830 Effect of the flavonoid catechin or epicatechin on the motility of extended cooled equine spermatozoa. C. A. Woodward*, S. A. Ericsson, P. H. Phurdy, and M. D. Fox, Jr., *Sul Ross State University, Texas, TX*.

The objective of this study was to determine if either of the flavonoids catechin or epicatechin were capable of maintaining the motility of extended cooled stallion spermatozoa. Semen samples were collected from 7 stallions using a teaser mare, phantom mount and artificial vagina. Samples were aliquoted (100 μl) into replicates of 3 and placed into Kenney's non-fat dried skim milk extender (400 μl) containing 0 (control), 25, 50, 75 or 100 μM catechin or epicatechin. Extended samples were cooled and maintained at 5°C prior to microscopic analysis (250X) of percent sperm motility at 24, 48, 72 and 96 hr. Percentage data for motility were arcsine transformed and analyzed using ANOVA for differences among levels of flavonoid. The percentage of motile spermatozoa in the extender containing no flavonoid (mean ± SD: 23% ± 14) at 48 hr was significantly lower than those containing 25 μM (31% ± 18, $P < 0.004$), 50 μM (34% ± 18, $P < 0.0004$), 75 μM (34% ± 17, $P < 0.0001$) or 100 μM (31% ± 19, $P < 0.005$) of catechin. Samples treated with 25 μM (33% ± 16, $P < 0.0001$), 50 μM (35% ± 17, $P < 0.0001$), 75 μM (37% ± 20, $P < 0.0001$) and 100 μM (31% ± 17, $P < 0.007$) of epicatechin had significantly higher percentages of motile spermatozoa than the extender containing no flavonoid. The extender without flavonoids at 72 hr contained fewer motile spermatozoa (15% ± 11) than extenders with 50 μM (24% ± 15, $P < 0.008$) or 75 μM (20% ± 13, $P < 0.05$) of catechin or 50 μM (26% ± 16, $P < 0.002$) or 75 μM (22% ± 18, $P < 0.03$) of epicatechin. Likewise, the control (8% ± 8) at 96 hr contained fewer motile spermatozoa than extenders with 25 μM (12% ± 8, $P < 0.008$), 50 μM (15% ± 11, $P < 0.003$), 75 μM (12% ± 10, $P < 0.01$) or 100 μM (11% ± 8, $P < 0.009$) of catechin and 25 μM (13% ± 11, $P < 0.002$) or 50 μM (15% ± 13, $P < 0.006$) of epicatechin. These results suggest that there is a dose and time related effect of catechin and epicatechin on the motility of extended equine spermatozoa.

Key Words: Flavonoids, Sperm, Equine

831 The effect of 2-bromo-ergocriptine on LH secretion in mature breeding stallions. K. Bennett-Wimbush*[1] and D. Keisler[2], [1] *Ohio State University Agricultural Technical Institute, Wooster, Ohio,* [2] *University of Missouri, Columbia, Missouri*.

Five mature stallions (ages 4-20) were used in this paired t-test experiment to determine the effect of daily administration of the dopamine agonist, 2-bromo-ergocriptine on LH secretion patterns during the physiological breeding season. Prior to treatment, stallions were fitted with indwelling jugular catheters at approximately 0800 hour. After an initial 30 minute adjustment period, blood samples were collected every 15 minutes for 8 hours into sodium heparinized Vacu-tainer tubes. Catheters were removed after this collection period. Samples were immediately placed on ice and held until the blood was centrifuged and plasma was decanted off. Plasma was stored at -10° C until it was assayed for LH. Following this pre-treatment bleed, stallions were treated with .08 mg/kg metabolic body weight ($kg^{.75}$) of 2-bromo-ergocriptine, IM, twice daily for 30 days. On day 28 of treatment, jugular catheters were again used to repeat the intensive bleeding procedure as described earlier. Plasma prolactin concentrations were determined from daily samples collected one week prior to treatment and again during week 4 of treatment Prolactin and LH concentrations were determined using homologous and heterologous radioimmunoassays respectively. LH pulse frequency, amplitude and means were determined using PC PULSAR. Differences between pre-treatment and post-treatment LH pulse frequency, amplitude, means and prolactin were analyzed using GLM, SAS. Mean plasma prolactin decreased ($p < .01$) from 7.9 ng/ml before treatment to 3.6 ng/ml after treatment. There was no change in mean LH, LH pulse frequency or average amplitude following treatment with bromocriptine. Before treatment LH pulse frequency, average pulse amplitude and mean LH was 2.9 ± .7 pulses/8 hr., 2.9 ± 1.0 ng/ml and 5.9 ± 1.0 ng/ml respectively vs. 2.4 ± .9 pulses/8 hr., 1.5 ± 1.3 ng/ml and 3.8 ± 1.3 ng/ml following 28 day administration of bromocriptine. In conclusion, the administration of bromocriptine decreased plasma prolactin, however neither the decrease in prolactin or alteration of the dopaminergic system resulted in any change in the LH secretion patterns in mature stallions.

Key Words: Stallion, Prolactin, LH

832 Effects of group size and bull-to-heifer ratio on sexual behavior, and concentrations of LH and testosterone in yearling beef bulls. R.D. Smith*, D.B. Imwalle, A.L. King, and K.K. Schillo, *University of Kentucky, Lexington, KY*.

We tested the hypothesis that expression of sexual behavior in bulls is dependent on both the number and type (male and female) of cattle present during behavior tests. Eighteen Black Angus bulls were halter broken and assigned randomly to one of three treatments at approximately 12 months of age. In treatment A, a bull was tested with one heifer. In treatment B, a bull was tested in the presence of three heifers. In treatment C, a bull was tested in the presence of three heifers and two other bulls. Each bull was subjected to all treatments. Order of treatments was random for each bull. Heifers were ovariectomized and injected with 2 mg estradiol benzoate in order to induce estrus at the time of testing. During testing, heifers and bulls were allowed to roam freely in a paddock. Test periods lasted 15 min, during which the following traits were recorded: number of successful and aborted mounts, Flehmen responses, chin rests, and ejaculations. Blood samples were collected by jugular venipuncture 2 min before and 2 min after testing and analyzed for LH and testosterone. Number of aborted mounts for treatment A was less than ($P<.05$) those for treatments B and C. Expressions of other behaviors were not affected by treatment ($P>.1$). Neither LH nor testosterone were affected by treatments ($P>.1$). However, between 2 min before and 2 min after testing, testosterone decreased in treatments A and B, but increased in treatment C ($P<.05$). Neither LH nor testosterone was correlated with any of the behavioral traits ($P>.1$). Overall, the social conditions tested in this experiment did not dramatically alter sexual behavior in bulls.

Key Words: Sexual behavior, LH, Testosterone

833 Peripubertal testicular characteristics in various hair sheep and meat goat breeds. B. L. Sayre* and S. Wildeus, *Virginia State University*.

Reproductive organ weights and histomorphometric dimensions were evaluated in peripubertal, co-raised ram lambs (Katahdin - KA and Barbados Blackbelly - BB; 5/breed) and bucklings (Myotonic - MY, Nubian - NU, Pygmy - PY, and Spanish - SP; 6/breed) recruited from a December-born crop, weaned at 8 weeks of age, and fed for moderate growth. Scrotal content was collected at necropsy at 6 mo of age, after determination of BW and scrotal circumference (SC). Testicular weight and dimensions, and segmented epididymal weights were recorded. Parts of testicular parenchyma, and epididymal segments were fixed and processed for histology (H+E staining) to determine seminiferous and epididymal tubule diameter and epithelial height. Statistical comparisons were performed between species and among breeds within species. Final BW and SC were greater ($P>.001$) in hair sheep than goats (32.8 vs 24.7 kg; 25.9 vs 20.8 cm, respectively), with KA heavier ($P>.001$) and a greater ($P>.05$) scrotal circumference than BB, while goats breeds differed in BW ($P<.001$), but not SC. Testicular and epididymal weights and dimensions were consistently larger in hair sheep than goats ($P<.01$); there were no differences between KA and BB, however, SP had longer testis ($P<.05$) and NU lower caput epididymal weights ($P<.05$) than the other goat breeds. Two BB and 6 bucklings (representing all breeds) had incomplete seminiferous epithelial development. Seminiferous tubule diameter and epithelial height were not affected by species, however, KA (172 μm) had a larger ($P<.01$) diameter than BB (150 μm), and PY had a larger diameter (180 μm) and height (56 μm) than the other goat breeds (156 and 51 μm, respectively). Epididymal tubule diameter was not affected by species or breed within species, whereas epithelial height was greater ($P<.001$) in the cauda region of hair sheep than goats (40 vs 33 μm, respectively). Data indicated differences between species and breeds within species, independent of species and breed variation in BW.

Key Words: Goat, Sheep, Testis

834 Post-treatment reproductive characteristics of boars fed ractopamine hydrochloride during the finishing period. D. J. Jones*[1], D. H. Mowrey[1], W. P. Waitt[1], and W. L. Singleton[2], [1]*Elanco Animal Health, Greenfield, IN,* [2]*Purdue University, West Lafayette, IN.*

Thirty-six crossbred (Hampshire × Yorkshire) boars, approximately 17 wk of age at trial initiation, were used to evaluate the effect of feeding ractopamine hydrochloride (R) during the finishing period on subsequent reproductive characteristics. Boars were fed R at 0 (n=18) or 20 ppm (n=18) in an 18% crude protein (CP) corn-soy diet, *ad libitum*, from approximately 67 to approximately 116 kg body weight. A non-medicated 18% CP diet was fed at 2.27 kg/day during the remainder of the trial. Boars were trained to a dummy sow at approximately 33 wk of age and semen was collected twice weekly at approximately 36, 40, and 44 wk of age. Least squares means for selected parameters are shown in the table. Boars fed 20 ppm R had increased (P<0.01) average daily gain and improved (P<0.01) feed to gain ratio during the finisher period with no difference (P>0.05) in daily feed intake. No treatment differences were detected (P>0.05) in the following post-treatment reproductive characteristics: testes volume, total semen volume, fluid volume, sperm concentration, total sperm per ejaculate, sperm motility, percent normal sperm, number of boars which mounted, or number of boars collected. In summary, R fed at 20 ppm to boars during the finisher period caused no adverse effects on subsequent reproductive characteristics evaluated.

Parameters	0 ppm R	20 ppm R	SD
Average Daily Weight Gain, kg	1.05	1.16	0.12
Average Daily Feed Intake, kg	2.83	2.75	0.27
Feed to Gain Ratio	2.73	2.38	0.23
Testes Volume, cm^3	3021	3158	1068.4
Total Semen Volume, ml	233.1	223.8	166.4
Fluid Volume (minus gel), ml	196.4	188.2	138.1
Sperm Concentration (no. cells/ml × 10^8	3.37	3.53	1.90
Total Sperm/Ejaculate (× 10^9)	62.5	60.5	29.5
Sperm Motility, %	80.0	78.1	9.7

Key Words: ractopamine, boars, reproduction

835 Insemination of lactating Angus cows with sexed sperm. S. P. Doyle[1], K. D. McSweeney*[1], J. L. Schenk[2], R. D. Green[1], and G. E. Seidel, Jr.[1], [1]*Colorado State University, Fort Collins,* [2]*XY, Inc., Fort Collins, CO.*

Insemination of flow-sorted sperm has been reasonably successful in heifers. The objective was to determine pregnancy rates in lactating beef cows following AI of frozen Y-chromosome-bearing sperm. Sperm were sorted with a flow cytometer/cell sorter on the basis of DNA content, targeting 90% Y chromosome-bearing sperm. Angus cows 2 to 15 y of age and in good body condition were synchronized with GnRH/prostaglandin F2a and inseminated (N=105) 12 or 24 h after detected estrus with the following treatments balanced over 3 Angus bulls and 2 inseminators: 1) unsorted, frozen control (20 x 10^6 sperm/dose) deposited in the uterine body; 2) sorted, frozen sperm (3 X 10^6 sperm/dose), half deposited into each uterine horn using a side-opening embryo transfer sheath (sexed:horn); and 3) sorted, frozen sperm (3 X 10^6 sperm/dose) deposited in the uterine body (sexed:body). The day 60 pregnancy rate for sexed:body (55%) was not different (P>.1) from the frozen control (71%) or sexed:horn (45%); however, there was a difference between the frozen control and sexed:horn (P<.05). There were significant AI sire effects on pregnancy rates (P<.05) which were magnified if only sorted treatments were included (39, 43, and 68% pregnant for sexed sperm from 3 bulls). There were no significant interactions. Sex ratios determined by ultrasound of 2-mo fetuses for the sorted treatments (95% males) were different from the frozen control (53% males; P<.01). Fertility of sorted sperm varied among bulls; when low numbers of sperm are inseminated, practical application of current sexing technology may require identification of bulls whose sperm tolerate the stress of sorting and cryopreservation.

Key Words: Beef cattle, Sex control, Fertility

836 X and Y chromosome specific duplex PCR standardized to quantify sex ratio variation in bulls and boars. J. B. Paul*, A. M. Canal, and J. E. Chandler, *LSU Agricultural Center, Baton Rouge, LA.*

Sex ratio manipulation in farm animals is a tool to increase production and hasten genetic improvement. X and Y-bearing spermatozoa variation between ejaculates has not been clearly defined. This describes a duplex PCR assay standardized to quantify X- and Y-bearing spermatozoa ratio variation in bull and boar. PCR primers were designed for single copy genes specific to the X (factor IX) and Y (SRYB) chromosomes. Individually amplified X and Y products were extracted from agarose gels and quantified at 260 nm. Extract volume containing 1 ng of product was calculated and log diluted from 1 ng to 10 fg. Each dilution was subjected to a duplex PCR reaction with the original primers. Each reaction was duplicated, sampled twice and electrophoresed on agarose gels containing ethidium bromide. Gels were viewed on a UV transilluminator and photographed with a silicon intensified camera capable of detecting 10 $^{-7}$ lux. Gel were imaged with image analysis software to determine the bands intensity density (IDEN). A dilution that yielded intensities near a grayscale value of 125 were selected as a midpoint for the construction of a standard curve. One hundred percent each product was double the product amount selected as midpoint. Treatment combinations (Rx) on the curve were assigned as X to Y percent product (0:100, 20:80, 40:60, 60:40, 80:20, and 100:0). Duplex PCR was performed with the same primers and the gel images were analyzed in order to quantify varying X and Y template concentrations. Intensity densities were analyzed by multivariate regression methods. Linear combinations (Li) were formed from the maximal variance-covariance eigenvector and the IDENs for the appropriate Rx. Simple linear regression of Li on Rx yielded coefficients of determination for bovine and porcine analyses of 0.90 and 0.71. Bovine and porcine Li slopes were different from zero (P<0.0001). Thus, Li can be used to discriminate between ejaculates based on duplex PCR screening .

Key Words: PCR, Bovine, Sex ratio

837 Cryopreservation of flow cytometrically sorted boar sperm: effects on in vivo embryo development. L. A. Johnson*[1], H. D. Guthrie[1], P. Fiser[1], W.M.C. Maxwell[2], G.R. Welch[1], and W.M. Garrett[1], [1]*USDA Beltsville Agricultural Research Center, Beltsville, MD USA,* [2]*University of Sidney, Australia.*

Cryopreservation of sorted boar sperm would enhance the feasibility of IVF for producing sexed embryos. Aliquots of neat semen from mature boars were diluted and stained with Hoechst 33342, incubated at 35 C, and sorted at room temperature (SORT) into TEST-Yolk (20%)with out regard to separation of the X and Y sperm populations (Theriogenology 52:1323-1341;1999). Samples were centrifuged, 3% glycerol added, and loaded into .25 ml straws. After cooling the straws were frozen using a controlled rate freezer. Control semen had been frozen previously from another boar using 11% lactose as the freezing extender UNSORT). Ovulation was controlled in 11 gilts by an altrenogest-PMSG-hCG regime. Gilts were laparotomized at 44 hr post-hCG and inseminated with 200,000 SORT or UNSORT into the isthmus of respective oviducts. Ten gilts were also inseminated in both oviducts using only SORT to determine litter size at term. Gilts (N=11) for embryos were slaughtered and embryos recovered at 43 hr post insemination. The embryos were fixed in 4% paraformaldehyde, and stained with Texas Red-x phalloidin and Hoechst 33342. Confocal laser microscopy was used to visualize actin cytoskeleton and chromatin configuration. A total of 287 oocytes and embryos were evaluated. Cryopreservation and the subsequent thawing of the SORT sperm had a negative impact on fertilization and embryo development compared to UNSORT sperm as indicated by the incidence of sperm penetrated ova and total embryos (41.2 vs 96.5%, P<.001), normal embryos without fragmentation or polyspermy (4.36 vs 10.5/oviduct, P<.001), incidence of normal embryos (55.4 vs 83.2%, P=.05), and the proportion of 5-9 cell embryos of normal embryos (2.7 vs 21.4%, P<.05) Of 10 gilts allowed to go to term, 1 farrowed two pigs and 4 are at 73 to 94 days of gestation. These results demonstrate the reduced developmental potential of embryos produced from sorted and frozen boar sperm, but that pregnancies can be established.

Key Words: Flow cytometric sorting, Boar spermatozoa, Sperm cryopreservation

838 Effects of transforming growth factor β (TGF) on development of bovine embryos *in vitro*. A.L. King*, D.L. Funk, R.D. Smith, D.B. Imwalle, L.A. Anderson, and K.K. Schillo, *University of Kentucky, Lexington, KY.*

The objective of this experiment was to determine the dose-response relationship between TGF and development of bovine embryos following collection from donor cows. Estrous cycles of six Angus cows were synchronized, and animals were treated with FSH to induce superovulation. Cows were inseminated artificially and embryos were collected nonsurgically six days after breeding. A total of 52 viable embryos were collected (8.6 ± 2.9 embryos per cow). Embryos were sorted by grade and quality and then cultured in 2 ml of a comercial holding medium containing TGF at concentrations of 0(n=12), .03(n=8), .3(n=12), 3.0(n=8), and 30ng/ml(n=12) for 96 h. Media was changed and embryos re-evaluated every 12 h. There was a significant (P<.01) linear relationship (R^2 = .964) between the log of the dose of TGF and percentage of embryos reaching the expanded blastocyst stage. Ratio of embryos reaching this stage in the 0 ng/ml dose (5/12), was not different from those of the .03 (4/8), .3 (7/12), or 3 (5/8) ng/ml doses, but was different (P<.05) from that of the 30 ng dose (9/12). These results are consistent with the hypothesis that TGF may enhance development of bovine embryos. Whether or not this peptide can improve efficacy of embryo transfer in cattle remains to be determined.

Key Words: TGF, Embryo Transfer, Bovine

839 The effects of phytohemagglutinin and pokeweed mitogen on bovine oocyte maturation *in vitro*. S. Wang[1], K.E. Panter*[2], J.N. Stellflug[3], R.C. Evans[1], and T.D. Bunch, [1]*ADVS Department, Utah State University, Logan,* [2]*USDA-ARS, Poisonous Research Laboratory, Logan, UT,* [3]*3USDA-ARS, U.S. Sheep Experiment Station, Dubois, ID.*

Phytohemagglutinin (PHA) and pokeweed mitogen (PWM), the extracts from *Phaseolus vulgaris* and *Phytolacca americana*, respectively, are two mitogenic agents causing blastoid reaction to produce a short-lived burst of mitosis involving small lymphocytes. This study investigated the effects of PHA and PWM on bovine oocyte maturation as indicated by the subsequent preimplantation embryo development *in vitro* using a randomized complete block (5 replications) design with three treatments (TRT). Bovine oocytes (n = 736) were aspirated from abattoir ovaries and *in vitro* matured (IVM) in the media containing PWM (Gibco, Cat. No. 15360-019) 10 μl/ml (TRT1), PHA (Gibco, Cat. No. 10576-015) 10 μl/ml (TRT2) or none of them (Control), respectively. The IVM culture lasted for 24 h in a humidified 5% CO_2 atmosphere at 39 °C. The IVM oocytes were fertilized *in vitro* (IVF) and the IVM/IVF derived zygotes were then *in vitro* cultured (IVC) in modified CR2 medium. Embryo development was evaluated at d 2, d 6, and d 8 (IVF = d 0). Percentage data were angularly transformed and analyzed by ANOVA. The cleavage rates were 83.5%, 90.0% and 88.9%. The percentage of morulae at d 6 was 26.6, 53.9 and 54.4; and the percentage of blastocysts at d 8 was 1.4, 12.7 and 12.9 for TRT1, 2 and 3, respectively. The cleavage rate and the numbers of embryos developed to morulae and blastocyst stages in PWM medium were significantly lower than those in PHA and Control ($P < 0.05$), indicating that PWM adversely influenced bovine oocyte maturation and the effects are extended to subsequent embryo development. However, there was no difference ($P > 0.05$) between PHA and Control. In conclusion, bovine oocyte *in vitro* maturation may be adversely affected by mitogenic agents, depending on their sources, as shown by the subsequent development of preimplantation embryos *in vitro*.

Key Words: *In vitro* maturation, Phytohemagglutinin, Pokeweed mitogen

840 *In vitro* maturation medium supplemented with bovine follicular fluids on subsequent embryo development. S. Wang*[1], Y. Liu[1], K.E. Panter[2], J.N. Stellflug[3], R.C. Evans[1], and T.D. Bunch[1], [1]*ADVS Department, Utah State University, Logan,* [2]*USDA-ARS, Poisonous Research Laboratory, Logan, UT,* [3]*USDA-ARS, U.S. Sheep Experiment Station, Dubois, ID.*

This study investigated the effects of bovine follicular fluids (bFF) collected from various sizes of follicles on bovine embryo development *in vitro* using a randomized complete block (8 replications) design with five *in vitro* maturation (IVM) treatments (TRT). Follicular fluids were aspirated from follicles of abattoir ovaries. Oocytes (n = 3904) were aspirated from 5 to 7 mm follicles, washed in hepes-TALP five times and moved into IVM medium supplemented with 20% (v/v) bFF from follicles < 2 mm (TRT1), 3-7mm (TRT2), 8-15 mm (TRT3), and >15 mm (TRT4) in diameter. The IVM medium without bFF served as control (TRT5). The IVM culture lasted for 24 h in a humidified 5% CO_2 atmosphere at 39 °C. The IVM cultured oocytes were then subjected to *in vitro* fertilization (IVF) and *in vitro* culture (IVC). Cleavage rates were determined at 45 h after IVF. Embryo development was evaluated at d 6 and d 8 (IVF = d 0). Percentage data were angularly transformed and analyzed by the ANOVA. The cleavage rates were 82.1%, 81.9%, 83.5%, 81.4% and 82.8%; the percentage of morulae at d 6 was 41.2, 41.6, 41.1, 48.9 and 49.5; and the percentage of blastocysts at d 8 was 25.4, 25.0, 26.7, 29.3 and 29.6, for TRT1, TRT2, TRT3, TRT4 and control, respectively. There was no significant (P>0.05) difference with respect to oocyte cleavage. However, there were significantly (P<0.05) fewer embryos that developed to morula and blastocyst stages after matured in the medium supplemented with bFF from small follicles (<2mm and 3-8 mm) than those supplemented with bFF from large follicles (>15 mm), indicating that bFF from small size follicles (<15 mm) adversely influence *in vitro* development of bovine oocytes to the morulae and blastocyst stages. There was no significant difference in embryo development between TRT4 and control. These results suggest that bovine embryo development was affected by the bFF from various size of the follicles supplemented to IVM medium.

Key Words: Bovine, Follicular fluid, *In vitro* maturation

841 Embryonic development in beef cattle administered ergotamine tartrate to simulate fescue toxicosis. M. E. Hockett*, T. M. Towns, J. L. Edwards, N. R. Rohrbach, and F. N. Schrick, *University of Tennessee, Knoxville.*

A replicated experiment was performed to determine the effects of simulated fescue toxicosis on early embryonic development in beef cattle. Beef cows and heifers were randomly allotted by age, breed and body condition to be fed a 2:1 ground corn/soybean meal mix supplemented with either 0 (CON, n=38) or 40 μg/kg BW ergotamine tartrate (TRT, n=37). Following a 30-d adjustment period on respective diets, animals were artificially inseminated at estrus with semen of known fertility. Weekly BW, body temperatures and blood samples were collected throughout the study. Six days following mating, blood was collected and ovaries were scanned with ultrasonography to determine volume and location of the corpus luteum. Single embryos were flushed from the uterine horn ipsilateral to the corpus luteum and scored for quality (scale of 1, excellent to 4, degenerate) and development (scale of 1, unfertilized to 9, expanded hatched blastocyst). Prolactin concentrations were decreased by d 20 of feeding ergotamine tartrate in TRT (83.4 ± 15.7 ng/mL) compared to CON animals (127.8 ± 15.7 ng/mL; P < .05). Concentrations of progesterone at embryo recovery (2.9 ± .2 ng/mL) did not differ. Embryo recovery tended to be greater (P = .08) for CON (68.4%) than for TRT (48.7%); but flush scores (range of 1, excellent to 3, poor) did not differ. Embryo development to stage 4 (compact morula) or higher was greater for embryos collected from CON (88% or 22/25) compared to TRT animals (56% or 10/18; Fisher's Exact Test, P = .02). The percentage of transferrable embryos (quality score 1,2 or 3 on the 1-4 scale) tended to be higher (P = .09) for CON (88% or 22/25) than for TRT (61% or 11/18). Degenerate embryos were recovered more frequently from TRT (39% or 7/18) than CON animals (12% or 3/25). These results demonstrate that ergotamine tartrate administration decreased embryo quality and development prior to day 6 after mating. Fescue toxicosis may negatively impact reproduction through alterations in early embryo development.

Key Words: Beef cattle, Fescue toxicosis, Embryo

842 Interaction of endophyte-infected fescue and heat stress on ovarian function in the beef heifer. J. M. Burke*[1], F. N. Kojima[2], B. E. Salfen[2], S. L. Wood[2], D. J. Patterson[2], M. F. Smith[2], M. C. Lucy[2], W. G. Jackson[1], and E. L. Piper[3], [1]*USDA, ARS, Booneville, AR,* [2]*University of Missouri, Columbia,* [3]*University of Arkansas, Fayetteville.*

Objective was to examine interaction of fescue and heat stress (HS) on ovary development. Heifers were fed endophyte-free (E-) or endophyte-infected seed (E+; 18.4%) at thermoneutral (S-) or HS (S+; E-S-, E-S+, E+S-, E+S+; n = 6/treatment) 4 wk before and 3 wk after synchronized

ovulation (7-11 Synch; D 0 was d of expected ovulation). Serum was collected and ovaries were monitored every other d after PGF until D 20 or ovulation. Size of follicles > 4 mm and CL were recorded. Heifers were subjected to 19°C, 50% RH from D -8 to -2 and S+ heifers to 31°C/25°C from D -1 until D 20. Feed intake was not different between E- and E+ heifers, but was reduced in S+ vs S- heifers (HS x d, P < .01). Water intake was not different among treatments. There was an interaction between diet, HS, and d for AM (P < .001) and PM (P < .001) rectal temperature and a diet x d (P < .001, AM; P < .01, PM) and HS x d (P < .001, AM; P < .001, PM) interaction for respiration rate. Serum P4 was reduced in S+ heifers and to a greater extent in E+S+ heifers (HS x d, P < .06; y_{E-S-} = -3.6 - 2.3x - .21x^2 - .0049x^3, y_{E-S+} = -1.7 - 1.3x^2 - .0023x^3, y_{E+S-} = -1.7 -1.8x -.17x^2 - .0043x^3, y_{E+S+} = -.66 - .72x - .061x^2 - .0013x^3, where y = P4 and x = d; P < .003). Diameter of the CL was reduced in S+ heifers (y_{S-} = 13.5 - 1.5x - .067x^2, y_{S+} = 14.8 - .77x - .034x^2, where y = CL diameter and x = d; P < .07). Serum E2 was reduced in E+ heifers (y_{E-S-} = 6.6 + .67x + .026x^2, y_{E-S+} = 2.6 - .0035x - .0024x^2, y_{E+S-} = 5.9 + .72x + .029x^2, y_{E+S+} = 4.6 + .49x + .019x^2, where y = E2 and x = d; P < .001). Similarly, diameter of ovulatory dominant follicle was reduced in E+ heifers (diet x d; P < .07). Intake of E+ reduced P4 only during HS, but reduced E2 under S- and S+ conditions. Impaired follicle function may explain reduced pregnancy rates commonly observed in heifers grazing E+ pasture.

Key Words: Beef, Fescue, Ovary

843 Enhancement of superovulatory response using a norgestomet implant during the FSH treatment period. G. W. Bednar* and J. R. Pursley, *Michigan State University, East Lansing*.

