to measure ambient temperatures. Semen was collected from each of two stallions diluted at a ratio of 3:1 (extender: semen) with a commercially available stallion semen extender (Exodus Breeders Supply, York, PA) and packaged into each of the containers. Three sets of containers per season (winter and summer) where commercially shipped from Tucson, AZ to Springfield, MO with overnight delivery. Spermatozoal motilities were evaluated utilizing CASA following shipment. Containers were exposed to ambient temperatures that range from a high of 41°C during the summer to a low of 5.5°C during flight in the winter. Values for progressive motility of spermatozoa following 24 h of storage and shipment were similar ($P > .05$) for both cooling devices. Final storage temperatures following 24 h were similar ($P > .05$) for both devices. These data suggest that during both the winter and summer months in the southwestern United States, disposable cooling devices can be used to ship or cool stallion spermatozoa for 24 h.

Key Words: Stallion, Semen, Shipment

M247 Massage as a recovery method in exercising horses. C. A. S. Porr* and K. Bennett-Wimbush, *Ohio State University, Wooster*.

Delayed onset muscular soreness (DOMS) is a painful condition which often occurs after unaccustomed or intense exercise. While no current therapy prevents DOMS, massage is a one that may alleviate pain more quickly, potentially allowing athletes to compete more effectively in subsequent exercise that may occur within a short time period. With regard to horses, the weight and ability of a rider can compromise proper back function, resulting in shortened strides, stiffened posture, and resistance to riders cues. The objective of this research was to evaluate the effects of massage as a recovery aid on subsequent performances of an exercise test in horses. Ten geldings, five Thoroughbreds (TB) and five Quarter Horses (QH), were randomly divided into two groups and subjected to two exercise tests. A test consisted of two exercise bouts with a 30 min recovery between them and a two hr data collection period following the second bout. Recovery between bouts was either control (walking and standing) or 30 min massage. Massage included a combination of Swedish and sports techniques, and was performed by a single certified equine massage practitioner. Horses in Group 1 received a massage recovery during the first test and a control recovery during the second while horses in Group 2 had reversed treatments. All horses received the same recovery after the second bout of exercise, and 10-12 d elapsed between exercise tests. Heart and respiration rates and plasma for lactate were collected before and after each exercise bout and at 5, 10, 20, 30, and 120 min post exercise. Serum for creatine kinase was collected before and after each exercise bout and at 120 min post exercise. Horses showed lower heart rate (46 ± 8 vs 52 ± 7; $P<.03$) and respiration rate (57 ± 15 vs 69 ± 13; $P<.05$) during the second exercise test, most likely due to lower environmental temperatures (24.9 vs. 20.8). Also, QH had higher serum lactate concentrations than TB ($P<.01$). There were no effects of massage on any parameters measured, though there were expected effects of exercise on each ($P<.0001$).

Key Words: Massage, Exercise, Horses

M248 Comparisons of behavioral testing on Morgan horses at different training levels. K. M. Holt and M. C. Nicodemus*, *Mississippi State University, Mississippi State*.

Horses with an early, successful show career are more profitable and while locomotive studies have been able to predict show performance, limited research has been done on behavioral characteristics that demonstrate show aptitude. The objectives were to quantify behavior in 2 training levels of Morgan horses by performing standardized behavioral tests: 1) 2-year olds in the initial stages of show training (Group 1) and 2) Adult horses (7-13 years old) successfully showing at an advanced level (Group 2). Comparisons of tests results should indicate that if Group 1 has similar results as Group 2 then they are more likely to demonstrate the same success in the future training process as Group 2. 6 registered Morgan horses, 3 for each group, were selected according to the following criteria: 1) consistently managed in the same program (W.H. Miner Agricultural Research Institute); 2) similar training and training levels within each group; and 3) were clinically healthy and sound. The following standardized behavioral tests were randomly performed in a consistent manner on the same day and location: 1) Learning retention- time for the horse to select a previously taught symbol; 2) Problem solving- time to find a reward placed under an obstacle; 3) Touch stimulus- amount of pressure on the skin before the horse reacts; 4) Auditory stimulus- time to re-approach a point where a loud noise was sounded; and 5) Visual stimulus- time to re-approach a point where an unfamiliar object was introduced. If the horse did not immediately and correctly perform test 1 or 2, "failing" was assigned. Both groups were similar in their results except for the learning retention test in which the adult horses were disinterested in the test, both the teaching and testing process, while the young horses were curious. While the similarity between group reponses may indicate that the young horses, similar to the adult horses, should be successful in their future training process leading to a more promising show career, unforeseen events may hinder or further future performance. Larger sample populations with different backgrounds may result in more variations.

Table 1. Means (SD) of behavioral testing results for the young minimally trained (Group 1) and adult advanced-trained (Group 2) Morgan horses.

	Group 1	Group 2
Learning Retention	100% Pass	100% Fail
Problem Solving	67% Fail	67% Pass
Touch Stimulus (psi)	2.7(.8)	2.7(1.2)
Sound Stimulus (s)	3.7(4.1)	2.5(.5)
Visual Stimulus (s)	5.6(.8)	5.2(.9)

Key Words: Morgan Horses, Behavioral Testing, Training

Physiology and Endocrinology: Female Reproduction

M249 Residual feed intake (RFI) and serum concentrations of insulin in developing Brangus heifers from sires with differing EPDs for growth and scrotal circumference. K. L. Shirley*[1], M. G. Thomas[1], D. H. Keisler[2], D. M. Hallford[1], D. M. Montrose[1], G. A. Silver[1], and M. D. Garcia[1], [1]*New Mexico State University, Las Cruces,* [2]*University of Missouri, Columbia*.

Objectives were to evaluate growth, feed intake characteristics, and metabolic hormones related to puberty in Brangus heifers from a desert breeding program. Heifers were from sires with EPDs (accuracy) for yearling weight and scrotal circumference of 28.5 (0.55) and 0.2 (0.44) for a large growth-moderate scrotal circumference sire (LG-MSC), 17.2 (0.61) and 1.0 (0.49) for a moderate growth-large scrotal circumference sire (MG-LSC), and 19.5 (0.54) and 0.5 (0.44) for a sire with balanced EPD values. Eight heifers per sire were weaned at 6 mo of age, trained to a Calan gate system for daily feed intake evaluation, and fed a diet of 11.6% CP and 79.4% TDN until 15 mo of age. Heifers were bled twice weekly to determine concentrations of progesterone and metabolic hormones via RIA and BW was measured every two weeks. In 2003, results of ANOVA among sires were reported for growth traits, level of feed intake, serum concentrations of leptin, and puberty (J. Anim. Sci. 86(Suppl.1):236). These analyses suggested that heifers from the Balanced EPD sire achieved puberty (i.e., progesterone concentrations > 1ng/ml) 1 mo earlier than heifers from LG-MSC or MG-LSC sires. Heifers from the Balanced EPD sire also consumed less feed from 11 to 13 mo of age, but no differences in serum concentrations of leptin were observed among sires. Further analyses suggest serum concentrations of insulin were lower ($P < 0.05$) in heifers from a sire with balanced EPDs relative to heifers from LG-MSC ($2.8 < 5.1 \pm 0.7$ ng/mL). Since a strong ($R^2 = 0.96$) regression was observed between BW and time, residual feed intake (RFI) was estimated by predicting daily feed intake with metabolic midweight ($BW^{0.75}$) and ADG. In heifers born to the sires with Balanced and LG-MSC EPDs, RFI was less ($P < 0.05$) than in heifers born to the MG-LSC sire ($-0.59 = -0.38 < 0.70 \pm 0.30$ kg/d). Results suggest Brangus heifers from a sire with balanced EPDs for growth and scrotal circumference appear to have concentrations of

insulin and RFI favorable for earlier onset of puberty than heifers from LG-MSC or MG-LSC sires.

