for high [45.9 ± 0.8 kg/d; $n=49$] vs. 10.7 ± 0.8 h for low [33.7 ± 0.3 kg/d; $n=135$] producers; $P < 0.0001$) and multiparous (6.4 ± 0.6 h for high [46.6 ± 0.5 kg/d; $n=98$] vs. 11.9 ± 1.4 h for low [33.0 ± 0.6 kg/d; $n=41$] producers; $P < 0.0001$) cows. Milk production for 10d before the day of estrus was correlated with estrous duration for primiparous ($r = -0.54$; $P < 0.0001$) and multiparous ($r = -0.48$; $P < 0.0001$) cows. E2 concentrations were lower (6.8 ± 0.5 ; $n=31$ vs. 8.6 ± 0.5 pg/ml; $n=40$; $P = 0.01$) for high than low producing cows in spite of larger preovulatory follicle diameter (18.6 ± 0.3 ; $n=31$ vs. 17.4 ± 0.2 mm; $n=40$; $P = 0.004$). Level of milk production was correlated with E2 concentrations ($r = -0.56$; $P < 0.0001$) and diameter of the preovulatory follicle

($r = 0.44$; $P < 0.0001$). Thus, high level of milk production decreases duration of estrus probably due to decreased concentrations of E2 at estrus.

Key Words: Dairy cow, Estrous behavior, Milk production

T13 Milk urea nitrogen and conception rate: a population study using test-day records. J. E. Vallimont¹, G. W. Rogers^{*2}, L. A. Holden¹, M. L. O'Connor¹, J. B. Cooper², C. D. Dechow², and J. S. Clay³, ¹Penn State University, ²University of Tennessee, ³Dairy Records Management Systems.

Reproductive failure is costly to dairy producers, and high milk urea nitrogen (MUN) levels are known to affect reproduction. Dairy Records Management Systems, Raleigh, NC, provided records for 15,191 test days with a first service within 30 d of a MUN test to determine the relationship between MUN and conception rate (CR) in Holstein cows. Conception rate data were included from October 1998 to December 2000; seasons were summer (April to September) and winter (October to March). Days to first service (DFS) was limited to 25 to 200 d. Herds were required to have a first service CR between 10% to 65%. Data were analyzed with SAS using the PROBIT model of PROC LOGISTIC. Analyses included wet chemistry alone (WC) and WC plus infrared (ALL). Variables in the final model were herd, year-season of insemination, parity group (1, 2, and 3+), and MUN as a continuous variable or MUN grouped (<6 , 6 to 7, 8 to 9, 10 to 14, 15 to 16, 17 to 18, and >18 mg/dl). Milk yield did not change the impact of MUN on CR, and DFS was not significant. Milk urea nitrogen approached significance at the 0.10 level. Cows were 22% more likely to conceive if they had a WC MUN of 8 to 9 mg/dl ($n=181$) in the period of the week preceding or following a service compared to MUN of 10 to 14 mg/dl ($n=1690$, $P < 0.08$). Cows with WC MUN 6 to 7 mg/dl ($n=57$) had a 22% better likelihood of conception than those with MUN 10 to 14 mg/dl ($n=1638$) when inseminated within a two-wk period after MUN test ($P < 0.09$); cows with WC MUN <6 mg/dl or >18 mg/dl ($n=14$, $n=621$) were 27% and 13% less likely to conceive than those with MUN 10 to 14 mg/dl for the same period ($P < 0.09$). The continuous MUN variable in WC and ALL models predicted pregnancy outcome for services within two wk after MUN test, but was not significant for services within two wk before MUN sample date ($P < 0.06$). Lower MUNs, with the exception of <6 mg/dl, at the time of insemination were associated with improved CR.

Key Words: Milk urea nitrogen, Conception rate

T14 The effect of daily drenching with propylene glycol during the transition period on LH pulsatility and the fate of the first follicle wave in dairy cows. S. T. Butler^{*} and W. R. Butler, *Cornell University*.

The early postpartum period in high producing dairy cows is characterized by chronic severe negative energy balance, hypoinsulinemia, hypoglycemia, and inadequate LH pulse frequency resulting in a varying duration of anovulation. This experiment was carried out to determine if a daily transient elevation in insulin and glucose could ameliorate the detrimental effects of negative energy balance on LH pulsatility and the fate of the first follicle wave. Mature Holstein cows were drenched with either 500 ml of propylene glycol (PG; $n=30$) or water (CTL; $n=29$) daily from day -10 prior to parturition until day 25 postpartum. Transrectal ultrasound examinations of ovarian follicle development were carried out on 3 days per week from day 10 until day 30. Frequent blood samples (every 30 minutes) were collected via indwelling jugular catheters from a sample of 10 cows from each treatment group on day -10, 2, and 25 to assess the glucose and insulin response to the treatments. In addition, on day 10 postpartum blood samples were collected at 10 min intervals for 12 hours to determine treatment effects on LH pulse profiles. Both insulin and glucose were elevated on day -10, 2, 10 and 25 following PG administration (treatment by time, $P < 0.01$). On day 10 postpartum the number of LH pulses (7.8 ± 0.5 vs. 7.1 ± 0.5 pulses per 12 hours; $P > 0.1$), mean LH (0.56 ± 0.05 vs. 0.46 ± 0.05 ng/ml; $P > 0.1$) and pulse amplitude (0.56 ± 0.07 vs. 0.50 ± 0.7 ng/ml [peak # base]; $P > 0.1$) were not different between CTL and PG cows respectively. The proportion of dominant follicles that became ovulatory (10/29 vs. 11/30), non-ovulatory (13/29 vs. 15/30) and cystic (6/29 vs. 4/30) between day 10 and 30 postpartum were not different between CTL and PG cows respectively. The results indicate that daily drenching from day -10 to

25 relative to parturition with propylene glycol had little effect on LH pulsatility or on the outcome of the first follicle wave.