Results from studies predicting or controlling the onset of a new follicular wave for superovulation are inconsistent. The objectives of this experiment were: 1) to determine if a norgestomet implant inserted during the FSH injection period would allow more follicles to reach the ovulatory pool when FSH was initiated during follicular dominance, and 2) to determine the fate of the 1st wave DF and its effect on ovulation rates. Beef cows were synchronized with two injections of PGF2α 14 days apart. Cows detected in estrus (n = 28) were assigned to three groups; GROUP 1 cows began FSH on d of emergence of the 2nd follicular wave (ranged from d 7 to 9 of the estrous cycle) and served as the control. GROUP 2 and 3 cows began FSH during follicular dominance (d 5 or 6 of estrous cycle). In addition, Group 2 cows received a norgestomet implant during the FSH treatment period. All cows received a total of 380 mg Folltropin-V® during a 4.5 d period in decreasing 2x/d doses. Cows received 25 mg PGF2α on d 3.5 and 4. Follicles and CL were mapped daily using ultrasonography. All cows (19/19) in GROUP 2 and 3 responded to FSH with emergence of a new follicular wave. Numbers of new follicles 2 d after initiation of FSH were not different (P > 0.05) between treatments, 39 ± 6.0, 37 ± 4.9, 35 ± 5.2, for GROUPS 1, 2, and 3, respectively. Number of follicles ovulating following FSH treatment in cows with > 1 ovulation was greater (P < 0.05) in GROUP 1 (25.9 ± 2.9) and GROUP 2 (29.9 ± 3.2) compared to GROUP 3 (16.4 ± 5.0). Of the cows that did not respond with multiple ovulations, the 1st wave DF ovulated either during (4 of 4 cows in GROUP 2) or after (2 of 2 in GROUP 3) the FSH treatment period. In cows with multiple ovulations, the 1st wave DF ovulated in 6/8 cows in GROUP 2 and 0/5 cows in GROUP 3. In summary, the fate of the 1st wave DF appeared to play a key role in determining whether follicles were allowed to ovulate. Also, it appears the norgestomet implant allowed sufficient additional time for superstimulated follicles to reach the ovulatory pool when the DF remained functional and ovulated following FSH.

Key Words: Dominant follicle, FSH, Norgestomet

844 Follicular dynamics and oocyte quality during early lactation in Holstein cattle. A.H. Walters*, T.L Bailey, J. Strauss, and F.C. Gwazdauskas, *Virginia Polytechnic Institute & State University, Blacksburg*.

Ultrasound-guided transvaginal follicular aspiration was used to obtain oocytes from cows to study follicular development and oocyte morphology. Follicular aspiration was conducted once during wk 1 to 12 postpartum on 102 lactating cows with 6 groups, separated by biweekly intervals. Approximately one half of the aspirated cows at each session were from the early groups (wk 1-2, 3-4, or 5-6) and the other half from the later groups (wk 7-8, 9-10, or 11-12). On the day of aspiration the number of follicles on each ovary and their sizes, small (2-5 mm), medium (6-10 mm) and large (≥ 11 mm), were recorded. The collected oocytes were morphologically classified into 4 grades, with A = excellent, B = good, C = fair, and D = poor. A Chi square analysis revealed a significant (P < .01) difference in the distribution of small follicles over time between first lactation and older cows (≥ 2nd lactation). The percentage of small follicles increased with week postpartum in first lactation cattle (3 % at wk 1-2 to 57% at wk 11-12), while older cows peaked at 58% in wk 7-8, and then decreased to 34% at 11-12 wk. Medium and large follicles followed similar patterns. When compared across time postpartum, the distribution of A and B quality oocytes was cyclical, peaking in wk 7-8 and wk 11-12. This was different from the distribution of D quality oocytes (P < .01) which peaked at 70% in wk 1-2, decreased to 27% in wk 7-8, and then increased to 55% in wk 11-12. The distribution of oocyte quality also differed across parity, where the percentage of A quality oocytes was higher (12% vs 9%) for older cows compared to first lactation cattle (P < .05). Additionally, first lactation cattle had a higher percentage of B quality oocytes than older cows (18% vs 14%; P < .05). The changes in follicular dynamic patterns and oocyte quality in younger and mature cattle may be an indication of the impact of early lactational performance and growth demands on subsequent fertility.

Key Words: Follicle, Oocyte, Lactation

845 Effects of follicle stimulating hormone (FSH) treatment on follicular development and oocyte retrieval in seasonally anestrous ewes. T.K. Stenbak*, L.P. Reynolds, D.A. Redmer, and A.T. Grazul-Bilska, *Department of Animal and Range Sciences, North Dakota State University, Fargo, ND, USA*.

To determine the effects of FSH and Syncro-Mate-B (SMB) treatment of seasonally anestrous ewes on the number of follicles, and the recovery and quality of oocytes, a 2 x 3 factorial study was designed. Half of the ewes were implanted with SMB for 14 days and the other half of the ewes were not implanted. SMB-implanted and non-implanted ewes were then assigned to one of three treatments: no treatment (control, n = 12), FSH-injected for two days (2d, n = 21) or FSH-injected for 3 days (3d, n = 15). Ewes received twice daily (morning and evening) intramuscular injections of FSH, beginning on day 12 (3d) or day 13 (2d) after SMB implantation. Ewes were laparotomized 15 h after the last FSH-injection to determine number of small (<3 mm) and large (>3 mm) follicles and to retrieve oocytes. Oocytes were evaluated as atretic or healthy on the basis of morphology. Ewes treated with SMB had similar (P > 0.05) numbers of follicles, numbers of oocytes, and numbers of healthy oocytes compared with ewes that did not receive SMB; therefore, data were combined across SMB implanted and non-implanted ewes. FSH-treatment increased (P < 0.05) the number of large (1.5±0.3 vs 13.0±1.3 for control and FSH-treated ewes, respectively) and the total number (6.9±0.9 vs 18±1.5) of follicles, and the number of oocytes recovered from large follicles (1.3±0.3 vs. 11±1.4) and total follicles (5.6±1.0 vs. 15.3±1.3). There was no difference between the control and the FSH-treated ewes in the number of oocytes recovered from small follicles (4.3±0.9 and 3.7±0.7). The recovery rate of oocytes (number of oocytes recovered divided by the number of follicles; 79.5%±9.0 and 83%±2.4) and the percent of oocytes that were healthy (84%±5.0 and 84%±2.4) did not differ between the control and the FSH-treated ewes. However, the number of healthy oocytes was greater (P < 0.05) for the FSH-treated ewes than the control ewes (11.8±1.8 vs. 4.6±0.8). These data indicate that SMB has no effect on follicular development, but that FSH-treatment improves follicular development and the number of healthy oocytes retrieved from seasonally anestrous ewes. Supported by NDSU RDSP1172 grant.

Key Words: Ewes, Follicle Stimulating Hormone (FSH), Oocyte collection

846 Effect of FSH on in vitro growth of early antral follicles from bovine fetal ovaries. K. R. Chohan and A. G. Hunter*, *Dept. of Animal Science, University of Minnesota, Saint Paul, USA*.

Effect of FSH was evaluated on in vitro growth of bovine early antral follicles. Ovaries of 7 to 9 month old fetuses were dissected and antral

follicles between 500 μm to 1.3 mm were selected. Follicles similar in diameter were divided into two equal groups in each replicate. The culture medium was Minimal Essential Medium supplemented with 1% ITS (Insulin 6.25μg, Transferrin 6.25μg, Selenium 6.25μg), 3 mg/ml BSA (F-V), 2 mM glutamine, 2 mM hypoxanthine, 0.23 mM sodium pyruvate, 100 μg/ml streptomycin, 100 IU/ml penicillin, 25 μg/ml of fungizone, 1 μg /ml estradiol, 10% FCS and 15 mM HEPES. Follicles were cultured individually in 96 U-shaped well plate for 15 days in 70μl of medium with 100 ng/ml FSH (n=102) or without FSH (n=92) under 20 μl of mineral oil at 39°C and 5% CO_2. Follicles were transferred to new wells with pre-equilibrated medium after every 24 hrs and follicle diameters were recorded at 48 hrs intervals. Data were analyzed using repeated measures analysis in PROC MIXED procedure of SAS system. No effect of FSH was observed on development in follicle size (P>0.05). The follicles appeared morphologically healthy at 15 days culture as no signs of atresia were observed. However, follicle diameter decreased significantly (P<0.05) with time in both groups. These results indicated that refinements in culture conditions are needed for full follicle development.

Group	D 1	D 3	D 5	D 7	D 9	D 11	D 13	D 15
FSH	866.7 ± 20.2	877.3 ± 20.2	847.3 ± 20.3	828.6 ± 20.3	814.9 ± 20.3	798.7 ± 20.2	791.3 ± 20.2	749.6 ± 20.1
CONT	892.8 ± 21.3	893.2 ± 21.3	855.2 ± 31.3	840.5 ± 21.3	820.5 ± 21.3	806.2 ± 21.3	778.5 ± 21.3	745.4 ± 21.3

Follicle diameters shown in table are in μm and Mean ± SE.

Key Words: Bovine, Follicle, Culture

847 Effect of culture duration on germinal vesicle and meiotic development of bovine fetal oocytes isolated from small antral follicles.

K. R. Chohan and A. G. Hunter*, *Dept. of Animal Science, University of Minnesota, Saint Paul, USA*.

The effect of duration of culture was observed on germinal vesicle and meiotic development of oocytes from < 2 mm follicles from ovaries of 7.5 months to term bovine fetuses. Oocytes were evaluated at isolation (0 hrs) and after 24 and 48 hrs of *in vitro* culture. The culture medium was M199 supplemented with 10 μg/ml FSH, 10 μg/ml LH, 1.5 μg/ml estradiol, 75 μg/ml streptomycin, 100 IU/ml penicillin, 10mM hepes and 10% FCS. Cumulus cells were removed using 3 mg/ml hyaluronidase and repeated pipetting. Denuded oocytes were fixed in 3% glutaraldehyde, stained with DAPI and evaluated for stage of germinal vesicle (GV) and subsequent meiotic development. Data were analyzed using Chi Square. The majority of oocytes were at GV-I (36.4%) and II (49.7 %) at isolation (0hrs). A shift was observed (P<0.05) from the early to final stages of GV and meiotic development after 24 hrs of culture as 22.8% oocytes were observed at GV-V, 14.9% at pre-metaphase and 17.5% at metaphase-I. Only 7.9% of the oocytes were able to reach the metaphase-II stage. A significant difference (P<0.05) was observed in GV morphology and meiotic development after 48 hrs of culture compared to results at 0 hrs as more oocytes (18.3%) reached metaphase-II. The degeneration rates increased from zero percent at isolation (0hrs) to 4.4% and 13.5% after 24 and 48 hrs of culture (P<0.05), respectively. Although the fetal oocytes from < 2 mm follicles showed improvement in germinal vesicle development and meiotic competence with an increase in culture duration, these results are still too poor to be considered for in vitro fertilization procedures. Therefore, it will be necessary to determine if fetal oocytes from < 2 mm follicles, need a modified medium to achieve *in vitro* developmental competence.

Oocytes (#)	GV-I (%)	GV-II (%)	GV-III (%)	GV-IV (%)	GV-V (%)	GV-BD (%)	Premet (%)	Met-I (%)	Met-II (%)	Degn (%)
0 H	36.4^a	49.7^a	5.6^a	3.5^a	4.9^a	0.0^a	0.0^a	0.0^a	0.0^a	0.0^a
24 H	0.0^b	7.0^b	9.6^a	7.9^a	22.8^b	7.9^b	14.9^b	17.5^b	7.9^b	4.4^b
48 H	0.0^b	1.9^b	3.8^a	2.9^a	15.4^b	6.7^b	18.3^b	$19 .2^b$	18.3^c	13.5^c

Key Words: Fetal, Oocyte, Development

848 Temporal and spatial expression of tissue inhibitor of matrix metalloproteinase 1 (TIMP-1) in bovine corpus luteum.

B. Zhang* and P.C.W. Tsang, *Department of Animal & Nutritional Sciences, University of New Hampshire, Durham NH 03824*.

The corpus luteum (CL) is a dynamic endocrine gland that undergoes dramatic structural and functional changes that are associated with various phases of its life span. The matrix metalloproteinases (MMPs) and TIMPs, which we have shown to be present in CL obtained over the estrous cycle, may mediate these changes. In order to gain a greater understanding of the role of TIMPs in luteal function, we explored the temporal and spatial expression of TIMP-1 in bovine CL. Luteal tissues were collected from early (4 days old, estrus= day 0), mid (10-11 days old) and late (16 days old) phases (n=3 for each phase) of the estrous cycle. Proteins were extracted from all samples, and analyzed by Western blotting and reverse zymography. In addition, frozen tissue sections were also prepared for in situ hybridization and immunocytochemistry. Reverse zymography indicated that TIMP-1 was the major TIMP present in bovine CL. Western blotting showed no differences in TIMP-1 protein levels between early and mid phases. However, TIMP-1 levels decreased (p<0.05) from mid to late stage CL. In situ hybridization and immunocytochemistry revealed that TIMP-1 mRNA and protein had a similar cellular distribution. Large luteal cells from all three ages of CL expressed TIMP-1, with the highest level of expression detected in tissue of the mid cycle phase. Furthermore, TIMP-1 was also localized in capillary smooth muscle cells, but expression levels were greater in late than early CL. Overall, these results indicated that the variations in TIMP-1 expression may suggest diverse roles for this multifunctional protein in the physiology of CL during the estrous cycle. (Supported by Hatch 343 to PCWT)

Key Words: corpus luteum, TIMP-1, bovine

849 Immunization of sheep against homologous placental lactogen: effects on lamb birth weight, milk production and conception rate.

H. Leibovich*[1], A. Gertler[1], F.W. Bazer[2], and E. Gootwine, [1] *The Hebrew University of Jerusalem, Israel,* [2] *Texas A&M University System Health Science Center,* [3] *Institute of Animal Sciences, ARO, The Volcani Center, Israel*.

The effect of active immunization of sheep against recombinant ovine placental lactogen (oPL) on reproduction and production performances was examined. Five month old Booroola-Assaf (n=16) and Assaf (n=35) ewe lambs were immunized against oPL. Anti oPL antibody titers, maternal serum levels of oPL, reproductive performances and lamb birthweight were followed up to the second lambing in the immunized ewes and in control non-immunized ewes (n=129). All the immunized ewes developed anti oPL antibodies which could interfere with oPL bioactivity in in-vitro cell proliferation assays. Conception rate did not differ (P>0.05) between immunized and non immunized ewes. Presence of abundant antibody-bound non-active oPL in the sera of immunized ewes, as was shown by Western analysis indicates enhanced oPL production by the placenta during pregnancy following the immunization. Birthweight of lambs born to the immunized ewes was higher (P<0.01) than birthweight of lambs born to control ewes on the average by 0.2, 0.5 and 0.9 kg for singles, twins and triplets, respectively. Immunized ewes produced in the first 3.5 months of the lactation 17% and 25% more milk than control ewes (P<0.02) in the first and the second lactations, respectively. These findings suggest that oPL may not play a major role in maternal recognition of pregnancy but plays an important role in fetal growth and mammogenesis. Immunization against oPL may have a potential application in manipulating fetal growth in prolific sheep and milk production of both dairy and mutton ewes.

Key Words: Placental Lactogen, Milk production, Lamb birth weight

850 Lambing rates after oxytocin-induced cervical dilation, cervical manipulation, and laparoscopic artificial insemination.

J. Stellflug*[1], M. Wulster-Radcliffe[2], E. Hensley[2], and G. Lewis[1], [1] *USDA-ARS US Sheep Experiment Sation, Dubois ID,* [2] *Virginia Tech University, Blacksburg*.

Exogenous oxytocin (OT) dilates the cervix and facilitates transcervical, intrauterine AI in sheep. Even though OT and cervical manipulation (CM) have not affected ovum fertilization rates, their effects on lambing rates have not been adequately evaluated. Thus, to further evaluate OT

and CM, multiparous white-faced ewes (n = 220) were assigned to a 2 x 2 factorial array of treatments: 1) saline-sham CM, 2) saline-CM, 3) OT-sham CM, and 4) OT-CM. Progestogenated pessaries were used to synchronize estrus, and 400 IU of eCG were injected i.m. at pessary removal. Between 48 and 54 h later, 200 USP units of OT or 10 mL of saline were injected i.v., approximately 25 min before sham CM or CM. Immediately after sham CM or CM, laparoscopy was used to AI all ewes with frozen thawed-semen composited from several rams (50 x 10^6 to 75 x 10^6 spermatozoa/200 μL diluent). At 10 to 12 d after AI, ewes were mated with black-faced rams for 21 d. Jugular blood samples were collected between 24 and 26 d after AI for pregnancy-specific protein B (PSPB) RIA. A PSPB-positive sample indicated that a ewe was, or had been, pregnant from AI. Time of lambing and type of lamb were recorded. Chi-square procedures were used. The PSPB pregnancy rate and lambing rate were both 62% in saline-sham controls. The CM did not affect either pregnancy (69%) or lambing rate (64%). The OT decreased (P < 0.05) PSPB pregnancy rate (59%), lambing rate (56%) in OT-sham ewes, and pregnancy and lambing rate in CM ewes (both 43%). In ewes not pregnant from AI, neither CM nor OT affected lambing rates to the next estrus, indicating that there was no long-term damage to the cervix or uterus. In summary, CM did not affect fertility after laparoscopic AI, but OT caused decreases in lambing rate independent of CM. Reduced doses of OT are being tested, but, in the event OT will not be usable for AI, it may still be a valuable tool for training AI personnel.

Key Words: Sheep, Artificial Insemination, Cervix

851 Progesterone down regulates the uterine immune response to infectious bacteria in gilts.

M. C. Wulster-Radcliffe*[1], R. C. Seals[1], and G. S. Lewis[2], [1]*Virginia Polytechnic Institute and State University, Blacksburg, VA,* [2]*USDA Sheep Experiment Station, Dubois, ID.*

A progestogenated uterus is susceptible to infections, but this is not well characterized in gilts. Therefore, the effects of day of the estrous cycle (DOC) and progesterone (P4) were evaluated. Gilts (n = 5/group) were assigned to treatments in 2×2 factorial arrays. In Exp. 1, DOC and bacterial challenge (BC) were main effects. On d 0 or 8, uteri were inoculated with either 70×10^7 cfu of *Escherichia coli* and 150×10^7 cfu of *Arcanobacterium pyogenes* in PBS (10 mL) or PBS. In Exp. 2, ovariectomy (OVEX) and P4 supplementation were main effects. On d 0, gilts were OVEX or a sham procedure was performed. After surgery, gilts received i.m. injections of P4 (10 mg/5 mL) or 5 mL of safflower oil diluent twice daily. On d 8, gilts were inoculated with the same doses of bacteria as in Exp. 1. In Exp. 1 and 2, vena caval blood was collected for 4 d, gilts were killed, and uteri were collected. Sediment (packed-cell volume; PCV) and ability to culture *E. coli* and *A. pyogenes* from uterine flushings were used to diagnose infections. Differential white blood cell counts and lymphocyte blastogenic response to mitogens (i.e., concanavalin A [Con A] and lipopolysaccharides [LPS]) were used to evaluate immune function. In Exp. 1, d-8 gilts receiving bacteria developed infections (PCV > 5%), but d-0 gilts receiving bacteria did not (PCV < 1%). Daily percentages of neutrophils and lymphocytes changed (P < .05) in response to DOC and BC. Basal and Con A-stimulated blastogenesis were greater (P < .05) for d-0 than for d-8 gilts. In Exp. 2, gilts exposed to P4 (i.e., ovarian or injected) had infections (PCV > 5%). Daily percentages of neutrophils and lymphocytes changed (P < .01) in response to OVEX, and neutrophils changed (P < .05) in response to P4. The Con A- and LPS-stimulated blastogenesis increased in response to OVEX (P < .05) and decreased in response to P4 (P < .05). Day of the estrous cycle and P4 supplementation changed the uterine immune response to infectious bacteria in gilts.

Key Words: Gilt, Progesterone, Uterine infections

852 Prostaglandin modulation of the uterine responses to infections in ewes.

R. C. Seals*, M. C. Wulster-Radcliffe, and G. S. Lewis, *Virginia Polytechnic Institute and State University, Blacksburg.*

Luteal-phase uteri are susceptible to infections, and exogenous progesterone (P_4) can down-regulate, whereas $PGF_{2\alpha}$ can up-regulate, uterine immune functions. Thus, we evaluated these factors. In Exp. 1, ewes (n = 6/group) were assigned to treatments in a 2 × 2 factorial; stage of estrous cycle and bacterial challenge were main effects. On d 0 or 6, uteri were inoculated with either 35 × 10^7 cfu of *Escherichia coli* and 75 × 10^7 cfu of *Arcanobacterium pyogenes* in 5 mL of PBS or PBS. Vena caval blood was collected for 3 d, ewes were killed, and uteri were collected. Lymphocytes were assigned to treatments in a 2 × 2 factorial; 10^{-7} M $PGF_{2\alpha}$ and 10^{-7} M indomethacin (INDO), a PG-synthesis inhibitor, were main effects. Amount of sediment and ability to culture *E. coli* and *A. pyogenes* from uterine flushings were used to diagnose infections. Lymphocyte blastogenesis ([^{3}H]thymidine incorporation) was used to evaluate immune function, P_4 was quantified, and white blood cells (WBC) were counted. The P_4 was greater ($P < .01$) for d 6 than for d 0 (6.6 vs 1.3 ng/mL). Neutrophil numbers were greater ($P < .01$, 53 vs 44/100 WBC, SEM = .4), but lymphocytes were less ($P = .04$, 21 vs 34/100 WBC, SEM = .5), after bacteria than after PBS. Bacteria on d 6, but not on d 0, induced infections. Blastogenesis was greater for d 0 than for d 6 in response to concanavalin A (Con A) alone ($P < .05$, 4.1 vs 3.1 pmol, SEM = .5) and to Con A + $PGF_{2\alpha}$ ($P < .01$, 3.5 vs 2.7 pmol, SEM = .4), but INDO did not affect blastogenesis. In Exp. 2, uteri (n = 6 ewes/group) were inoculated with bacteria on d 6. Ewes were treated with 15 mg of Lutalyse or PBS on d 9, killed on d 11, and uteri were collected. Protocols were the same as in Exp. 1. The $PGF_{2\alpha}$ reduced ($P < .01$) P_4 (1.5 vs 11.7 ng/mL, SEM = .8), tended ($P = .06$) to increase neutrophils (48, 54, and 42/100 WBC, SEM = 4, on d 9, 10, and 11, respectively), allowed ewes to clear infections, but did not affect blastogenesis. Stage of the estrous cycle of ewes seems to have a greater effect on uterine immune function than $PGF_{2\alpha}$.

Key Words: Sheep, Uterine Infections, Prostaglandin F2alpha

853 Effects of selection for ovulation rate or uterine capacity on gravid uterine, farrowing, and weaning traits in swine.

R. K. Christenson*[1] and K. A. Leymaster[1], [1]*USDA, Agricultural Research Service, U.S. Meat Animal Research Center, Clay Center, NE.*

Eleven generations of selection for ovulation rate (OR) or uterine capacity (UC) increased (P < 0.01) OR (3.2 ova) or UC (1.1 pigs per uterine horn) in swine [Leymaster & Christenson, J. Anim. Sci. 78(Suppl. 1), 2000]. Our objective was to explore the effects of enhanced OR or UC on gravid uterine, farrowing, and weaning traits. Littermate control- (CO), OR-, or UC-line gilts were randomly assigned either to be unilaterally hysterectomized-ovariectomized, mated within line, and slaughtered at 105 days of gestation or to remain intact, mated within line, and farrowed. At slaughter, CO line (n = 80) means for gravid uterine horn, total fetal, total placental, and empty uterine weight were 10.6 ± 0.4, 5.4 ± 0.2, 1.31 ± 0.05, and 1.87 ± 0.05 kg, respectively. Gilts selected for OR (n = 78) or UC (n = 62) deviated from the CO gilts by -0.9 and 1.5**, -0.5 and 0.8**, -0.21** and 0.16**, and 0.04 and 0.15* kg, respectively (*P < 0.05, **P < 0.01). UC exceeded CO line gilts by 8 to 15% for these reproductive traits. At farrowing, CO line (n = 84) means for total number of pigs born, litter birth weight, number of pigs weaned, and litter weaning weight were 10.6 ± 0.3 pigs, 13.6 ± 0.3 kg, 8.9 ± 0.3 pigs, and 40 ± 1.3 kg, respectively. Gilts selected for OR (n = 66) or UC (n = 62) deviated from the CO gilts by 0.7 and 0.9* pigs, -0.3 and 0.8* kg, 0.1 and 0.3 pigs, and 0 and 1 kg, respectively (*P < 0.05). OR and UC line gilts produced 7 and 9% more pigs at birth than CO line gilts. Simultaneous increases in both OR and UC are necessary to produce substantial increases in litter size at birth and subsequent farrowing and weaning traits.

Key Words: Swine, Selection responses, Reproductive traits

854 Estrus and ovarian responses to P.G.600 administered after Regumate withdrawal in gilts.

M.J. Estienne*, A.F. Harper, C.E. Estienne, and J.W. Knight, *Virginia Polytechnic Institute and State University, Blacksburg.*

The effects of exogenous gonadotropins on the treatment-to-estrus interval and ovulation rate were assessed in gilts following termination of orally administered progestogen treatment. Randomly-cycling, crossbred gilts (n = 64; 143.1±.9 kg BW; mean±SE) were fed 2.7 kg of a complete ration containing 15 mg Regumate (Hoechst Roussel Vet, Warren, NJ) daily for 18 d. Twenty-four h after the last feeding of Regumate, 32 gilts received an i.m. injection of P.G.600 (400 I.U. PMSG and 200 I.U. HCG; Intervet Inc., Millsboro, DE) and 32 control gilts received an i.m. injection of deionized water. All gilts were checked for estrus twice daily (at approximately 0700 and 1900) in the presence of a mature boar, and were sacrificed 9 to 11 d after the onset of estrus. Ovaries were removed

and weighed, and corpora lutea (CL) were excised and weighed. Remaining ovarian tissue was minced and blotted and weight of follicular fluid determined. Follicles with a diameter of 12 mm or greater were considered cysts. The percentage of gilts displaying estrus and ovulating within 7 d after treatment and the treatment-to-estrus interval were similar (P > .1) for P.G. 600 treatment (93.8% and 4.1±.1 d, respectively) and controls (90.6% and 4.3±.1 d, respectively). Number of CL (indicative of ovulation rate) (P < .01) and mean CL weight (P < .08) differed between P.G. 600-treated gilts (28.8±1.1 and .48±.01 g, respectively) and controls (17.4±1.1 and .51±.01 g, respectively). Follicular fluid weight was greater (P < .09) for P.G. 600-treated gilts (4.4±.2 g) than for control gilts (3.8±.2 g). The percentage of gilts with follicular cysts and the number of cysts in these gilts were similar (P > .1) for P.G. 600 treatment (30% and 3.6 ±.9, respectively) and controls (17% and 1.4±.2, respectively). In summary, withdrawal of Regumate after 18 d of exposure resulted in a synchronous onset of estrus. Ovulation rate was enhanced by treating gilts with P.G.600 24 h after progestogen withdrawal.

Key Words: P.G. 600, Regumate, Gilts

855 Effectiveness of an early pregnancy test for cows. L. A. Pagels*, M. G. Daves, and C. S. Whisnant, *North Carolina State University, Raleigh*.

Early pregnancy tests are available for some species including humans. These depend upon the presence of a factor in pregnant animals that is not found in non-pregnant females. No such factor has been identified in cows. Recently, a kit has become commercially available (ECF^{TM}, Concepto-Diagnostics, Knoxville, TN) that purports to be able to identify a factor secreted by the early bovine conceptus. The literature accompanying the kit states that the factor can be detected in blood or milk of pregnant cows from 48 hours to 20 days after breeding. The presence of two lines on the test indicates pregnancy while one line indicates an open cow. No independent verification of the accuracy of this kit has been reported. The purpose of this experiment was to test the accuracy of the kit using ultrasound between days 25-30 or palpation on days 35-42 for comparison. Blood samples were taken by tail vein puncture from 46 heifers or cows. All animals were between 9 and 15 days post-insemination. All tests were conducted according to the instructions provided by the manufacturer. All test reagents were stored refrigerated and allowed to come to room temperature before testing. Blood samples were allowed to clot and a single drop of serum was placed into the receptacle of the test cassette using the disposable pipettes provided. Then the wash solution was placed on the receptacle and the tests were allowed to sit for two hours at room temperature before being examined for the results. As per instructions the presence of a second line however faint was regarded as a sign of conception. Ultrasonography was performed on days 25-30 post-insemination or rectal palpation was performed between days 35-42. The test results corresponded with the results of the ultrasound examination or palpation 55% of the time. Of the 45% that disagreed, 21% were diagnosed pregnant by the test and were found to be open by palpation or ultrasonography. Twenty four % were diagnosed as open by the test and were later found to be pregnant. These data suggest that the current level of accuracy in the kit is insufficient.

Key Words: Pregnancy, Cows

856 Evaluation of an early conception factor test for use in dairy cattle. B. Gandy*[1], W. Tucker[1], P. Ryan[1], A. Williams[1], A. Tucker[1], A. Moore[1], R. Godfrey[2], and S. Willard[1], [1]*Dept. of Animal and Dairy Sciences, Mississippi State Univ.,* [2]*Agric. Exp. Station, Univ. of Virgin Islands, St. Croix*.