Key Words: Heifer, Puberty, Intake

M250 Inhibition of development of bovine embryos by gossypol timing of inhibitory effects and possible involvement of apoptosis. J. Hernández-Cerón[1], F. D. Jousan*[2], P. Soto[2], and P. J. Hansen[2], [1]*Universidad Nacional Autónoma de México, México,* [2]*University of Florida, Gainesville.*

Gossypol, a polyphenolic pigment in cottonseed, inhibits development of bovine preimplantation embryos. The embryonic stage most sensitive to gossypol is not known, however, nor the mechanism by which gossypol blocks development. Objectives were to characterize embryonic stages at which gossypol inhibits development and test involvement of apoptosis in actions of gossypol. For Exp. 1, presumptive one-cell embryos were cultured continuously in medium containing 0, 2.5, 5, and 10 μg/ml gossypol. Cleavage rate was not reduced by gossypol but the percent of one-cell embryos that became blastocysts 8 d post-insemination (pi) was reduced ($P<0.05$) by 10 μg/ml gossypol (14.4, 14.3, 12.2, and 7.6% for 0, 2.5, 5 and 10 μg/ml; 6 replicates with 180-242 oocytes/group, SEM=1.5%). For Exp. 2 (6 replicates, 237-268 oocytes/group), presumptive one-cell embryos were exposed to 0 or 10 μg/ml gossypol for either 24 h or 8 d. The proportion of embryos developing to the blastocyst stage was not reduced by 24-h exposure to gossypol (18.4 vs 17.7% for 0 or 10 μg/ml) but was reduced when gossypol exposure was for 8 d (13.0 vs 3.1%; gossypol x incubation time, $P<0.03$, SEM=1.5%). For Exp. 3 and 4, gossypol did not affect development to the blastocyst stage when two-cell embryos were collected at 30-31 hpi and cultured with gossypol until 8 dpi (Exp. 3) or for 24 h (Exp. 4). In Exp. 5, embryos > 16 cells were collected at 5 dpi, cultured with 0 or 10 μg/ml gossypol for 24 h, and analyzed by the TUNEL procedure to determine apoptosis (n=6 replicates/99-100 embryos/group). There was no effect of gossypol on cell number (51.0 $\pm$ 2.8 vs 51.9 $\pm$ 2.6 for control and gossypol, respectively) or the percentage of cells that were apoptotic (8.2 $\pm$ 0.7% vs 9.2 $\pm$ 0.6%). Results indicate that embryonic development can be disrupted by long-term exposure to 10 μg/ml gossypol and that exposure at the one-cell stage is required for disruption in development. Inhibition of development does not appear to involve induction of apoptosis. (USDA NRICGP 2002-35203-12664).

Key Words: Gossypol, Embryo, Apoptosis

M251 Distinct steroidal regulation of mRNAs for aromatase and gonadotropin receptors in bovine granulosa cells. W. X. Luo* and M. C. Wiltbank, *University of Wisconsin-Madison, Madison.*

Many genes are regulated through estrogen and androgen receptors; however, regulation of gene expression by these steroids in follicular cells has not be completely defined. Granulosa cells of 5 mm bovine follicles were cultured in Medium 199 with 1% fetal bovine serum. Real-time PCR was used to determine mRNA concentrations. In expt. 1, cells were treated with eight different doses of 17 beta-estradiol (E_2), testosterone (T), or 5 alpha-dihydrotestosterone (DHT) for 5 days. Aromatase mRNA was induced by all doses of E_2, but only by high doses (300, 100, 30, 10ng/ml) of T and DHT. Concentrations of LH receptor mRNA increased only after treatments with high doses of E_2, T, and DHT. FSH receptor mRNA was increased by high doses of T and DHT, but not by any dose of E_2. In expt. 2, cells were treated with 30 ng/ml of E_2, T, or DHT for 1, 3, or 5 days. Aromatase mRNA was increased by E_2 and T at all days, but only after 5 d of DHT treatment. The mRNA for LH receptor was not increased by any steroidal treatment on day1. E_2 and T stimulated LH receptor expression on days 3 and 5. FSH receptor mRNA was induced by T and DHT on all days, but by E_2 only on day 1. In expt. 3, ICI (specific estradiol receptor antagonist) inhibited E_2-induced aromatase and LH receptor mRNA expression. In expt. 4, cells were treated with different combinations of FSH (F) (0.3 ng/ml), E_2 (30 ng/ml), and DHT (30 ng/ml) to investigate synergistic effects. F + E_2 + DHT dramatically synergized to induce aromatase mRNA expression compared with other treatments (F + E_2 + DHT = 2373%; FSH = 166%; E_2 = 519%; DHT = 92%; F + E_2 = 1022%; F + DHT = 254%; E_2 + DHT = 760% of control). Thus, androgen from the thecal cells specifically induces FSH receptor and synergizes with FSH and estradiol to induce aromatase expression in granulosa cells. These inter- and intra-cellular regulatory mechanisms may be critical for normal follicle growth and dominant follicle selection.

Key Words: Granulosa Cell, mRNA, Steroid

M252 Expression of genes encoding steroidogenic enzymes and *in vitro* steroidogenesis by dominant bovine follicles during the 1st follicular wave. K. E. Valdez*[1], S. P. Cuneo[2], P. J. Gorden[3], and A. M. Turzillo[4,5], [1]*Physiological Sciences,University of Arizona, Tucson,* [2]*Department of Veterinary Science and Microbiology, University of Arizona, Tucson,* [3]*Dairy Veterinary Services, Chandler, AZ,* [4]*Department of Physiology, University of Arizona, Tucson,* [5]*Department of Animal Sciences, University of Arizona, Tucson.*

The hallmark of follicle health is the capacity to produce estradiol (E2), which requires supply of androgen from theca interna. During the 1st follicular wave following ovulation, concentrations of E2 in follicular fluid (FF) of the dominant follicle peak around Day 4 of the wave and then quickly decline. The purpose of this study was to elucidate mechanisms responsible for this abrupt decline in E2 production. Follicular dynamics were monitored by ultrasonography and dominant follicles were collected from Holstein heifers on Day 4, 6, or 8 of the 1st follicular wave (n= 4/Day). Steady-state levels of mRNA encoding 17α-OH in theca interna and aromatase in granulosa cells were determined by ribonuclease protection assay. Theca interna and granulosa cells were cultured for 3 h. Granulosa cells were cultured in the presence or absence of 10^{-7} M testosterone as substrate for aromatase. Decreased ability of granulosa cells to produce estradiol on Day 6 preceded a loss in aromatase mRNA on Day 8. However, this decrease was not accompanied by significantly lower amounts of 17α-OH mRNA or decreased androstenedione (andro) production by theca interna. From these results we conclude that the decline in FF E2 observed on Day 6 of the follicular wave is not due to lack of androgen substrate for aromatization or down-regulated expression of the aromatase gene, but instead may be the direct result of decreased activity of the aromatase enzyme within the granulosa cells. Supported by USDA 35203-9167.

Day of wave	FF E2 (ng/ml)[a]	17α-OH mRNA in theca interna[b]	Andro secretion (pg/μg protein)[a]	Aromatase mRNA in granulosa cells[b]	Aromatase secretion (pg/10^5 cells)[a]
4	320 $\pm$ 42[c]	97$\pm$37[c]	0.12 $\pm$ 0.02[c,e]	194$\pm$ 37[c,d]	3.5 $\pm$ 0.8[c,e]
6	98 $\pm$ 33[d]	45 $\pm$25[c,d]	0.26 $\pm$ 0.08[d]	264$\pm$ 29[c]	1.8 $\pm$ 0.6[d]
8	83 $\pm$ 17[d]	13 $\pm$4[d]	0.19 $\pm$ 0.03[d,f]	138$\pm$ 44[d]	2.2 $\pm$ 0.2[d,f]

[a] Mean $\pm$ SEM. [b]Mean CPM $\pm$ SEM.
[c,d]Values within each column with no common superscripts differ ($P< 0.05$).
[e,f]Values within each column with no common superscripts tend to differ ($P< 0.08$).

