810 Plasminogen activation system in milk from Friesian and Jersey cows. F. Fantuz[1], A. Baldi[1], O. Pedron[1], D. Tedesco[1], P. Giorgi*[2], and V. Bontempo[2]*, [1]*Institute of Animal Nutrition, Veterinary Medicine, University of Milan, Italy and* [2]*Dip S.A.V.A., University of Molise (CB), Italy.*

Aim of study was to evaluate the effects of breed and stage of lactation on plasminogen activation system in milk from Friesian and Jersey cows. Correlations between plasminogen activator (PA), plasmin (PL) and plasminogen (PG) and some milk components were also determined. Eight Friesian and 8 Jersey cows, kept in the same herd, were used to provide milk samples throughout lactation (up to 9th month in milk). Breed did not affected PA, PL and PG activities. PG/PL ratio resulted lower (P<0.05) in milk from Friesian cows, suggesting accelerated conversion of PG to PL. PL and PG increased (P<0.05) with advancing lactation, from 3.89 U/ml and 25.54 U/ml to 12.57 U/ml and 60.71 U/ml, respectively for PL (SEM 1.71) and PG (SEM 3.74). PA activity shown the same tendency, from 288,34 U/ml to 514.27 U/ml (P>0.05)(SEM 51.33). As expected, PA activity was positively correlated (P<0.05) with SCC in both Friesian (r=0.39) and Jersey cows (r=0.41). The negative correlation observed between PA activity and PG/PL ratio reached the significant threshold only for milk from Jersey cows (r=-0.33; P<0.05). A significant negative correlation was observed between PA and casein/total protein for Friesian cows (r=-0.32; P<0.05). The latter correlation for Jersey cows was: r=-0.28; P>0.05). It appears that the plasminogen activation system would be a better predictor of cheese-making efficiency for Friesian than Jersey cows.

Item	Friesian	Jersey	SEM
PA, U/ml	398.98	335.71	28.09
PL, U/ml	9.75	9.09	0.90
PG, U/ml	38.75	44.67	2.13
PG/PL	3.97^b	4.91^a	0.59

^{a,b}: Different supescripts indicate significant differences, (P<0.05)

811 Direction of mammary venous circulation and parity of lactating Holstein cows. M. C. Thivierge*[1], D. Petitclerc[2], J. F. Bernier[1], and H. Lapierre[2], [1]*Université Laval, Quebec, Canada,* [2]*Dairy and Swine R & D Centre, Lennoxville Qc Canada.*.

Seven Holstein cows, averaging 28 ± 4 kg milk/d were used to evaluate the effect of parity on valvular competence of the external pudic vein. At least 34 d before the beginning of the experiment, catheters were surgically implanted in the left external pudic artery, left external pudic vein, and the left subcutaneous mammary vein; an ultrasonic flow probe was also placed around the left external pudic artery. In a first study, a pulse dose (15 ml) of para-amino hippuric acid (PAH, 10%) was injected into the external pudic vein of 3 cows. Right after the injection, blood samples were collected simultaneously from both left external pudic artery and mammary vein every 10 s for the first 2 min and then every 20 s during the following 2 min. For cows in their 2nd parity (n = 2), PAH appeared first in the arterial pudic plasma within 20 s and then in the mammary venous plasma 30 s later. For the third cow, in her 3rd parity, PAH injected into the pudic vein appeared first in the mammary vein (15 s) and afterwards was observed in the external pudic artery (15 s later). For the second experiment, an occlusion Fogarty catheter was surgically placed in the external pudic vein of 4 cows. Two treatments were assigned according to a switchback design and consisted of the effect of occlusion of the pudic vein compared with no occlusion on mammary vein plasma PAH concentration. A PAH solution (10%) was infused (2.8 ml/min) into the external pudic artery at least 45 min before the beginning and during the experiment. Samples were taken every 3 min for 15 min (n = 5), during each treatment period. Occlusion of the pudic vein in cows of 2nd parity (n = 2) did not alter PAH concentration in the mammary vein plasma (63.8 ± 2.2 mg/l). In contrast, mammary vein PAH concentration increased (P= 0.03) from 51.5 to 72.7 ± 2.2 mg/l when the pudic vein was occluded in cows of 3rd and 4th parity (n = 2). These results suggest that pudic vein valves may lose their effectiveness and blood flow reverse when parity is greater than 2.