The ability to detect conception or conception failure post-breeding would be beneficial to producers in decreasing the number of days a cow remains open. To this end, the objective of this study was to evaluate a commercially available early conception factor (ECF) test for detecting the non-pregnant cow. Lactating Holstein cows (n = 40) were synchronized using an OvSynch protocol. Cows were inseminated using timed AI (day 0), and blood samples were collected daily through d 15 and on alternate days thereafter until d 30. Milk samples were also collected at varying intervals. Serum and milk ECF tests were conducted following the manufacturer's guidelines (Concepto Diagnostics, Knoxville, TN) on d 9, 15, 21 and 30. Ultrasonography was performed on d 30, palpation on d 51 and serum samples analyzed for progesterone to confirm pregnancy. Pregnancy diagnosis revealed that 50% of cows were pregnant to AI, while serum and milk ECF analysis indicated a 100% and 37.5% pregnancy rate (d 30), respectively. Results of the serum and milk ECF tests disagreed with one another 36.9% of the time overall. Agreement between ECF and pregnancy diagnosis was 50.6% and 45.6% overall for serum and milk, respectively. The accuracy of a negative ECF result in identifying a non-pregnant cow varied greatly (0 to 100%) depending on the day of test. Regardless of accuracy by day, a negative ECF result could only identify 5% and 28.8% of non-pregnant cows overall for serum and milk ECF tests, respectively (i.e., true negatives). Moreover, the incidence of a false positive ECF result was 47.5% and 31.3% for serum and milk, respectively. Post-study re-testing of the d 9 serum samples (optimal test d) revealed that a negative ECF result was only 52.6% accurate in detecting the non-pregnant cow. Based upon these data, the current ECF test does not accurately identify the non-pregnant cow with the precision needed by the producer. [Supported in part by Concepto Diagnostics]

Key Words: Conception, Pregnancy, Cattle

857 The use of estrous synchronization, artificial insemination and a cow side early conception test on Holstein heifers in the tropics. R.W. Godfrey*[1] and S.T. Willard[2], [1]*Agricultural Experiment Station, University of the Virgin Islands, St Croix ,* [2]*Department of Animal and Dairy Sciences, Mississippi State University, Mississippi State*.

The use of AI and estrous synchronization in the US Virgin Islands would be more acceptable to producers if producers could determine conception within a few days after AI due to limited availability of liquid nitrogen locally for long-term storage of frozen semen. This study was conducted to evaluate the use of an early conception test in conjunction with AI and estrous synchronization. Holstein (n = 18) heifers were treated with PG (Lutalyse®, 25 mg, i.m., 11 d apart) to synchronize estrus. Heat detection was done with the use of Kmar patches. Heifers were inseminated using the am-pm rule. At 24 h after insemination a blood sample was collected, serum was harvested and used in an early conception test following the manufacturers instructions (ECF^{TM}, Concepto Diagnostics, Knoxville, TN). Rectal palpation was done at 45 d after AI associated with a positive ECF test. Heifers that had a negative ECF test were treated with PG a 6 or 7 d later and bred by AI based on detection of estrus. This was repeated until there were no more heifers to breed. Accuracy of palpation and ECF tests were based on calving dates. Based on the final AI of each heifer, the ECF test yielded a true positive 38.9%, false positives 44.4%, a true negative 11.1% and a false negative 5.6% of the time. Over the course of this project there were 26 inseminations of 18 heifers and ECF tests 48 h after AI yielded true positive results in 26.9%, false positive results in 30.8%, true negative results in 38.5% and false negative results in 3.8% of the tests. Based on these results it does not appear that the ECF test (at 24 to 72 h post-AI) can be used to accurately differentiate between pregnant and open dairy heifers in the tropics. The low rate (true negative: 11.1 and 38.5%) of detecting open heifers would not be acceptable to the producer. [This study was supported in part by Concepto Diagnostics].

Key Words: Artificial Insemination, Cattle, Conception

858 Leptin secretion in heifers approaching puberty. C. S. Whisnant, H. Lowman, A. N. Elias, and L. A. Pagels, *North Carolina State University, Raleigh*.

Research in rodents suggests that leptin may be involved in regulating age at puberty. Underfed rats had delayed puberty but treatment with leptin reversed this effect. Little is known about leptin secretion in cattle. The objective of the current experiment was to examine changes in leptin secretion in heifers from weaning to puberty in two groups of heifers fed to gain at different rates. The diets contained adequate energy and protein for the rates of growth as determined by NRC. Two weeks after weaning twenty Angus or Simmental heifers (n=10 of each breed) were assigned by breed and body weight to be fed to gain either (high H) 0.91 kg/head/day or (moderate M) 0.45 kg/head/day. Initial weights were not different between groups (H 285.6 ± 19 kg vs. M 286.1 ± 24.6 kg). Calan gates were used to allow individual feeding. Animals were on dietary treatments for 84 days. Heifers were weighed every 14 days and blood samples were collected weekly for measurement of serum leptin and progesterone concentrations. Every 28 days five heifers from each group were cannulated and samples taken for 6 h at 15 min intervals. Serum leptin concentrations were determined in these samples as

well using the Linco Multi-Species kit. Data were analyzed using PROC GLM with treatment and breed in the model. Average daily gain was greater (P<.01) in H heifers (1.02 ± 0.17 kg) than M heifers (0.65 ± .04 kg). No breed difference in serum leptin concentrations was detected. There was no difference between treatments in the number of heifers reaching puberty by the end of the study (10 in H group; 9 in M group) or age at which puberty occurred. Mean serum leptin concentrations were lower in M heifers (3.6 ± 0.3 ng/ml, P<.01) than in H heifers (6.4 ± 0.7 ng/ml) from 14 days after dietary treatment began until the end of the study. These data suggest that level of dietary energy can affect leptin secretion in heifers. At the rates of growth used in this study the difference in serum leptin concentrations was unrelated to age at puberty, which did not differ between treatments.

Key Words: Leptin, Puberty, Heifer

859 Plasma leptin concentrations in dairy cows: I) Effect of short-term fasting and refeeding. P.K. Chelikani*[1], J.D. Ambrose[2], D.R. Glimm[1], T.J. Kieffer[1], and J.J. Kennelly[1], [1]*University of Alberta, Edmonton, Canada.*, [2]*Alberta Agriculture,Food & Rural Development, Edmonton, Canada.*.

In humans and rodents, leptin levels in circulation are reduced with fasting and rebound to prefasting levels on refeeding. We hypothesized that a similar physiological response occurs in dairy cows. Five Holstein dairy cows (BW 603±30kg; BCS 2.48±0.05) were fasted for 48 h and subsequently refed. Blood samples were taken at 0 (baseline), 10, 22 and 46 h of fast, and at 4, 8, 12, 18 and 24 h after refeeding. Plasma leptin concentrations were determined using a multispecies double antibody assay kit (Linco Research, St. Louis, MO). Serial dilutions of a pooled bovine plasma sample were found to be parallel to the human leptin standard curve. Human leptin was quantitatively recovered from a bovine plasma sample. Assay sensitivity was 1ng/ml. The intra- and interassay CV were 7.8% and 14.67% respectively. There was a significant decrease (P=0.03) in plasma leptin concentrations at 10h fast (2.83±0.23 ng/ml) compared to baseline (3.61±0.23ng/ml). However, there were no changes (P>0.05) in leptin concentrations either at 22 and 46 h of fast, or during the refeeding period. Despite an initial decrease, the gradual rise in plasma concentrations of leptin with fasting is in contrast to the response observed in humans and rodents and warrants further study.

Key Words: Leptin, Fasting, Dairy cows

860 Concentrations of leptin in serum and milk from sows that differed in body condition at farrowing. M.J. Estienne*[1], A.F. Harper[1], C.R. Barb[2], and M.J. Azain[3], [1]*Virginia Polytechnic Institute and State University, Blacksburg,* [2]*USDA-ARS, Athens, GA,* [3]*University of Georgia, Athens.*

Leptin concentrations in serum and milk were assessed for Yorkshire x Landrace sows (parity = 2) that were classified as FAT (over 25 mm; n = 7), MEDIUM (20 to 25 mm; n = 8) or THIN (less than 20 mm; n = 9) based on last-rib backfat thickness measured ultrasonically immediately after farrowing. Body weight (kg) and backfat thickness (mm), differed (P < .001) among FAT (236.3±4.4 and 28.1±.9, respectively), MEDIUM (235.5±6.0 and 22.4±.3, respectively) and THIN (217.9±6.5 and 15.5±.8, respectively) sows. Loss of backfat (mm) from farrowing to weaning (21.7±.5 d of lactation) differed among groups (P < .01) and was 6.7±1.1, 4.7±.6, and 1.8±1.0, for FAT, MEDIUM, and THIN sows, respectively. Within 24 h after farrowing, on d 7 and d 14 of lactation, and on the day of weaning, blood samples were collected via jugular venipuncture, and milk samples were obtained following i.m. administration of 1.5 cc oxytocin. At farrowing, serum concentrations of leptin were higher (P < .01) for FAT sows (4.9±.2 ng/mL) compared to MEDIUM (3.7±.2 ng/mL) or THIN (2.8±.2 ng/mL) sows, and across groups, serum leptin levels were correlated (r^2=.67; P < .01) with backfat thickness. Concentrations of leptin in serum decreased (P < .01) by d 7 of lactation in FAT (to 3.2±.2 ng/mL) and MEDIUM (to 2.8±.2 ng/mL) sows but remained at similar levels (P > .1) throughout lactation in THIN sows. At weaning, serum leptin levels were similar (P > .1) among groups and were 2.7±.1 ng/mL. Concentrations of leptin in whole milk were not affected (P > .1) by body condition or stage of lactation and overall averaged 30.5±3.0 ng/mL. In whole milk, levels of leptin and lipid content (81.2±9.1 mg/mL) were not correlated (r^2=.25; P > .1). There was no effect (P > .1) of body condition on skim milk leptin concentrations. Among groups, leptin levels in skim milk decreased (P < .01) from farrowing (21.0±1.0 ng/mL) to d 7 of lactation (14.4±1.0 ng/mL) and remained at similar levels (P > .1) for the remainder of lactation. In summary, circulating, but not whole or skim milk, leptin concentrations are related to adiposity in lactating sows. The physiological role of leptin in the postpartum female warrants investigation.

Key Words: Leptin, Body Condition, Sows

861 Effect of leptin on release of luteinizing hormone from bovine anterior pituitary cells in vitro. T.D. Ridgway*, R.P. Wettemann, and L.J. Spicer, *Oklahoma Agricultural Experiment Station, Stillwater.*

Enzymatically dispersed bovine anterior pituitary cells were used to determine the effects of leptin on release of luteinizing hormone in vitro. Steer pituitaries were obtained immediately after slaughter on two days (reps). Connective tissue was removed from pituitaries and slices of tissue were exposed to collagenase. Cells (approximately 100,000/well) were plated in 24-well culture plates and incubated at 38.5° C with 95% air and 5% CO_2. Dulbecco's modified Eagle medium with 2% newborn calf serum, 2% glutamine and antibiotics was used for culture of cells. After 72 h of incubation, medium was removed and plates were rinsed three times with medium and cells were treated with 0, 10^{-10}, 10^{-9} or 10^{-8} M concentrations of recombinant mouse leptin (Exposure I). After 4 h, medium was removed and frozen for LH analysis and new medium without leptin was added. After 20 h, medium was removed and frozen (post-leptin) and the same leptin treatments were given. After 4 h, the medium was removed and frozen (Exposure II). Leptin at 10^{-8} and 10^{-9} M suppressed LH release (P < .002) by 42% and 35%, respectively, compared with control cells during rep 1 of Exposure I. However, during rep 2 leptin did not alter LH release. During the 20 h post-leptin incubation, LH release was 35% greater from previously leptin treated cells than from control cells (P < .0001). Exposure II with leptin, in both reps, for 4 h resulted in a 41% decrease of LH release (P < .002) compared with control cells. The decrease in LH release during exposure to leptin followed by increased LH release after leptin treatment was removed indicates that leptin directly affects the release of LH by bovine pituitary cells.

Key Words: Bovine, Leptin, Luteinizing Hormone

862 Relationship of leptin and puberty in performance-tested bulls. T. M. Towns*[1], F. N. Schrick[1], F. M. Hopkins[1], F. D. Kirkpatrick[1], A. M. Saxton[1], K. W. Thompson[1], M. E. Hockett[1], and C. S. Whisnant[2], [1]*University of Tennessee, Knoxville,* [2]*North Carolina State University, Raleigh.*

The relationship of leptin and onset of puberty was assessed in Angus bulls at the University of Tennessee Bull Performance Evaluation Center. Sixty one bulls (**SR**, n = 24, 273.9 ± 5.4 d of age; **JR**, n = 37, 237.0 ± 4.4 d of age) with a scrotal circumference (SC) less than 28 cm were allotted to a 112-day test. Bulls were acclimated for two weeks prior to test following arrival in July (SR) and September (JR). Bulls were fed a diet containing 70% TDN. Blood samples for concentrations of serum leptin and testosterone and measurements of BW, backfat (BF), rumpfat (RF), and SC were collected on days -14 (arrival), 0 (on-test), 28, 56, 84, and 112 (off-test). On-test SC was smaller in JR (23.5 ± .3 cm) than SR bulls (25.7 ± .4 cm; P < .001); however, on-test BW (324.9 ± 5.7 kg), BF (2.8 ± .3 mm) and testosterone concentrations (2.6 ± .4 ng/mL) were similar between bull types. Concentrations of leptin were lower in JR (2.5 ± .3 ng/mL) at d 0 than SR bulls (3.5 ± .3 ng/mL; P < .05). Bulls were considered pubertal when SC ≥ 28 cm. Age at puberty was lower in JR (281.7 ± 4.9 d) than SR bulls (323.6 ± 6 d; P < .001). At puberty, bull types were similar in BW (408.5 ± 9.0 vs. 421.3 ± 11.1 kg), BF (5.7 ± .2 vs. 5.6 ± .3 mm), and RF (7.4 ± .4 vs. 7.1 ± .5 mm) for JR and SR bulls, respectively. Concentrations of testosterone tended to be higher at puberty in JR (6.3 ± .5 ng/mL) than SR bulls (4.8 ± .7 ng/mL; P = .09). Leptin also tended to be higher in JR compared to SR bulls at puberty (3.5 ± .3 vs. 2.6 ± .3 ng/mL, respectively; P = .07). At d 112 (off-test), SR bulls had larger SC than JR bulls (36.4 ± .5 vs. 34.8 ± .4 mm, respectively; P < .001); however, backfat (11.1 ± .3 mm) and BW (540 ± 5.7 kg) did not differ between bull types. In conclusion, leptin and testosterone concentrations tended to differ at

onset of puberty between JR and SR bulls; however, body weight and fat measurements were similar.

Key Words: Bulls, Puberty, Leptin

863 Developmental changes in the gene expression of the long form leptin receptor(Ob-Rl) and related neuropeptides in the pig hypothalamus. J. Lin[1], J.B. Barrett[2], C.R. Barb[2], R.R. Kraeling[2], G.J. Hausman[2], and G.B. Rampacek[1], [1]*Department of Animal and Dairy Science, University of Georgia, Athens,* [2]*USDA, Agricultural Research Service, Athens, GA.*

The hypothalamus is the key site of central regulation of energy homeostasis, appetite and reproduction. The Ob-Rl is localized within the hypothalamus along with several other neuropeptides that are involved in regulation of the neuroendocrine axis. In the present study, developmental changes in gene expression of the Ob-Rl, preproorexin, proopiomelanocortin (POMC), corticotropin releasing factor (CRF), somatostatin, gonadotropin releasing hormone (GnRH) in the hypothalamus was studied. Hypothalami were collected from 106 d-old fetus (n=3) and 7 d-old (n=3), 3.5 month-old (n=3) and 6 month-old (n=2) gilts and expression of Ob-Rl and neuropeptide mRNA examined by semi-quantitative RT-PCR. In addition, back fat leptin mRNA expression in the first three ages was examined. Leptin mRNA expression increased (P<.05) by 7d postnatal, but Ob-Rl mRNA expression increased (P<.05) by 3.5 month. Expression of preproorexin, somatostatin and GnRH mRNA peaked by 3.5 months of age (P<.05) while POMC mRNA expression increased markedly (P<.01) by 6 months of age. The CRF mRNA expression did not change across ages. These findings suggest that Ob-Rl and hypothalamic neuropeptides are associated in development of the neuroendocrine axis in the gilt.

Key Words: leptin receptor, pig, development

864 The insulin-like growth factor system and leptin: role as possible metabolic signals for regulating puberty and growth in dairy heifers. G. Luna-Pinto*[1] and P. B. Cronje[1], [1]*Department of Animal and Wildlife Sciences, University of Pretoria.*

The aim of this experiment was to study how changes in the blood concentrations of insulin-like growth factor-1 (IGF-1), IGF binding proteins (IGFBP), and leptin mediate the effects of food restriction and compensatory growth on the age of puberty in heifers. Fresian heifers were allocated to one of two dietary treatments. Treatments were designed to result in two different growth rates during the first 13 weeks of the experiment: 0.3 kg/d (restricted treatment) or 0.6 kg/d (control treatment). From weeks 14 to 30, the restricted group received the same amount of food as was fed to the control group. Heifers in the control treatment reached puberty sooner (P < 0. 01) than the restricted group (43.8 +/- 0.75 wk. vs 47 +/- 0.63 wk). Mean body weight at puberty was 256.3 +/- 5.22 kg and was not affected by treatment (P > 0.05). Growth rate differed between treatments (P < 0.01) from week 0 to 25. By the end of the restriction period there were differences (P < 0.01) between dietary treatments for plasma leptin, IGF-1 and IGFBP-3 concentrations. Leptin concentration was higher (P < 0.01) in the control group animals than in the restricted animals at weeks 7, 13, 15, 17 and 21. Within the control group, there was a sequential increase (P < 0.01) in plasma leptin concentration between weeks 0, 7, 13, 15 and 17 respectively. Plasma IGF-1 concentration was higher in the control group than in the restricted group at the end of the restriction phase. Higher IGF-1 concentrations were observed in the restricted group during week 15, when growth rates were at their highest (P < 0.01). Plasma IGFBP-3 concentration increased (P < 0.05) during compensatory period from week 13 until week 21. The present results show that concentration of IGF-1 and IGFBP-3 were directly affected by the restriction program, suggesting that these factors may be involved in mediating the effects of nutrition on the onset of puberty.

Key Words: Compensatory growth, Puberty, Insulin-like growth factor system

865 The short-term feeding responses by the changes of amino acid concentrations in plasma and brain. B.W. Kim*[1], C.H. Kim[1], J.S. Shin[1], and H. Tanaka[2], [1]*Kangwon National University, Korea,* [2]*Utsunomiya University, Japan.*

This study examined the mechanism of feed intake regulation by investigating the relationship between the amino acid (AA) concentrations in feed and those in plasma as well as brain-prepyriform cortex (PPC). Forty male Wistar rats averaging 140g were randomly assigned into the 9 dietary treatment groups and the 1 control group, with 4 rats in each group. After a 10-day adaptation period, the 9 treatment groups were fed an amino acid-balanced diet for 11days followed by an amino acid-imbalanced diet which lacks one essential AA in each group. Control group was fed only the amino acid-balanced diet. The feed intake and the AA concentrations in plasma and PPC were measured during the periods in which both amino acid-balanced and -imbalanced diets were fed. Overall, the results indicated that the AA concentrations in plasma and PPC were decreased when the amino acids-imbalanced diets were fed. Specifically on the first day of diet change, the reduction of initial feed intake was greatest in the group with methionine-limited diet and least in the group with threonine-limited diet. On the 3rd day of diet change, in comparison with control, the reduction of feed intake was 71% in the group with histidine-limited diet, 68% in leucine, 66% in isoleucine, 63% in threonine, 61% in tryptophan, 55% in valine, 52% in phenylalanine, 51% in methionine, and 44% in lysine, respectively. The lysine concentrations in plasma and PPC significantly (P<0.05) decreased 2 hours after replacing the amino acid-balanced diet with the lysine-limited diet. These results suggest that the short-term feed intake responses are intimately involved in the AA concentrations in plasma and PPC which are derived from the AA concentrations in feed.

Key Words: Amino acids, Prepyriform cortex, Feed intake

866 Effect of selection for milk yield on hepatic gene expression in the Holstein cow: the growth hormone (GH) receptor and insulin-like growth factor-I (IGF-I). B. A. Crooker*, L. S. Ma, W. J. Weber, L. B. Hansen, and H. Chester-Jones, *University of Minnesota, St. Paul.*

Cows from a breeding project initiated in 1964 to develop a stable control line (CL) that represented US breed average in 1964 and a select line (SL) that represented contemporary US Holsteins were used to evaluate changes in hepatic GH receptor and IGF-I gene expression. Milk yield of the two lines currently differs by more than 4,500 kg/305 d lactation. Cows from both lines were housed together in a free stall barn and fed ad libitum diets designed to meet their nutritional requirements. Hepatic biopsy samples were obtained from multiparous (10 CL and 13 SL) and primiparous (8 CL and 8 SL) cows at -12 ± 1 (range -1 to -29), 20 ± 0.4 (range 16 to 24) and 68 ± 0.5 (range 63 to 76) d postpartum (PP). Ribonuclease protection assays were conducted to determine GH receptor and IGF-I gene expression. Gene expression results are presented as pixel density relative to an internal control (GADPH) and were analyzed as repeated measures using PROC MIXED of SAS. Milk yield of primiparous and multiparous CL cows was 18.7 and 28.4 kg/d while SL cows produced 29.9 and 44.6 kg/d. Expression of the genes for the liver specific GH-1A receptor (1.67^a, 1.38^b, 1.64^a) and for IGF-I (0.69^a, 0.49^b, 0.65^a) were less (P < 0.001) at 20 d PP than at -12 d PP and returned to prepartum amounts by 70 d PP. Parity did not affect expression of these genes. The SL and CL cows expressed similar amounts of the IGF-I gene. Expression of the liver specific GH-1A receptor was less in SL than CL cows at -12 (1.55, 1.79; P = 0.08) and 20 (1.23, 1.52; P = 0.04) d PP, similar at 68 (1.59, 1.69) d PP and less (P = 0.04) during the overall study (1.46, 1.67). Expression of the common GH-1B receptor was not affected by genetic line, parity, or day of lactation. These results indicate selection for milk yield has reduced expression of the liver specific GH-1A receptor during the periparturient period but has had no affect on expression of the genes for IGF-I or the GH-1B receptor.

Key Words: Genetic Selection, Gene Expression, Dairy Cattle

867 Evaluation of insulin receptor mRNA levels at different days pre and postpartum in liver tissue of genetically selected dairy cattle. J.A. McMullen*[1], J.H. White[1], J.R. Knapp[1], W.J. Weber[2], H. Chester-Jones[2], L.B. Hansen[2], and B.A. Crooker[2], [1]*University of Vermont, Burlington,* [2]*University of Minnesota, St. Paul.*

The objective of this study was to clone the bovine insulin receptor cDNA and quantitatively analyze the expression levels of bovine insulin receptor mRNA in liver tissue. The hybridization probe was cloned using primers designed from homologous regions in human and mouse insulin receptor cDNAs. A 287 base pair clone was isolated and sequenced and found to be 95% homologous to the mouse insulin receptor cDNA at the DNA level and 100 % homologous at the amino acid level. This bovine specific clone was then used to evaluate the expression levels of mRNA in liver tissue biopsied from two lines of dairy cows from the Waseca Experiment Station, University of Minnesota. The control line has been genetically maintained at the 1964 Predicted Transmitting Abilities (PTA) for milk production by being bred in a rotating fashion to the same 20 bulls. The select line has been bred each year to sires with the top 1% PTA for milk production and there is now a 12,000 lbs milk yield difference between the two lines. Liver biopsies were taken from 5 cows in each line at 14d prepartum, 14, 21 and 70d postpartum and an equal amount of tissue from each cow within a group were pooled together. Total RNA was isolated, purified, and analyzed by Northern blotting hybridization techniques. mRNA expression levels were quantified using a STORM phosphorimager system. No significant differences were found in the expression levels of insulin receptor mRNA between the two lines. However, there was an interaction between the stage of gestation/lactation and line. mRNA levels for insulin receptor tended to be higher than those of controls at 14d prepartum ($p \leq 0.10$). The RNA of the individual cows that made up the pooled samples were analyzed and were found to contribute 3.8 - 38.1% of the variation in the pooled control samples and 8.7 - 22.5% in the pooled select samples. Genetic selection of dairy cattle alters expression levels of the liver insulin receptor in late gestation; however, this change was not observed in lactation.

Key Words: Insulin Receptor, Lactation, Dairy Cattle

868 Effect of bST and monensin on lipogenesis and gene expression in adipose tissue during the transition to calving. S.S. Donkin*[1], C. Agca[1], A. Arieli[2], J.E. Vallimont[3], and G.A. Varga[3], [1]*Purdue University, West Lafayette, IN,* [2]*Hebrew University of Jerusalem, Israel,* [3]*Pennsylvania State University, University Park, PA, USA.*

Enhancing lipogenesis to counteract adipose tissue mobilization in late gestation may serve to alleviate the metabolic pressures experienced by the transition dairy cow. To test this possibility the effects of prepartum monensin (300 mg/head), somatotropin (bST), and their combination were measured on lipogenesis and gene expression in dairy cattle. Beginning 28 d prior to expected calving 30 multiparous Holstein cows were assigned to either a control (n=10), monensin (n=6), bST (n=7), or monensin and bST (n=7) treatment groups. The bST (POSILAC, 500 mg) was injected 28-d and 14-d prior to calving. Adipose tissue biopsies were obtained on -14 days and +14 days relative to calving. Lipogenesis (nmol [1-^{14}C] acetate incorporated into total lipid $\cdot$ g tissue^{-1} $\cdot$ 2 h^{-1}) was determined using explant cultures. Total RNA extracted from adipose tissue was analyzed for fatty acid synthase (FAS), acetyl-CoA carboxylase (ACC), growth hormone receptor (GHR), and leptin mRNA. Lipogenesis was 5 percent of prepartum levels at 14 days of lactation (6464 and 30; prepartum vs postpartum) and was increased prepartum by bST (2186 vs 4307) and monensin (1663 vs 4831) but these effects were not additive. The effects of bST were sustained through calving. Treatments did not alter ACC or FAS mRNA during the prepartum period but ACC and FAS mRNA were decreased to 10 to 20 percent of prepartum levels after calving. Monensin retarded the postpartum decrease in ACC mRNA. The effect of bST to increase GHR mRNA ($P < .10$) is observed at both sampling times. Leptin mRNA was reduced ($P < .05$) by 72 percent after calving but was unaffected by the treatments. The data indicate bST or monensin enhances lipogenesis during late gestation and bST does not change expression of lipogenic genes yet GHR mRNA changes indicate tissue responsiveness.

Key Words: Transition dairy cattle, Adipose tissue, Gene expression

869 Acute effects of estrogen on lactotroph abundance in GH_1, GH_3, GH_4C_1, MMQ and GC pituitary cell lines. X. Fu* and T. E. Porter, *University of Maryland, College Park.*

Estrogen is a very important regulator of prolactin (PRL) production. Previous work indicated that estrogen could increase lactotroph abundance in GH_4C_1 cells after 2 days of treatment. Recent experiments imply that estrogen might have more rapid effects. The purpose of this study was to explore the existence of short-term effects of estrogen on lactotroph abundance in five pituitary cell lines, GH_1, GH_3, GH_4C_1, MMQ and GC. GH_1, GH_3 and GH_4C_1 secrete both growth hormone (GH) and PRL. MMQ and GC cells secrete only PRL and GH, respectively. All cells were cultured in Ham's F-10 medium, containing 5% charcoal-treated Fetal Bovine Serum, to remove endogenous estrogen. In order to study the short-term effects of estrogen on lactotroph abundance, 4 and 24 hour treatments were evaluated. PRL containing cells were assessed using immunocytochemistry after treatment with estradiol-17β(E_2, 10^{-10} and 10^{-8}M). E_2 had no effect on the percentage of PRL containing cells in GC and MMQ cells. For the 24 hour treatment, E_2 increased the percentage of PRL containing cells in GH_1 cells ($P<0.05$) to 54 $\pm$1.3% of all cells, relative to the control of 43 $\pm$1.2%. In GH_3 cells, E_2 increased the PRL containing cells ($P<0.05$) to 57$\pm$0.6%, relative to the control of 46$\pm$1.4%. In GH_4C_1 cells, the PRL containing cells increased to 53$\pm$1.2% ($P<0.05$), relative to the control of 34$\pm$0.6%. For the 4 hour treatment, E_2 increased the abundance of PRL containing cells in GH_1 cells ($P<0.05$) to 50$\pm$0.9%, relative to the control of 43$\pm$1.2%. In GH_4C_1 cells, the population of PRL containing cells increased ($P<0.05$) to 38$\pm$1.4%, relative to the control of 34$\pm$0.6%. In GH_3 cells, however, E_2 decreased the population of PRL cells ($P<0.05$) to 36$\pm$1.5% compared to the control of 46$\pm$1.4%. The results indicate that estrogen can affect lactotroph abundance following both long-term and short-term treatments. As the percentage of PRL cells increased within 4 hours following E_2 treatment of GH_1 and GH_4C_1 cells, these cell lines will be good models for the rapid recruitment of lactotrophs in response to estrogen.

Key Words: estrogen, prolactin, lactotroph

870 Sensitive sandwich assay for the determination of bovine growth hormone in blood and milk. P. Lovendahl*[1], J. Adamsen[1], R. Lund[2], and P. Lind[2], [1]*Danish Institute of Agricultural Science,* [2]*Danish Veterinary Laboratory, Denmark.*

Monoclonal antibodies against synthetic bovine growth hormone were raised and selected for binding to bGH. The catching mab-GH1 was coated on microtiter plates and used to immobilize bGH to plates. Bound bGH was quantitated by adding labelled mab-GH2 during incubation for 9 hours at 25C. Labelling of mab-GH2 was either directly with Europium (method A) or with biotin (method B). If biotin was used, a second step followed, where Europium labelled streptavidin was allowed a further 0.5 hours incubation to bind to biotin. Time resolved fluorescence from Europium was read following chelation with an enhancement solution. The sensitivity of the assay is less than 0.1 ng/mL in both blood and milk. Milk and EDTA containing plasma can only be measured by method B, because of interactions between Europium and EDTA. The working range is 0.2 to 200.0 ng/mL (method A), or 0.3 to 30.0 ng/mL (method B). Variation is generally low (CV within assay $< 6\%$ at 4 ng/mL; $< 3\%$ at 20 ng/mL). Results correlate perfect with RIA results $r > 0.99$. Growth hormone in plasma from sheep and goats can also be mesured. We conclude that the developed assay is sensitive, accurate, fast and eliminates the hazards attached to radio labelling.