Key Words: LH, Ovary, Propylene glycol

T15 Reproductive and metabolic parameters associated with low postovulatory progesterone secretion in lactating dairy cows. G. E. Mann^{*1}, L. M. Hicking¹, and D. Blache², ¹University of Nottingham, Sutton Bonington, UK, ²University of Western Australia, Nedlands, Australia.

In dairy cows, inadequate progesterone secretion following mating is an important cause of early pregnancy loss though the reasons for this reduced secretion of progesterone are not known. The aim of this study was to determine the reproductive consequences of low postovulatory progesterone secretion and to identify parameters associated with this problem. Milk progesterone concentrations were determined on day 5 following first insemination in 96 lactating Holstein Friesian dairy cows. Low progesterone was empirically defined as a milk progesterone concentration of <3ng/ml while high progesterone was defined as any concentration greater than this value. Of the 96 cows sampled, 15 (15.6%) had low milk progesterone concentrations (<3ng/ml) and 81 cows (84.4%) had high milk progesterone. Mean milk progesterone concentration was 1.9±0.2ng/ml in the low group and 6.8±0.3ng/ml in the high group. Conception rate in the low progesterone group (13.3%) was significantly lower ($P<0.01$) than in the high progesterone group (58.8%). However, there was no difference between low and high progesterone cows in the days from calving to first insemination or in the day of initiation of first luteal activity. Milk yield in the low progesterone group (36.9±1.5 l/d) was not significantly different to that in high progesterone group (34.9±0.9 l/d). Body condition score (0 to 5 scale) in the low progesterone group (1.4±0.1) was significantly lower ($P<0.01$) than in the high progesterone group (1.8±0.1) as was plasma leptin concentration (1.4±0.2 vs 2.2±0.2 ng/ml; $P<0.05$). There were no significant differences in plasma concentrations of urea, beta hydroxybutyrate or glucose between the low and high progesterone groups. In conclusion, low day 5 progesterone resulted in a severely reduced pregnancy rate and was associated with reduced condition score and plasma leptin concentration but was not associated with increased milk yield or altered blood metabolites.

Key Words: Milk progesterone, Cow, Pregnancy

T16 Effect of gossypol intake on plasma and uterine gossypol concentrations and on embryo development and viability in vivo and in vitro. M. Villaseñor*, A. C. Coscioni, K. N. Galvao, S. O. Juchem, J.E.P. Santos, and B. Puschner, University of California - Davis.

Forty-eight postpubertal Holstein heifers (13 mo; 380 kg of BW) were blocked by age and BW and randomly assigned to one of three isocaloric and isonitrogenous diets differing in their free gossypol (FG) content: control (C; 0 mg of FG/kg of BW); medium (M; 20 mg of FG/kg of BW); and high (H; 40 mg of FG/kg of BW). Cracked Pima cottonseed was used as a source of gossypol. Heifers were fed diets for 70 d prior to superovulation and embryo collection. Heifers were flushed on d 5.5 after the initial AI and embryos were evaluated. Plasma gossypol concentrations were measured 1 d prior to uterine flush. Embryos with grades 1 to 3 were either stained, to determine the number and proportion of live and dead cells, or randomly assigned to culture in vitro for 96h in a medium containing either 0 (MC) or 10 µg/ml (MG) of gossypol acetic acid (GAA). Continuous, binomial, and count data were analyzed by the GLM, LOGISTIC, and GENMOD procedures of the SAS (2001) program. Number of embryos grades 1 and 2 was similar for C, M, and H and averaged 3.3 ($P=0.29$), but number of unfertilized oocytes, grade 3 and degenerated embryos was higher for H than C and M (5.6 vs 2.9 vs 0.5; $P<0.01$). Total number of cells in fresh stained embryos were similar for C, M, and H (16.6 vs 14.8 vs 16.4; $P=0.40$). However number of live cells was higher for C compared to M, but did not differ from H (13.5 vs 10.4 vs 12.2; $P=0.05$). Similarly, percent of total live cells was higher for C compared to M, but did not differ from H (80.2 vs 72.2 vs 78.1; $P=0.02$). Hours of development in in vitro cultured embryos was reduced by GAA (77.1 vs 64.9h; $P=0.05$), but no interaction between heifer diet and culture medium was observed ($P=0.50$). An interaction between heifer diet and culture medium was observed for the proportion of live embryos after 96h of culture ($P=0.10$). Number of cells after culture was higher for C than M and H (23.1 vs 20.3 vs 14.6; $P<0.01$), but

culture medium did not influence cell numbers ($P=0.50$). High gossypol diet and GAA in the medium influenced negatively embryo quality and development.