Key Words: Bovine, Ovary, Steroidogenesis

M253 Effects of dietary supplemental fat on reproductive performance and body composition in pre-puberal beef heifers. A. R. Dos Santos*[1], S. T. Willard[2], O. J. Sharpe[3], and R. C. Vann[1], [1]*Brown Loam Experiment Station, Raymond, MS,* [2]*Mississippi State University, Mississippi State,* [3]*Sharpe Farm, Rolling Fork, MS.*

Varying results have been reported on use of fat in animal diets to improve growth and influence body composition. Effects of feeding fat on reproduction may occur through stimulation of ovarian function in association with increased dietary energy. Previous data by our laboratory has shown a relationship between body composition (percent intramuscular fat; %IMF) and reproductive cyclicity in beef heifers. The objective of this study was to evaluate supplemental fat sources, tallow vs Extruded-expelled Soybean meal (ESBM), on reproductive performance and body composition traits in pre-puberal beef heifers. Angus-crossbred heifers (n=63; grazing Ryegrass/bermudagrass) were assigned to two diet groups: corn and commercial protein pellet (G1; n=31) with 3.6% fat (tallow; ME=4.70 Mcal/kg), and corn and ESBM (G2; n=32) with 4.2% fat (ESBM; ME=4.75 Mcal/kg). Diets were formulated to be isonitrogenous (CP 15%) and fed daily at 2.3 kg/hd for 70 d. Ultrasound measurements for body composition traits were taken at d 0, 23, 46, and 70 for ribeye area (REA), back fat (BF), rump fat (RF), gluteus medius depth (GMD) and %IMF. Blood was analyzed for serum progesterone

(P4), total (CH) and HDL-cholesterol. After d 70, estrus was synchronized using CIDR^{TM} and heifers were bred AI. As determined by P4 and estrus detection through d 70, a similar proportion (P>0.10) of heifers G2 and G1 were cycling (78% vs 71%) prior to synchrony; however G2 had greater (P<0.05) luteal phase serum P4 pre-synchrony than G1. CH increased over the feeding period for both G1 and G2 (P<0.05). IMF stress score was negatively correlated to %IMF (-0.24, P<0.07), and GMD was negatively correlated to GMD stress score (-0.34, P<0.006). REA, BF, RF and GMD did not differ (P>0.10) relative to the two groups at any time. Pregnancy rate was 54.4% for G2 vs 45.7% for G1 (P>0.10). In summary, ESBM as a supplemental fat source vs tallow did not influence body composition or overall reproductive performance post-breeding (AI).

Key Words: Reproduction, Body Composition, Fat Supplement

M254 Lipid transport in the developing bovine follicle: mRNA expression for selective uptake receptors increases and for endocytosis receptors decreases. N. Argov*[1], U. Moallem[2], and D. Sklan[1], [1]*Hebrew University, Rehovot, Israel,* [2]*ARO, Bet Dagan, Israel.*

Differences in rates of steroid production and secretion will, eventually, determine the developmental rates of ovarian follicles. The major supply of cholesterol, the precursor for steroid and androgen biosynthesis, to ovarian cells is from circulating lipoproteins via membrane receptors from the low density lipoprotein receptor (LDL) superfamily. This occurs by either endocytosis, which has been described for very low density lipoprotein receptors (VLDLr) and for LDL receptors, (LDLr) and by the selective uptake pathway described for the scavenger receptor class B type 1 receptor and the recently described ovarian receptor, lipoprotein receptor related protein 8 (LRP8). The objective of this study was to determine mRNA expression of these four cholesterol receptors in bovine ovarian cells at four different stages of follicular development by semi-quantitative PCR. A different pattern of expression was found for the two types of receptors. In small antral follicles, mRNA expression of the endocytosis receptors was higher than in large antral follicles. Expression of LRP8 mRNA increased linearly with follicular size (P<0.01), together with an increase in LDL, VLDL and cholesterol concentrations in the follicular fluid (P<0.01). Expression of SRB1 mRNA tended to increase with follicular diameter (P<0.1). Since different mRNA expression patterns were found for the two types of receptor, this may imply different regulation of cholesterol supply at different stages of follicular development. Accumulation of LDL and VLDL particles in the follicular fluid of large antral follicles enhances cholesterol availability for the intense steroidogenic activity which is essential at these stages. These processes are vital for normal estrous cycles, follicular function and conception.

Key Words: Bovine, Follicular Development, Cholesterol

M255 The expression and localisation of angiogenic growth factors in the porcine corpus luteum. R. S. Robinson*, A. J. Hammond, G. E. Mann, and M. G. Hunter, *Division of Animal Physiology, University of Nottingham, Sutton Bonington Campus, Loughborough, Leics, UK.*

The growth and development of the corpus luteum (CL) is dependent on angiogenesis. Studies in ruminants have shown that vascular endothelial growth factor (VEGF) and fibroblast growth factor 2 (FGF-2) are likely to be the most important factors controlling angiogenesis in the CL. However, to date, FGF-2 and VEGF protein have not been measured or localised in the porcine CL. The objective of this study was to determine VEGF and FGF-2 concentrations in the porcine CL throughout the oestrous cycle and localise components of the VEGF system. Ovaries were collected from the slaughterhouse and were divided into early, mid and late luteal phase based on their visual appearance (n=4 per group). From each animal, 3 CL were homogenised and VEGF and FGF-2 concentrations were determined by ELISA. VEGF and its 2 receptors, VEGFR-1 and VEGFR-2 were localised by immunocytochemistry. Both VEGF and FGF-2 concentrations varied with stage of the oestrous cycle (P<0.05). The highest concentrations of VEGF and FGF-2 were observed in the early luteal phase, at the time of the peak of angiogenesis, supporting the hypothesis that FGF-2 and VEGF are key angiogenic factors in the porcine CL. VEGF was localised to both small and large luteal cells at all stages. The strongest VEGFR-1 immunostaining was observed in the endothelial cells of larger capillaries, but was also present in the cytoplasm of large and small luteal cells at all stages of the cycle. There was moderate staining of VEGFR-2 in the endothelial cells of both larger capillaries and luteal parenchyma throughout the cycle. Since VEGF was localised to the hormone producing cells and the VEGF receptors to endothelial cells, this suggests that VEGF stimulates angiogenesis in a paracrine manner. VEGFR-2 staining was also seen on the membrane and in the cytoplasm of luteal cells, pre-dominantly in the mid and late luteal phase CL, which suggests that VEGF may also regulate luteal cell function. In conclusion, FGF-2 and VEGF system are present in the porcine CL and are likely to play an active role in porcine CL angiogenesis.