Key Words: somatotropin, ELISA, bovine

871 Effects of antiserum against adipocyte plasma membrane proteins on body composition of passively immunized Sprague-Dawley rats. K. H. Paik, E. J. Kwon, T. H. Kwak, S. H. Chae, K. K. Jung, and C. B. Choi, *Department of Animal Science, Yeungnam University, Korea.*

The objectives of the current study were to develope polyclonal antibodies in sheep against adipocyte plasma membrane(APM) proteins isolated from Sprague-Dawley(SD) rat, to investigate tissue specificity, and to determine cytotoxic effects of antiserum on rat adipocyte metabolism *in vitro* and *in vivo*. Plasma membrane proteins from adipocyte, heart, kidney, liver, muscle and spleen of SD rats were isolated using an isoosmotic sucrose self-forming gradient. Adult male sheep was immunized

three times at three week interval with the purified rat APM proteins. Antisera were taken from immunized sheep at 10, 12, and 14 days after the third immunization. Antiserum expressed strong antigen-antibody reactivity with APM proteins determined by ELISA, and the reactivity could be detected at dilutions in excess of 1:81,000. Tissue specificity of the antisera was confirmed by Western immunoblotting. Although not entirely specific, the antisera demonstrated remarkably higher degree of reactivity with proteins isolated from adipocytes compared with the other tissues. The reactivity of antiserum to the external surface of the fixed rat adipocytes could be confirmed by using a fluorescent material, anti-sheep immunoglobulin G-FITC. Confluent rat adipocytes in culture were lysed by antisera treatment and cytosolic lactate dehydrogenase was released as a dose-dependent patterns while adipocytes treated with non-immunized serum maintained their integrity. Furthermore, passive immunization of male SD rats with antisera significantly($p<.01$) reduced subcutaneous(21.9%) and perirenal+mesentric+epididymic(36.0%) adipose tissue mass. These results implicate that the antisera raised against APM proteins isolated from SD rats could be used to reduce body fat contents in meat animals. Further studies, however, are necessary for the practical applications of the current results.

Key Words: Adipocyte, Antiserum, Passive immunization

872 Insulin sensitivity (IS) and endocrine responses to insulin in ewe lambs. S.E. Recabarren, A. Lobos, C. Vilches*, M.J. Nuñez, and P. Muñoz, *Lab. of Animal Physiology and Endocrinology, Fac. Vet Med. Universidad de Concepcion, Chillán, Chile*.

In the growing female sheep, glucose(G)and insulin(INS)may be used as metabolic signals to inform to the GnRH neurons about the metabolic status of the growing sheep. Peripheral IS could change during prepubertal development to allow more G or INS available to the brain. A study was conducted to determine the IS in 20 and 30-week ewe lambs (n=6), using the insulin tolerance test, and to explore LH, cortisol(C)and leptin(L) changes in response to insulin. Lambs received an iv bolus of human INS (0.1 IU/kgBW). Blood samples were collected by means of an indwelling jugular vein catheter at 0, 3, 5, 7, 10, 13, 15, 17, 20, 30, 40, 50, 60, 80, 100 and 120 min after INS administration. Plasma G,INS, C were measured in all samples, LH and L in selected samples. IS was calculated using formula IS=(G20-G0)/G0 where G0 is G at 0 min and G20 is G at 20 min derived from a regression plot.IS was similar at both ages:0.18±0.03 and 0.17±0.03. Basal G levels were 0.68±0.03 and 0.72±0.03(g/l)respectively. G significantly descended after 10 min of INS injection, reached a nadir after 30 min and remained low until 120 min. Plasma C levels decreased after 20 min of INS administration ($P\leq0.05$) and increased thereafter, with a peak at 80-100 min. Plasma INS increased from 0 to 3 min and returned to basal levels at 120 min. In 20-week lambs,LH increased from 0.44±0.14 at time 0 to 1.6±0.47 ($P\leq0.05$) at time 30 min. In 30-week lambs, plasma LH reached a peak at 30 min but without statistical significance. Plasma L did not change after INS administration. Results suggest that the IS does not change during prepubertal development but INS had a stimulatory effect on LH secretion even when G levels were low and C was increasing. Supported by DIUC 98.153.009-1

Key Words: Insulin sensitivity, LH, Puberty

873 Effects of colostral immunity on lifetime performance of the female dairy cattle. R. Kliks and R. Skrzypek*, *Agricultural University, Poznan, Poland*.

Concentrations of total protein, globulins and immunoglobulins were measured on the second day of life in blood serum of 327 Black and White females, born on the university farm from 1986 to 1990. The relationships between passive immunity and performance parameters were most evident for immunoglobulins; therefore only results obtained for this trait will be presented. We found significant partial correlation coefficients between concentration of colostral immunoglobulins and the first lactation milk yield ($r = -.26$; $P \leq .01$) as well as calving intervals throughout the period of life (r from -.20 to -.35; $P \leq .05$ and $P \leq .01$, respectively). As regression analysis showed in many instances presence of significant quadratic effects, three levels of passive immunity were assigned taking in account the first and the third quartiles as limit values; low (L; .4 to 6.0 g/l), medium (M; 6.1 to 22.4 g/l) and high (H; 22.5 to 49.0 g/l). All important lifetime performance traits subjected to the analysis reached the highest values in M group, while the lowest values were in L group. For instance, lifetime milk yield was 13397 kg in L group, 16682 kg in M group and 14904 kg in H group; and functional length of life was 1628 days in L group, 1848 days in M group and 1782 days in H group. This finding seems to confirm recent reports by other authors, indicating that high intake of maternal immunoglobulins by the newborn calf may have a depressing effect on active humoral immunity acquired during the later period of life. It is concluded, that colostral immunity affects performance of female dairy cattle throughout life.

Key Words: Dairy females, Colostral immunity, Lifetime performance

874 Protein nitrotyrosine residues are associated with the nonmastitic bovine mammary gland. T. K. Ledbetter*[1], M. J. Paape[2], and L. W. Douglass[1], [1]*University of Maryland, College Park,* [2]*USDA-ARS, Beltsville, MD*.

Nitrated tyrosine residues accumulate at sites of inflammation, and are an indicator of the presence of reactive nitrogen species. Nitration of tyrosine residues in bovine blood and mammary polymorphonuclear neutrophils (PMN) and milk proteins during acute mastitis was examined. Blood and milk were collected before and 12 h after intramammary injection of 50 g *Escherichia coli* lipopolysaccharide (LPS) (n = 3 cows), and milk was collected after oxytocin injection. Nitrotyrosine residues in isolated PMN lysates and whey proteins were visualized by immunoblotting and quantitated by ELISA. Binding of the anti-bovine PMN mAb 36H10 (used as a positive control) to blood and milk PMN induced tyrosine phosphorylation and reversed tyrosine nitration after 20 s. Blood and milk PMN lysates had moderate and high levels, respectively, of nitrotyrosine before LPS injection. Nitrotyrosine levels tended to decrease in blood PMN ($p < 0.2$) and decreased significantly in milk PMN ($p < 0.001$) 12 h after LPS injection. Phosphotyrosines in blood and milk PMN increased after LPS injection. Nitrotyrosines and phosphotyrosines were present in whey proteins before injection. Tyrosine nitration of milk proteins decreased significantly in whey 12 h after LPS injection ($p < 0.05$) and remained low in residual milk. Milk protein tyrosine phosphorylation increased 12 h after LPS injection and remained elevated in residual milk. In a second experiment, the statistical relationship between whey protein nitrotyrosine concentration and somatic cell count (SCC) was determined using composite milk from 42 cows. Nitrotyrosine concentration was negatively correlated with $\log_{10}$SCC ($p < 0.001$). Tyrosine nitration in blood and milk PMN and whey proteins was associated with gland normality and low SCC. Tyrosine nitration in bovine PMN is reversible, and may represent the normal state of many proteins in whey and PMN.

Key Words: Nitrotyrosine, Mastitis, Polymorphonuclear neutrophil

875 Evaluation of udder health using differential inflammatory cell count. S. Pillai*, B. Jayarao, S. Senh, E. Kunze, K. Shafer-Weaver, and L. Sordillo, *Pennsylvania State University, University Park*.

A flow cytometric technique called Differential Inflammatory Cell Count (DICC) to identify leucocyte cell types was standardized using monoclonal antibodies to leucocyte cell surface antigens CD3, CD11b and CD18. DICC was then evaluated using individual quarter milk samples from 13 cows. Cows were sampled at weekly intervals for 3 weeks and total cell count (TCC), Lymphocyte/Monocyte count (LMC), and polymorphonuclear cell count (PMNC) were determined and compared to somatic cell count (SCC). There was a strong positive correlation between SCC and TCC (r=0.8, 0.76,0.76 at day1, 7, 14 respectively). TCC was also observed to be positively correlated with PMNC (r=0.96, 0.93, 0.9 at day 1, 7, 14 respectively). A consistent and significantly higher mean TCC (3.76,3.86,3.86 log units respectively) and PMNC (3.32, 2.85, 3.45 log units respectively) was observed in quarters with SCC > 5.4 log units as compared to quarters with SCC <5.4 log units (TCC 2.95, 2.8, 3.23 log units respectively and PMNC 2.05, 1.41, 2.63 log units respectively). Quarters with high TCC and PMNC also had a higher number of quarters that were culture positive (62 - 87%) as compared to quarters with low TCC and PMN (37-51%). The findings of the study suggest that DICC has the potential to evolve as a new technique for evaluation of udder health status in dairy herds.

Key Words: Differential inflammatory cell count, somatic cell count, total cell count

876 Expression of angiogenic growth factors throughout lactation and during the dry period. L. Varticovski[1], A.V. Capuco[2], and R.A. Christensen*[1], [1]*St. Elizabeth's MC, Tufts U. School of Medicine, Boston, MA,* [2]*USDA-ARS, Beltsville, MD.*

A non-lactating "dry" period of 40-60 days prior to parturition is required to maximize milk production in the subsequent lactation. Without this dry period, milk production may be reduced by as much as 20%. The mechanism whereby the dry period is required for subsequent maximal milk production is unknown. Angiogenic growth factors, including vascular endothelial growth factor (VEGF), angiopoietin 1 (Ang 1) and angiopoietin 2 (Ang 2)levels increase as a result of hypoxia during wound healing, tumor development and other conditions which are associated with new blood vessel development. We hypothesize that angiogenic growth factors and their receptors will be elevated during the dry period. RT-PCR was performed after total RNA isolation from samples obtained from dairy cows which were in early (n=3), mid (n=3), late (n=3) lactation (14, 120, and 300 days, respectively) or 40 days into the dry period (n=3). Data were analyzed by ANOVA and treatment means were compared by Bonferroni test. Expression of β-actin was used as the control. We amplified all four isoforms [121, 165, 189, and 206 amino acids (aa)] of VEGF. The freely soluble isoform (121 aa) was similar (P>.2) in cows at different stages of lactation and the dry period. There was a tendency (P=.14) for a difference in the cell surface and extracellular matrix bound isoform (165 aa). The two isoforms which bind almost exclusively to the extracellular matrix (189 and 206 aa) also tended (P < .08 and .09, respectively) to be different. The differences in 165, 189, and 206 aa isoforms of VEGF primarily resulted from higher expression of these isoforms in non-lactating cows when compared to cows in late lactation. Ang 1 and 2 and their receptor, Tie2, expression was also highest in non-lactating cows. In conclusion, the changes in VEGF, Ang 1, Ang 2, and Tie2 receptor support the hypothesis that hypoxia, which is associated with involution, stimulates production of angiogenic growth factors. These data suggest that angiogenic growth factors may play an important role in the development of the microvascular network in the mammary gland during involution.

Key Words: Mammary, Angiogenesis, Vascular endothelial growth factor

877 Dopamine antagonist affects cortisol secretion in lactating dairy cows. A. Ahmadzadeh*, M. A. Barnes, F. C. Gwazdauskas, and A. H. Walters, *Virginia Polytechnic Institute and State University, Blacksburg, Virginia.*

The role of dopamine in regulation of glucocorticoid secretion was investigated by characterizing serum cortisol response to fluphenazine (FLU), a dopamine receptor antagonist, in lactating dairy cows. In Exp. 1, 12 anovulatory primiparous cows received (i.v.) either saline (SAL; n=6) or .3 mg/kg BW FLU (n=6) in wk 2 postpartum. Blood samples were collected every 30 min for 4 h before and 4 h after SAL or FLU. In Exp. 1, mean serum progesterone concentration was .13 ± .1 ng/ml and there was no difference in mean serum cortisol concentrations between groups before treatments (5.9 ± 2.0 in FLU vs 6.9 ± 2.0 ng/ml in SAL). FLU caused an increase (P < .01) in mean serum cortisol concentration (from 5.9 ± 2.0 to 18.2 ± 2.0 ng/ml) in primiparous cows. Serum cortisol level increased within 30 min after FLU administration and remained elevated throughout the sampling period. Mean serum cortisol remained unchanged in SAL-treated cows (6.9 ± 2.0 before vs 4.0 ± 2.0 ng/ml after SAL). To eliminate possible parity effects that may have confounded Exp. 1, a second experiment was conducted. In Exp. 2, 6 anovulatory multiparous cows (wk 2 postpartum) were used and all cows received FLU (.3 mg/kg BW). Experimental procedures were the same as used in primiparous cows. In Exp. 2, mean serum progesterone concentration was .12 ± .05 ng/ml. Similar to Exp. 1, FLU increased (P < .01) mean serum cortisol concentration (from 13.2 ± 6.5 to 45.4 ± 6.5 ng/ml) in multiparous cows. These results indicate the stimulatory role of dopamine antagonist in cortisol secretion regardless of parity and presumably by affecting pituitary ACTH secretion, and suggest that endogenous dopamine, at least in part, regulates cortisol secretion in lactating dairy cows.

Key Words: Dopamine, Glucocorticoids, Dairy cow

878 Dexamethasone treatment at birth enhances neonatal growth in swine. J.A. Carroll* and R.L. Matteri, *Animal Physiology Research Unit, Agricultural Research Service, USDA, Columbia, MO.*

Previously, we have reported that elimination of the periparturient events associated with the natural birth process through caesarian delivery reduces average daily gain (ADG) and serum concentration of insulin-like growth factor 1 (IGF-1; J Anim Sci 76 (Suppl 1):126, 1998). Therefore, the objective of the present study was to determine if dexamethasone (Dex; a potent synthetic glucocorticoid) treatment at birth would alter postnatal growth in neonatal pigs. Forty crossbred pigs were injected i.m. with either sterile saline (Cont; n=10 males and 10 females) or Dex (1 mg/kg; n=10 males and 10 females) within 1 hr of birth. All pigs remained with their respective dams until 18 d of age. Body weights were recorded weekly and on d 18. On d 17, all pigs were non-surgically fitted with an indwelling jugular catheter and placed back with the sows. On d 18, all pigs were placed in individual pens for serial blood collection. Birth weights (1.53 ± .04 kg) did not differ between birth treatments or sex classes (P > 0.70 and 0.89, respectively). A time by birth treatment effect was detected (P < 0.007) for body weight such that those pigs which received Dex at birth had the greatest body weights during the 18-day period. Average daily gain was increased (P < 0.017) by 12.2% in those pigs which received Dex at birth (.286 ± .007) as compared to the Cont pigs (.255 ± .01 kg/day). Serum concentration of IGF-1 was influenced by both birth treatment (P = 0.0006) and sex class (P < 0.017). In the male pigs, Dex increased (P = 0.02) serum concentration of IGF-1 by 40.5% as compared to Cont male pigs, whereas in the females, Dex increased (P < 0.007) serum concentration of IGF-1 by 33.5% as compared to Cont female pigs. Based on the results of the present study, as well as previous reports from our laboratory (J Anim Sci 76 (Suppl 1):99, 1998; J Anim Sci 76 (Suppl 1):126, 1998), the early neonatal period may be an opportune time to permanently alter physiological factors which influence the growth biology of swine. Also, the present study indicates that early neonatal hormonal therapies may be useful in achieving the actual genetic potential for growth in pigs.

Key Words: Neonatal Pigs, Dexamethasone, Growth

879 Estradiol/progesterone treatment inhibits nitric oxide production in endotoxemic cattle. J. L. Sartin*[1], T.H. Elsasser[2], S. Kahl[2], D.D. Schwartz[1], J. Baker[1], M.A. Shores[1], and B. Steele[1], [1]*Auburn University,* [2]*ARS/USDA.*

Estradiol/progesterone (EP) implants have been shown to decrease clinical signs of pathologic responses of cattle to coccidiosis and endotoxemia. We proposed that EP acts to minimize effects of endotoxemia in cattle through a reduction of nitric oxide (NO) production, a key step in mediating cytokine actions on target tissues. Holstein steers were divided into 4 groups: control, EP, endotoxin, and EP-endotoxin (n=5/group). Cattle were provided EP implants (Synovex S; Ft. Dodge) at 4 mos. of age and one week later injected with endotoxin (0.6 μg/kg BW; Sigma). Body temperature was measured at 0,2,4,6, and 8 h. Plasma was collected at time 0 and hourly thereafter for 8 h and assayed for nitrate/nitrite (stable end product of NO metabolism), prostacyclin, cortisol and tumor necrosis factor (TNF). Body temperature was increased in both endotoxin and endotoxin-EP calves, but had returned to normal by 6 and 8 h in the endotoxin-EP group (P<0.05). Plasma nitrate/nitrite levels were elevated (P<0.01) in calves treated with endotoxin alone, whereas treatment with EP blocked the endotoxin-mediated increase in plasma nitrate/nitrite levels. Plasma TNF and cortisol concentrations were increased by endotoxin (P<0.01), but were unaffected by EP. Likewise, EP did not affect the increases in endotoxin-induced prostacyclin concentrations, indicating EP does not affect this indicator of oxidative stress. These data indicate that EP can reduce the production of NO in calves stimulated with endotoxin. The mechanism for this inhibition is not known, but based on in vitro studies in lymphocytes and with studies of the hypothalamus, EP would be expected to inhibit NO synthase activity and/or synthesis.

Key Words: endotoxin, nitric oxide, estradiol

880 Neonatal Quipazine treatment induced a cortisol release in pigs but did not increase hippocampal glucocorticoid receptor levels. S Weaver*[1] and M.J.M. Meaney[2], [1]*Livestock Behavior Research Unit, USDA-ARS, West Lafayette, IN,* [2]*Douglas Hospital Research Centre, Montreal, Quebec, Canada.*

Improved well-being for swine in commercial production would result from decreased exposure to or response to stressors. In the rat, hippocampal glucocorticoid receptors (GR) regulate the duration of stress responses. The number of GRs in the hippocampus of the rat is positively regulated by the serotonin agonist quipazine in vitro. Postnatal antagonism of serotonin signaling prevented neonatal handling-induced increases in hippocampal GR in the rat. The objectives of this study were:1) to determine if in vivo quipazine administration would alter GR levels in the hippocampus of neonatal pigs, 2) induce a cortisol release, and 3) affect body weights prior to weaning. Eight litters of PIC pigs were assigned to one of 3 doses of quipazine dissolved in saline: 0, .5, 1.0, 2.0 mg/kg (n=16 pigs per treatment) which was balanced across sex and litter. Subcutaneous injections were given for the first 7 days of life. On day 5 blood samples were obtained. At 14 days of age 2 males and 2 females from each treatment were euthanized and the pituitary gland, frontal cortex, hypothalamus and hippocampus were collected. Data were analyzed as a split-plot design with sex as the whole plot, drug treatment as the sub-plot and litter as the replication followed by Student-Newman Keuls post hoc tests. A dose related cortisol response to quipazine administration was detected with significantly higher (P<.05) plasma cortisol levels in pigs given 2.0 compared to .5 or 0 mg/kg quipazine (2.0 mg 21.22 ± 2.40; .5 mg 12.50 ± 2.07; 0 mg 11.39 ± 1.16). There was no significant effect of quipazine on immunoreactive GR levels as determined using western blotting. Weekly body weights, up to 28 days of age, were not affected by quipazine treatments. In conclusion, hippocampal GR levels were not altered by postnatal administration of quipazine. Quipazine administration did induce a cortisol release in neonatal pigs that did not alter body weights prior to weaning.

Key Words: Glucocorticoid receptor, Serotonin, Neonate

881 Molecular cloning of bovine corticotropin releasing factor receptor 1 (CRFR1) cDNA: Tissue distribution and regulation of CRFR1 mRNA expression in the anterior pituitary of endotoxemic steers. I.M. Qahwash*, C.A. Cassar, R.P. Radcliff, and G.W. Smith, *Michigan State University, East Lansing.*

Actions of corticotropin releasing factor (CRF) in rodents are mediated through two distinct receptor subtypes, CRFR1 and CRFR2, which differ in pharmacological properties and distribution within the brain and peripheral tissues. The distribution, regulation, and physiological roles of specific CRF receptor subtypes in cattle are unknown. Our objectives were to clone and sequence a bovine CRFR1 cDNA, to characterize distribution of CRFR1 mRNA in the brain and peripheral tissues, and to examine CRFR1 mRNA regulation in the anterior pituitary (AP) during systemic inflammatory stress. A 1,248 bp cDNA encoding the entire CRFR1 open reading frame was isolated from bovine AP and sequenced. The nucleotide and predicted amino acid sequence of bovine CRFR1 was 89 and 95% similar to the rat CRFR1. A prominent 2.5 kb CRFR1 mRNA transcript and a second 8.0 kb minor transcript were detected in specific regions of the brain including the cerebellum, cerebral cortex and hypothalamus, and also in the AP. Expression was not detected in peripheral tissues tested (liver, spleen, heart, kidney, and adrenal gland). We then examined the regulation of CRFR1 mRNA expression in AP collected from Holstein steers at 0, 2 and 4 h after bacterial endotoxin administration (n = 8). All endotoxin treated animals responded with the increase in body temperature and elevation of plasma ACTH and cortisol characteristic of a systemic inflammatory response. AP CRFR1 mRNA decreased by 4 h after endotoxin administration (P = 0.003). In summary, bovine CRFR1 mRNA was detected in specific brain regions but not in the periphery. Expression in AP was decreased during systemic inflammatory stress. The contribution of CRFR1-mediated pathways to specific components of the stress response in cattle awaits further investigation.

Key Words: Corticotropin releasing factor receptor 1, Bovine anterior pituitary, Stress

882 Relationships of plasma cortisol and corticosteroid-binding globulin (CBG) concentrations, and hepatic CBG mRNA expression levels in fetal and postnatal pigs. J. Heo*[1], H. G. Kattesh[1], R. L. Matteri[2], and M. P. Roberts[1], [1]*University of Tennessee, Knoxville,* [2]*Animal Physiology Unit, Agricultural Research Service, USDA, Columbia, MO.*

The concentration of CBG in circulation is determined by hepatic synthesis, peripheral degradation, and/or transfer to the extravascular system. The purpose of this study was to evaluate the relationships among hepatic CBG mRNA expression and plasma concentrations of cortisol and CBG during fetal and postnatal periods in the pig. A partial CBG cDNA (500bp) probe was developed from porcine liver RNA using RT-PCR. The PCR product was subcloned into the pGEM-T easy vector. The porcine partial CBG cDNA sequence showed 77% and 82% homology with human and sheep CBG cDNA, respectively. Blood and liver tissue were collected from fetal pigs (n=7-14) on d 50, 70, 80, 90, and 104 of gestation, as estimated by fetal length, and from postnatal pigs (n=7-8) on d 1, 3, 10, 20, 30, and 40 following birth. Plasma cortisol and CBG concentrations were determined by RIA and ELISA, respectively. Total RNA was isolated from liver tissue. Levels of CBG mRNA were determined by Northern blot analysis and expressed relative to β-actin. In fetal pigs, CBG mRNA expression was highest (p<.005) on d 50 compared to d 90 exhibiting a negative relationship (r=-.63, p<.001) with estimated fetal age. Plasma CBG concentrations were correlated (r=.90, p<.01) to CBG mRNA levels. Plasma cortisol concentrations were not different over this same period. In postnatal pigs, CBG mRNA expression increased (p<.001) from d 3 to d 40. Plasma CBG concentration increased (p<.001) from d 1 (6.07±3.37 ug/ml) to d 10 (15.15±3.72 ug/ml). Plasma cortisol concentrations remained constant. The present study represents the first determination of the relationship between the abundance of hepatic CBG mRNA and circulating CBG concentrations in the pig. An understanding of these relationships provides an important fundamental understanding of the mechanism determining the bioavailability of cortisol necessary in prenatal development and the conservation of cortisol during postnatal development.

Key Words: CBG, Cortisol, Fetal and postnatal pig

883 Adrenocortical function in nutritionally restricted female Nubian goats. R. M. Melendez-Soto*[1], M. Gomez-Pasten[1], L. Zapata-Salinas[2], and H. R. Vera-Avila[2], [1]*FES Cuautitlan-UNAM, Mexico,* [2]*CNIFyMA-INIFAP, Mexico.*

To test the effect of nutritional restriction on adrenocortical function, 19 mature female Nubian goats (51±1.2 kg) were randomly assigned to be fed a maintenance diet (NR0, n=5) or to be 20 (NR20, n=7) or 40 % (NR40, n=7) nutritionally restricted. On weeks 9 (early winter), 18 (early spring), 27 (mid-spring) and 36 (early summer) animals were weighed, exposed to transport stress and fitted with jugular catheters for intensive bleeding (IB). BW changes with respect to d0 (BWC) were estimated and compared between treatments. Blood was collected before and after transport and analyzed for cortisol (CT). Differences between pre- and post-transport CT were considered adrenocortical response (CTR). IB was initiated at 5:00 AM and blood samples were collected every 80 min over a 24 h period. Plasma was obtained and analyzed for CT. BW was essentially maintained in NR0 and gradually decreased in NR20 and NR40 (-.5±.25,-1.2±.31,-.8±.63 and +.4±.22, -2.2±.35,-3.4±.69,-3.2±.84 and -4.0±.96, -4.4±.63,-7.3±.87,-7.7±.97 and -11.7±1.1 kg BWC for NR0, NR20 and NR40 on weeks 9, 18, 27 and 36; treatment x week, P<.001). CTR was affected by week (P=.02) but not by treatment or treatment x week (P>.05). A random variation between weeks was observed on CTR (4.4±.42,3.6±.37,4.3±.40 and 3.3±.28 ng/ml for weeks 9, 18, 27 and 36). Mean CT during IB was affected by week (P<.01) but not by treatment or treatment x week (P>.05). A significant increase in mean CT was observed on weeks 18 and 27 as compared to weeks 9 and 36 (1.2±.16,1.7±.28,1.8±.14 and 1.1±.13 ng/ml for weeks 9, 18, 27 and 36). CT profile during IB was affected by week, time and week x time (P<.01) but not by treatment or any interaction with treatment (P>.05). Peak plasma CT concentrations, which best reflected differences in the week x time interaction during IB were observed between times of collection 2 to 4 (2.2±.55,3.3±.84 and 2.3±.67, 3.7±.24,3.7±.38 and 3.6±.50, 4.8±.45,5.0±.35 and 4.3±.41, 1.8±.21,1.5±.38 and 1.1±.39 ng/ml for times 2, 3 and 4 on weeks 9, 18, 27 and 36). Overall, nutritional restriction did not alter adrenocortical function. A trend for a seasonal effect on adrenocortical function was observed.

Key Words: Adrenal Gland, Undernutrition, Goats

884 Influence of Bos taurus and Bos indicus breedtype on production of cortisol. J.W. Koch*[1], T.H. Welsh[1], J.O. Sanders[1], D.G. Riley[1], D. Lunt[2], J.W. Holloway[3], T.D.A. Forbes[3], H. Lippke[3], F.M. Rouquette[4], and R.D. Randel[4], [1]*Texas Agricultural Experiment Station, College Station,* [2]*McGregor,* [3]*Uvalde,* [4]*Overton.*

Both the plasma concentration of cortisol and adrenal gland weight have been reported to be greater for Bos taurus (Angus) than Bos indicus (Brahman) cattle. The objective of this experiment was to determine if differences exist in proportional response (fold increase) of plasma cortisol following an injection of ACTH (0.1 IU/kg of bodyweight). Full blood Bos taurus (BT, n = 58), 3/4 Bos taurus - 1/4 Bos indicus (.25 BI, n = 137), 1/2 Bos taurus - 1/2 Bos indicus (.5 BI, n = 96), and full blood Brahman (BI, n = 24) stocker steers and heifers owned by the Texas Agricultural Experiment Station were utilized for this study. A blood sample was taken via tail venipuncture and followed immediately by an intravenous injection of ACTH into the jugular vein. A second blood sample was taken via tail venipuncture 30 min following ACTH injection. There were no differences ($p > .10$) in proportional response of cortisol (PR) between steers and heifers within breedtype. The PR was higher ($p \leq .0028$) in the BT cattle compared to the .50 BI cattle and the BI cattle (2.48 ± 0.20 vs. 1.69 ± 0.15; 1.37 ± 0.31 respectively). There was no difference ($p = .31$) in PR between the BT cattle and .25 BI cattle (2.48 ± 0.20 vs. 2.24 ± 0.13, respectively). The PR was higher ($p \leq .010$) in the .25 BI cattle compared to the .50 BI cattle and BI cattle (2.24 ± 0.13 vs. 1.69 ± 0.15; 1.37 ± 0.31, respectively). The proportional response was not different ($p = .36$) between the .50 BI cattle and the BI cattle (1.69 ± 0.15 vs. 1.37 ± 0.31, respectively). These results indicate animals of various percentages of Bos indicus and Bos taurus breedtype respond differently in production of cortisol after exogenous ACTH.