Key Words: Gossypol, Embryo, Heifers

T17 Effect of gossypol intake and plasma gossypol concentrations on follicle development and luteal function in dairy heifers. A. C. Coscioni*, K. N. Galvao¹, M. Villaseñor¹, J.E.P. Santos¹, B. Puschner¹, and L.M.C. Pegoraro², ¹University of California - Davis, ²EMBRAPA - Brazil.

Twenty-seven postpubertal Holstein heifers (13 mo; 380 kg of BW) were blocked by age and BW and randomly assigned to one of three isocaloric and isonitrogenous diets differing in their free gossypol (FG) content: Control (C; 0 mg of FG/kg of BW, N=8); medium (M; 20 mg of FG/kg of BW; N=9); and high (H; 40 mg of FG/kg of BW; N=10). Cracked Pima cottonseed was used as a source of gossypol. Heifers were fed diets for 30 d and then estrus was synchronized with an injection of GnRH (Cystorelin- Merial Ltda) and insertion of a progesterone implant (CIDR- Pharmacia Animal Health), followed 7 d later by an injection of PGF2a (Lutalyse-Pharmacia Animal Health). Heifers were ultrasounded every 24 h during an entire estrous cycle. Follicle and CL development, and plasma progesterone concentrations were evaluated daily, and plasma gossypol concentrations were evaluated once at the end of the cycle. Continuous data were analyzed by the GLM procedure of SAS (2001) and repeated measurements over time were analyzed by the MIXED procedure of SAS (2001). Emergence of first and second follicular waves (FW) were similar ($P>0.15$) for C (1.1 and 9.1 d), M (1.0 and 8.9 d), and H (1.9 and 8.8 d). Deviation of the dominant follicle (DF) after emergence for the first (C=3.5 vs M=3.5 vs H=3.4; $P=0.99$) and second FW (C=4.0 vs M=4.6 vs H=4.5; $P=0.61$) was not affected by treatments. Treatment had no effect on CL growth throughout the estrous cycle ($P=0.68$). Estrous cycle length, maximum follicle diameter for the DF of the first and second FW, period of follicle dominance for the DF of the first and second FW, and diameter of ovulatory follicle were not influenced by dietary gossypol intake. Results indicate that consumption of up to 40 mg of FG/kg of BW does not influence follicle and CL development in dairy heifers. A.C. Coscioni: Supported by CAPES, Brazil

Key Words: Gossypol, Heifer, Follicle development

T18 Effect of gossypol intake on plasma and uterine gossypol concentrations and on embryo quality and development in superovulated Holstein dairy heifers. A. C. Coscioni*, M. Villaseñor¹, K. N. Galvao¹, R. C. Chebel¹, J.E.P. Santos¹, J. H. Kirk¹, B. Puschner¹, and L.M.C. Pegoraro², ¹University of California - Davis, ²EMBRAPA - Brazil.

Seventy-four postpubertal Holstein heifers (13 mo; 380 kg of BW) were blocked by age and BW and randomly assigned to one of three isocaloric and isonitrogenous diets differing in their free gossypol (FG) content: Control (C; 0 mg of FG/kg of BW, N=23); medium (M; 20 mg of FG/kg of BW; N=26); and high (H; 40 mg of FG/kg of BW; N=25). Cracked Pima cottonseed was used as a source of gossypol. Heifers were fed diets for 60 d prior to superovulation. Superovulatory treatment with 8 decreasing doses of FSH started on d 9 of the estrous cycle. Heifers were inseminated twice, 12 h apart, with the first insemination when estrus was first detected. Heifers were flushed on d 7.0 after the initial AI and embryos evaluated. Blood and uterine flush samples were collected and evaluated for the gossypol concentration. Embryos graded as 1 to 3 were frozen and evaluated again after thawing. Continuous, binomial, and count data were analyzed by the GLM, LOGISTIC and GENMOD procedures of SAS (2001), respectively, and heifer was the experimental unit for analyses. Number of structures collected per heifer were, respectively, 9.4, 8.4, and 8.8 for C, M, and H ($P=0.88$). Number of embryos grades 1 and 2 were similar for all treatments ($P=0.87$), and averaged 3.5, 3.6, and 3.3 for C, M, and H, respectively. However, heifers receiving the H diet (5.8) had higher number ($P<0.01$) of grade 3 and degenerated embryos than those receiving the C (3.6) and M (3.2) diets. Number of unfertilized oocytes were higher ($P<0.01$) for M (2.4) than C (2.0) and H (1.0). Heifers fed C and M had a lower proportion of embryos classified as Morula than those fed H (33.3 vs 20.2 vs 47.7%; $P<0.02$). Feeding a diet with 40 mg of FG/kg of BW increased the number of degenerated embryos and hindered embryo development by day 7 after AI.

A.C. Coscioni: supported by CAPES, Brazil.

Key Words: Gossypol, Embryo quality, Heifer

T19 Enhancing ability of bovine sperm to survive cryopreservation with cyclodextrin and cholesterol. A. Kaya^{*1,2} and J. J. Parrish¹, ¹*University of Wisconsin Madison, Wisconsin*, ²*University of Selçuk Konya, Turkey*.