Key Words: Blood Circulation, Mammary Glands, Cows

812 Isotope dilution models for partitioning leucine uptake by the liver and mammary gland of the lactating dairy cow. L. A. Crompton*[1], J. France[1], M. D. Hanigan[2], C. K. Reynolds[1], J. Dijkstra[3], J. A. Maas[1], B. J. Bequette[4], and D. E. Beever[1], [1]*Department of Agriculture, University of Reading, Reading, Berks, UK,* [2]*Purina Mills Inc., St. Louis, MO,* [3]*Animal Nutrition Group, Wageningen Institute of Animal Sciences, Wageningen, The Netherlands,* [4]*Rowett Research Institute, Aberdeen, UK.*

Prediction of dietary nitrogen utilization for milk production in dairy cows relies on an understanding of amino acid (AA) absorption and metabolism by the gut, liver (LIV) and mammary gland (MG). Isotope dilution models for partitioning leucine (LEU) uptake by the LIV and the MG of lactating cows have been constructed assuming steady state and used to resolve *in vivo* stable isotope data. The models require measurement of blood flow rate across the tissue, LEU, α-ketoisocaproate (KIC) and CO_2 concentrations and plateau isotope enrichments in the arterial supply, venous drainage and plateau enrichments (LEU and KIC) in the tissue intracellular pool, during a constant infusion of [^{13}C]- LEU tracer. With limited assumptions, solution of the models allows calculation of the rates of LEU uptake, release, oxidation and transamination, and LEU fluxes representing the synthesis and degradation of constitutive (CON) and export (EX) proteins. Using the model solutions and literature values for organ protein mass and LEU content, total (CON+EX) protein, fractional synthesis rates (FSR) were estimated for LIV and MG. LEU kinetics were measured in Holstein-Friesian cows during mid-lactation (cow average: 600 kg BW; 16 kg/d DMI (60:40 concn:grass silage, 135 gCP/kg DM); 24 kg/d milk). Total LIV protein FSRs ranged from 23–55%/d, with assumed export protein contributing between 9–21% of total protein synthesis (TPS) and for the MG, total protein FSRs ranged from 39–53%/d, with measured export protein (milk) contributing between 60–88% of TPS. Both models, when applied to other AA with similar metabolic fates in the LIV and the MG, could provide estimates of the impact of LIV metabolism on AA availability to peripheral tissues and the partition of AA between milk protein synthesis and other metabolic fates in the MG.

Key Words: Isotpe Dilution, Leucine, Dairy Cow

813 Responses in mammary gland metabolism of lysine in lactating goats infused with lysine and methionine. S. J. Mabjeesh[1]*, C. E. Kyle[2], J. C. MacRae[2], and B. J. Bequette[2], [1]*Department of Animal Science, The Faculty of Agriculture, Rehovot, Israel;* [2]*Rowett Research Institute, Aberdeen, UK.*

An experiment was conducted to examine the response of lactating goats given a forage concentrate diet including soyabean meal and fishmeal (16% crude protein) to an intravenous infusion of lysine and methionine in either early (80±17 DIM) and later (233±14 DIM) lactation. Opportunity was taken to examine the intramammary gland metabolism of lysine using [2-^{15}N] and [1-^{13}C; 6,6-$^2H_2$] lysine tracers. The animals were prepared with pudic artery and mammary vein catheters across one half of the gland and tracer studies were performed on the last day (d5) of a saline (SAL) infusion and an infusion of lysine (3.2 mmol/h) and methionine (0.8 mmol/h). Dry matter intake, milk yield and milk protein output were higher (P< 0.005) in early than in late lactation (116 vs. 92 g/kg $BW^{0.75}$ 4.6 vs. 2.2 kg/d; and 138 vs. 86 g/d, respectively), but amino acid infusion had no effect on milk yield or milk protein output at either stage. Although whole body lysine flux (15.7 vs. 11.8 and 11.5 vs. 7.9 mmol/h in early and late lactation, respectively) and plasma lysine concentration (+60% and +80% in early and late lactation, respectively) increased (P < 0.005) on amino acid infusion, net extraction of plasma lysine across the gland did not change significantly. Furthermore, fractional oxidation rate of lysine by the gland increased (P < 0.005) from 0.17 to 0.31 at both stages of lactation. These are thought to be the first data reporting substantial oxidation of lysine by the lactating mammary gland. The fact that this oxidation increased (P < 0.005), but milk protein output remained unchanged as lysine and methionine availability was increased indicates that the gland partitioned the extra lysine into catabolic rather than anabolic processes, suggesting that methionine and lysine were not limiting milk protein synthesis on this particular ration.