Key Words: Bos taurus, Bos indicus, cortisol

885 Does pretreatment with GnRH prior to a GnRH-PGF$_{2\alpha}$ (PG) protocol improve synchronization of estrus in beef cattle? F. N. Kojima*, S. L. Wood, M. F. Smith, and D. J. Patterson, *University of Missouri, Columbia.*

This experiment was conducted to determine whether addition of GnRH (100 μg Cystorelin®) administered 7 d prior to a GnRH-PG protocol (GnRH followed in 7 d with 25 mg PG, Lutalyse®) would improve estrous response and/or synchrony of estrus in postpartum suckled beef cows. Primiparous and multiparous Angus crossbred cows were assigned by days postpartum (dpp) to a GnRH-PG (n=29 and n=26, respectively) or GnRH-GnRH-PG protocol (2xGnRH-PG: n=28 and n=26, respectively). Average BCS and dpp for GnRH-PG and 2xGnRH-PG treated cows at the initiation of treatment was 4.6 and 52 d, and 4.4 and 53 d, respectively. Multiparous cows in the 2xGnRH-PG group had lower ($P < .05$) BCS than contemporaries in the GnRH-PG group (4.2 and 4.7, respectively). Pretreatment cyclicity status did not differ ($P > .10$) between GnRH-PG (62%) and 2xGnRH-PG groups (69%), determined by two blood samples obtained 7 d prior to and on the day treatments were initiated. Cows were observed for signs of behavioral estrus for 7 d following PG, and inseminated 12 h after detected estrus. Synchrony of estrus was analyzed by ratio of variance (F-test) for mean time interval to onset of estrus. Estrous response (75% vs. 80%), synchronized conception rate (76% vs. 67%), synchronized pregnancy rate (56% vs. 54%) and pregnancy rate during the breeding season (87% vs. 89%) did not differ ($P > .10$) between GnRH-PG and 2xGnRH-PG treated cows, respectively. Furthermore, there were no significant differences among these response variables ($P > .10$) between primiparous and multiparous cows. Estrus synchrony among multiparous cows was greater ($P < .01$) for 2xGnRH-PG treated cows (124.2, df=24) than for GnRH-PG treated cows (453.3, df=21), indicated by the lower variance for mean interval to estrus determined by F-test. In summary, the addition of GnRH prior to a GnRH-PG protocol improved synchrony of estrus in multiparous cows with no reduction in fertility.

Key Words: Estrus synchronization, Artificial insemination, Beef cows

886 Estrus and fertility in beef heifers synchronized with melengestrol acetate (MGA) and prostaglandin F$_{2\alpha}$ (PG) with or without GnRH. S. L. Wood*, M. C. Lucy, M. F. Smith, R. F. Randle, D. K. Hardin, and D. J. Patterson, *University of Missouri, Columbia.*

The objective of this field trial was to determine whether addition of GnRH to a MGA-PG estrus synchronization protocol would improve synchrony of estrus and maintain high fertility in beef heifers. Angus beef heifers (n=690) were assigned to one of two treatments by age and reproductive tract score (RTS). Two age groups (older, n=320; younger, n=370) were used in this study. Heifers were fed MGA (.5 mg·hd^{-1}·d^{-1}) for 14 d followed by an injection of PG (25 mg Lutalyse®) 19 d after MGA withdrawal (d 33). MGA was delivered in a supplement of 2.25 kg·hd^{-1}·d^{-1}. One-half of the heifers (n=344) received an injection of GnRH (100 μg Cystorelin®) 12 d after MGA withdrawal (d 26) and 7 d prior to PG (MGA-GnRH-PG). The control group (n=346) received only MGA-PG. Heifers were monitored for signs of behavioral estrus continuously for 7 d with the Heatwatch® Estrus Detection System (DDx, Inc.) beginning on the day PG was administered. Heifers were inseminated 12 h after onset of estrus. Average age, estrous response and conception rates are summarized in the table below. The peak synchronized period was 48-72 h after PG for the older heifers and 36-60 h for the younger heifers. Total and peak estrous response for the older heifers differed between treatments ($P<.05$, $P<.01$, respectively). There were no differences in conception rate between treatments for either age group. These data suggest that addition of GnRH to a 14-19 d MGA-PG estrus synchronization protocol failed to improve total estrous response or synchrony of estrus in replacement beef heifers.

	Older heifers		Younger heifers	
	MGA-PG	MGA-GnRH-PG	MGA-PG	MGA-GnRH-PG
Age (mo)	21	21	15	15
Peak estrus response	126/160 79%^{a}	110/160 69%^{b}	124/186 67%	116/184 63%
Total estrus response	158/160 99%^{a}	148/160 93%^{b}	170/186 91%	161/184 88%
1st service conception	105/159 66%	102/153 67%	109/175 62%	109/166 66%

^{a,b} Values with different superscripts in same row and age group are significantly different ($P<.05$).

Key Words: Heifer, Estrus synchronization, Progestin

887 Effect of estradiol benzoate (EB) administered at insertion of an intravaginal progesterone releasing insert (CIDR) on pregnancy rates in crossbred *Bos indicus* cows. C. R. Barthle*, J. R. Kempfer, J. K. Fullenwider, J. W. Lemaster, C. L. Barnett, G. E. Portillo, and J. V. Yelich, *University of Florida, Gainesville.*

Crossbred, lactating cows of *Bos indicus* type breeding were used to evaluate pregnancy rates of cows receiving EB or No-EB at CIDR insertion. Cows were randomly assigned to each of six treatments by body condition score (scale 1-9) and postpartum interval at CIDR insertion (d 0 of experiment). All cows received a 7 d CIDR (EAZI-BREED™ CIDR®) and 25 mg of PG (Lutalyse) on d 7. Treatments were: 1) CIDR + 1 mg EB 24 h after CIDR removal and AI 12 h after exhibiting estrus (C); 2) 2 mg EB on d 0 + CIDR + 1 mg EB 24 h after CIDR removal and AI 12 h after exhibiting estrus (EBC); 3) CIDR + 1 mg EB 24 h after CIDR removal and timed-AI (TAI) 48 h after CIDR removal (CTAI); 4) 2 mg EB on d 0 + CIDR + 1 mg EB 24 h after CIDR removal and TAI 48 h after CIDR removal (EBCTAI); 5) CIDR + 100 μg GnRH (Cystorelin) at TAI 48 h after CIDR removal (CGTAI), and 6) 2 mg EB on d 0 + CIDR + 100 μg GnRH at TAI 48 h after CIDR removal (EBCGTAI). Estrus was detected twice daily for 5 d following CIDR removal. Calves remained with cows throughout the experiment. Pregnancy was determined by ultrasonography 50-60 d after AI. Cows receiving EB at CIDR insertion had greater ($P<.05$) conception (56.7 vs 41.2%) and pregnancy rates (53.5 and 37.9%) than No-EB, respectively. Estrous rates were greater ($P<.05$) for the C (92.6%) and CTAI (84.6%) than CGTAI (35.8%) within the No-EB treatments, and were

also greater (P<.05) for the EBC (95.7%) and EBCTAI (80.5%) than EBCGTAI (32.9%) within the EB treatments. For No-EB treatments, conception (42.7, 35.9, 38.3%) and pregnancy (39.5, 35.9, 38.3%) rates were similar (P>.05) between the C, CTAI and CGTAI, respectively. For EB treatments, conception (59.1, 50.6, 52.9%) and pregnancy (56.5, 50.6, 52.9%) rates were similar (P>.05) between the EBC, EBCTAI and EBCGTAI, respectively. EB administered at CIDR insertion increased pregnancy rates in lactating cows of *Bos indicus* type breeding.

Key Words: *Bos indicus*, Estradiol Benzoate, GnRH

888 Effect of estradiol benzoate in combination with progesterone to induce follicular turnover at varying stages of the estrous cycle. V.L. Bogacz*[1], J.E. Huston, D.E. Grum, and M.L. Day, [1]*The Ohio State University, Columbus.*

An experiment was performed to test the efficacy of estradiol benzoate (EB) to induce atresia of the dominant follicle and emergence of a new wave of follicular growth at three stages of the estrous cycle. Thirty-five cyclic beef heifers were observed for estrus twice daily for 30 d. The d of detection of estrus was used to assign heifers to receive treatments at one of three stages of the estrous cycle: early (d 2 to d 6), mid (d 11 to d 13) and late (d 16 to d 21). At the initiation of treatment, (d 0) all heifers received an intra-vaginal progesterone insert (IPI; CIDR-B®) for 7 d and 25mg of PGF2α at the time of IPI removal. Heifers within each stage were assigned to receive either 1mg EB/500 kg bodyweight (EB; n=13) or no further treatment (0EB; n=14). Ultrasonography was performed daily from d -3 through ovulation. Diameter of the dominant follicle on d 0, was 8.1 $\pm$ .6 mm, 9.3 $\pm$ 1 mm and 14.1 $\pm$ .7 mm for heifers in the early, mid and late stages respectively with a range of 4 mm to 17 mm. Follicle turnover occurred between d 0 and d 7 in all but one heifer in the late stage that received the 0EB treatment. New wave emergence occurred earlier (P<.05) in the 0EB (2.6 $\pm$ .47 d) than in the EB (3.9 $\pm$ 0.4 d) treatment with no significant treatment by stage interaction. Across treatments, emergence occurred later (P<.05) in the early (4.2 $\pm$ .55 d) as compared to the late (2.6 $\pm$ .43 d) and mid (2.3 $\pm$ .43 d) stages. Consequently, diameter of the dominant follicle on d 7 was smaller for heifers in the early (8.9 $\pm$ .85 mm) then the late (13.6 $\pm$ 1.3 mm) stage. EB treatment tended to reduce (P=.13) variation in follicle diameter on d 7 as compared to the 0EB treatment (early 8.2 $\pm$ .9 vs. 9.7 $\pm$ 1.5; mid 10.0 $\pm$ 0 vs. 11.8 $\pm$ 1.2; and late 11.8 $\pm$ .48 vs. 15.5 $\pm$ 1.8) respectively. It is concluded that 1mg EB/500 kg bodyweight in conjunction with a progestin induces atresia and new wave emergence and may aid in the standardization of follicle diameter throughout varying stages of the estrous cycle.

Key Words: Estradiol Benzoate, Follicle, Beef Cattle

889 Association of PGF$_2\alpha$ and estradiol-17β with function of induced corpora lutea and maintenance of pregnancy in beef cows. P. J. Bridges*[1], D. J. Wright[1], W. I. Buford[1], N. Ahmad[1], H. Hernandez-Fonseca[1], M. L. McCormick[1], F. N. Schrick[2], R. A. Dailey[1], P. E. Lewis[1], and E. K. Inskeep[1], [1]*West Virginia University, Morgantown,* [2]*University of Tennessee, Knoxville.*

Ability of induced (replacement) corpora lutea (CL) to maintain pregnancy was examined in cows without a primary CL, in which pregnancy had been maintained with exogenous progestogen. In preliminary experiments, more pregnancies were maintained when replacement CL were induced after (13/13), than before (7/14) d 36 of pregnancy (P < .05), if CL were adjacent to the gravid horn. An experiment was conducted to directly evaluate the effect of concentrations of PGF$_2\alpha$ and estradiol-17β on maintenance of pregnancy by replacement CL. In cows with replacement CL induced before d 36 of pregnancy, that maintained pregnancy while progestogen was provided, maintenance of pregnancy after withdrawal of exogenous progestogen tended to be greater with high (5/5) than low (2/6; P < .10) concentrations of PGF$_2\alpha$, and low (6/7) than high (2/6; P = .10) concentrations of estradiol-17β between d 31 and 35. In cows with high concentrations of PGF$_2\alpha$ between d 31 and 35, secretion of progesterone by replacement CL was increased (P < .05), but secretion of estradiol-17β did not differ, compared to cows with low PGF$_2\alpha$. Again, maintenance of pregnancy in cows without a primary CL was greater when replacement CL were induced after (9/9) than before d 36 (8/16; P < .05). In conclusion, maintenance of pregnancy after induction of replacement CL was increased when CL were induced after d 36 of pregnancy. When a CL was induced before d 36 of pregnancy, concentrations of PGF$_2\alpha$ affected the ability to form a functional CL. Higher concentrations of PGF$_2\alpha$ may increase maintenance of pregnancy in cows via facilitation of embryonic attachment, as has been reported for implantation in other species.

Key Words: Cattle, Embryo Mortality, Corpora Lutea

890 Use of bovine ovarian follicle wall in a culture system to study long-term steroidogenesis. M. Frajblat* and W. R. Butler, *Cornell University, Department of Animal Science, Ithaca, NY.*

The objective was to develop a long-term culture system for bovine follicle wall that maintains the normal interdependence of theca and granulosa tissues and produces increasing amounts of estradiol (E_2) without supplementation of androgen precursor. Theca and granulosa cells collaborate to produce E_2 and progesterone. Despite the close relationship between these cell types, most research has studied them separately. Ovaries were collected at a slaughterhouse and follicles (<5 mm) were dissected from the ovarian stroma. The follicular wall was peeled away and cut into several pieces for culture in 0.5 ml medium (3 pieces/well). Media was changed every 48 hrs for 6, 8 or 12 days according to the experiment.E_2 secretion increased linearly over 6 days of culture when the media included insulin (10 ng/ml). When IGF-I was also added (50 ng/ml), E_2 secretion was maintained at high levels for 12 days without androgen supplementation. A high E_2:progesterone ratio was also maintained during the 6 or 12 day cultures. Addition of testosterone (at a normal or high concentration, 0.5 and 2.5 μM, respectively) as substrate for aromatase activity, increased secretion of E_2 significantly (P<0.05). Despite increased E_2 secretion, lactate production was similar (P>0.18) between control and testosterone treatments suggesting that the increase in E_2 was independent of glucose consumption. However, addition of glucose to the culture increased E_2 and lactate production (P< 0.05) demonstrating a strong relationship between E_2 secretion and energy availability. All IGF binding-proteins (IGFBP 2,3, 4 and 5) were detected in the culture media despite the high levels of E_2 secretion. When the follicle wall was challenged with 50 or 100 ng/ml of bLH, progesterone secretion increased 4- fold suggesting a shift from estrogen to progesterone synthesis in response to LH. Therefore, in this culture system, the integrity of the follicle wall is maintained and secretion of E_2 increases over a period of 6 to 12 days in culture. This system provides a valuable physiological approach for studying hormonal or metabolic regulation of steroidogenesis in bovine follicles.

Key Words: Follicle, Bovine, Estradiol

891 Development of follicular cysts in cattle is due to an estradiol-induced GnRH/LH surge without subsequent progesterone exposure. A. Gumen*, R. Sartori, F. Costa, and M. C. Wiltbank, *University of Wisconsin, Madison.*

The hypothesis was tested that follicular cysts are due to lack of a GnRH/LH surge in response to estradiol. We further hypothesized that this condition developed due to estradiol induction of a GnRH/LH surge in the absence of subsequent ovulation. In expt 1, 7 cows were synchronized with an intravaginal progesterone implant (IPI) for 9 d with prostaglandin F2α treatment on d 7. All follicles were aspirated at the time of IPI removal using transvaginal follicular aspiration. Two days after aspiration cows were treated with 5 mg estradiol benzoate (EB) to induce a GnRH/LH surge in the absence of an ovulatory follicle. All cows had an LH surge following the estradiol treatment and 3 of 7 developed a large follicle anovulatory condition (LFAC) that resembled follicular cysts. The 4 cows that did not develop LFAC either ovulated or luteinized the cells of the aspirated follicle. Thus, all cows with a progesterone elevation after the estradiol/GnRH/LH surge had subsequent ovulatory cycles; whereas, the absence of progesterone was followed by LFAC. After 49 d the anovulatory cows were induced back to normal cyclicity by insertion of an IPI for 7 d. In two subsequent experiments, 9 of 26 cows were induced to LFAC using follicular aspiration followed by 5 mg EB similar to expt 1. After 26 d of observation all LFAC cows received a second treatment with 5 mg EB and none of the cows showed an LH surge or ovulation in spite of high (>30 pg/ml) serum estradiol concentrations. Cows with LFAC were divided into two groups: untreated (n=4 controls) or treated for 7 d with an IPI (n=5). All cows were subsequently treated for third time with 5 mg EB. All IPI cows had an LH surge in response to estradiol and ovulated; whereas, none of the control cows had an LH surge or ovulation after the estradiol treatment. Thus, LFAC, similar to follicular cysts, can be induced by

estradiol induction of a GnRH/LH surge in the absence of subsequent luteinization. Progesterone eliminates this condition by reinitiation of GnRH/LH surges in response to estradiol.

Key Words: Follicular cysts, progesterone, cattle

892 Differences between lactating cows and nulliparous heifers in follicular dynamics, luteal growth, and serum steroid concentrations. R. Sartori*, J. Haughian, G. J. M. Rosa, R. D. Shaver, and M. C. Wiltbank, *University of Wisconsin, Madison*.

Pregnancy rate per AI is much greater in nulliparous heifers (70%) than in lactating dairy cows (40%). This experiment was designed to compare follicular dynamics, luteal growth, and hormonal patterns in dairy cows with high milk production (C) and nulliparous heifers (H) with similar genetic potential. Holstein cows (n=14; 28 to 100 d postpartum; average milk production >40 Kg/d) and heifers (n=28; 10 to 16 months old) were examined for one normal interovulatory interval using daily ultrasound and blood samples. All the values presented below are expressed in mean±sem. Estrous cycle length was not different (p>.20) between H (23.0±.4 d) and C (23.9±.8 d). Animals in both groups had estrous cycles with 2 follicular waves (13 H and 10 C), 3 waves (11 H and 3 C), or 4 waves (4 H and 1 C). C had greater incidence of multiple ovulations than H (25.0 vs. 1.8%; p<.01). Mean size of ovulatory follicles from C that ovulated 2 or more follicles simultaneously (n=15 follicles) was smaller than the size of ovulatory follicles from H with single ovulation (n=55), which was smaller than the single ovulatory follicles from C (n=21) (13.1±.8; 14.8±.2; and 17.4±.5 mm, respectively; p<.01). Interestingly, serum estradiol near estrus was lower in single ovulating C as compared to H (7.1±.5 vs. 9.4±.6 pg/ml; p<.05) in spite of the larger follicular size in C. Similarly, serum progesterone (analyzed from d 1 to 14 of the cycle) was lower for C than H from d 5 to 14 (on day 14: 4.1±.3 vs. 6.1±.4 ng/ml; p<.01); whereas volume of luteal tissue was greater for C than H (on day 14: 8272±932 vs. 5415±276 mm^3; p<.01). Thus, differences between C and H in reproductive parameters, such as multiple ovulation rate and fertility, may be due to lower circulating steroid concentrations in spite of larger follicular and luteal sizes.

Key Words: Fertility, Estrous cycle, Dairy cow

893 The effects of body condition and protein supplementation of postpartum beef cows on estrous behavior and follicle size. C. A. Lents*, F. J. White, D. L. Lalman, and R. P. Wettemann, *Oklahoma Agricultural Experiment Station, Stillwater*.

Multiparous Angus x Hereford cows (n = 45) were fed to calve with a body condition score (BCS; 1 = emaciated, 9 = obese) of thin (< 5) or moderate (≥ 5). Cows were blocked by BCS and calving date (March and April) and randomly assigned to receive either low (1.2 kg/d) or high (2.5 kg/d) amounts of a 42% CP supplement. All cows grazed the same native grass pasture with native grass hay free choice, and were fed supplement in individual stalls for 49.2 ± 2.3 d. Beginning 20 d postpartum, blood samples were collected from each cow three times weekly, and estrous behavior was monitored continuously with the HeatWatch® system. Size of the dominant follicle at 4 to 14 h after the onset of estrus was determined by ultrasonography. Duration of luteal activity (LA) before and after estrus was characterized as short (plasma progesterone ≥ .5 ng/mL for < 5 consecutive samples) or normal (plasma progesterone ≥ .5 ng/mL for ≥ 5 consecutive samples). Body condition score of thin cows was less (P < .01) than moderate cows (4.3 ± .1 vs 5.0 ± .1). Cows on high and low nutrition had similar BCS after treatment (4.5 ± .1). Weight gains tended to be greater for high vs low cows (25 ± 3 vs 17 ± 3 kg; P < .13). Prior to the first estrus, short LA occurred in 65% of cows, normal LA occurred in 27% of cows, and LA was not detected in 8% of cows. First estrus with normal LA was earlier (P < .01) for cows with moderate BCS at calving (64 ± 7 d) compared with thin cows (93 ± 6 d). Duration and number of mounts at the first estrus were not influenced by BCS at calving or postpartum nutrition. Size of the dominant follicle was greater (P < .01) for moderate BCS cows than thin cows (15.3 ± .5 vs 13.4 ± .4 mm), and for high vs low postpartum nutrition (15.0 ± .4 vs 13.7 ± .5 mm). Concentrations of estradiol in plasma at estrus were not influenced by BCS or nutrition. In conclusion, postpartum nutrient intake and BCS at calving influenced the size of the dominant follicle at the first estrus in multiparous beef cows.

Key Words: Nutrition, Estrus, Follicle

894 Effects of urea infusion on uterine luminal pH, prostaglandins and proteins in lactating dairy cows. M. L. Bode*, R. O. Gilbert, and W. R. Butler, *Cornell University, Ithaca, NY*.

Recent studies have demonstrated that decreased fertility in lactating dairy cows is associated with high levels of plasma urea nitrogen (PUN), although the mechanism by which this occurs is unknown. The objective of this study was to monitor changes in the uterine environment during chronic elevation of PUN. Dairy cows (n=8) between 40 to 100 days of lactation were studied on day 7 after estrus. Cows were determined to have ovulated by observation of a corpus luteum (CL) using ultrasonography. Infusion through jugular vein catheters of either saline (n=4) or urea (.01 g urea/hr/kg bwt; n=4) was continued for 24 hrs. Treatments were then switched between groups for a second 24 hr infusion period. Blood samples were collected every 1 to 2 hours to monitor PUN levels. Uterine pH was recorded at 6-hr intervals by inserting a steel cannula containing a micro pH electrode through the cervix and into the uterine lumen. After the termination of each infusion period, 30 ml of sterile saline was flushed into the uterine lumen and immediately retrieved. During urea infusion, the mean PUN level increased from the baseline during saline treatment (16.1 ± 1.5 mg/dl) to that commonly found in high producing dairy cows fed a TMR (23.3 ± 1.0 mg/dl; P=.001). Uterine pH declined during urea infusion from a mean of 7.18 ± .03 at 0 hr to 6.88 ± .03 at 18 hr (P=.06), but remained unchanged in controls (7.16 ± .04 to 7.27 ± .04). PGE_2 and $PGF_{2\alpha}$ levels in uterine lavages were not different between treatments. Proteins in uterine lavage samples were characterized using SDS polyacrylamide gel electrophoresis (SDS-PAGE). Protein profiles appeared to be similar among cows with no obvious differences being observed. Decreases in uterine pH during the infusion periods were correlated with higher plasma progesterone concentrations (P=.04). This study demonstrates that increased PUN can exert direct effects on the uterine environment. However, the mechanism by which changes in the uterine environment may affect fertility in high producing dairy cows needs further study.

Key Words: Cows, pH, Urea

895 Effect of Menhaden fish meal on uterine secretion of $PGF_{2\alpha}$, dry matter intake, milk yield and milk composition. R. Mattos*[1], J. Williams[2], C. R. Staples[1], and W. W. Thatcher[1], [1]*University of Florida, Gainesville,* [2]*University of California, Davis*.

Objective was to determine whether increasing concentrations of dietary Menhaden fish meal (FM) attenuate uterine secretion of $PGF_{2\alpha}$ and alter milk production and composition. Cycling Holstein cows (n=33) averaging 116 d in milk were fed diets containing 0 (Control), 2.6, 5.2 or 7.8% FM (Omega Protein, LA) on a DM basis. Diets were 18.0 to 18.4% CP and 1.67 to 1.69 Mcal NEl/kg. Fish oil was added to the 7.8% FM diet (0.28% of diet DM) to increase intake of PUFA eicosapentaenoic (EPA, C20:5, n-3) and docosahexaenoic (DHA, C22:6, n-3). Average intake of EPA plus DHA were 0, 9.7, 18.3 and 40.1 g/d for cows fed 0, 2.6 5.2 and 7.8% FM diets. Average DM intake was 25.4, 25.4, 23.8 and 25.1 kg/d (S.E. = 0.9) and milk production was 40.1, 40.7, 35.1, and 40.3 kg/d (S.E. = 2.2). Cows fed the 5.2% diet tended (P = 0.09) to produce less milk. Milk protein concentration increased linearly with increasing intake of FM (b= 0.023 %protein/%FM, P<0.06). Diets did not affect milk fat or milk urea nitrogen concentrations (P > 0.1). At 30 to 34 d after initiation of diets, cows received an injection of GnRH (Cystorelin, Merial, 100 μg) followed by injection of $PGF_{2\alpha}$ after 7 (Lutalyse, Pharmacia & Upjohn, 25 mg i.m.) and 8 d (15 mg i.m.). Injection of hCG (Chorulon, Intervet, 3000 IU) at 24 h after the second $PGF_{2\alpha}$ injection induced a synchronous ovulation in 32 of 33 cows. Subsequent luteal phase concentrations of plasma progesterone did not differ. At 15 d after hCG injection, cows were injected with estradiol-17β (Steraloids, 3 mg i.v.) at 0900 h and oxytocin (100 IU i.v.) at 1300 h. Nineteen blood samples for PGFM analyses were collected from jugular catheters between 1 h before to 4 h after oxytocin injection. Response curves of plasma PGFM concentrations after oxytocin injection were reduced in cows fed FM diets (0% > 2.6, 5.2, 7.8% FM, P < 0.025; 2.6% vs. 5.2% and 7.8% not significant; 5.2% < 7.8% P < 0.05). Thus, increased concentrations of dietary fish meal reduced uterine secretion of $PGF_{2\alpha}$ and increased milk protein concentrations. NRIGCP 98 35203-6367.

Key Words: Fishmeal, Milk, PGFM

896 Characteristics of the reproductive biology of multiparous sows from a commercially relevant population. M.E. Wilson*[1], K.A. Vonnahme[1], G.R. Foxcroft[2], G. Gourley[3], T. Wolff[4], M. Quirk-Thomas[5], and S.P. Ford[1], [1]*Iowa State University, Ames,* [2]*University of Alberta, Edmonton,* [3]*SGE, Webster City,* [4]*Roche Vitamins Inc., Kansas City,* [5]*Pig Improvement Company, Frankfort, KY.*

A major component limiting litter size is conceptus mortality. In an effort to characterize the timing and pattern of conceptus development and mortality in a commercially relevant pig, we collected mid-pregnant reproductive tracts from 244 PIC Camborough sows representing parities 1 to 14. The animals were slaughtered on d25, 36, or 44 of gestation. Each uterus was trimmed, the length of each horn measured and CL dissected to determine ovulation rate (OR). Conceptuses were removed and fetal weight (FW) and placental weight (PW) were determined. Uterine length (UL) and OR were 434±5 cm and 26.6±.4. Conceptus number decreased (P<.05) from 15.8±.6 on d25 to 12.9±.5 and 12.1±.5 on d36 and 44. Conceptus survival to d25 was 60.2±.1% which then decreased (P<.05) to 50.1±.1% on d36 and 46.3±.1% on d44. On d25, FW was .57±.01 g and increased (P<.05) to 4.98±.06 g on d36 and to 19.2±.17 g on d44. PW increased (P<.05) progressively, averaging 6.1±.2, 47.2±1.2 and 66.2±1.6 g on d25, 36 and 44, respectively. Placental efficiency (FW/PW) was similar on d25 and 36 (.12±.01) and increased (P<.05) on d44 to .33±.01. There was a positive correlation between conceptus number and OR on d25 (r=.50, P<.05), but by d36 this association was lost. Conceptus number was not associated with UL on d25; however, by d36 there was a positive association (r=.36, P<.05) which remained on d44 (r=.40, P<.05). On all three gestation days there was a negative association between conceptus number and PW (r=-.33, P<.05), but no association between conceptus number and FW. These data indicate that, compared to commonly reported values, OR in these higher parity production animals is very high and conceptus survival as a result quite low. Additionally, although conceptus number present is related to the OR on d25, by d36 the size of the uterus begins to decrease conceptus survival irrespective of OR.