Key Words: Pig, Corpus Luteum, Angiogenesis

M256 Factors associated with multiple ovulation in lactating dairy cows. H. Lopez*[1], D. C. Caraviello[1], L.D. Satter[1,2], and M. C. Wiltbank[1], [1]*Dairy Science Department, University of Wisconsin,* [2]*US Dairy Forage Research Center, USDA-ARS, Madison.*

The objective of this study was to evaluate factors that might alter natural multiple ovulation rate in lactating dairy cows. Ovaries of cows (n=267;> 50 days in milk; DIM) were evaluated weekly by transrectal ultrasonography to determine natural (no hormonal treatment) ovulation rate. Cows were fitted with a transmitter to record standing estrous activity, were housed in a freestall barn, and were milked twice daily with milk production recorded. Serum concentrations of progesterone (P4) were recorded weekly starting at wk 1 postpartum and body condition scores (BCS) were determined for all cows monthly. There were 76 (28.5%) anovular and 191 (71.5%) ovular cows by 70 DIM. Lower BCS (P<0.001), but not level of milk production (P>0.10), was associated with the incidence of anovulation. Multiple ovulation rate for first ovulations in anovular cows that naturally recovered from the condition was 46.3% [19/41]. For second and subsequent ovulations (n=463), the level of milk production for 14 d before the d of estrus was associated with increased ovulatory rate (P<0.001). To illustrate, incidence of multiple ovulations was 1.6% [2/128], 16.9% [32/189], and 47.9% [70/146] for ovulations when cows were producing <35, 35 to <45, and #880545 kg/d, respectively. Duration of estrus (4.3±0.7 vs. 9.9±0.5 h; P<0.001) was shorter for multiple (47.2 ± 0.9 kg/d; 92 ± 5 DIM; n=48) than single ovulations (38.1 ± 0.5 kg/d; 95 ± 2 DIM; n=237). Serum concentrations of estradiol were lower (5.5 ± 0.3; n=15 vs. 7.8 ± 0.4 pg/ml; n=71; P=0.005) for estruses with multiple ovulations in spite of larger preovulatory follicle volume (4136 ± 123 vs. 3085 ± 110mm^3; P<0.001). Similarly, 7 d later, serum P4 was lower (2.5 ± 0.3 vs. 3.2 ± 0.1 ng/ml; P=0.04) in multiple than in single ovulators in spite of larger luteal tissue volume (8291 ± 516 vs 6405 ± 158 mm^3; P<0.001). In summary, first ovulation after an anovular condition, and level of milk production for 14 d before the day of estrus, were associated with increased rates of multiple ovulations. Additionally, multiple ovulations were associated with lower E2 at estrus, shorter duration of estrus, and lower P4 7 d after estrus.

Key Words: Dairy Cow, Multiple Ovulation, Milk Production

M257 What regulates ovine placental steroidogenesis? C. Weems*, Y. Weems*, and C. Yin, *University of Hawaii, Honolulu.*

By d 90, the placenta (PL) secretes half of the progesterone (P4) and 85% of the estradiol-17β in blood and ovariectomy (OVX) does not abort ewes (Y Weems et al., Prostaglandins (PG) 46:277, 1992; 48:139, 1994). Regulation of luteal P4 secretion at d 90 of pregnancy is by PGE_2 , not LH, in ewes (Y Weems et al., PG 53:337, 1997) and PGE concentration in uterine and ovarian venous plasma (6 ng/ml) is similar (Y Weems PG 46:277, 1993). Ovine PL PGE secretion is regulated by LH up to d 50 and by pregnancy specific protein B (PSPB) after d 50 of pregnancy (Y Weems et al., PG 71:55, 2003). Indomethacin (INDO), a PG synthesis inhibitor, lowers P4 and PGE in blood by half at d 90 (Bridges et al., PG 58:113; 58:267, 1999). Ovine PL from d 90 OVX pregnant ewes secrete 2-3 fold more estradiol-17 β, PSPB, PGE, and P4, but not $PGF_2\alpha$ in vitro than intact ewes (Y Weems et al., PG 58:139, 1999). The objective was to determine what regulates ovine PL P4 and estradiol-17β secretion at d 90 of pregnancy. In Expt. 1, PL slices from d 90 intact or OVX (d 83) pregnant ewes were incubated in vitro in M-199 with vehicle, LH, FSH, PL lactogen, PGE_1, PGE_2, PGD_2, PGI_2, IGF_1, IGF_2, LTC_4, PAF-16, or PAF-18 (1, 10, or 100 ng/ml) for 4 hr. In Expt.

2, PL slices from d 90 intact and OVX (d 83) ewes were incubated in vitro with vehicle, INDO, meclofenamate (MECLO), PGE_1, PGE_2, INDO+PGE_1, MECLO+PGE_1, INDO+PGE_2, or MECLO+PGE_2 for 4 hr. Media were analyzed for P4, estradiol-17β, PGE, or $PGF_2\alpha$ by RIA. Hormone data in media were analyzed in Expt. 1 by a 2x3x13 and in Expt. 2 by a 2x9 factorial ANOVA. In Expt. 1, PL P4, PGE, or estradiol-17β secretion was increased ($P<0.05$) by OVX (two-fold), P4 was not increased ($P>0.05$) by any treatment other than OVX, and only FSH increased ($P<0.05$) estradiol-17β secretion (two-fold) in both OVX and intact ewes. In Expt. 2, INDO or MECLO decreased ($P<0.05$) PL P4 secretion by 88% but did not decrease ($P>0.05$) PL estradiolβ secretion from intact or OVX ewes and PGE_1 or PGE_2 increased ($P<0.05$) P4 secretion by more than two-fold only in ewes also treated with INDO or MECLO. It is concluded that FSH probably regulates d 90 ovine PL estradiol-17β secretion, while PGE_1 or PGE_2 regulates d 90 PL P4 secretion.

Key Words: Placenta, Sheep, Steroidogenesis

M258 Evaluation of an SNP in steroidogenic acute regulatory (STAR) protein for reproductive traits in swine. J. G. Kim*, J. L. Vallet, R. K. Christenson, G. A. Rohrer, and D. J. Nonneman, *USDA, ARS, U.S. Meat Animal Research Center, Clay Center, NE*.

STAR transports cholesterol from the outer to the inner mitochondrial membrane, which is the rate limiting step for steroidogenesis. STAR mRNA is expressed in reproductive organs including the testis, ovary, and placenta of the pig. The porcine STAR gene was previously mapped by PCR-RFLP analysis to a region that is within one (chromosome 15; 95% confidence interval: 53-101 cM) of several QTL associated with ovulation rate in swine. However, sequence variation in the porcine STAR gene and its association with swine reproductive traits has not been studied. The objectives of this study were to 1) confirm the location of the STAR gene, 2) identify sequence variation in the coding region, and 3) determine the association of STAR gene single nucleotide polymorphisms (SNPs) with reproductive traits. Primers were designed to amplify and sequence the STAR gene. Two contiguous SNPs (G/C and G/T) in STAR were identified from genomic DNA of Meishan x White composite pigs. The G/T polymorphism changes amino acid 126 from valine to leucine. An assay was designed to genotype the SNP by mass spectrometry. Genotyping of the founders of the resource population indicated that the T allele was found only in Meishans (frequency = 0.81). F2, F8, and F9 gilts with phenotypic data were genotyped for association analysis. Linkage analysis mapped the STAR gene to chromosome 15 position 58.5 cM. Data were analyzed by the General Linear Models procedure of SAS. There was no significant association between the SNP and ovulation rate or age at puberty. Ovulation rate for TT homozygotes (n = 30), GT heterozygotes (n = 90), and GG homozygotes (n = 79) were 12.8 $\pm$ 0.53 (least squares mean $\pm$ standard error), 13.4 $\pm$ 0.32, and 13.4 $\pm$ 0.35, respectively. Age at puberty for TT homozygotes (n = 33), GT heterozygotes (n = 93), and GG homozygotes (n = 97) were 169 $\pm$ 6, 166 $\pm$ 3, and 170 $\pm$ 4 days, respectively. Although STAR is located in a QTL region for ovulation rate in the pig, this polymorphism does not appear to affect ovulation rate or age at puberty significantly.

Key Words: Ovulation Rate, Age At Puberty, Gene Mapping

M259 Ontogeny of uterine gene expression in the prepuberal pig. R. L. Richardson*[1], G. J. Hausman[1], R. Rekaya[2], L. Lee-Rutherford[1], R. R. Kraeling[1], and C. R. Barb[1], [1]*USDA-ARS, Athens, GA,* [2]*University of Georgia, Athens*.