Protection of plasma membrane integrity during cryopreservation of spermatozoa is important for their fertilizing ability. The objective of this study was to determine the optimal dose of cholesterol-loaded cyclodextrin (CLC) resulting in an increase in the post-thaw characteristics of bull sperm. Semen was initially diluted to 120×10^6 sperm/ml in a Na citrate buffer composed of 63 mM Na citrate, 55 mM glucose and at PH = 7.0. The CLC was dissolved in TALP medium containing 3 mg/ml of BSA. The solution was added to the sperm suspension at various doses of CLC (0, 1.25, 2.5, 3.75, 5 and 7.5 mg/ 120×10^6 sperm/ml) and incubated 15 min at room temperature. The sample was then processed by standard freezing procedures. Post-thaw sperm characteristics were evaluated for motility using time-lapse photography of fluorescently labeled sperm nuclei, sperm with intact acrosomes (wet mount, DIC) capacitation status in response to lysophatidylcholine (LPC), sperm viability assessment using SYBR-14 and propidium iodide (PI) and functional integrity of sperm membrane using hypoosmotic swelling (HOS) test. The CLC addition to the extender did not differ significantly in sperm motility, intact acrosome and capacitated sperm rates ($P > 0.05$). However sperm treated with 1.25 and 2.5 mg CLC had improved sperm viability (69.0 and 69.2 % vs. 58.5 %) and membrane functional integrity (59.8 and 51.8 % vs. 36.5 %) compared to control, respectively ($P < 0.01$). Additionally, 3.5 mg of CLC also resulted in an increase in the response to the HOS test (47.8 % vs. 36.5 %; $P < 0.05$). These results indicate that sperm treated with the lower doses of cholesterol-loaded cyclodextrin could modify sperm plasma membranes resulting in an increase post-thaw viability and osmotic responsiveness. Supported by State of Wisconsin and American Breeders Service Global.

Key Words: Cholesterol, Cyclodextrin, Semen freezing

T20 Wisconsin avian extender yields better post-thaw motility for rooster semen than Minnesota avian extender after cryopreservation. L. E. Enwall^{*1}, A. Kaya², L. N. Geiger¹, and J. J. Parrish¹, ¹*University of Wisconsin Madison, Wisconsin*, ²*Selçuk University Konya, Turkey*.

Reliable and consistent protocols for the successful freezing of avian sperm remain somewhat elusive especially in comparison to the success enjoyed by the bovine industry. We developed an extender, Wisconsin avian extender (WISA) and compared it to Minnesota avian extender (MNA) with differing final concentrations (6%, 11%, 14%, and 19%) of glycerol. WISA is a modification of a Tyrode's based medium used to incubate bovine sperm in an air atmosphere. Semen was pooled from 4-12 roosters, diluted 1:2 with extender, frozen in 0.5 ml straws over static nitrogen vapor, and then immersed in liquid nitrogen. Straws were thawed at 35C for 1 minute and then semen diluted 1:20 into the same base extender, either WISA or MNA. Two separate operators assessed motility of thawed semen visually. Sperm frozen without glycerol did not survive. At the lowest final concentration of glycerol, 6%, neither treatment yielded greater than 15% average motility and were not statistically different from one another although both were different from the WISA at 11%, 14%, and 19% glycerol concentrations ($p < .001$). At final glycerol concentrations of 11%, 14%, and 19%, WISA demonstrated a far more significant ($p < .001$) advantage in preserving sperm motility after thawing. None of the 11%, 14%, and 19% MNA/glycerol treatments exceeded 10% average motility while the comparable WISA treatments exceeded 30%, 40% and 40% motility respectively. Although motility is not necessarily indicative of fertility, these results indicate that WISA is a strong candidate for the cryopreservation of rooster sperm.

Key Words: Cryopreservation, Semen, Rooster

T21 The effect of time and fluid volume on the rate of boar sperm settling using a commercial extender. K. L. Willenburg^{*}, K. J. Rozeboom, B. R. Lindsey, and M. E. Wilson, *Minute of America, Verona, WI, USA*.

The objective of the study was to evaluate the effect of time and fluid volume on the rate of sperm settling using a commercial extender with special interest in uniform concentration distribution during semen dose preparation. To analyze sperm settling rate, three extended semen volumes (10, 80, and 1000 ml) contained in each of six 15 ml conical tubes, each of six 100 ml AI tube, and a 1000 ml glass beaker, respectively, were sampled from three areas of each container (top, middle, and bottom). Samples (0.5 ml) were drawn from each container at each of the three levels and each time point using a glass pipette. Samples contained 3.75×10^8 spermatozoa/ml from a pool of two boars of known fertility. Sperm concentration was determined with Sperm Vision, which was verified in an earlier study with a hemacytometer. Samples were taken at 0, 5, 10, 20, 40, and 80 minutes. However for statistical analysis, times were blocked into three groups, T1 = 0 and 5 m, T2 = 10 and 20 m, T3 = 40 and 80 m. Initial sperm concentration was similar among the three treatment volumes. Furthermore, settling rate by volume interactions, as measured by sampling the top, middle, and bottom, areas was not present ($P > 0.1$). However, semen concentration over time varied among the three areas sampled ($p < .01$). Top, middle, and bottom concentrations were similar for T1, however, for T2, more sperm were recovered from the bottom than from the top (3.1 vs 3.3×10^6 , respectively, $p < .01$). Similarly, sperm numbers were different at T3 for the top, middle, and bottom (2.5 vs. 2.9 vs. 5.1×10^6 , respectively, $p < .01$). In summary, sperm settling appears to depend upon time, but not necessarily volume. Based upon these conditions, settling occurred between 10 and 20 minutes regardless of volume. The results of this experiment show that extended semen should be remixed before dose distribution if the semen is left undisturbed for more than 10 minutes.