Key Words: Pig, Litter size, Pregnancy

897 Beta-Lactoglobulin as a modulator of intestinal activity and effects on immunoglobulin uptake in the gut of the neonatal pig. L. F. Sutton*[1] and B. Alston-Mills[1], [1]*North Carolina State University, Raleigh.*

The effects of the bovine milk whey protein Beta-Lactoglobulin (BLG) on IgG uptake, endogenous porcine production of IgA, IgM, and IgG, proliferation of total intestinal DNA, and intestinal enzymatic activity was investigated. A total of 18 piglets were taken from three sows following partuition and divided into three experimental groups: two removed from the sow immediately (colostrum deprived) and one remained on the sow (control, Treatment 3, n=6). The colostrum deprived piglets were further divided: one receiving commercial bovine colostrum that was supplemented with an extra 10% BLG (Treatment 1, n=6) and the other group receiving only commercial bovine colostrum (Treatment 2, n=6). After 36 hours, all piglets receiving bovine colostrum were placed onto a liquid neonatal diet without additional supplementation. All piglets were weaned at 18 days and placed onto a dry feed diet. Blood samples were collected daily for the first five days and then every three days until day 28 and cast against both porcine and bovine anti-IgA, IgG, and IgM for sera concentrations. Treatment 1 exhibited increased weight gain when compared to the other two groups, and displayed significantly higher concentrations (p<.001) of bovine IgG after 3 days. Endogenous production of porcine IgG was found to be highest in controls after 18 days. Levels of endogenous porcine IgG exhibited no differences between treatments after 21 days. Sera levels of IgA and IgM were low amongst all treatments with no treatment effect. Previous results indicate that total DNA concentration was highest in piglets receiving BLG supplementation after 5 days. There was no difference in maltase, lactase, and alkaline phosphatase activity irrespective of treatment. These results suggest that bovine BLG may facilitate uptake of bovine IgG prior to gut closure, have no effect on endogenous production of IgG, IgM, and IgA, and may induce DNA proliferation.

898 Pituitary adenylate cyclase-activating polypeptide induces release of similar amounts of growth hormone before and after meal feeding of steers. R. P. Radcliff*, L. T. Chapin, K. J. Lookingland, and H. A. Tucker, *Michigan State University, East Lansing.*

Pituitary adenylate cyclase-activating polypeptide (PACAP) induces growth hormone (GH) secretion from cultured bovine pituitary cells. Feeding steers ad libitum for 2 h each day reduces basal secretion of GH for 2 h after feeding. Serotonin (5-HT) induces GH secretion both before and after feeding, but the mediator of 5-HT-induced GH secretion is not known. We hypothesize that PACAP may mediate 5-HT-induced secretion of GH. Initially, five Holstein steers (100 ± 6 kg BW) were used in a 5 × 5 Latin Square design to determine a dose of PACAP that would induce GH secretion. Each steer was injected i.v. with either 0, 0.1, 0.3, 1, or 3 μg PACAP/kg BW 1 h before feeding on each day. Sampling days were separated by 48 h and each steer eventually received all doses. Blood was collected from jugular catheters at -60, -40, -20, 0, 5, 10, 15, 20, 30, 40, and 60 min relative to injection of PACAP. Compared with controls, 3 μg/kg BW increased area under the GH response curve (253 ± 40 vs. 407 ± 41 ng·ml^{-1} min; P = 0.04), as well as peak GH concentrations (10.7 ± 2.0 to 21.5 ± 2.1 ng/ml; P = 0.005), while doses of 0.1, 0.3 and 1 μg/kg BW were ineffective. Subsequently, we tested if PACAP would induce GH secretion to similar magnitudes before and after feeding as observed with 5-HT. Accordingly, four steers were injected with PACAP (3 μg/kg BW, i.v.) 1 h before feeding and four were injected 1 h after feeding. Forty-eight hours later treatments were reversed. Blood was collected at -60, -40, -20, 0, 5, 10, 15, 20, 30, 40, and 60 min relative to injection of PACAP. Compared to before feeding, injection of PACAP after feeding induced similar increases in areas under the GH response curve (392 ± 48 vs 275 ± 56 ng·ml^{-1} min; P = 0.16) as well as peak GH concentrations (19.9 ± 2.3 vs 14.1 ± 2.7 ng/ml; P = 0.14). Therefore, PACAP may mediate 5-HT-induced GH secretion.

Key Words: Pituitary adenylate cyclase-activating polypeptide, Growth hormone, Cattle

899 Pre-weaning growth hormone (GH) response to growth hormone-releasing hormone (GHRH) is indicative of on-test average daily gain (ADG): selection for GH response to GHRH increases its association with ADG. T. L. Auchtung*, S. M. Barao, and G. E. Dahl, *University of Maryland, College Park.*

Previously we have shown the GH response to GHRH challenge at weaning to be indicative of higher ADG during a standard performance test in Angus bulls and lower on-test ADG in heifers. In this study, we tested the hypothesis that GH response to GHRH would be predictive of ADG at an earlier age. Bulls with the highest and lowest GH responses to GHRH over three years were used as sires, allowing for examination of the persistence of GH response to GHRH through selection. Forty-nine Angus calves (bulls, n = 24; heifers, n = 25) were challenged with GHRH at 60, 105 and 150 d of age (SD = 15, 12 and 14 respectively) and at weaning (219 d; SD = 25). Blood samples were taken immediately prior to and 10 min following a clearance dose of 4.5 μg GHRH/100 kg BW and, 2 hr later, immediately prior to and 10 min following a challenge dose of either 1.5 or 4.5 μg GHRH/100 kg BW. Two hours later, the procedure was repeated, with each calf receiving the other challenge dose. All calves in this study were sired by one of four Angus bulls selected for their GH response to GHRH (HI, n = 2; LO, n = 2). BW was measured every 28 d and ADG was calculated over a 140-d growth performance test (heifers and bulls maintained separately). Response to GHRH was inversely related (P < .05) to on-test ADG in heifers of HI sires at 60 d (R^2 = .30), 105 d (R^2 = .22) and all heifers at 150 d (R^2 = .08) and at weaning (R^2 = .22). Response to GHRH was positively related (P < .05) to on-test ADG in all bulls at 105 d (R^2 = .28), 150 d (R^2 = .27) and at weaning (R^2 = .52). Inclusion of sire in the model improved the correlation between ADG and predicted ADG for both genders. In conclusion, the relationship between ADG and GH response to GHRH is consistent between calves from unselected and selected populations and the relationship is improved with selection. In addition, GH response to GHRH as early as 60 d of age is indicative of on-test ADG in beef cattle.

Key Words: Beef Cattle, Growth Hormone, Predicting Growth

900 Superovulatory responses in cows receiving bovine somatotropin. F. Moreira*, L. Badinga, C. Burnley, and W. W. Thatcher, *University of Florida, Gainesville.*

Objective was to determine effects of bST on early embryonic development in vivo. Lactating (n = 8) and dry (n = 4) Holstein cows were superovulated 2 to 10 times with the following protocol: at experimental d 0, all cows received 2.5 mg of estradiol-17β and 50 mg of progesterone (i.m.), and two progestin implants (Synchro-Mate-B; 6.0 mg; s.c.). On the afternoon of d 4 and then twice daily until the morning of d 8, cows received a sequence of eight decreasing doses of FSH (Folltropin; 20 mg/ml of NIH FSH-P1; i.m.): two doses each of 4, 3, 2, and 1 ml. On the afternoon of d 6 and morning of d 7, $PGF_{2\alpha}$ (Lutalyse; 25 mg; i.m.) was injected. Implants were removed in the afternoon of d 7. After d 8, cows were observed for estrus and received 1 to 4 artificial inseminations (AI) every 12 h while estrus was expressed. At AI, cows were assigned randomly to a non-treated control group (n = 26) or to receive a single injection of bST (n = 26; Posilac; 500 mg; s.c.). Embryos were non-surgically flushed 7 d after AI. Total structures per flush did not differ between bST and control groups (9.3 $\pm$ 1.5 vs 9.4 $\pm$ 1.5), but were greater for dry than for lactating cows (13.3 $\pm$ 2.0 $>$ 5.5 $\pm$ 2.4; $P < 0.03$). Unfertilized ova per flush was less for bST than for control cows (1.0 $\pm$ 0.9 $<$ 3.7 $\pm$ 0.9; $P < 0.04$) and less for lactating than for dry cows (0.9 $\pm$ 0.6 $<$ 3.9 $\pm$ 0.5; $P < 0.01$). Percentage of transferable embryos relative to total structures flushed was greater for bST than for control cows (77.2 % $>$ 56.4 %; $P < 0.01$). Distribution among stages of development differed between bST and control groups, respectively: morula, 18.4 and 28.0 %; early blastocyst, 41.3 and 53.4 %; blastocyst, 32.5 and 9.3 %; expanded blastocyst, 7.8 and 9.3 % ($P < 0.001$). Number of blastocysts per flush was greater for bST than for control cows (2.4 $\pm$ 0.7 $>$ 0.4 $\pm$ 0.7; $P < 0.04$). Administration of bST at AI decreased the number of unfertilized ova, increased the percentage of transferable embryos, and stimulated embryonic development to the blastocyst stage.

Key Words: Bovine somatotropin, Superovulation

901 Effects of melengestrol acetate (MGA) on sexual behavior, testosterone (T) and luteinizing hormone (LH) concentrations in mature beef bulls. D. B. Imwalle, R. D. Smith, A. L. King, J. D. Bailey, and K. K. Schillo, *University of Kentucky, Lexington.*

We investigated the effects of varying doses of MGA on the sexual behavior and concentrations of circulating T and LH in mature Angus bulls. Twenty-four bulls (12-14 mos. of age; 339.06 $\pm$ 6.47 kg body weight) were randomly assigned to four treatment (n=6; 0.0, 0.5, 1.0, 2.0 mg/hd/d) groups. MGA-treated bulls received MGA for 99 days; treatment began on day 0. Plasma was collected sequentially (every 12 minutes for 8 hours) to assess patterns of LH and T on days 18, 9, 37, 65 and 93. Sexual behavior was assessed for 30 minutes on days 13, 15, 43, 71, and 99 with 6 haltered, estradiol-primed, ovariectomized heifers. There were no differences in mounts, LH patterns or T concentrations before treatment (p>.1). Analysis of variance failed to detect a significant treatment by time interaction for LH pulse patterns or mean LH (p>.1) during MGA. Mean LH was lower (p<.05) in controls than in treated animals (0.99 vs. 1.35 ng/ml). Mean LH declined in a linear fashion in all treatment groups (p<.1). Numbers of LH pulses were greater (p<.06) in the 0.5 mg group than in the 1.0 and 2.0 mg groups (2.79 pulses vs. 2.00 pulses/8h). In addition, number of pulses were greater (p<.06) in the 1.0 mg group than the 2.0 mg group (2.46 vs. 1.54 pulses/8h). Analysis of variance revealed a significant treatment by time interaction for T (p<.01) and for mounts (p<.02). Although there were significant differences among treatments at various times there were no consistent dose response relationships between treatment and these traits during MGA. Therefore, it is unlikely that MGA can be used effectively to control sexual behavior in bulls.

Key Words: Sexual Behavior, Bull, MGA

902 Ovulation and synchronization rates in Holstein and crossbred lactating dairy cows during two seasons when receiving the PGF2α injection on d 6 or 7 of the Ovsynch protocol. J.L.M. Vasconcelos*, T.P.B. Araujo, R.L.A. Cerri, R.L. Valarelli, and F.S. Wechsler, *FMVZ, Botucatu-Brazil.*

Heat stress inhibits the growth and function of the dominant follicle, increasing the number of cows with three follicular waves. This study was designed to evaluate whether synchronization rate varied between seasons (winter vs. summer), breeds (Holstein vs. Holstein-Gir) and day of PGF2α injection (d 6 or 7 after the first GnRH injection). This trial was conducted in two commercial dairy herds (one with Holsteins and another with crossbred cows) in Brazil, in September, 1999 (winter) and January, 2000 (summer). Cows (n=159) were assigned randomly to receive PGF2α injection on d 6 at the acupuncture point BAI HUI, or on d 7 I.M., after the first GnRH injection. Both groups received a second GnRH injection 48h after the PGF2α. Ovulation after the first and second GnRH injections and structural regression of the corpus luteum (CL), were evaluated by ultrasonography. Data were analyzed using the logistic regression procedure of SAS. Ovulation rate after the first GnRH injection was higher ($P<0.05$) in winter than in summer, with 61.4% and 37.8% for Holsteins, and 58.3% and 45.2% for crossbred cows, respectively. The lower ovulation rate during the summer was probably due to rapid loss of ovulatory capacity by the dominant follicle during heat stress. In cows that ovulated following the first GnRH injection (n=81), day of PGF2α injection did not influence ($P>0.10$) CL regression, with 92% for d 6 and 100% for d 7. Summer had more detrimental effect on the synchronization of ovulation of Holstein cows ($P=0.07$; 62.2 vs. 72.7%) than crossbred cows (59.5 vs. 63.9%). The greater decrease in the synchronization rate of Holstein cows might have been caused by the higher sensitivity to heat stress. Injection of PGF2α on d 6 was not efficient in increasing the synchronization rate. Following PGF2α injection on day 6, 71.6%, 13.5%, and 14.9% of the cows were synchronized, ovulated between the first and second GnRH injections, or did not ovulate 48h after the second GnRH, respectively; for injection on d 7, the values were 58.8%, 16.5%, and 24.7%, respectively. In conclusion, lactating cows receiving the Ovsynch protocol during the summer suffer a decrease in ovulation and synchronization rates, which may be associated with an increase in the proportion of cows with three follicular waves.

Key Words: synchronization, heat stress, dairy cows

903 Evaluation of injection intervals in a modified ovulation-synchronization protocol in dairy heifers. C. Rose*[1], J. Fuquay[1], A. Moore[1], S. Whisnant[2], A. Williams[1], S. Willard[1], W. Tucker[1], and P. Ryan[1], [1]*Mississippi State University, Mississippi State,* [2]*North Carolina State University, Raleigh.*

Our objectives were to: 1) determine the most effective injection intervals for the administration of GnRH and $PGF_{2\alpha}$ to induce and synchronize ovulation of the dominant follicle in dairy heifers and, 2) assess the influence of the different injection intervals on subsequent fertility in response to timed-AI. Heifers (n=40) were synchronized on d 0 with a single GnRH injection (Cystorelin, 100 μg) then divided into two groups. Heifers were treated with $PGF_{2\alpha}$ (Lutalyse, 25 mg) either on d 7 or d 12 followed by a second dose of GnRH (100 μg) 48 h later. All heifers were bred by AI 16 h after the last GnRH injection. Ultrasonography was performed daily on 9 heifers from each group to monitor follicular dynamics and blood was collected from an additional six per group for LH analysis. All heifers had blood collected on Days 3, 6, 12, 21 and 45 post-AI for P_4 analysis. Peak LH concentrations of 15.3 $\pm$ 1.9 and 13.5 $\pm$ 2.2 ng/ml obtained 60 min after GnRH treatment were not different (d 7 vs d 12, respectively). Total LH concentrations were 49.5 $\pm$ 5.2 and 47.0 $\pm$ 7.6 ng/ml, and mean dominant follicle size after the second GnRH injection was 15.1 $\pm$ 1.9 and 17.0 $\pm$ 1.8 mm and did not differ for d 7 and d 12 heifers, respectively. Compared with d 12 heifers, d 7 heifers had higher conception rates (27% vs 7%) and reduced incidence of behavioral estrus (58.8% vs 87.5%) within 18 days post-insemination. Of the d 12 heifers scanned, 4 of 9 had persistent follicles. In addition, serum P_4 concentrations 21 d post-AI were higher (4.8 vs 0.7 ng/ml) for d 12 than for d 7 non-pregnant heifers. These data suggest that extending the interval between the initial GnRH and $PGF_{2\alpha}$ injections (12 vs 7 days) failed to reduce the variability in response of dairy heifers to synchronization of ovulation. [Supported by Mississippi Agricultural and Forestry Experiment Station]

Key Words: Dairy heifers, Ovulation-synchronization, Injection intervals

904 Concentrations of progesterone in lactating dairy cows with ovarian follicular cysts. I. Formation of cysts during the early postpartum period. W.J. Silvia*, L.F. Laranja da Fonseca, and S.H. Hayes, *University of Kentucky, Lexington*.

Progestational treatments that maintain concentrations of progesterone at an intermediate level (1 ng/ml) have been shown to inhibit the preovulatory surge of luteinizing hormone (LH) but not the tonic, pulsatile secretion of LH. This results in formation of a persistent follicle that does not ovulate. The objective of this experiment was to determine if an intermediate level of progesterone is associated with the formation of ovarian follicular cysts during the early postpartum period. Twenty-nine lactating Holstein cows were sampled for at least 20 days beginning 9-21 days postpartum. Ovaries were examined three times weekly by transrectal ultrasonography to monitor follicular growth and development. Venous plasma samples were collected daily for quantification of progesterone. Cows were classified into 3 groups based on the development of the first dominant follicle formed. Group 1 consisted of cows in which the follicle ovulated (n=11). Group 2 consisted of cows in which the follicle reached preovulatory size but failed to ovulate and underwent atresia (n=3). Group 3 consisted of cows that formed ovarian follicular cysts (follicle reached preovulatory size, failed to ovulate and persisted for at least 6 days; n=5). The concentrations of progesterone during the 7 days prior to ovulation or attainment of preovulatory size were compared among groups. Concentrations of progesterone were very low (at or below 0.1 ng/ml) in both Groups 1 and 2. In contrast, two of the five cows that formed ovarian follicular cysts (Group 3) had concentrations of progesterone that ranged from 0.1 to 1.0 ng/ml throughout the 7 day period prior to attainment of preovulatory size. The other three cows that formed cysts had concentrations of progesterone that were low and similar to the concentrations observed in Groups 1 and 2. Concentrations of progesterone increased to greater than 0.1 ng/ml in one of these cystic cows 5 days after the cyst was formed. In conclusion, an intermediate concentration of progesterone (0.1 to 1 ng/ml) is sometimes associated with the formation of ovarian follicular cysts but not with normal ovulation or normal turnover of follicles. (supported by the KY Agricultural Experiment Station and KABA/Select Sires)

Key Words: Cyst, Progesterone, Follicle

905 Concentrations of progesterone in lactating dairy cows with ovarian follicular cysts. II. Relationship to subsequent follicular development. T.B. Hatler*, D.W. Yelton, S.H. Hayes, A.M. Nugent, and W.J. Silvia, *University of Kentucky, Lexington*.

The first objective of this experiment was to determine the peripheral concentration of progesterone (P) in cows diagnosed with ovarian follicular cysts. Nonpregnant, lactating Holstein and Jersey cows were palpated per rectum at 10-day intervals as part of routine reproduction management. Cows suspected of having ovarian cysts were examined by transrectal ultrasonography for verification (follicle diameter > 20 mm, no luteal tissue). Following verification, cows (n=32) were examined by transrectal ultrasonography three times weekly and venous blood samples were collected daily for quantification of P. Concentrations of P at the onset of sampling were classified as low (<0.1 ng/ml), intermediate (0.1 to 1.0 ng/ml) or high (1.0 to 2.0 ng/ml). The percentages of cows with low, intermediate and high concentrations of P were 25%, 66% and 9%, respectively. Thus, 75% of follicular cysts are associated with intermediate or high concentrations of P. The second objective of this experiment was to determine the fate of follicles that reached preovulatory size (20 mm) in the presence of ovarian follicular cysts and to relate that to concentrations of P. Follicles were classified, retrospectively, as either ovulating (n=21) or failing to ovulate (n=36; either forming a cyst (persistant for at least 6 days; n=31) or undergoing normal follicular turnover (n=5)). Concentrations of P during the 7-day period prior to ovulation or attainment of preovulatory size were examined. Follicles that ovulated were associated with either of two patterns of P during the period. Some cows (n=13) had intermediate or high concentrations of P initially, then declined to 0.1 ng/ml or below for at least 1 sample prior to ovulation. Other cows (n=8) were low throughout. Follicles that failed to ovulate were associated with either of two patterns. Some (n=20 follicles observed in 11 cows) had intermediate or high concentrations of P throughout the 7 day period prior to attainment of preovulatory size. Others (n=17 follicles observed in 3 cows) had low concentrations of P throughout. Thus, intermediate or high concentrations of P were frequently associated with ovulation failure. (supported by the KY Agricultural Experiment Station and KABA/Select Sires)

Key Words: Cyst, Progesterone, Follicle

906 Effect of ergotamine on plasma metabolite concentrations in norgestomet-treated cows. R. Browning, Jr.*, *Tennessee State University, Nashville*.

The influence of ergotamine, a tall fescue ergopeptine alkaloid, on metabolic intermediates was assessed in six cycling Holstein F_1 cows (31 to 33 mo of age). On two occasions, paired cows were injected i.m. with 25 mg of $PGF_{2\alpha}$ and treated with a s.c. ear implant containing 6 mg of norgestomet. Ten day after implant insertion, plasma was sampled every 20 min for 1 h before an i.v. bolus of ergotamine tartrate (20 μg/kg body weight; ET) or saline vehicle (SV) and for 7 h after treatment. Mean ambient temperature was 31.6°C (SD = 2.2) and relative humidity averaged 50.1% (SD = 14.6) during data collection. Each heifer received one treatment per synchronized period and both treatments during the study; 6 wk separated the estrous synchronization periods. Treatment x time affected ($P < .001$) plasma concentrations of glucose, triglyceride, and total cholesterol and tended ($P = .09$) to influence urea nitrogen. All means were separated by protected LSD procedure at an alpha level of 1%. Glucose increased from 71.7 ± 3.1 mg/dL before ET to 107.3 ± 6 mg/dL 2 h after ET, but was unchanged after SV. Triglyceride increased from 27.3 ± .8 mg/dL before ET to 35.5 ± .9 mg/dL 1 h after ET, then declined to 16.3 ± .5 mg/dL by 7 h after ET, but was unchanged after SV. Total cholesterol for ET cows continuously increased from 114.9 ± 1.7 mg/dL before treatment to 122.6 and 134.9 ± 1.9 mg/dL by 2 and 6 h, respectively, after treatment. Plasma cholesterol increased gradually in SV cows from 113.4 ± 1.7 mg/dL before treatment to 121.7 ± 1.9 mg/dL by 6 h after treatment. From 2 to 7 h post-treatment, ET cows had higher ($P < .001$) total cholesterol concentrations than SV cows. Urea nitrogen increased from 10.7 ± .2 mg/dL before ET to 11.5 ± .2 mg/dL from 4 to 7 h after ET, but was unchanged after SV. Results indicate that ergotamine can alter plasma concentrations of intermediates that are involved in nutrient metabolism of cattle.

Key Words: Cows, Ergotamine, Metabolites

907 Evaluation of pregnancy rates in lactating dairy cows using two systematic breeding protocols for first and second service. B. G. Dransfield, R. L. Nebel*, J. H. Bame, and D. A. Henderson, *Virginia Polytechnic Institute and State University, Blacksburg*.

Six hundred and nine Holstein cows from 19 commercial dairy herds were used to evaluate pregnancy rates obtained following two systematic breeding protocols. Cows were assigned randomly at each location to either timed AI (TAI) consisting of 25 mg of $PGF_{2\alpha}$ (d -23) followed by 100 μg GnRH (d -9) then 25 mg of $PGF_{2\alpha}$ (d -2) and 100 μg GnRH (d 0) with AI 6 to 18 h later or Modified Targeted Breeding (MTB) that eliminated the second GnRH administration on d 0 and included periodic visual observation and AI following the detection of estrus or timed AI at 72 to 80 h if estrus was not detected. Initial $PGF_{2\alpha}$ administration occurred at approximately 45 DIM. If a cow was determined not to be pregnant during bi-monthly uterine palpation the initial protocol was repeated eliminating the first $PGF_{2\alpha}$ administration. There was no detectable difference in days to first AI for TAI (71.9 d) and MTB (73 d). First service pregnancy rates for TAI and MTB protocols differed across herds with a mean of 25.9% for MTB and 30.8% for TAI with a herd X protocol interaction ($P < 0.05$). Pregnancy rates for the MTB protocol increased if cows were detected in estrus (50%) versus cows bred on appointment (15.5%). Second service pregnancy rates improved for both protocols to 34.6% for MTB and 38.7% for TAI. Month of AI did influence pregnancy rates for both first and second service ($P < 0.01$). Pregnancy rates were highest for MTB in herds that had excellent estrus detection. However, TAI had an overall higher pregnancy rate with the elimination of estrus detection.

Month	Pregnancy rate first service (%) MTB	TAI
May	27.3	37.9
June	30.4	36.8
July	21.2	15.6
August	22.2	27.8
September	32.7	30.2
October	18.5	42.9

Key Words: Systematic breeding, Dairy cattle, Pregnancy rates

908 Comparison of modified target breeding and presynch-timed artificial insemination at first insemination postpartum. E. Jordan*[1], M. Schouten[2], J. Quast[3], A. Belschner[4], and M. Tomaszewski[1], [1]*Texas A&M University, College Station,* [2]*Hico, TX,* [3]*Comanche, TX,* [4]*Pharmacia & Upjohn, Kalamazoo, MI.*

A field trial was conducted on a large Central Texas dairy to evaluate the effectiveness of two synchronization regimes on first service pregnancy rate. For 11 months cows were grouped based on week of calving and assigned to one of two synchronized insemination groups. Cows on both groups received an injection of $PGF_{2\alpha}$ 21 ±3 days prior to the conclusion of the 60 day voluntary waiting period, followed by GnRH 14 days later and a second $PGF_{2\alpha}$ injection 7 days after the GnRH injection. During the two days following the second $PGF_{2\alpha}$ injection, cows in the Modified Target Breeding (MTB) treatment were inseminated based on signs of estrus. Any cow not inseminated by 72 hours after the second $PGF_{2\alpha}$ injection was inseminated at 72 hours. In the Presynch-Timed Artificial Insemination (Pre-TAI) treatment, cows were given a second GnRH injection 48 hours after the second $PGF_{2\alpha}$ injection and were inseminated 24 hours later. Injections, inseminations and estrous detection occurred in the morning throughout the trial, due to the potential impact of heat stress during the summer and for labor management reasons. During the trial 566 cows were assigned to MTB and 486 cows to Pre-TAI. Lactation number, purchased or raised, calving ease score, multiple births, abortions, gender of calf, retained placentas, mastitis prior to 70 DIM, whether the technician defined the cow in estrus at insemination (Estrus-B), number of estruses detected prior to insemination, season of calving, season of insemination, days dry, and previous gestation length were recorded. One hundred percent of the cows were inseminated in both treatments. The GENMOD Procedure in SAS was used to analyze the data. The first service pregnancy rate was 30.9% and 28.6% for MTB and Pre-TAI, respectively. Treatment group, Estrus-B, season, multiple births, retained placenta and mastitis prior to 70 DIM significantly ($p<.05$) impacted conception. Initiating a lactation with an abortion, calving ease score, lactation number, purchased or raised and the gender of the calf were not significant. Treatment X Estrus-B was the only significant interaction ($p<.01$).

Key Words: Modified Target Breeding, Estrous Synchronization, Pregnancy Rate

909 Evaluation of two timed artificial insemination (TAI) protocols for reproductive management of lactating dairy cows in grazing-based dairy systems. M. C. Cordoba* and P. M. Fricke, *University of Wisconsin, Madison.*

Lactating dairy cows (n=165) from two grazing-based dairies were randomly but unequally assigned to one of three groups to receive either Ovsynch (50 μg GnRH, Day -10; 25 mg $PGF_{2\alpha}$, Day -3; 50 μg GnRH, Day -1) and TAI on Day 0 (Group 1), a modified Ovsynch and TAI similar to Group 1 but with the addition of 25 mg $PGF_{2\alpha}$ 12 d before initiation of Ovsynch (Day -22; Group 2), or standard reproductive management (natural service and/or AI at estrus beginning on Day 0; Control). Ovulatory response at 48 h after the second GnRH injection (synchronization rate), conception rate after TAI at Day 32, and cumulative pregnancy rate at Day 32 and 60 was determined using transrectal ultrasound. Blood samples collected on Day -22, -21, and -11 were classified based on serum progesterone (P) concentrations as High (≥1 ng/ml) or Low (<1 ng/ml). Luteolysis (High P on Day -22, Low P on Day -21) was greater ($p<0.01$) for cows in Group 2 (86.4%; 19/22) compared with Group 1 (0%; 0/18). Synchronization rate (84.2%, 48/57 vs. 78.3%, 47/60), conception rate at Day 32 (43.4%, 23/53 vs. 41.0%, 25/61) and pregnancy rate at Day 60 (57.1%; 32/56 vs. 51.7% 30/58) was similar for cows in Group 1 and 2, respectively. The proportion of anestrus cows (Low P on Day -22 and -11) was similar for Group 1 (28.8%, 15/52) and 2 (26.7%, 16/60), and conception rate at Day 32 was similar (p=0.19) for cycling (45.6%, 36/79) and anestrus (33.3%, 10/30) cows receiving TAI. Cumulative pregnancy rate was greater ($p<0.01$) for cows receiving TAI (42.1%, 48/114) compared with Control cows (15.2%, 7/46) at Day 32 but did not differ at Day 60 (54.4%, 62/114 vs. 62.2%, 28/45 for TAI vs. Control, respectively). Synchronization of ovulation to initiate TAI at the onset of the breeding season resulted in earlier establishment of pregnancy compared with standard reproductive management. Administration of $PGF_{2\alpha}$ 12 d before initiation of Ovsynch did not improve synchronization, conception, or pregnancy rate compared with Ovsynch. *Supported by Hatch project WIS04222*

Key Words: Ovsynch, Timed AI, Grazing

910 Use of estradiol cypionate for timed insemination. F.L. Lopes*, D.R. Arnold, J. Williams, S.M. Pancarci, M-J. Thatcher, M. Drost, and W.W. Thatcher, *University of Florida, Gainesville.*

Four experiments (Exp) evaluated use of estradiol (E_2) cypionate (ECP) for timed insemination in dairy cattle. All animals were synchronized with injection of GnRH (100 μg) on d 0, feeding melengestrol acetate (MGA) on d 1 - 6 (0.5 mg/d) and injection of $PGF_{2\alpha}$ on d 7. Exp 1 examined plasma E_2 concentrations and ovulation after injection of 0 (n = 7), 0.5 (n = 7), 1 (n = 8) or 2 (n = 7) mg ECP on d 8. Blood was collected twice daily and ovulation time determined by ultrasound. Peak concentrations of E_2 occurred 1 - 1.5 d after ECP and differed between groups (12 pg/ml, 0 mg ECP < 25 pg/ml, 0.5 and 1.0 mg ECP < 35 pg/ml, 2 mg ECP; $P < 0.01$). Concentrations of E_2 differed at 4 d after ovulation (4 pg/ml, 0 ECP < 7 pg/ml, 0.5 and 1.0 mg ECP < 12 pg/ml, 2 mg ECP; $P < 0.01$). Ovulation time was shorter and better synchronized for 0.5 and 1 mg ECP than for 0 and 2 mg ECP groups (63 ± 5, 60 ± 2 versus 83 ± 13, 81 ± 6 h; $P < 0.01$). In Exp 2, plasma LH was characterized after ECP (1 mg, n = 6; 24 h after $PGF_{2\alpha}$) versus GnRH (100 μg, n = 6; 48 h after $PGF_{2\alpha}$). Blood was collected every 2 h for 50 h (ECP) or at 15 - 30 min intervals from -1 to 6 h (GnRH). After ovulation, cows were bled daily until next ovulation, and first wave follicular dynamics examined at 1, 3, 5, 7 d. ECP treated cows had a longer LH surge (10 h > 4 h; $P < 0.01$) and no differences were detected in plasma progesterone or follicular dynamics. In Exp 3, ovulation in 14 heifers treated with 0.5 mg of ECP occurred at 62 ± 2 h after ECP. In Exp 4, heifers (n = 158) were assigned to control or ECP timed insemination groups in August. Following synchronization (GnRH, MGA, $PGF_{2\alpha}$), control heifers were inseminated at estrus, and ECP treated heifers were injected with 0.5 mg ECP on d 8 and timed inseminated 48 h later. Pregnancy rates (PR) did not differ between control (39.2%) and ECP treated (39.2%) heifers. In conclusion, ECP in multiparous dry cows (1 mg) and heifers (0.5 mg) was effective to induce a LH surge, a synchronized ovulation with a subsequent normal luteal phase, and normal PR in heifers.