The molecular mechanisms that regulate uterine development in the pig are not understood. To better understand physiological pathways controlling uterine function, a custom microarray was developed to profile differential gene expression. Oligonucleotides (70 mer) were produced from sequenced ESTs from the Meat Animal Research Center, ARS, USDA libraries that had at least 90% homology to known genes in The Institute of Genome Research pig gene index. Total uterine RNA was isolated from gilts at 90, 150, and 210 days (d) of age and dye labeled cDNA probes were hybridized to arrays representing about 600 pig genes involved in growth and reproduction. Quantitative analysis using a mixed linear model statistical program identified ($P<0.01$) 45 genes differentially expressed from 90 to 210 d of age, which included growth factor/hormone receptors, growth and cell cycle regulators, apoptosis and seven transcription factors. The gene bone morphogenetic protein 4, a structural protein and signaling growth factor, was down regulated at 90 d and up-regulated at 150 d. The gene for zona pellucida glycoprotein 3A (sperm receptor) was up-regulated by 150 d and oviductil glycoprotein 1 was up-regulated by 210 d. Both of these genes play a role in the fertilization process and/or early embryonic development. A number of these differentially regulated genes expressed in the pig have not been reported in the human or the pig, including melanocortin 3 receptor and PPAR gamma coactivator 1.These results demonstrate, for the first time, differentially expressed uterine genes in the prepuberal pig. The temporal expression of these key genes that regulate uterine development and function during the prepuberal period will lead to a better understanding of the molecular mechanisms controlling fertilization and early embryonic development.

Key Words: Pig, Uterus, Gene

M260 Observed frequency of monozygotic twinning in lactating Holstein cows. N. Silva Del Rio*, B. W. Kirkpatrick, and P. M. Fricke, *University of Wisconsin - Madison, Madison*.

Bonniers equation has been used to mathematically estimate the frequency of monozygotic twinning in epidemiologic studies of twinning in dairy cattle. Our objective was to empirically determine the frequency of monozygotic twinning in lactating Holstein cows and compare it with that predicted by Bonniers equation. Ear biopsies were collected from 102 sets of Holstein twins from 6 Wisconsin dairies resulting in a sex ratio (Bull:Heifer) distribution of 39% B:H, 27% B:B, 34% H:H. The frequency of monozygotic twinning estimated using Bonniers equation based on the deviation from the expected sex ratio distribution of 50% B:H, 25% B:B, 25% H:H was 34.6% of same-sex twins and 21.0% of all twins. To empirically determine the frequency of monozygotic twinning, DNA was extracted from ear biopsies from same-sex twins (62/102) and was PCR amplified using primers for seven polymorphic microsatellite markers (BM4305, BM6425, BM9289, BMS360, BMS650, BMS823, UW20). Mixed-sex and same-sex twins differing in at least one microsatellite marker genotype were classified as dizygotic twins. Same-sex twins were classified as monozygotic when genotypes were identical for at least 5 microsatellite markers. Of the 62 same-sex twins, 58 were classified as dizygotic and 4 were classified as monozygotic resulting in a monozygotic twinning frequency of 6.5% of same-sex twins and 3.9% of all twins. Complete twinning data was collected throughout a 12-mo period on two of the farms representing a total of 1,291 calvings. Both farm ($P<0.0001$) and season of calving ($P<0.005$) affected twining rate. The overall twinning rate was 12.1% for farm 1 and 3.9% for farm 2. Twinning rate was 8.3% from April to June and 9.8% from July to September, both of which were greater ($P<0.0005$) than the twining rate of 5.3% from October to December and 2.3% from January to March. We conclude that Bonniers equation overestimated the frequency of monozygotic twinning in this population of Holstein cows. *Supported by USDA NRICGP grant 2002-02033 to PMF*

Key Words: Twinning, Dairy Cattle, Monozygotic

M261 Ontogeny of ovarian gene expression in the prepuberal pig. R. L. Richardson[1], C. R. Barb[1], R. Rekaya[2], L. Lee-Rutherford[1], R. R. Kraeling[1], and G. J. Hausman*[1], [1]*USDA-ARS, Athens, GA,* [2]*University of Georgia, Athens*.

The molecular mechanisms that regulate ovarian development in the pig are complex. To better understand physiological pathways controlling functions of the ovary, a custom microarray was developed to profile differential gene expression. Oligonucleotides (70 mer) were produced from sequenced ESTs from the Meat Animal Research Center, ARS, USDA libraries that had at least 90% homology to known genes in The Institute of Genome Research pig gene index. Total ovarian RNA was isolated from gilts at 90, 150 and 210 days (d) of age and used to prepare dye labeled cDNA probes, which were hybridized to arrays representing about 600 pig genes involved in growth and reproduction. Quantitative analysis using a mixed linear model statistical program identified ($P<0.01$) 41 genes differentially expressed from 90 to 210 d of age, which included genes involved in lipid/steroid metabolism, cell growth and regulation, and extracellular matrix adhesion. The gene, protein kinase-c theta, involved in apoptosis was up-regulated at 210 d. The transcription factor, PRDM2, was up-regulated at 90 and 150 d, but down regulated at 210 d. One unknown gene was down-regulated at 90d and up-regulated by 210 d. A number of these differentially regulated genes which were

expressed in the pig ovary had not been reported in the human, including transcription factors, ATP binding cassette B member 11 and CAAT/enhancer binding protein alpha. These results demonstrate, for the first time, differentially expressed pig genes in the prepuberal pig. The ontogeny of expression of these key genes that regulate ovarian development and function leading to onset of follicular growth during the prepuberal period will lead to a more detailed understanding of the molecular mechanisms controlling follicular development and ovulation.

Key Words: Pig, Ovary, Gene

M262 Ovarian activity of dairy cows fed two amounts of phosphorus.

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Multiparous Holstein cows were used to determine the effect of dietary P on ovarian activity and reproductive performance. Cows were assigned randomly at calving to either a 0.35% (n = 27) or 0.47% (n = 27) P (DM basis) TMR; the high P diet was obtained by adding monosodium phosphate to the low P diet. Ovarian activity was monitored three times weekly by ultrasonography starting 10 DIM until the end of a 60-d voluntary waiting period, after which cows were synchronized and bred using the Ovsynch protocol. During wk 2 of lactation, the number of small (3-5 mm) or large (> 9 mm) follicles and the diameter of the dominant follicle did not differ between groups, but the number of medium (6-9 mm) follicles was lower for the low P diet than for the high P diet. Days to first ovulation and the diameter of the ovulating follicle were not affected by dietary P. During the first wave of follicular growth following the first ovulation, dietary P did not affect the number of follicles of all size categories or the diameter of the CL. The diameter of the dominant follicle was greater for the low P group during this period. Multiple ovulation rate or the proportion of cows that were anovulatory or developed follicular cysts did not differ between groups. The first service conception rate and pregnancy loss during 30-60 d after AI were not influenced by dietary P treatment. Milk production for the first 170 d of lactation, based on DHIA records, was not different between groups. Increasing dietary P from 0.35 to 0.47% did not improve ovarian activity.

Item	0.35% P	0.47% P	SEM	*P*
Number of small (3-5 mm) follicles[a]	4.6	4.4	0.6	0.81
Number of medium (6-9 mm) follicles[a]	1.2	1.9	0.3	0.06
Number of large (> 9 mm) follicles[a]	1.0	1.0	0.2	0.82
Diameter of dominant follicle[a], mm	12.5	12.4	1.0	0.95
Days to 1st ovulation	24.2	28.7	2.4	0.20
Diameter of 1st ovulating follicle, mm	16.8	15.9	0.8	0.48
Diameter of 1st wave dominant follicle[b],mm	16.5	13.9	0.9	0.06
Diameter of 1st estrous cycle CL, mm	23.6	21.9	1.3	0.33
Multiple ovulation rate[c], %	38.1	30.0	...	0.58
Proportion of cows with follicular cysts[c], %	11.5	14.8	...	0.72
Proportion of anovulatory cows[c], %	19.2	25.9	...	0.56
First service conception rate,%	32.0	44.4	...	0.36
Pregnancy loss (30-60 d)[d], %	25.0	33.3	...	0.69
Milk yield, first 170 d, kg/d	46.8	45.0	1.4	0.37

[a]During wk 2 of lactation; [b]First estrous cycle;
[c]During the voluntary waiting period; [d]First service only.