Key Words: AI, Boar, Sperm concentration

T22 Boar seminal plasma effects on AI outcomes. A.L. Ruiz-Sanchez^{*}, R. O'Donoghue, and G. Foxcroft, *University of Alberta, Edmonton, Alberta, Canada*.

Rozeboom et al. (Swine AI News Bulletin vol. IX. 2000) suggested a minimum requirement of 10 to 12% seminal plasma (SP) in semen diluted for AI use to maintain high fertility. As part of an ongoing study of ejaculate quality and boar fertility, the impact of differing percentages and absolute amounts of seminal plasma on fertility outcomes was examined. The first sperm rich fraction of ejaculates collected from nine boars twice weekly over 7- to 8-month periods was diluted to 1.5 billion morphologically normal sperm in 50 mL BTS extender, and used to breed at least 55 gilts. Boars differed consistently for pregnancy rate (73 to 98%; $P < 0.0003$) and farrowing rate (71 to 98%; $P < 0.0003$) and two boars (G1, R1) were identified as being less fertile. Total born was affected by both boar (8.8 to 12.0; $P < 0.001$) and time (9.5 to 11.1; $P = 0.038$), with no boar x time interaction. In contrast, a boar x time interaction ($P < 0.0001$) existed for percentage of SP (range 4.9 to 20.7%) and total volume of SP (range 2.5 to 10.3 mL) per AI dose, and a lack of significant correlations between SP inclusion and proven fertility suggests that even at low sperm numbers, the amount of SP per AI dose did not critically affect fertility. Unpublished data in the boar and other domestic species suggest that specific boar SP proteins make be related to differences in boar fertility. Our initial results indicate that although total protein concentration in raw semen SP was different among boars (19.13 to 37.97 mg/mL; $P = 0.029$), total SP protein in diluted semen did not differ among boars or times, and hence showed no meaningful correlations with proven differences in boar fertility. Associations with specific SP proteins are presently being evaluated.

Key Words: Seminal plasma, AI, Boar Fertility

T23 Evaluation of post-thaw boar semen characteristics of two genotypes using three extenders. H. D. Blackburn^{*}, *USDA-ARS-National Animal Germplasm Program*.

The National Animal Germplasm Program's (NAGP) charge is to develop cryopreserved collections of animal genetic resources. Effective implementation of swine cryopreservation in the program requires freezing protocols that can be employed across genotypes. Therefore, semen was collected from Yorkshire (YK, n=4) and Composite (CP, n=5)

boars to evaluate three extenders: BF5, LEY and BF5 containing 2-hydroxypropyl-beta-cyclodextrin added (BF5CD). The literature has suggested adding cyclodextrin to boar semen extenders improves post-thaw viability. Post-thaw measurements were performed with computer assisted sperm analysis (CASA) for motility (MOT), cell area, track speed (VCL) and straightness of cell movement (STR). CASA readings were taken on each extender breed combination from post-thaw time 0 (T-0) and a subsequent reading at 105 minutes (T-105). Post-thaw CASA characteristics were evaluated using a mixed model (SAS, 2002). Model main effects were: extender, breed and extender*breed as fixed effects, while boar nested within breed was random. For evaluating pre-freeze and post-thaw cell area the inverse of cell area was used to normalize the data. The effect of extender*breed for MOT and VCL were highly significant at T-0. The YK-BF5 combination caused the interaction by increasing MOT to 52.3% vs. 30.6% and VCL to 145.6 vs. 121.3 the mean for all other extender breed combinations. For the response variables STR and cell area the BF5 extender yielded better post-thaw performance ($P < .01$). Boar nested within breed was found to be significant at T-0, with MOT ranging from 4 to 70%. However, by T-105 boar within breed was not significant for any of the traits measured. At T-105 BF5 held a significant advantage over LEY and BF5CD. These results do not support previous work showing BF5CD as affording better cryoprotection when compared to BF5. Given these results the extender of choice for preserving boar semen in the NAGP repository is BF5. As new extenders become available additional testing of genotypes and extenders will be performed.

Key Words: Cryopreservation, Boars, Genetic conservation

T24 Effect of fetal bovine serum on the development of in vitro produced porcine embryos. J. N. Caamano^{*1}, J. Mao¹, T. C. Cantley¹, A. R. Rieke¹, R. Farwell¹, C. Murphy¹, B. A. Didion², and B. N. Day¹, ¹University of Missouri, Columbia MO, ²Monsanto, St. Louis, MO.