Key Words: Timed Insemination

911 Stage of cycle, incidence and timing of ovulation, and pregnancy rates in dairy cattle after three timed breeding protocols. J.A. Cartmill*[1], S.Z. El-Zarkouny[1], B.A. Hensley[1], G.C. Lamb[2], and J.S. Stevenson[1], [1]*Kansas State University, Manhattan,* [2]*University of Minnesota, Grand Rapids.*

In Exp. 1, estrous cycles in dairy cows (n = 705) were synchronized prior to timed AI. The first treatment ($PGF_{2\alpha}$+OVS) consisted of one injection of $PGF_{2\alpha}$ given 12 d (d -22) before initiating the Ovsynch (OVS) protocol (GnRH given 7 d before [d -10] and 48 h [d -1] after $PGF_{2\alpha}$ [d -3]) with the object to increase the percentage of cows in their early luteal phase before the onset of the OVS protocol (d -10). We compared the latter two protocols to a third protocol in which cows were given two injections of $PGF_{2\alpha}$12 d apart (2×PG12; d -15 and -3), followed 48 h later by an injection of GnRH (d -1). In all three protocols, one timed insemination (d 0) was made 16 to 20 h after the single or second injection of GnRH (d -1). Blood was collected on d -22, -15, -10, -3, and -1 before hormone or placebo injections and assayed for progesterone. More ($P < 0.05$) cows treated in the $PGF_{2\alpha}$+OVS (36%) or 2×PG12 (29%) protocols were in their early luteal phase (d 5 to 9) at the onset of the OVS protocol than cows in the OVS protocol (18%). Cows in their first lactation had greater ($P < 0.01$) pregnancy rates 28 d after insemination than cows in their second or greater (L2+) lactation (41 vs. 32%). Cows (L2+) had greater ($P = 0.08$) pregnancy

rates in the $PGF_{2\alpha}$+OVS (42%) treatment than their contemporaries in the OVS (28%) and 2×PG12 (27%) treatments. Embryo survival (67 to 74%) between d 28 and d 38-58 of pregnancy depended on cycling status of cows before treatment. In Exp. 2, follicle diameter in a sub-group of cows from each treatment was determined via ultrasonography prior to GnRH injection (48 h after $PGF_{2\alpha}$) and between 32 and 48 h after GnRH or until ovulation occurred. Mean preovulatory follicle diameters were larger ($P < 0.05$) in 27 spontaneously ovulating controls and in the 2×PG12 cows and less ($P < 0.05$) variable in diameter in controls than in all treated cows. More ($P < 0.05$) control cows (93%) ovulated by 32 h than cows in the OVS (55%), PG+OVS (64%), and 2×PG12 (56%) treatments. We concluded that a presynchronizing injection of $PGF_{2\alpha}$ given 12 d before OVS increased pregnancy rates in older cows.

Key Words: Timed AI, Ovulation synchronization, Embryonic survival

912 Reproductive characteristics of dairy cows following early nonpregnant diagnosis by ultrasonography on days 27 to 29 after AI and subsequent treatments with $PGF_{2\alpha}$ and(or) GnRH. J.S. Stevenson*, J.A. Cartmill, B.A. Hensley, and S.Z. El-Zarkouny, *Kansas State University, Manhattan*.

Our objective was to determine the feasibility of prompt reinsemination of dairy cows once diagnosed not pregnant 27 to 29 d after AI when a first-wave dominant follicle was present that could ovulate after precocious luteal regression occurred in response to $PGF_{2\alpha}$. Cows diagnosed not pregnant by ultrasonography on either 27, 28, or 29 d after AI (corresponding to d 6, 7, or 8 of the estrous cycle for cows not detected in estrus after first insemination [assuming a 21-d estrous cycle]) were treated with 25 mg of $PGF_{2\alpha}$. Approximately half of the cows were then treated with 100 μg of GnRH 48 h later and inseminated 16 to 20 h after GnRH (PG+GnRH; n = 147). Cows treated only with $PGF_{2\alpha}$ were inseminated 8 to 16 h after detected estrus (PG; n = 120). For comparisons of reproductive characteristics, 390 control cows were reinseminated after nonpregnant diagnosis based on detection of natural estrus ±14 d of reinsemination dates of treated cows. Blood samples were collected at the time of pregnancy diagnosis in nearly all cows and again 48 h later in fewer cows. Treated cows (30 ± 1 d) were re-inseminated earlier ($P < 0.05$) after last AI than controls (40 ± 2 d) but conception rates were not different: 27.4, 25.0, and 24.5% in control, PG, and PG+GnRH cows, respectively. Open cows treated 27 d after AI (85 ± 6) had more ($P < 0.05$) days from treatment to conception than cows treated on d 28 or 29 (58 ± 10). More ($P < 0.05$) control than treated cows (61 vs. 19%) were eventually culled from the herds. Just over 70% of the cows had elevated (≥1 ng/mL) concentrations of progesterone before treatment and 80% of cows given $PGF_{2\alpha}$ were synchronized based on decreased concentrations of progesterone by 48 h after $PGF_{2\alpha}$. We conclude that treating nonpregnant cows with $PGF_{2\alpha}$, GnRH, or both on d 27 to 29 after insemination produced conception rates equal to that of controls. Cows treated on d 28 or 29 conceived earlier than those treated on d 27 probably due to poorer rates of $PGF_{2\alpha}$-induced luteolysis on d 27.

Key Words: Early Pregnancy Diagnosis, Resynchronization, GnRH

913 Progesterone increases pregnancy rates and embryo survival in lactating dairy cows. S.Z. El-Zarkouny*, J.A. Cartmill, B.A. Hensley, and J.S. Stevenson, *Kansas State University, Manhattan*.

Our objective was to investigate the effect of progesterone (P4) on pregnancy rates and embryo survival in cows treated with P4 concurrent with the Ovsynch protocol. Lactating Holstein cows were assigned randomly to three treatments: 1) Ovsynch (OVS; n=91; GnRH given 7 d before [d -10] and 48 h [d -1] after $PGF_{2\alpha}$ [d -3]); 2) OVS+CIDR (n=90; same as OVS with an intravaginal CIDR insert containing 1.9 g of P4 in situ for 7 d [d -10 to -3]); and 3) control (n=80). Controls received no treatment but had HeatWatch® patches attached to their rumps containing pressure-sensitive radiotelemetric transmitters to enable detection of estrus. Cows in the first two treatments were inseminated 17 to 19 h after the second GnRH injection (d 0), whereas controls were inseminated 8 to 16 h after detected estrus. Blood samples were collected on d -20 and prior to each hormone treatment (d -10, -3, and -1) for determination of P4. Pregnancy status was verified on d 28 and 56 after AI by ultrasonography of uterine contents and by palpation once between 40 and 53 d. Concentrations of P4 on d -20 and -10 revealed that only 44.4% of the cows had at least one elevated (≥1 ng/mL) P4 sample and were considered to be cycling. Only 58, 67, and 36% of the OVS+CIDR, OVS, and controls had elevated concentrations of P4 at the time of $PGF_{2\alpha}$ injection. Diameter of the preovulatory follicle was greater ($P < 0.05$) on d -1 in older cows treated with the CIDR (17.4 ± .6 mm) than in older cows (15 ± .6 mm) in the OVS protocol, whereas no differences were detected among first-lactation cows. Over 80% of the treated cows ovulated by 48 h after GnRH. Pregnancy rates on d 28 (58 vs. 36%) and d 56 (44 vs. 20%) were greater ($P < 0.05$) in the OVS+CIDR than in the OVS cows, respectively, indicating greater ($P < 0.05$) embryo survival (75.6%) due to P4 treatment than in OVS alone (56.1%). At palpation, pregnancy rates were 25% in controls, 39% in OVS+CIDR, and 21% in OVS cows. We concluded that P4 treatment in conjunction with the OVS protocol improved pregnancy rates in high-producing dairy cows despite <50% of the cows cycling at the onset of treatments.

Key Words: Progesterone, Pregnancy Rate, Ovsynch Protocol

914 An estrous synchronization field study comparing estradiol benzoate (EB) and GnRH in combination with an intravaginal progesterone insert (CIDR) for timed-AI in crossbred *Bos indicus* cows. J. K. Fullenwider, J. R. Kempfer, C. L. Barnett, G. E. Portillo, C. R. Barthle, and J. V. Yelich*, *University of Florida*.

Two estrous synchronization experiments were conducted in crossbred *Bos indicus* cows to compare pregnancy rates of two timed-AI protocols. In both experiments, cows were body condition scored (BCS; scale 1-9) and received 2 mg of EB on d 0 of a 7 d CIDR (EAZI-BREED™ CIDR®) and 25 mg of PG on d 7. Cows were randomly allotted to one of two treatments: 1) 1 mg EB 24h after CIDR removal (CEB) and 2) 100μg GnRH (Cystorelin) at AI (CGnRH). All cows were AI 48-54 h after CIDR removal. Semen from multiple sires were equally allotted to each treatment and randomly assigned within a treatment. Pregnancy was determined by ultrasonography 50-60 d after AI. In experiment 1, dry cows at four locations were used. Pregnancy rates were similar between CEB (126/267 = 47.2%) and CGnRH (131/281 = 46.6%) and not affected ($P > .10$) by location, BCS or technician. Pregnancy rates were influenced by sire ($P < .001$) but there was no sire by treatment ($P > .10$) effect. Sire pregnancy rates were: A) 14/56 = 25.0%; B) 18/56 = 32.1%; C) 22/51 = 43.1%; D) 25/58 = 43.1%; E) 20/46 = 43.5%; F) 27/55 = 49.1%; G) 28/55 = 50.9%; H) 34/59 = 57.6%; I) 31/52 = 59.6%; J) 35/57 = 61.4%. In experiment 2, 387 postpartum-lactating cows were used with 48 h calf removal initiated on d 7. Cows were managed in two age groups, ≤ 3 (BCS = 4.3; n = 125) and ≥ 4 (BCS = 5.0; n = 262). Overall, pregnancy rates were greater ($P < .05$) for CEB (54.0%) than CGnRH (43.9%). Pregnancy rates tended ($P < .06$) to be influenced by sire and there was a treatment by sire effect ($P < .01$). Pregnancy rates were greater ($P < .05$) in the ≥ 4 age group for CEB (61.4%) than CGnRH (46.6%) but similar ($P > .05$) in the ≤ 3 age group ($P > .05$) for CEB (40.0%) than CGnRH (38.3%). In conclusion, timed-AI protocols were effective in lactating and dry crossbred *Bos indicus* cows although effectiveness may be limited by semen fertility.

Key Words: *Bos indicus*, Estradiol Benzoate, GnRH

915 Effect of dosage of gonadotropin-releasing hormone (GnRH) in an estrus synchronization protocol on conception rates to a fixed-time insemination in postpartum beef cows. D.L. Funk* and L.H. Anderson, *University of Kentucky, Lexington*.

A major limitation to the implementation of estrus synchronization systems which include GnRH is the high expense of GnRH. Previous research has suggested that 50 μg of GnRH will induce ovulation of a dominant follicle as effectively as 100 μg in yearling dairy heifers. The objective of this experiment was to determine whether decreasing the dosage of GnRH from 100 to 50 μg in the CO-Synch estrus synchronization protocol would alter pregnancy rates to a fixed-time insemination in postpartum beef cows. Postpartum cows (n = 494) at 5 locations were randomly assigned by calving date and age to receive either 50 or 100 μg of GnRH on D 0. Because a percentage of postpartum cows will show estrus on D 6 or 7, cows were observed for estrus on these days. Cows showing estrus on D 6 or 7 were inseminated 12 hours after observed estrus and received no further treatment. On D 7, to regress both the initial and any accessory corpus luteum, the remaining cows received 25 mg of prostaglandin $F_2\alpha$ (Lutalyse; Pharmacia & UpJohn, Kalamazoo, MI). On D 9, cows were administered a second injection of the same dose of GnRH and artificially inseminated. Beginning on D 23, all cows

were exposed to a 45-day natural breeding season. Date of conception was estimated using transrectal ultrasonography on approximately D 90. Conception rates to the fixed-time insemination were significantly lower ($P < .05$) for cows receiving 50 μg GnRH (32%; 76/239) than those cows receiving 100 μg GnRH (42%; 106/255). Although treatments varied by location ($P < .05$), no treatment by location interaction was observed ($P > .10$). We conclude that 50 μg GnRH is likely inadequate to synchronize estrus and ovulation and therefore reduces the conception rates of postpartum beef cows to a fixed-time insemination.

Key Words: GnRH, Estrus Synchronization, Cattle

916 Addition of GnRH or an intravaginal progesterone insert to a GnRH-PGF synchronization system to enhance response to timed breeding in postpartum beef cows.

S.K. Johnson*[1], D.E. Grum[2], and M.L. Day[2], [1]*Kansas State University, Colby,* [2]*The Ohio State University, Columbus.*

The objective of this study was to determine if the responsiveness of postpartum (pp) beef cows to a GnRH-PGF synchronization system for timed AI could be improved by addition of a progestin or an additional injection of GnRH. Postpartum beef cows (n=1035) from two herds (10 groups) were assigned by calving date and age to one of four treatments. All cows received an injection of $PGF_2\alpha$ (25 mg, i.m.) on d 0 and GnRH (100 ug, i.m.) on d 2 at timed insemination. Treatments were as follows: 1) GnRH on d -7 (GnRH-PGF); 2) GnRH on d -14 and d -7 (+GnRH); 3) GnRH on d -7 and an intravaginal progesterone insert (CIDR) from d -7 to 0 (GnRH/CIDR); 4) CIDR from d -7 to 0 (CIDR). On d 0, cows averaged 71 d pp. Pregnancy was diagnosed by transrectal ultrasound between 75 and 50 d following timed AI. Cow age, service sire, AI technician and the treatment by location interaction were not significant for any of the variables tested and were removed from the statistical analyses. Pregnancy rate to timed AI was lower ($P<.05$) in CIDR (39%) compared to GnRH-PGF (47%). Pregnancy rates in +GnRH (50%) and GnRH/CIDR (45%) were not different from GnRH-PGF. Cows less than 60 d pp on d 0 had a lower ($P<.05$) pregnancy rate (39%) compared to cows 60-80 d pp (50%) or cows > 80 d pp (47%). For cows < 60 d pp (n=297), pregnancy rate was lower ($P<.05$) for GnRH-PGF cows compared to +GnRH (36% vs 53%, respectively) and did not differ from GnRH/CIDR and CIDR. Ovarian status (cyclic vs anestrus) prior to the start of treatments (determined in fifty 2- yr-old cows and 98 later calving cows in one herd) did not influence response to treatments. An additional injection of GnRH one week prior to the GnRH-PGF treatment improved pregnancy rates to timed AI in cows less than 60 d pp at breeding. The addition of a CIDR to the GnRH-PGF system did not influence pregnancy rates, whereas removal of the initial injection of GnRH on d -7 reduced pregnancy rates in the timed AI program evaluated.

Key Words: Timed insemination, GnRH, Progestin

917 Effect of an orally active progestin on follicular dynamics in cycling and anestrous postpartum beef cows.

G. A. Perry*[1], F. N. Kojima[1], B. E. Salfen[1], J. F. Bader[1], D. J. Patterson[1], and M. F. Smith[1], [1]*Department of Animal Science, University of Missouri, Columbia.*

Melengestrol acetate (MGA) is an orally active progestin that is widely used for synchronizing estrus in beef cattle. Although treatment of cycling cows with low concentrations of a progestin (i.e. MGA) results in formation of persistent follicles in the absence of corpora lutea, it is not known whether persistent follicles form in anestrous cows in response to a similar treatment. The objective of this experiment was to determine the effect of MGA on follicular dynamics in anestrous postpartum beef cows. Treatment groups included the following: anestrous control (AC), anestrous MGA (AM), and cycling MGA (CM; positive control). Angus-crossbred cows were assigned to treatment by age and days postpartum. Cows were fed carrier (AC group) or 0.5 mg MGA·hd^{-1}·d^{-1} (AM and CM groups) for 14 d beginning approximately 38 d postpartum. Cows allotted to the CM group were injected with $PGF_{2\alpha}$ on the first day of MGA treatment to induce luteolysis. The preceding treatment (CM) results in formation of persistent follicles and secretion of elevated concentrations of estradiol. Ovaries of each cow were examined daily by transrectal ultrasonography beginning 5 d preceding the initiation of feeding MGA or carrier until ovulation or 7 d following MGA feeding. There was no difference among groups in the stage of follicular wave or diameter of the largest follicle at the start of carrier or MGA feeding. The length of the follicular wave present at the start of MGA was greater ($P<.02$) for cows in the CM (14.5 d) group compared to the AM (9.4 d) or AC (9.7 d) groups. Maximum follicular diameter was greater ($P<.02$) for the CM (18.9 mm) group than the AM (15.8 mm) or AC (16.0 mm) groups. Circulating concentrations of estradiol were also increased ($P<.05$) in the CM group compared to the AM or AC groups. In summary, MGA treatment did not increase the duration of the follicular wave, maximum follicular diameter, or secretion of estradiol in anestrous postpartum cows.

Key Words: Progestin, Follicle, Beef Cows

918 Inclusion of an intravaginal progesterone insert plus GnRH and prostaglandin $F_2\alpha$ for ovulation control in postpartum suckled beef cows.

G. C. Lamb*[1], J. S. Stevenson[2], D. J. Kesler[3], H. A. Garverick[4], D. R. Brown[1], and B. E. Salfen[4], [1]*North Central Research and Outreach Center, University of Minnesota, Grand Rapids,* [2]*Department of Animal Sciences and Industry, Kansas State University, Manhattan,* [3]*Department of Animal Sciences, University of Illinois, Urbana,* [4]*Department of Animal Sciences, University of Missouri, Columbia.*

Four experiment stations (IL, KS, MN, and MO) conducted an experiment to determine the effects of introducing an intravaginal progesterone insert (IPI; CIDR-B, InterAg, Hamilton, NZ) into a synchronization system for postpartum suckled beef cows. Five hundred and forty-four cows were assigned randomly to two synchronization treatments: 1) 100 μg of GnRH (i.m.) followed in 7 d with 25 mg of $PGF_2\alpha$, followed by a second injection of GnRH and one fixed time insemination (Cosynch; n = 279); or 2) Cosynch plus one IPI during the 7 d between the first injection of GnRH and $PGF_2\alpha$ (Cosynch + IPI; n = 265). Most cows were inseminated at the second GnRH injection (n = 460), but 84 cows were inseminated 16 to 18 h after that injection at one station. Blood samples were collected at d -17, -7, 0, and +2 relative to $PGF_2\alpha$ to determine concentrations of progesterone. Ultrasonography was used to determine the presence of a fetus at 30 to 35 d after insemination. Pregnancy rates tended ($P = .13$) to be greater for Cosynch + IPI (58%) than for Cosynch (48%) treated cows. No station × treatment interaction occurred; however, MO (63%) and KS (60%) had greater ($P < .05$) pregnancy rates than IL (48%) and MN (44%). Cows in greater body condition at the onset of the breeding season experienced improved ($P < .001$) overall pregnancy rates. Pregnancy rates for cows that calved >50 d before the onset of the breeding season were greater ($P < 0.01$) than those which calved ≤50 d. Thus, treatment of suckled cows with Cosynch yields acceptable pregnancy rates; addition of an IPI may improve overall pregnancy rates. Body condition and days postpartum at initiation of the breeding season affect overall efficacy of the Cosynch and Cosynch + IPI protocols.

Key Words: Beef Cows, Estrous Synchronization

919 Improved synchronization of estrus in postpartum suckled beef cows with a progestin-GnRH-prostaglandin $F_{2\alpha}$ (PG) protocol.

D. J. Patterson*, S. L. Wood, F. N. Kojima, and M. F. Smith, *Univeristy of Missouri, Columbia.*

This experiment was conducted to determine whether treatment with the oral progestin, melengestrol acetate (MGA), prior to GnRH and PG would improve estrous response and synchrony of estrus in postpartum suckled beef cows compared to cows synchronized with GnRH and PG. Multiparous Angus crossbred cows (n=110) were assigned by age, body condition score (BCS) and days postpartum (dpp) to one of two treatments. Cows were fed a ground corn supplement for 14 d (1.8 kg·hd^{-1}·d^{-1}) with or without MGA (0.5 mg·hd^{-1}·d^{-1}). GnRH (100 μg Cystorelin®) was administered to all cows 10 d after MGA or carrier withdrawal and 7 d before PG. Mean BCS and dpp for MGA and control cows at the initiation of treatment was 5.1 and 5.0, and 44 and 42 dpp, respectively. Blood samples were collected from all cows 10 d before and on the first day of MGA or carrier, and 7 d before and on the day PG was administered. Concentrations of P_4 in serum at the initiation of treatment were elevated (>1 ng/ml) in 49% of the MGA and 43% of the control cows. Cows were observed for signs of behavioral estrus for 7 d after PG and inseminated 12 h after observed estrus. Estrous response was higher ($P<.09$) among MGA treated cows compared with controls [89% (50/56), and 77% (42/54), respectively]. However, there was no difference between treatments in the total number of cows that exhibited estrus during the peak synchronized period from 48 to 96 h

after PG (MGA-77%, controls-68%). Synchronized conception (SCR:no. pregnant/no. inseminated) and pregnancy rate (SPR:no. pregnant/no. treated) did not differ between treatments [SCR=78% (39/50), 76% (32/42); SPR=70% (39/56), 59% (32/54) for MGA and control cows, respectively]. In summary, this sequential approach to estrous cycle control of postpartum suckled beef cows (progestin-GnRH-PG) offers significant potential to effectively synchronize estrus with resulting high fertility.

Key Words: Progestin, Postpartum cow, Estrus synchronization

920 Reduced dose GnRH in Select Synch and CO-Synch protocols to synchronize estrus or ovulation in beef cows. B.W.P. Sasongko[1], J.C. Whittier*[1], T.W. Geary[2], D.N. Schutz[1], E.R. Downing[1], P. Burna[1], R.G. Mortimer[1], and G.E. Seidel, Jr., [1]*Colorado State University, Ft. Collins,* [2]*Livestock and Range Research Center, Miles City, MT.*

Two studies were conducted using half dose (50μg) GnRH injections with Select Synch and CO-Synch synchronization protocols in beef cows. In Trial 1 (Select Synch), primiparous (P1) and multiparous (Pn) crossbred cows at two locations (L1, n = 241; L2, n = 334) received 50 μg or 100 μg GnRH on d 0 of the study. On d 7, all cows received 25 mg of PGF2α. Estrous detection and am/pm AI took place from d 7 to d 12; clean-up bulls were turned in on d 13. Location had no effect on number of cows observed in estrus, therefore data were pooled across location. There was no difference due to treatment (Trt) in synchronization rate (P=.96; 100 μg = 54.9% (173/315), 50 μg = 55.1% (171/292)). AI conception rate determined by ultrasound 45 d after AI at L2, was not affected by the Trt (P=.22; 100 μg = 59.6% (55/94), 50 μg = 50.5% (47/93)). In Trial 2 (CO-Synch), cows received either 50 μg or 100 μg GnRH on d 0 and d 9, and 25 mg PGF2α on d 7. At L3, 204 P1 and Pn Angus cows were assigned to one of four Trt in a 2x2 factorial with day and dose as factors. (i.e. 50:50, 50:100, 100:50, 100:100). At L4, 98 Pn cows received 100 μg or 50 μg of GnRH on both d 0 and d 9 (i.e. 50:50 or 100:100). Calves were removed from d 7 to d 9, cows were mated by am/pm AI up to 48 h post PGF2α, then cows not observed in estrus were AI mated. Clean-up bulls went in 14 d after timed AI. Transrectal ultrasonography was used to determine pregnancy at 40 d after timed AI at L4 and 61 d at L3. Location did not affect synchronization or AI pregnancy rates; therefore data were pooled. There was no difference in synchronization rate between Trt. Dose reductions of GnRH had no effect on AI pregnancy rate (P=.65; 100:100 = 46.2% (48/104), 100:50 = 42.3% (22/52), 50:100 = 49.0% (24/49), 50:50 = 39.2% (38/97)). We conclude that 50 μg of GnRH was sufficient to induce fertile estrus and ovulation in beef cows in both synchronization protocols.

Key Words: Synchronized Breeding, GnRH Dose, Beef Cows

921 Effects of estradiol benzoate (EB) on ovulation of newly emerged and mature dominant ovarian follicles in prepubertal heifers. C.R. Burke*[1,2], M.L. Mussard[1], and M.L. Day[1], [1]*The Ohio State University, Columbus,* [2]*Dairying Research Corporation, Hamilton, New Zealand.*.

Prepubertal heifers were used in a 2 x 2 factorial design to test the hypotheses that: I) an injection of estradiol benzoate (EB) 24 h after withdrawal of an intravaginal progesterone insert (CIDR) will increase the incidence of a detected estrus independent of maturity of the dominant follicle (DF); but that, II) newly emerged DF will not ovulate despite estrus having been induced with EB. Prepubertal beef heifers (n=31) weighing 323 ± 5 kg (mean ± SEM) and 306 ± 6 d of age each received a CIDR and an i.m. injection of 1 mg EB/500 kg BW. Daily ultrasonography detected the emergence of a new follicle wave 3.1 ± .1 d after treatment initiation. Allocation to the 4 treatment groups was balanced for day of new emergence. The CIDR was removed 1.3 ± .1 d (Young DF; n=15) or 4.2 ± .1 d (Mature DF; n=16) after emergence of the new follicle wave, and heifers within each group received either an injection of EB (.75 mg/500 kg BW) 24 h after CIDR withdrawal, or no further treatment. Expression of behavioral estrus was monitored by twice daily observations. Diameter of DF at the time of CIDR withdrawal was greater (p<.01) in heifers of the Mature DF (9.1 ± .4 mm) than the Young DF Group (6.7 ± .2 mm). Most heifers receiving EB 24 h after progesterone withdrawal were detected in estrus (15/16) and ovulated (12/15). Maturity of the DF did not affect (P>.1) these responses. Age of the DF at ovulation was 3.6 ± .2 d and 6.4 ± .2 d for heifers in Young DF and Mature DF groups, respectively (p<.01). Among animals that did not receive EB, a single heifer ovulated and none were detected in estrus within 5 d of CIDR withdrawal. We conclude that EB is effective in inducing estrus, and ovulation of newly emerged or mature DF in prepubertal heifers previously treated with progesterone and EB.

Key Words: Puberty, Cattle, Estradiol

922 Evaluation of four selective media for isolation of catalase-negative gram-positive cocci from bulk tank milk. B. Jayarao*, S. Pillai, and A. Sawant, *Pennsylvania State University, University Park.*

Four selective media (Edward's agar, TKTFC agar, COEBA agar, and Streptosel Agar) were tested on three separate occasions with a battery of gram positive (cocci and bacilli) and gram negative bacteria. Based on experimental inoculation, it was inferred that COEBA agar was highly selective for Streptococci. Growth of organisms belonging to the genera Lactococcus, Enterococcus and Aerococcus were not inhibited by the media, however the colony size was considerably smaller as compared to that on regular 5% sheep blood agar plates. All of the 9 Staphylococcal organisms and gram positive bacilli tested were completely inhibited by COEBA agar. Further, when farm bulk tank samples (n = 105) were examined for Streptococci using COEBA agar, the medium permitted growth of catalase negative bacteria belonging to the genera Streptococcus, Enterococcus, Lactococcus and Aerococcus. Growth of Staphylococci, gram-positive bacilli, and gram-negative bacteria present in bulk tank milk were completely inhibited on COEBA agar. Results of the study suggest that although COEBA agar does not exclusively select for Streptococci, COEBA agar does give a differential count of catalase-negative gram-positive cocci, many of which are useful indicators of milk hygiene and milking practices followed on the farm. It is recommended that COEBA agar can be used for isolation of catalase-negative gram-positive cocci from bulk tank milk.