Key Words: Phosphorus Requirement, Reproductive Performance, Dairy Cows

M263 Association between plasma urea nitrogen levels and reproductive fluid urea nitrogen and ammonia concentrations in lactating dairy cows..

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Two experiments were conducted to study the relationship between blood plasma urea N (PUN) and ammonia (NH_3), urea N concentrations in fluid of preovulatory follicles and in uterine fluids in lactating dairy cows. In Experiment 1, mean PUN levels were used to distribute cows into two groups: (1) cows with PUN #8805 20 mg/dl (HPUN), and (2) cows with PUN < 20 mg/dl (LPUN). Blood and follicular fluids from preovulatory follicles of 38 early lactation dairy cows were collected on the day of estrus (d 0) 4 hr after feed was offered. Follicular fluid NH_3 was significantly ($P < 0.01$) higher in HPUN cows (308.05 ± 72.27) compared to LPUN cows (93.88 ± 13.09). Follicular fluid urea N was significantly ($P < 0.001$) higher in HPUN cows (22.36 ± 0.44) compared to LPUN cows (17.0 ± 0.34). PUN and follicular fluid urea N were correlated (r = 0.93) within cows. In Exp 2, blood and uterine fluids were collected from 30 cows on d 0 and on d 7. Uterine fluid NH_3 was significantly ($P = 0.05$) higher in HPUN cows (1562 uM ± 202) than in LPUN cows (1082 uM ± 202) on d 7 d, but not on d 0. Uterine fluid urea N was significantly ($P < 0.001$) higher in HPUN cows than in LPUN cows on d 0 (26.9 ± 1.3, 20.4 ± 0.7) and d 7 (26.5 ± 1.1, 21.4 ± 1.1). There was a significant ($P < 0.01$) correlation (r = 0.41) between PUN and uterine fluid urea N within cows. The results of this study indicate that high PUN concentrations were associated with elevated NH_3 and urea N concentrations in the preovulatory follicular fluids on the day of estrus and in the uterine fluid during the luteal phase of the estrous cycle in early lactation dairy cows. Elevated NH_3 or urea N concentrations in the reproductive fluids may contribute to reproductive inefficiency in dairy cows with elevated plasma urea nitrogen.

Key Words: Cattle, Urea, Ammonia

M264 Gonadotropin secretion and ovarian activity in non-pregnant mares treated continuously with GnRH during the anovulatory season.

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Objectives were to determine if low-dose, continuous infusion of GnRH from Fall to Spring would prevent seasonal anovulation in mares. Twenty Quarter Horse mares, ages 18 mo to 24 yrs, were stratified by age and body condition score and assigned randomly to either a saline Control (n = 9) or GnRH (n = 11) treatment group. Treatments were instituted between September 23 and October 9. Gonadotropin-releasing hormone was delivered in 0.9% physiological saline via Alzet osmotic minipumps (Model 2004) placed s.c. at the base of the neck, with Silastic sham pumps placed in control mares. Pumps were inserted on day 3 following ovulation or during the follicular phase if ovulation did not occur by October 9. Delivery rate of GnRH was 2.5 μg/h (60 μg/d) for the first 60 d, followed by 5.0 μg/h (120 μg/d) thereafter, with all pumps replaced every 30 d. By December 1, all mares had become anovulatory and remained anovulatory until February. Mean serum concentrations of LH were not affected by treatment in anovulatory mares. In contrast, control mares that exhibited ovulatory cycles after study onset had higher ($P < 0.05$) mean concentrations of LH during all phases of the estrous cycle except diestrus. Mean serum concentrations of FSH were not affected by treatment, but were lower ($P < 0.05$) from November though January relative to all other months in anovulatory mares. Interovulatory intervals in mares that cycled temporarily did not differ between groups. Ovulatory control mares had slightly larger ($P < 0.10$) follicles overall than GnRH-treated mares; however, ovulatory follicle diameters for control and GnRH-treated mares did not differ. Ovulatory control mares had higher ($P < 0.10$) mean concentrations of progesterone during metestrus and late diestrus. In a subgroup of control (n =5) and GnRH-treated (n = 5) mares, total releasable pools of LH in response to 1 mg GnRH did not differ between groups. Ovulation resumed in 3 control and 3 GnRH-treated mares by March 30. Results indicate that continuous infusion of native GnRH at the doses employed in this study is not sufficient to maintain ovulatory cycles during the anovulatory season.

Key Words: GnRH, Seasonal Anovulation, Equine

M265 Effect of genetic strain, feed allowance, and parity on interval to first ovulation and the first estrous cycle in pasture-managed dairy cows.

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Cows with North American (NA) pedigrees in traditional New Zealand (NZ) dairy herds may have poor reproductive performance. The objective was to measure postpartum anovulatory interval (PPAI) and the length of the first postpartum estrous cycle in strains of NA and NZ cows managed in a NZ pasture system. The Overseas90 strain (OS90; n=76) were modern NA cows [high breeding worth (BW) with predominately NA pedigree]; the NZ90 strain (n=86) were modern NZ cows (high BW with predominately NZ pedigree); and the NZ70 strain (n=45) were low BW cows with NZ pedigree. Cows (first, second, or third parity) were

allocated to different pasture-based feeding levels (tons DM per cow annually). Milk was sampled twice weekly and analyzed for progesterone (P4) to determine PPAI (interval to first milk P4 > 2 ng/ml) and the interval between first and second ovulations (cycle one). There was an effect of parity (P<.001), strain (P<.02) and a feed by strain interaction (P<.01) for PPAI. First parity cows had a longer PPAI (40±3 d) compared with second (28±3 d) or third (28±1 d) parity cows. The NZ90 strain had a longer PPAI (35±2 d) compared with either OS90 (27±2 d) or NZ70 (30±3 d). Increasing feed allowance did not decrease PPAI for OS90 or NZ70 cows but led to a shorter PPAI for NZ90 cows (44, 40, 27, and 30±4 d for 5.0, 5.5, 6.0, and 6.5, ton DM/cow, respectively). Cycle one was longer (P<.001) for OS90 (18±1 d) compared with NZ90 (13±1 d) or NZ70 (13±1 d) cows. Both parity and strain affect PPAI in dairy cattle. First parity cows had longer PPAI. Selection for production in NZ (NZ90 vs. NZ70) has led to longer PPAI that can be shortened by increasing feed allowance. OS90 cows have BW similar to NZ90 but shorter PPAI and a longer first cycle. Independent physiological mechanism may control PPAI and the first luteal phase in dairy cattle selected for production in NA and NZ.

Key Words: Postpartum, Dairy, Ovulation

M266 Effect of ovulation rate on development of the ovine corpus luteum.