The objective of the experiments was to assess the effect of adding Fetal Bovine Serum (FBS) to the embryo culture medium (NCSU-23) on days 3, 4 or 5 after in vitro fertilization (IVF) on embryo development of in vitro produced porcine embryos. In vitro maturation, fertilization and embryo culture were performed following established procedure (Abeydeera et al., 2000; Theriogenology 54:787-797) in our laboratory. In Experiment 1, embryos were selected and placed in a well after 72 h in culture. From this pool, embryos were randomly allocated to treatment groups. Groups of embryos were placed on day 3, 4 or 5 after IVF in NCSU-23 medium with the addition of 0.4% BSA or 10 % FBS. Blastocyst formation was assessed on day 6 to 9 after IVF. Only excellent/good quality blastocysts were included in the analysis. Results are presented in Table 1. There was an advantage for FBS when added on day 4 or 5 to the culture medium but not when was added on day 3 after IVF. In Experiment 2, FBS/BSA were added on day 4 and blastocyst cell number was assessed on day 6 after IVF. Average embryonic cell number was higher ($P < .05$) in embryos cultured in the presence of FBS (41.6 2.1) than in the presence of BSA (35.4 2.2). It was concluded that FBS could exert a differential effect on embryo development depending on the day that it was included in the culture medium. Table 1: Embryo development of in vitro produced embryos in NCSU-23 with the addition of Fetal Bovine Serum or BSA

Media	Day	No.	Day 6* %Total Blast. x±SEM
NCSU-BSA	3	57	52.0±6.4 ^{ab}
NCSU-BSA	4	57	57.9±6.4 ^{ab}
NCSU-BSA	5	46	49.1±6.4 ^{ab}
NCSU-FBS	3	57	33.2±6.4 ^a
NCSU-FBS	4	57	67.3±6.4 ^b
NCSU-FBS	5	55	65.3±6.4 ^b
Day 6*	Day 7*	Day 7*	Day 8
%Hatched Blast x ± SEM	%Total Blast. x ± SEM	%Hatched Blast x ± SEM	% Total Blast x ± SEM
0	46.9±6.7 ^{cd}	0.0±2.7 ^e	41.3±8.8 ^{gh}
0	60.3±6.7 ^{cd}	0.0±2.7 ^e	27.2±8.8 ^f
0	50.2±6.7 ^{cd}	0.0±2.7 ^e	31.1±8.8 ^{gh}
0	33.2±6.7 ^c	0.0±2.7 ^e	33.7±8.8 ^{gh}
0	68.7±6.7 ^d	18.6±2.7 ^f	66.4±8.8 ^h
0	67.6±6.7 ^d	3.1±2.7 ^d	67.6±8.8 ^h
Day 8	Day 9	Day 9	
% Hatched Blast** x ± SEM	% Total Blast* x ± SEM	% Hatched Blast** x ± SEM	
0.0±3.2 ⁱ	13.0±7.3 ^l	0.0±3.1 ⁿ	
0.0±3.2 ⁱ	12.2±7.3 ^l	0.0±3.1 ⁿ	
0.0±3.2 ⁱ	9.9±7.3 ^l	0.0±3.1 ⁿ	
5.1±3.2 ^{ik}	32.8±7.3 ^l	6.4±3.1 ⁿ	
28.1±3.2 ^j	66.4±7.3 ^m	35.4±3.1 ^o	
16.5±3.2 ^{jk}	66.6±7.3 ^m	33.6±3.1 ^o	

*Within columns, Least Squares Means without a superscript in common are different ($P < .05$). ** Within columns, Least Squares Means without a superscript in common are different ($P < .01$).

Key Words: Embryo culture, Porcine embryos, Fetal bovine serum

T25 Effect of follicular size on developmental competence of porcine oocytes in vitro. J. Mao^{*}, J. N. Caamano, T. C. Cantely, R. Farwell, A. R. Rieke, M. F. Smith, and B. N. Day, University of Missouri-Columbia.

The developmental competence of the oocyte is acquired progressively during late follicular growth. In conventional in vitro porcine oocyte maturation and fertilization procedures, immature oocytes are aspirated from 2- to 6- mm follicles. This experiment was conducted to test the hypothesis that the developmental potential of oocytes collected from different-sized follicles was different. Prepubertal gilt ovaries were obtained from a local abattoir. Oocytes were aspirated from three groups of follicles: 2.0-3.0 (small), 3.1-5.0 (medium), and 5.1-7.0 mm (large) in diameter. Oocytes were cultured in basic maturation medium (TCM199) supplemented with 0.5 µg/ml FSH and 0.5 µg/ml LH for 22 h, then transferred to TCM199 without hormones and cultured for another 22 h. After culture, oocytes were stripped of cumulus cells and fertilized with cryopreserved ejaculated spermatozoa for 6 h. In part 1 of the experiment, oocytes were cultured until day 6 post fertilization. Cleavage rate at 48 h and the percentage of blastocysts on day 6 was determined. Data were analyzed using SAS general linear model. The cleavage rate and proportion of oocytes developed to blastocyst stage on day 6 were 55.1 2.5% (n = 290), 50.4 1.5% (n = 969), and 44.2 3.9% (n = 188); 33.4 2.7%, 26.3 1.6%, and 13.8 4.2% for the large, medium and small follicle groups, respectively. Cleavage rate in the large follicle group was higher than that in the small follicle group ($P < 0.05$). The percentage of oocytes developed to blastocyst in large and medium follicle groups were higher than the small follicle group ($P < 0.05$). Blastocyst cell numbers in the large and medium follicle groups were also higher than those in the small follicle group (35.1 4.1 and 38.3 3.2, vs 25.2 4.2, $P < 0.05$). In part 2 of the experiment, 12 h post in vitro fertilization, oocytes were fixed in 25% acetic alcohol for 48-72 h and the number of pronuclei was determined. The polyspermic fertilization rate (3 or more pronuclei in the oocyte) was 42.9, 40.0 and 48.8% for the large, medium and small follicle groups ($P > 0.05$). It was concluded that oocytes aspirated from medium- and large-size follicles are more competent compared those collected from 2-3 mm follicles. However, the incidence of polyspermic fertilization is not different among them.