Key Words: COEBA agar, Streptococci, bulk tank milk

923 Physiological responses of Holstein cows to bovine somatotropin (bST) treatments during the transition period. M. S. Gulay*[1], A. Garcia-Gavidia[2], C. J. Wilcox[1], J. M. Hayen[1], and H. H. Head[1], [1]*University of Florida, Gainesville,* [2]*Universidad del Zulia, Maracaibo, Venezuela.*

Objectives were to determine effects of linear doses of bST prepartum and postpartum on DMI, BCS, BW, MY and some metabolic and lactogenic hormones and P_4 in plasma collected thrice weekly during experiment. Associations among metabolic hormones, IGF-I, P_4 and postpartum production and reproductive activity were evaluated. Twenty-three Holstein cows were assigned randomly to four groups (7, 5, 6, and 5 cows/group). Treatments included prepartum and postpartum biweekly injections of bST (I-IV; 0, 6, 12, or 18 mg bST/d) and evaluation of DMI, BCS, BW, MY and metabolic hormones and metabolites. Mathematical models included main effects of treatment, calving month, the 2-way interaction, and cow nested in treatment-calving month. Data were divided into prepartum and postpartum sets. Prepartum bST treatments for I to IV, respectively, resulted in higher mean concentrations of ST (4.0±0.8, 3.5±0.9, 15.1±0.8, and 16.0±1.2 ng/ml, P<0.06), PRL (19.8±2.5, 40.1±2.8, 31.4±2.2, and 25.8±3.9 ng/ml, P<0.09), and IGF-I (109.2±4.4, 153.7±5.2, 158.4±5.1, and 185.6±6.9 ng/ml, P<0.07). Postpartum treatments (12 and 18 mg bST/d) resulted in increased concentrations of ST (6.2± 0.5, 4.4± 0.6, 18.3±0.5, and 20.1±0.8 ng/ml, P<0.005), IGF-I (73.9±2.1, 69.9±2.6, 85.5±2.2 and 83.0±4.4 ng/ml, P<0.09), T_3 (524.7±14.7, 503.3±17.5, 598.6±16.2 and 992.0±21.7 pg/ml, P<0.08) and numerically greater MY (30.93, 31.23, 32.03, and 34.93 kg/d). Treatment affected mean DMI during early postpartum period (0-21d; P<0.08). Cows in groups III and IV maintained BCS better (P<0.025) than groups I and II. Treatment group IV had numerically higher mean plasma glucose concentrations. Early postpartum treatments (d 7 to d 21) increased mean plasma concentrations of NEFA for high doses of bST (III and IV vs I and II; 342.1 ±9.3 vs 241.4 ±8.2 μeq/ml). No effects of bST treatment were observed on plasma concentrations of INS, P_4, plasma protein or hematocrit. No adverse effects of bST treatment were observed in cows during either the prepartum or postpartum periods.

Key Words: bST, Transition, Lactation

924 Responses of Holstein cows to prepartum and postpartum injections of bovine somatotropin (bST). A. Garcia-Gavidia[2], M. S. Gulay*[1], M.J. Hayen[1], C.J. Wilcox[1], T.I. Belloso[1], and H.H. Head[1], [1]*University of Florida, Gainesville,* [2]*Universidad delZulia, Maracaibo, Venezuela.*

Objectives were to evaluate effects of bST treatment prepartum and/or postpartum and postpartum diets on DMI, energy status, BCS, BW, MY, metabolic and lactogenic hormones, and P_4 in plasma. Four to 5 wk prepartum 48 Holstein cows were assigned to two groups of 24 cows each. Prepartum treatments were bST (∼6 mg bST/d) or no bST beginning 3 wk before expected calving. Postpartum the experiment was arranged as a 2x2x2 factorial considering bST prepartum and postpartum and two postpartum diets (0 and 15% whole cottonseeds). Pre- and postpartum data were analyzed separately. Mathematical model for prepartum period included effects of bST, cow in treatment, and days to cubic order polynomial. For data from calving to 65 d postpartum the model included prepartum and postpartum bST treatment, postpartum diet, appropriate 2 and 3-way interactions, cow nested in treatment groups, effects of BW and BCS, and days to cubic order polynomial. Associations among metabolic and lactogenic hormones, IGF-I, P_4, and postpartum MY were evaluated. For prepartum period, mean DMI for groups did not differ (∼15kg/d) but decreased in both groups ∼35% (backslash) during week before calving. Energy status was positive and not different due to treatment (2.04 and 2.40 Mcal/d for bST and no bST groups). Mean concentrations of ST and glucose were greater ($P<0.002$ and $P<0.03$) in bST-treated cows. No effects of bST on mean concentrations of PRL, INS, T_4, T_3, IGF-I were detected during the prepartum period, but concentrations of IGF-I and INS decreased as parturition approached. For the postpartum period, no significant effects of prepartum bST, diet or their interaction were detected on DMI, MY, energy status or concentrations of ST, INS, or IGF-I during first 65 d of lactation. Postpartum bST treatment increased ST concentration ∼2-fold vs uninjected. Ovarian activity, measured by accumulated P_4 during 65 d postpartum, did not differ due to treatment or diet. MY was numerically greater (39.27 vs 37.69 kg/d) in cows treated with bST postpartum and also for cows fed whole cottonseed diet.

Key Words: Transition, bST, Lactation

925 Effect of fan and sprinkler configuration upon summer milk production of cows housed in 2-row freestall barns. M.J. Brouk*, J.F. Smith, J.P. Harner III, and J.E. Shirley, *Kansas State University, Manhattan.*

One hundred fifty-nine Holstein cows (66 primaparous and 93 multiparous) were blocked by parity, milk production and DIM then assigned to one of three cooling systems installed in 2-row freestall barns. Barn one (F&S) was equipped with a row of five 122 cm fans mounted every 12 m over the freestalls and a row of ten 91 cm fans mounted every 6 m over the feedline. Barn two (S) was equipped with five 122 cm fans mounted every 12 m over the freestalls. Both barn one and two were equipped with a feedline sprinkler that delivered 95 l of water in 3 min during a 15 min cycle. Sprinklers cycled every 15 min when the temperature exceeded 24°C. Barn 3 (S+) was equipped with five 122 cm fans mounted every 12 m over the freestalls and a sprinkler line on both the feedline and rear alley. The sprinkler operation was the same as barns one and two except 132 l of water were applied during each cycle. Fans in all treatments operated when temperatures exceeded 21°C. Cows were milked 2x and production for a 24-hour period was recorded every two weeks during the study. Pen intakes were recorded daily and body condition score was determined at the start and end of the study. Respiration rates were recorded on three separate days during heat stress. Temperature and humidity data were recorded continuously in each barn throughout the study. Average milk production during the 85 day trial was similar ($P>.05$) for all treatments (36.6, 36.4, and 35.1 kg/cow/day for F&S, S and S+ treatments, respectively). Primaparous and multiparous animals did not differ ($P>.05$) in milk production response to the systems. Afternoon respiration rates did not differ among treatments (83.2, 82.8 and 80.0 breaths/min, respectively). Cows housed in 2-row freestall barns did not produce more milk when fans were installed over the feedline in addition to freestall fans. Increasing sprinkler area did not increase milk production in 2-row freestall barns.

Key Words: Heat Stress, Cow Cooling, Dairy Facilities

926 Effect of fan configuration on summer milk production of dairy cows housed in a 4-row freestall barn. M.J. Brouk, J.F. Smith, J.P. Harner III, and J.E. Shirley, *Kansas State University, Manhattan.*

Ninety-three multiparous Holstein cows averaging 130 DIM at the start of the study were housed in a 4-row freestall barn. Cows were blocked by parity and production and allotted to one of three cooling treatments. Treatment one (2S) was a double row of 91 cm fans, spaced 7.3 m apart over the freestalls. Treatment two (F&S) was a single row of 91 cm fans, spaced 7.3 m apart over the freestalls and feedline. Treatment three (F&2S) was a double row of 91 cm fans spaced 7.3 m apart over the freestalls and a single row of 91 cm fans, spaced 7.3 m apart over the feedline. Fans operated when air temperatures exceeded 21°C. All three treatments had feedline sprinkler systems that delivered 189 l of water during a 3 min of a 15 min cycle. Sprinklers cycled every 15 min when air temperature exceeded 24°C. Cows were milked 3x and milk weights recorded for a 24-hour period every two weeks throughout the 85 day trial. Daily pen feed intake was also recorded. Respiration rates were observed 3 times during the trial and body condition scores were evaluated at the beginning and end of the trial. Temperature and humidity in each pen and outside the barn were recorded continuously throughout the trial. Cows cooled with treatment F&S produced more ($P<.05$) milk (44.8 vs 42.6 kg/c/d) than 2S while F&2S (43.8 kg/c/d) was intermediate and similar ($P>.05$) to both. Afternoon respiration rates (83.9, 72.3 and 70.6 breaths/min, respectively) were greater ($P<.05$) for cows cooled with 2S compared to the other treatments. In a 4-row freestall barn equipped with a feedline sprinkler system, installing fans over the feedline and over the freestalls increased milk production when compared to fans just over the stalls.

Key Words: Heat Stress, Cow Cooling, Dairy Facilties

927 Endocrine changes associated with the effect of nutrition on postpartum anestrus and reconception in dairy cows. G. Luna-Pinto* and P.B. Cronje, *University of Pretoria, Gaugteng, South Africa.*

The aim of this study was to determine whether plasma concentrations of IGF-1, IGFBP-3, leptin or glucose were associated with postpartum reconception in Fresian cows fed at two different planes of nutrition. Multiparous Fresian cows were fed one of two diets: 80 % or 120 % of requirements from calving until conception. Blood samples were collected weekly until conception. Cows fed at 120 % of requirements were heavier ($P < 0.05$) than cows fed at 80 % of requirements at the time of conception. Cows fed at 80 % of requirements lost 119 g/d body weight ($P < 0.05$) and 26 % of initial body condition score ($P < 0.01$) from week 2 to conception. Nutritional treatment influenced the interval from calving to conception ($P < 0.05$). Cows fed at 80 % of requirements expressed estrus and became pregnant at 118 +/- 11 d postpartum and cows fed at 120 % of requirements at 48 +/- 10 d. Neither level of nutrition nor sampling period had any effect ($P > 0.05$) on plasma concentration of IGF-1 (51.1 +/- 6.68 ng/ml) or glucose (3.8 +/- 0.3 mmol/l). Plasma IGFBP-3 concentration increased from week 2 to conception for both treatments ($P < 0.01$), but concentrations in cows fed at 120 % of requirements were higher than those fed at 80 % of requirements in both instances ($P < 0.01$). Plasma IGFBP-3 concentration increased from week 2 to conception by 133 % in cows fed at 120 % of requirements and 85 % in cows fed at 80 % of requirements. At week 2, cows fed at 120 % of requirements had 85 % greater plasma IGFBP-3 concentrations than cows at 80 % of requirements. At conception, plasma IGFBP-3 concentrations were 115 % greater in cows fed at 120 % of requirements than those fed at 80 % of requirements. There were no differences in plasma leptin concentrations between dietary treatments ($P > 0.05$), but concentrations were higher at conception ($P < 0.05$) than at week 2 postpartum. This study demonstrates that plasma IGFBP-3 concentration can be used as a predictor of days open in cows fed different diets.

Key Words: Reconception, Insulin-like growth factor system, Leptin

928 Effect of fat on plasma hormones and metabolites in early lactation grazing dairy cows. G.F. Schroeder*[1], D. Becu-Villalobos[2], I. Lacau-Mengido[2], and G.A. Gagliostro[3], [1]*CONICET- Fac. Cs. Agrarias UNMdP,* [2]*IBYME-CONICET,* [3]*INTA EEA Balcarce, Argentina.*

Thirty-seven multiparous Holstein cows were studied during the first 70 days of lactation in a complete randomized design with three treatments: 0 kg (T0, 12 cows), 0.5 kg (T0.5, 12 cows) and 1 kg (T1, 13 cows) of dried oil (30% C16:0 and 60% C18:0). Fat was added to a basal concentrate (5 kg/d ground corn, 0.4 kg/d fish meal and 20 g/d chloride calcium) starting 2 weeks before the expected calving date. Cows grazed a spring pasture (1.98±0.82 tDM/ha, 30±8 kgDM/cow/d) with 24.4% DM, 38.3% NDF, 23.4% CP and 73.2% IVDMD. Jugular blood was sampled on days 15, 30, 45 and 60 of lactation. Plasma immunoreactive growth hormone (GH), insulin (INS) and insulin-like growth factor-I (IGF-I) were analyzed at days 30 and 60 postcalving. On day 34, jugular blood samples were taken before and 15 min after an intravenous injection of isoproterenol (ISO). On day 37, samples were taken before and 15, 30 and 60 min after INS injection. Glucose and NEFA responses were evaluated. Treatment x time of sampling interaction was not detected for metabolite concentrations (P≥.1). Glucose, plasma urea nitrogen and NEFA were not affected by fat. NEFA decresed gradually as lactation advanced. Plasma triglycerides were lower (T1= 271a, T0.5= 292ab and T0= 318b mg/dl, P≤.05) and total cholesterol higher (T1= 236a, T0.5= 218ab and T0= 185b mg/dl, P≤.02) in T1 compared to control. Responses to ISO or INS injection were not affected by treatments. Average plasma hormone concentrations were not changed by fat (P≥.1). Treatment x time of sampling interaction was not detected and plasma GH and IGF-I concentration increased whereas INS decreased (P ≤.05) as lactation advanced. In early lactation, saturated fat did not reduce circulating NEFA, responsiveness of adipose tissue to lipolytic stimulation and the hypoglycemic or antilipolytic action of INS. Concentrations of NEFA and hormones were affected by lactation stage but not by saturated fat feeding.

Key Words: Fat supplementation, Early lactation, Metabolites and hormones

929 Effect of level of feed intake on plasma progesterone concentrations in deslorelin-implanted dairy cows treated with a CIDR device. A.R. Rabiee*[1], K.L. Macmillan[1], and F. Schwarzenberger[2], [1]*University of Melbourne, Melbourne, Australia,* [2]*University of Veterinary Medicine, Venna, Austria.*

The amount of feed, frequency of feeding and diet composition may influence concentrations of plasma progesterone (P4) by altering the metabolic rate rather than P4 production rate. In the present study, an external source of P4 was administrated to investigate the effect of the level of feeding on P4 concentrations. Twelve non-lactating Holstein-Friesian cows, 4-9 years old were randomly allocated to a restricted or ad libitum group. The ad libitum group had unrestricted access to graze irrigated pasture, whereas the restricted group had access for only 2h/day. Each animal was drenched orally twice daily with a chromic oxide capsule to allow daily feed intake to be estimated from fecal output. Endogenous P4 production was eliminated by subcutanously implanting a capsule containing 6mg of a potent GnRH agonist (deslorelin) into the ear of each animal 3 weeks before inserting a CIDR device containing 1.9g P4 into the vagina. Each device was removed after 11 days and residual P4 measured. Plasma from blood samples taken daily was assayed for P4. The average daily dry matter intake of pasture was higher for cows in the ad libitum group (15.9 vs 6.3 kg DM, P<0.01) but their plasma P4 concentrations were lower (1.08 vs 1.71 ng/ml; p<0.05) even though the residual P4 content of the used CIDR devices was not affected by feed intake (1.20 vs 1.25g; P>0.3). Plasma P4 concentrations in blood samples taken the day before device insertion or following removal were below the sensitivity of the assay. These results show that there was a negative relationship between feed intake and plasma P4 concentrations in these CIDR-treated GnRH downregulated Holstein cows.

Key Words: Progesterone, Feed intake

930 Effect of level of feed intake on the concentration and yield of fecal progesterone metabolites in deslorelin-implanted dairy cows treated with a CIDR device. A.R. Rabiee*[1], K.L. Macmillan[1], and F. Schwarzenberger[2], [1]*University of Melbourne, Melbourne, Australia,* [2]*University of Veterinary Medicine, Vienna, Austria.*

Progesterone is rapidly metabolized by the liver then excreted into the bile and voided in the feces. In the present study, an external source of progesterone (P4) was administrated to investigate the effect of level of feeding on the concentrations and daily yield of fecal P4 metabolites (FP4M). Twelve non-lactating Holstein-Friesian cows, 4-9 years old were randomly allocated to a restricted or ad libitum group. The ad libitum group had unrestricted access to graze pasture, whereas the restricted group had access for only 2h/day. Each animal was drenched orally twice daily with a chromic oxide capsule to allow daily feed intake to be estimated from fecal output. Endogenous P4 production was eliminated by subcutanously implanting a capsule containing 6mg of a potent GnRH agonist (deslorelin) into the ear of each animal 3 weeks before inserting a CIDR device containing 1.9g P4 into the vagina. Each device was removed after 11 days and residual P4 content measured. Fecal samples were taken daily and assayed for pregnanes containing a 20-oxo- a 20α- or a 20β-OH group with EIAs. The average daily dry matter intake of pasture and fecal output were higher for cows in the ad libitum group (feed intake:15.9 vs 6.3, fecal output: 5.4 vs 2.5 kg DM; P<0.01). The residual P4 content of the used CIDR devices was not affected by feed intake (1.20 vs 1.25g; P>0.3). The concentrations of FP4M were not affected by level of feed intake (20-oxo-: 3.3 vs 1.7, 20α-: 3.5 vs 3.7, 20β-: 2.1 vs 3.2 mg/ g DM; P>0.05). Daily yields of 20-oxo- and 20α- were higher in ad libitum cows (20-oxo-: 17.8 vs 4.3; P<0.05, 18.2 vs 8.9 mg/day; P<0.001) whereas daily yield of fecal 20β- was not affected by feed intake (11.9 vs 8.6 mg/day; P>0.5). These data showed that FP4M concentrations were not affected by level of feed intake or faecal output, but the volume of feces altered the daily excretion rate of FP4M.

Key Words: Fecal progesterone metabolite, Feed intake

931 Liver blood flow and steroid metabolism are increased by both acute feeding and hypertrophy of the digestive tract. S. Sangsritavong*, D.K. Combs, R.F. Sartori, and M.C. Wiltbank, *University of Wisconsin, Madison.*

Steroid metabolism primarily occurs in the liver. We hypothesized that changes in liver blood flow are due to acute feed consumption or growth of the digestive tract that could produce important alterations in steroid metabolism. To test this hypothesis, liver blood flow and steroid metabolism were evaluated in 2 experiments. Exp.1 evaluated the acute (0-6 h post feeding) effect of feeding in high producing lactating cows. Exp.2 evaluated the chronic effect of high feed consumption by comparing weight-matched lactating (high feed consumption) and dry (low feed consumption) cows. In both experiments liver blood flow was measured by continuous infusion of bromosulfothalein (BSP), which is specifically metabolized in the liver. Steroid metabolism was measured by continuous infusion of progesterone and estradiol 17β. Stable steady state serum concentration of all three compounds were achieved within 0.5 h of infusion. The steady state concentration was used to calculate the clearance rate for each compound. Acute feeding increase liver blood flow (BSP clearance) from 1035 to 1637 l/h (158%) by 4 h after feeding (n=2). In contrast, liver blood flow decreased (83% of control) when cows were left unfed during the same time. In Exp.2, lactating (n=4) and dry (n=3) cows had liver blood flow and steroid metabolism measured on 3 consecutive days at 3-5 h after feeding. Although variability between cows was substantial (CV=13.5%in lactating cows and 27.7% in dry cows) the variability between days for an individual cow was relatively low (CV=4.8%in lactating cows and 5.5% in dry cows). Lactating cows had greater (p<0.01) liver blood flow (1184±69 vs.756±120 l/h) and lower serum concentration of progesterone (2.3±0.13 vs. 3.55±0.25 ng/ml) and estradiol (265±2.7 vs. 352±6.8 pg/ml). Thus liver blood flow and concomitant steroid metabolism is acutely (>50% within 4 hour) and chronically (>50% higher in high feed intake cows) increased by feeding. Decreases in reproductive efficiency in high producing dairy cows may be mediated by high steroid metabolism due to high feed consumption and elevated liver blood flow.

Key Words: Liver blood flow, Steroid metabolism, Reproductive efficiency

932 Effect of conjugated linoleic acids (CLA) on parameters of adipose tissue metabolism in the lactating dairy cow. L.H. Baumgard*, B.A. Corl, S.S. Block, D.A. Dwyer, Y.R. Boisclair, and D.E. Bauman, *Cornell University, Ithaca, NY.*

CLA have profound impact on lipid metabolism. Feeding CLA at 1-2% of the diet dramatically reduced body fat content in growing mice, rats and pigs. Effects are also observed in lactating dairy cows where CLA at 10 to 20-fold lower concentrations markedly reduces milk fat synthesis. This reduction in milk fat is specific for the *trans*-10,*cis*-12 CLA isomer and shifts milk fat composition toward greater proportions of long chain fatty acids. The mechanism(s) by which CLA affects lipid metabolism is not clear, but postulated effects have included increased lipolysis. Our objectives were to evaluate the effects of CLA isomers on circulating concentrations of nonesterified fatty acids (NEFA) and the lipolytic response to a β-adrenergic challenge. We also evaluated circulating leptin given the reported role of this signal in storage and use of energy. Three multiparous Holstein cows (183 ± 8 DIM) were utilized in a 3x3 Latin square design. Treatments consisted of 5-d abomasal infusion of 1) control, 4L of skim milk/d, 2) 9,11 CLA, 10 g/d of *cis*-9,*trans*-11 CLA isomer and 3) 10,12 CLA, 10 g/d of *trans*-10,*cis*-12 CLA isomer. Basal concentrations of NEFA were determined on d 4. Epinephrine challenges (1.4 μg/kg body weight) were intravenously administered at 9:00 am and 3:00 pm on d4. Plasma samples (n=19) were obtained from a jugular catheter and area under the NEFA response curve (0 to 30 min, AUC) was quantified after correcting for basal concentrations. Basal NEFA concentrations (139 μmol/l) were not altered by treatment. NEFA response to epinephrine challenge was effected by treatment being reduced by 39% for the 10,12 CLA as compared to the other treatments($P<0.05$). Treatments had no effect on plasma leptin concentrations which averaged 3.1 ng/ml. Overall, data demonstrate that the *trans*-10,*cis*-12 CLA isomer which caused milk fat depression had no chronic effect on NEFA concentrations (basal lipolysis) or circulating leptin concentrations, but did give a modest reduction in the lipolytic response to an epinephrine challenge.

Key Words: CLA, NEFA

933 Acute nutritional restriction alters endocrine function and causes anovulation in beef heifers. F.J. White*, C.A. Lents, L.N. Floyd, L.J. Spicer, and R.P. Wettemann, *Oklahoma Agricultural Experiment Station, Stillwater.*

Effect of acute nutritional deprivation on ovarian luteal function and plasma concentrations of glucose, insulin, IGF-I, NEFA, and thyroxine was determined in 14 mo old Angus x Hereford heifers (BCS=5.7; 317 ± 2 kg BW). Heifers (n=19) were housed in individual pens in a barn and fed a diet supplying 1.2 x maintenance (1.2 M) for 1 wk to allow adaptation. Heifers were then randomly allotted on d 0 to either a diet supplying .4 x maintenance (.4 M) or 1.2 M. Heifers were treated with PGF on d -10, 0, and 10. Blood plasma was collected via tail venipuncture on alternate days. Heifers with plasma progesterone less than .5 ng/mL on d 15 to 21 were classified as anovulatory. Heifers on .4 M (306 ± 2 kg) lost BW ($P < .01$) while 1.2 M heifers (321 ± 2 kg) maintained BW. Seventy percent of .4 M heifers did not ovulate on d 14 while all 1.2 M heifers had normal luteal function. Nutritional restriction for 14 d did not alter plasma glucose or insulin. Heifers on .4 M had less plasma thyroxine (32.3 ± 1.7 ng/mL) than 1.2 M heifers (40.9 ± 1.7 ng/mL; $P < .01$). A day x diet ($P < .01$) effect on plasma NEFA was due to 1.2 M heifers maintaining NEFA concentrations while .4 M heifers had increased concentrations. Nutritional restriction decreased concentrations of plasma IGF-I in .4 M heifers from 50.5 ± 2.3 ng/mL on d 0 to 28.5 ± 2.3 ng/mL on d 14; however, 1.2 M heifers maintained concentrations (54.9 ± 2.3 and 58.4 ± 2.3 ng/mL on d 0 and 14, respectively; day x diet; $P < .01$). Acute nutritional restriction induced anovulation in 70% of heifers within 14 d, and plasma concentrations of NEFA increased while IGF-I decreased.

Key Words: Beef heifer, IGF-I, NEFA

934 Effect of source of Romosinuano germplasm and preweaning creep grazing on postweaning growth and puberty in heifers. C. C. Chase, Jr.*[1], M. J. Williams[1], A. C. Hammond[2], and T. A. Olson[3], [1]*USDA, ARS, Brooksville, FL,* [2]*USDA, ARS, Albany, CA,* [3]*University of Florida, Gainesville.*

Postweaning growth and puberty were determined for Romosinuano heifers from germplasm collected in Costa Rica (CR; n = 17) and Venezuela (VE; n = 34) that 98 d prior to weaning were either allowed to creep graze rhizoma peanut (*Arachis glabrata*; RP; n = 26) or no creep (n = 25). Postweaning, heifers were initially managed as a single group, then as two groups during a 90 d breeding season, and thereafter as a single group until the end of the study. Heifers were maintained on mixed bahiagrass (*Paspalum notatum*) and RP pastures (and hay) and fed 4.5 kg/d concentrate. Growth measurements were collected at the start of the study and at 28-d intervals for 358 d. Beginning three wk before the start of the breeding season and at 7-d intervals thereafter, blood was collected and plasma progesterone determined by EIA to assess puberty. At the start of the study, heifers from CR were older (236 vs 208 d; $P < .01$), heavier (218±7.1 vs 183±5.0 kg; $P < .001$), and taller (113±1.2 vs 110±.8 cm; $P < .05$) than heifers from VE. At the end of the study, heifers from CR were heavier (432±9.1 vs 387±6.4; $P < .001$) and taller (132±1.1 vs 129±.8 cm; $P < .05$) than heifers from VE. During the study, however, neither gain in BW (214±5.9 vs 204±4.2 kg, for CR vs VE, respectively) nor gain in hip height (19.0±1.0 vs 19.5±.7 cm) were affected by source of germplasm. Preweaning treatment did not affect BW or hip height at the start or end of the postweaning study; but non creep calves gained more ($P < .05$) BW than creep calves during the postweaning study (216±.8 vs 202±.8 kg). Age at puberty was similar between heifers from CR (428±8.5 d) and VE (419±6.0 d) and between creep (419±3.3 d) and non creep (427±3.4 d) heifers. Hip height ($P < .10$) and BW ($P < .05$) at puberty were influenced by the interaction of germplasm source x preweaning treatment. This appeared due to taller hip heights and heavier BW observed at puberty for CR-non creep heifers than for any of the other source-treatment combinations. Data from this study suggest that CR and VE heifers have similar postweaning growth rates and ages at puberty and that preweaning creep may depress postweaning gain in heifers but did not affect age at puberty.

Key Words: Heifers, Tropics, Puberty

PRODUCTION AND MANAGEMENT

935 Effects of two winter feeding methods on growth, conception, and cost of developing beef replacement heifers. C. L. Gasser*, E. W. Hawkins, R. W. Silcox, and C. W. Wiltbank, *Brigham Young University, Provo, UT.*

Performance of beef replacement heifers is crucial to a cow-calf operation, but heifer development is a major cost. The objective of this study was to determine whether beef heifers could be developed using low-cost winter pasture without compromising growth and reproductive performance. Crossbred Angus heifers were wintered on pasture residue with supplemented grass hay (P; n=40) or in drylots fed grass hay (D; n=40) for 77 days. Heifer age on d 1 ranged from 7.5 to 9.5 mo. Initial BW for P and D was 268 kg and 266 kg, respectively. Beginning on d 78, all heifers were sorted by weight and fed the same ration to reach breeding weight at d 120. Heifers were weighed on d 1, 23, 44, 65, 79, 107, and 121. Body condition score was determined on d 1 and 44. Blood samples taken on d 121 were assayed for progesterone level. Heifers were synchronized for breeding using MGA in the feed (d 89 to 103) followed by a single Lutalyse^{TM} injection on d 121. Heifers detected in estrus between d 122 and 126 were artificially inseminated using the AM/PM rule. All heifers were exposed to a bull from d 141 to 210. Pregnancy was determined by ultrasound on d 167 and 210. The treatment groups did not differ in BW over the course of the study or in blood progesterone levels. The number of heifers that exhibited estrus and received AI did not differ between treatments (p=0.147). D heifers had a higher conception rate from AI (P=46%; D=73%; p=0.033). However, overall pregnancy rate on d 210 did not differ between treatments (P=86%; D=95%). The overall cost of development was higher for D than P. There is evidence that beef replacement heifers may be developed using low-cost winter pasture without a significant change in overall growth and conception rates. However, to better ensure earlier conception, heifers should be fed a higher quality ration or a better supplement with pasture to avoid weight loss during periods of harsh winter weather.

Key Words: Heifer Development, Conception, Management