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Relationships among ovulation rate (OR), corpus luteum (CL) weight (wt), and numbers of small (< 3mm dia.) and medium (3-6 mm) antral follicles were evaluated for Rambouillet (Ram), 1/2 Romanov x 1/2 Dorset (RxD), Romanov (Rom), and Synthetic III (1/2 Columbia x 1/4 Suffolk x 1/4 Hampshire; SIII) ewes. Ovaries were collected at slaughter on d 14 to 17 of pregnancy, and numbers of CL and of visible follicles were recorded. Ovaries and excised CL were weighed individually. None of the variables differed (P > 0.1; NS) between the left and right ovaries and data were combined. Means (± sem) for OR, ovarian wt (less CL), total CL wt, mean CL wt and number of small and medium follicles are reported in Table 1 for the four genetic groups. OR ranged from 2 to 10. Rom ewes had the largest (P < 0.01) OR but the lightest (P < 0.01) individual CL; RxD ewes were intermediate for OR and CL wt relative (P < 0.01) to Rom or to Ram and SIII. Thus, OR and individual CL wt correlated negatively (r = -0.72; P < 0.01). However, total CL wt and number of antral follicles did not differ (P > 0.1) among the genetic groups. SIII ewes had heavier (P < 0.01) ovaries than Rom or RxD. Variables did not differ (P > 0.1) between Ram and SIII. The negative relationship between OR and individual CL weight implies that either 1) the number of follicular cells and(or) their luteinization is compromised in prolific ewes or 2) the progesterone threshold level for negative feedback on LH secretion is similar among individual ewes regardless of prolificacy; thus, development of individual CL is limited by lower LH stimulation in ewes with increased OR. Table 1. Genetic effects on OR, ovarian wt, total and individual CL wt, and follicle number

Breed	n	(OR) (No. CL)	Ovarian wt (g)	Total CL wt (g)	Individual CL wt (g)	Small follicles (n)	Medium follicles (n)
Ram	7	2.3±0.4	4.03±0.33	1.83±0.14	0.80±0.05	42.8±6.0	7.0±1.4
RXD	17	3.2±0.3	3.66±0.22	1.59±0.11	0.53±0.03	43.6±3.8	7.2±0.9
Rom	26	4.8±0.3	3.46±0.17	1.60±0.09	0.35±0.02	39.3±3.1	7.5±0.7
SIII	17	2.4±0.3	4.91±0.22	1.73±0.11	0.71±0.03	37.4±3.8	9.2±0.9
P <		0.01	0.01	NS	0.01	NS	NS

Key Words: Ovulation Rate, Corpus Luteum, Sheep

M267 A comparison of the anti-luteolytic activities of recombinant ovine IFN-tau and alpha in sheep.

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Interferon-tau (IFN-τ) is well established as the maternal recognition signal produced by ruminant conceptuses at the peri-implantation period. The question as to whether IFN-τ is superior as an anti-luteolytic agent to closely related Type I IFNs, such as IFN-α, however, remains unanswered. Previous studies have employed both intramuscular and intrauterine administration of recombinant IFN-τ or IFN-α and demonstrated that both proteins can extend estrous cycle length in cattle and sheep, but a concurrent comparison of these IFNs has not been undertaken. Thus the aim of this study was to determine whether equivalent antiviral units of ovIFN-τ and ovIFN-α are equipotent in extending estrous cycle length. Recombinant ovine IFNs (ovIFN-τ4 and ovIFN-α1) were expressed in yeast (*Pichia pastoris*) and purified from the culture media. The anti-viral activity of the IFNs was determined on MDBK and ovine uterine epithelial cells by a cytopathic protection assay. Indwelling uterine catheters were fitted into crossbred ewes (n=16) and on d3 post-estrus (d0=estrus). Between d10 to 18 post-estrus, ewes received twice daily infusions of $0.7x10^7$U of either IFN-τ or -α in PBS (n=6 ewes/treatment), adjusted for protein concentration by addition of serum albumin. Control ewes (n=4) received an equivalent amount of serum albumin. Daily blood samples were collected and ewes were monitored twice daily for estrus. Mean estrous cycle lengths were 16.3±0.9, 21.7±4.0 and 33.3±13.9 days for control, IFN-α and IFN-τ treated ewes respectively. Recombinant IFN-τ (p=0.01) and to a lesser extent IFN-α (p=0.04) increased cycle length. Number of CL, d10 temperature and progesterone concentrations throughout the cycle were not significantly (p>0.1) different between the three groups. These data demonstrate ovIFN-α to be an anti-luteolytic agent, but not as potent of one as ovIFN-τ. *Supported by NIH Grant HD21896.*

Key Words: Interferon, Infusion, Ovine

M268 Effects of monensin supplementation peripartum in metabolic and reproductive parameters in anestrous postpartum Nellore cows.

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The aim of this study was to evaluate the effects of monensin supplementation peripartum on plasma concentrations of insulin-like growth factor I (IGF-I), nonesterified fatty acids (NEFA), follicular diameter and response to GnRH in anestrous postpartum Nellore cows. Primiparous (n=123) and multiparous (n=179) cows were allocated randomly to 2 groups: G1 = 100g/d of a mineral supplement and G2 = 350g/d of a mineral supplement mixed with citrus pulp and monensin (150mg/d), from 30d prepartum to 90d postpartum. Blood samples, follicular diameter, and body condition score (BCS, scale of 1-5 adapted for 0.25 points) were determined at 54.3±23.08d postpartum, the d of GnRH injection (Cystorelin®, 50μg, i.m). IGF-I and NEFA were analyzed in a sub-group of animals (n=120) by RIA and enzymatic methods, respectively. Follicular diameter and ovulation to GnRH injection were evaluated by ultrasound. Data were analyzed using a general linear models procedure. Cows supplemented with monensin had greater concentrations of IGF-I (112.0±6.91 vs 65.4±6.89ng/mL, P<0.01) and lower of NEFA (956.8±51.52 vs 1131.2±54.12μmol/L, P<0.05) in relation to the controls. Monensin ingestion increased (P<0.05) the average diameter of the largest follicle in the animals that did (10.0±0.18mm) or did not (9.5±0.17mm) recieve monensin. Despite evidence of better metabolic status and larger follicular diameters, monensin did not increase the frequency of ovulation to GnRH (67.5±0.09 vs 63.7±0.09%) nor BCS (3.0±0.02 vs 3.1±0.02). Monensin influenced positively follicular diameter, probably due to effects on energy metabolism, which may have increased LH pulsatility and delayed the turnover of the dominant follicle. These data suggest that monensin ingestion can be a good strategy to improve energy status of peripartum cows and follicular development. This also could improve the responses to synchronization protocols and/or induce cyclicity in anestrous postpartum Nellore cows.

Key Words: Nellore Cows, Anestrus, Monensin

M269 Progesterone intravaginal device and/or calf removal on anestrous Angus/crossbred cows during a 60-day breeding season.

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The aim was to evaluate if treatment with a progesterone device and/or calf removal before the beginning of the 60-day breeding season (BS) could improve reproductive parameters in anestrous Angus/crossbred cows. Anestrous cows (n=286), 53±5 days postpartum (DPP), body condition scores (BCS) between 2.5 to 3.5 (1-5) were assigned to 4 groups: G1 (n=73) Control; G2 (n=70) calf removal (CR) for 48h; G3 (n=73) intravaginal progesterone device (CIDR(r)) for 7 days; G4

(n=70) CIDR for 7 days and CR for 48h. All groups were subject to AI 12h after heat detection. Ovarian structures and pregnancy were evaluated by ultrasound (Aloka SSD-500). BCS and cyclicity (evaluated by two ultrasound examinations 10 days apart) were determined before the beginning of BS. Data were analyzed by GLM, and the parameters included in the model were DPP, treatment, calf gender, BCS, and interactions. Cows bred during the BS were not affected by treatment: G1-53.3%; G2-64.1%; G3-56.5%; G4-64.5%. Number of days and conception to first IA were affected (P<0.01) by treatment: G1-28.5d; 45.7%; G2-13.3d; 29.3%; G3-26.9d; 50.9%; G4-9.6d; 76.6%, respectively. Treatment affected the number of days to conception (G1-34.3d; G2-40.5d; G3-36.4d; G4-13.0d; P<0.01), but not the percentage of cows pregnant at the end of the BS (G1-43.5%; G2-41.1%; G3-38.8% G4-45.5%). Conception at first AI and percentage of pregnant cows at the end of the BS were affected by BCS (2.5-40.2%; 20.4%; 2.75-42.5%; 37.5%; 3.0-41.6%; 40.7%; 3.25-56.8%; 54.1%; 3.5-72.0%; 58.3%; P<0.05), respectively. These data suggest that treatments G2 and G4 decrease the number of days to first AI, comparing with G1 and G3 (13.3d; 9.6d vs. 28.5d; 26.9d) respectively, but G2 had a decrease in conception (29.3%). Thus combining calf removal with CIDR insertion is a good strategy to induce fertile estrous and to enhance early season pregnancy. The responses may be even higher in cows with better body condition score.