Key Words: In vitro maturation, Follicular size, Porcine

T26 Effects of bovine somatotropin (bST) on IGF-I and IGF-binding proteins in non-lactating cyclic and pregnant Holstein cows on day 17 after estrus. T. R. Bilby*, A. Guzeloglu, S. Kamimura, F. Michel, and W. W. Thatcher, *University of Florida, Gainesville, FL, USA.*

Non-lactating cows were used to examine effects of bST on uterine proteins and gene transcripts encoding components of the IGF system. Cows (n=85) were injected on dV10 (d0 = timed insemination [TI]) with GnRH (100µg) followed 7d later (d-3) by PGF_{2α} (25 mg). At 48h after injection of PGF_{2α}, GnRH (d-1) was administered, and 16 h later (d0) 55 cows were TI and 29 cows were not TI (cycling). On d0 and d11, cows received either bST (500 mg, n=52) or no bST (n=33). A follicular cyst was detected on d0 in 7 cows and on d 7 in 5 cows. CL regression prior to d16 was observed in 2 cows. These 14 cows were not slaughtered; 22 cyclic and 49 TI cows were slaughtered on d17. Uteri were flushed with 40 ml of PBS to recover uterine flushings and verify presence of a conceptus. Ligand Blot analyses for IGFs in uterine flushings were done on 19 cyclic and 18 pregnant cows. Endometrial tissues were collected from 14 cyclic and 16 pregnant cows for Northern blot analyses. Ligand blots revealed IGFBP-3, 4, 5 and molecular weight protein 28-29 in flushings from all cows. IGF-binding protein-3 (IGFBP-3) was higher in bST treated pregnant cows (P < 0.05) compared to pregnant control cows. The IGFBP-4, IGFBP-5 and molecular weight 28-29 proteins were higher in cyclic versus pregnant cows (P < 0.001). Northern blot analyses detected IGF-I, IGFBP-3, IGF-II and IGFBP-2 mRNAs in endometrial tissues from all cows. However, growth hormone receptor (GHR-1a) mRNA was undetectable in all cows. Interactions between status and bST (P < 0.01) were detected for the mRNAs encoding IGF-I, IGFBP-3, IGF-II and IGFBP-2. The mRNAs for IGF-I, IGFBP-3, IGF-II and IGFBP-2 increased in bST treated cyclic cows; furthermore, pregnancy increased IGF-II and IGFBP-2 in control cows. In conclusion, differential uterine responses were detected in response to bST and pregnancy status in non-lactating Holstein cows.

Key Words: Cycle-pregnancy, bST, IGF-family

T27 Molecular characterization and endometrial expression of porcine Smad1. J. G. Kim*, J. L. Vallet, D. Noneman, G. A. Rohrer, and R. K. Christenson, ¹USDA, ARS, U.S. Meat Animal Research Center, Clay Center, NE.

Uterine capacity contributes to litter size in swine. Previous gene mapping analyses revealed a quantitative trait locus (QTL) for uterine capacity on chromosome 8. Comparison of porcine and human genetic maps suggests that the Smad1 gene is located near this region. Smad1 mediates signal transduction from TGF-β family ligands, including TGF-β and bone morphogenetic proteins. In addition, Smad1 mutation in mice causes defects in allantois formation. To further explore Smad1 as a candidate gene for the uterine capacity QTL, we 1) cloned and sequenced the full coding region for Smad1, 2) examined endometrial expression of Smad1 during the estrous cycle and early pregnancy, and 3) mapped the Smad1 gene. By iterative screening of a porcine expressed sequence tag library, we obtained 2161 and 2077 bp cDNA clones containing the full coding region of Smad1. The two clones differed in their 5' untranslated regions while their coding regions were identical, suggesting differential splicing. The inferred amino acid sequence of porcine Smad1 was 99.8% identical to human Smad1. Endometrial expression of Smad1 mRNA in White composite gilts (n = 3 to 4) was determined by Northern blotting using total RNA from d 10, 13 and 15 cyclic and from d 10, 13, 15, 20, 30 and 40 pregnant gilts, followed by densitometry. Endometrial expression of Smad1 mRNA was greater (P < 0.05) on d 10 (47.6 ± 3.8 arbitrary units) of pregnancy than d 10 (36.2 ± 3.8) of the estrous cycle. In both pregnant and cyclic gilts, endometrial expression of Smad1 mRNA was lower (P = 0.03) on d 15 (33.8 ± 3.8, 40.2 ± 3.8) than d 13 (44.0 ± 3.8, 48.4 ± 4.4), respectively. The Smad1 gene was mapped to chromosome 8, position 78 cM, near the peak of the uterine capacity QTL (71 cM). Elevated endometrial expression of Smad1 mRNA on d 10 of pregnancy suggests a role for Smad1 in signal transduction in endometrium during this period and the location of the Smad1 gene in the porcine genome is consistent with it being a candidate gene for the uterine capacity QTL.