Key Words: CIDR, Calf Removal, Short Cycle

M270 Effect of subluteal concentrations of progesterone on an estradiol cypionate induced LH surge in lactating Holstein cows. T. B. Hatler*, D. L. Ray, S. H. Hayes, and W. J. Silvia, *Department of Animal Science, University of Kentucky, Lexington*.

Intermediate circulating concentrations of progesterone (INT P4: 0.1-1.0 ng/ml) are often associated with ovarian follicular cysts in lactating dairy cows. The following experiment was conducted to determine if INT P4 during the follicular phase affected the occurrence of a LH surge induced by estradiol cypionate (ECP). Eazi-Breed CIDRs (1.38 g of progesterone) were preincubated in host cows for either 0, 14 or 28 days (CIDR-0, CIDR-14, CIDR-28, respectively) for subsequent use in this experiment. Ovaries of lactating Holstein cows were examined by transrectal ultrasonography to identify those with a corpus luteum (CL). Luteal function was later verified by RIA for P4 (>3.0 ng/ml). Within 24 hours of CL detection, preincubated CIDRs were inserted into 15 cows (n=5 per preincubation time). The day of CIDR insertion was designated as experimental day -1. Four cows received no CIDRs and served as controls (CONT). CIDRs remained in place for the following 3.5 days. Luteolysis was induced with 2 injections of PGF2α (Lutalyse, 25 mg, i.m.) given at 6 PM on day -1 and at 6 AM on day 0. Plasma samples were collected from day -1 to 2.5 at 12 hour intervals to measure P4. At 6 AM on day 1, an injection of ECP (3 mg, i.m.) was administered. Blood samples were collected via jugular venous catheter every 2 hours for the next 36 hours to detect the LH surge. The average circulating concentration of progesterone in the four samples collected on days 1 and 2 was calculated for each cow. The mean P4 concentrations were 1.20, 0.78, 0.45, and 0.11 ng/ml for cows treated with CIDR-0, CIDR-14, CIDR-28 and CONT, respectively (P<0.01). Treatment altered the occurrence of the ECP-induced LH surge (P<0.01). In all 4 CONT cows, the LH surge was detected an average of 18 hours after ECP injection. LH surges were not detected in any of the 5 CIDR-0 cows. The surge of LH was detected in 2 of 5 CIDR-14 and 4 of 5 CIDR-28 cows, respectively. It was concluded that intermediate progesterone during the follicular phase may contribute to cyst formation by blocking the estradiol-induced LH surge.

Key Words: Progesterone, Ovarian Follicular Cyst, LH Surge

M271 Effect of subluteal concentrations of progesterone on follicular phase events in lactating Holstein cows. T. B. Hatler*, D. L. Ray, S. H. Hayes, and W. J. Silvia, *Department of Animal Sciences, University of Kentucky, Lexington*.

Intermediate circulating concentrations of progesterone (INT P4: 0.1-1.0 ng/ml) are often associated with ovarian follicular cysts in lactating dairy cows. The following experiment was conducted to determine if INT P4 during the follicular phase prevented ovulation. In order to synchronize ovarian events, lactating Holstein cows were administered 2 injections of PGF2α (Lutalyse, 25 mg, i.m.) 14 d apart. An injection of GnRH (Factrel, 100 μg, i.m.) was administered 12 d later (designated experimental d 0). Daily transrectal ultrasonography of ovaries and collection of blood samples (for quantification of P4) began on d 0 and continued for the next 22 d. Eazi-Breed CIDRs (1.38 g of progesterone) were preincubated in host cows for either 0, 14 or 28 d (CIDR-0, CIDR-14, CIDR-28, respectively) for subsequent use in this experiment. On d 7(AM), preincubated CIDRs were inserted (CIDR-0 (n=5), CIDR-14 (n=5), CIDR-28 (n=6)) and left in place until the conclusion of the experiment. Cows without CIDRs served as untreated controls (CONT; n=4). Luteolysis was induced with 2 injections of Lutalyse (25 mg, i.m.) given 12 h apart on d 7 and 8. Jugular venous blood samples were collected every 4 h from 8 AM on d 9 to 8 AM on d 14 to detect the LH surge. Treatment with preincubated CIDRs during the follicular phase altered (P>0.01) the frequency of ovulation. Ovulation occurred in 0/5 and 1/5 cows treated with CIDR-0 and CIDR-14 while ovulation occurred in 3/6 and 4/4 cows treated with CIDR-28 and CONT, respectively. Preovulatory surges of LH were detected in all cows that ovulated and were not detected in any cows that did not ovulate. On average, LH surges were detected on d 11. Follicular phase concentrations of P4 were determined by calculating the average of P4 concentrations on d 9 thru 11. Circulating concentrations of P4 during the follicular phase were 1.25, 0.71, 0.36 and 0.14 ng/ml for CIDR-0, CIDR-14, CIDR-28 and CONT, respectively. Intermediate progesterone during the follicular phase may contribute to the formation of cysts by blocking the LH surge and thus, ovulation.

Key Words: Progesterone, Ovarian Follicular Cyst, LH Surge

PSA-Environment and Management

M272 Comparative study of body characteristics of broiler chickens from different rearing systems. A. O. Best*, W. L. Willis, and C. Murray, *Department of Animal Sciences, North Carolina A&T State University, Greensboro*.

An experiment was conducted to investigate the relationship between certain body characteristics and growth performance of broiler chickens subjected to different systems of rearing. Two hundred forty female day old broiler chicks were obtained from a local commercial hatchery, and divided into groups of 40. Each group was subjected to one of the following rearing systems: 1) battery cage-no heat; 2) battery cage-heat; 3) floor pens-no heat; 4) floor pens-heat; 5) pasture poultry-no heat and 6) floor pens/pasture. At seven weeks of age, whole carcass, bursa, gizzard, liver, spleen, stomach, and heart weights were taken. Abdominal and leg lengths were measured, and intestines lesion scores were observed. Although all birds were of the same age, their body characteristics were variable for each rearing system. Treatment 4, floor pens-heat, had the highest average body carcass weight (2.53 kg) and the lowest came from treatment 1, battery cage-no heat (1.37 kg). The bursa weights which reflect the health status of broilers in most treatments were above average, ranging from 0.20 (trt. 4) to 0.37 gms (trt.1). The mortality rate was highest in the pastured poultry system (trt. 5). The results of this study indicate that different rearing systems influence the body characteristics such as organ weights, carcass weights and abdominal length.

Key Words: Body Characteristics, Broilers, Rearing Systems

M273 *Campylobacter Jejuni* Asssessment in Organic vs. Conventional Reared Broiler Chickens. L. T. Donaldson*, W. L. Willis, and C. Murray, *Department of Animal Sciences, North Carolina A&T State University*.

An experiment was conducted to investigate the prevalence of *Campylobacter jejuni* in broiler chickens that were produced and marketed as organic or conventional. Trials were performed during the summer and winter utilizing three farms, each of which practice, organic or conventional rearing. Ten broilers from each farm were collected in the processing plant and subjected to carcass rinse (serial dilutions), crop swabbing, ceca swabbing and drug sensitivity prior to evisceration and after chilling. The prevalence of *Campylobacter jejuni* in organically