Key Words: Uterine capacity, Coding region, Mapping

T28 Relative amounts of mRNA encoding endothelial and inducible nitric oxide synthase in the bovine corpus luteum. W. D. Bryant*, K. K. Kane, J. S. Nelson, A. Ramos III, and D. E. Hawkins, *New Mexico State University.*

Nitric oxide (NO) has emerged as a molecular messenger that mediates biological processes in several mammalian tissues. Nitric oxide is synthesized from L-arginine by one of three different NO synthase (NOS) isoforms, two constitutively expressed, endothelial (eNOS) and neuronal nitric oxide synthase, and one inducible (iNOS). Nitric oxide has been implicated in the regression of the corpus luteum (CL). Therefore, relative amounts of mRNA encoding eNOS and iNOS were examined in the bovine CL by real-time RT-PCR during the early- and mid-luteal stages and in response to PGF_{2α}. Twenty cows were synchronized with 25 mg PGF_{2α} (i.m., Lutalyse®) and randomly assigned to one of four treatments (estrus = d 0). Corpora lutea (n = 5/treatment) were collected via ovariectomy during early-luteal (d 5; early), mid-luteal (d 12; mid), mid-luteal, 6 h after PGF_{2α} injection on d 12 (mid-6), and mid-luteal, 24 h after PGF_{2α} injection on d 12 (mid-24). Immediately after collection, CL were snap frozen in liquid nitrogen and stored at -80°C. Total RNA was extracted from each CL and amplified in duplicate by real-time RT-PCR. Corpora lutea collected in the early-luteal stage tended to have less mRNA encoding eNOS than those from mid-luteal stage cows (0.07 vs 0.22 ± 0.05 arbitrary units, early and mid, respectively; P = 0.09) whereas early and mid mRNA encoding iNOS did not differ (P = 0.90). When the CL in mid, mid-6, and mid-24 were compared for mRNA encoding iNOS, mid and mid-24 were greater than mid-6 (0.45, 0.14, 0.47 ± 0.09 arbitrary units, mid, mid-6, and mid-24, respectively; P = 0.03). A similar trend was also observed for eNOS (0.22, 0.02, 0.27 ± 0.09 arbitrary units, mid, mid-6, and mid-24, respectively; P = 0.14). Relative amounts of mRNA encoding iNOS were similar whereas mRNA encoding eNOS tended to differ in the early and mid-luteal bovine CL. Moreover, mRNA encoding iNOS differed, and eNOS tended to be altered, in response to PGF_{2α}, suggesting that PGF_{2α} may regulate amounts of mRNA encoding NOS in the bovine CL.

Key Words: Nitric oxide synthase, Bovine, Corpus luteum

T29 Factors affecting postpartum placental blood volume. A. L. Riddle* and H. D. Tyler, *Iowa State University, Ames, IA.*

The objective of this study was to determine factors affecting the volume of blood retained in the placenta following delivery of the calf. In addition, we developed a technique for accurately measuring the volume of blood retained in the placenta following delivery. Optimal delivery conditions can improve both the short term and long term health status of the calf. Fifteen Holstein cows and heifers were placed in a maternity barn approximately 3-4 d prior to their estimated delivery date. An electronic birth monitoring system was used to determine the initiation of stage 2 labor. The umbilical cord was clamped during the delivery process. Calves were separated into two groups: those with cords clamped prior to the first breath or simultaneous with the first breath (n=7) and those with cords clamped approximately one-minute after the first breath (n=8). The first breath was considered as the first inspiration (gasp) of air. Blood samples were collected from the jugular vein of the calf immediately after birth to determine hemoglobin concentrations. Placentae were evaluated within 12 h after expulsion. Cotyledon color, cotyledon number, hemoglobin concentration from all cotyledons, placental weight, and cotyledon weight were recorded. Blood remaining within the placenta was calculated using an algorithm that included cotyledonary weight, cotyledonary [Hb], and calf blood [Hb]. Multiple regression analysis was used to identify explanatory variables associated with each response variable. Response variables included placental blood volume and placental expulsion time. Factors that affected placental blood volume included cotyledon hemoglobin concentration (P < 0.001), placental weight (P < 0.01), and calf hemoglobin concentration (P < 0.01). The only factor that significantly affected placental expulsion time was the weight of the placenta (P < 0.01). These data suggest that placental blood transfer does not appear to affect placental expulsion time in cattle.

Key Words: Placenta, Placental blood volume