Key Words: Mammary Gland, Lysine, Metabolism

814 Responses in mammary leucine metabolism and blood flow due to unilateral frequent versus incomplete milking of the mammary glands of the goat. B. J. Bequette*[1], C. E. Kyle[1], and V. Volpe[2], [1]*Rowett Research Institute, Aberdeen, Scotland.*, [2]*Universiti' degli Studi di Udine, Italy.*.

The efficiency of milk removal from the mammary glands alters milk production. To delineate the mechanisms of this response, changes in leucine metabolism and blood flow (BF) across each udder half of goats were examined in response to unilateral milking frequency. Mid to late lactation goats (n=4) had the vascular systems between the udder halves separated and flow probes fitted around each external pudic artery. The following milking regimes were imposed on each goat in three consecutive periods (5-d each): 1) both glands milked twice daily (CON1), 2) one gland milked eight times (8X) and the other once incompletely (1X, 70% of CON1 milk removed) daily (UNI) and 3) both glands milked twice daily (CON2). On day 5 of CON1 and UNI, arteriovenous [1-^{13}C]leucine kinetics were monitored simultaneously across each gland. Compared to CON1, milk and protein yield increased (+15% and +16%) for the 8X gland and decreased (−30% and −27%) for the 1X gland, and for both glands milk production returned to CON1 levels in CON2. Similarly, BF (recorded 24 h/d) increased (+19%, $P<0.01$) for the 8X gland but decreased (−22%, $P=0.15$) for the 1X gland, and for both glands BF returned to CON1 values in CON2. Leucine data were covariate adjusted to CON1 to compare effects of 8X and 1X milking. Net uptake and oxidation of leucine (mmoles/h) were higher ($P<0.05$) for the 8X than the 1X gland (2131 vs 1504, 467 vs 340), but milking frequency only tended to affect fractional oxidation rate of leucine (0.198 vs 0.172, $P=0.20$), total mammary protein (mmoles leucine/h) synthesis (PS, 2117 vs 1633, $P=0.16$) and protein turnover (PS:net loss, 1.26 vs 1.38, $P=0.13$). Thus, independent of arterial substrates and hormones, regulation of BF by the mammary gland, rather than changes in amino acid (leucine) metabolism, appears to be a principle mechanism for increasing or decreasing amino acid availability for mammary uptake and utilisation for milk PS when the frequency and extent of milk removal are altered.

Key Words: Mammary Gland, Leucine, Blood Flow

815 Somatotropin augments milk yields of cows induced into lactation. R. S. Kensinger*, R. Graboski, and A. L. Magliaro, *Pennsylvania State University, University Park*.

Significant culling of good cows with low fertility reduces profitability on dairy farms as those cows are replaced with heifers. An improved method to induce open cows into lactation would be useful in this regard. The present study evaluated the efficacy of somatotropin to increase milk production in cows induced into lactation. Cows (n=28) were multiparous open Holsteins given a dry period of 50 d or more, and otherwise healthy. The cows were induced in 6 different groups with 4-6 cows/group. Cows were treated with estradiol-17B (.075 mg/kg BW/d) and progesterone (.25 mg/kg BW/d) for 7 d and milked beginning on d 18. Cows were randomly assigned to either control or bST treatment groups and milk production was compared for 70 d. After 70 d all cows were treated with bST. Cows in bST group produced more milk (28.3 kg/d) than control cows (24.1 kg/d). In spite of bST, milk yields varied significantly from cow to cow. Daily milk yields of cows increased gradually and did not attain peak milk production until 140 days. Evaluation of mammary secretions by gel electrophoresis showed initial colostrum which evolved into mature milk by 7 or 14 d of milking. Mean milk yield for cows completing lactations was 27.1 kg/d for 305 days. Pregnancy was confirmed in 14 of the first 20 cows, with 2.0 services per conception. Most cows remain in the herd, with several in subsequent natural lactations. A reliable method to return problem breeders to the milking herd could improve the financial status of many farms.

Key Words: Somatotropin, Induced, Lactation

816 Effect of Prolactin on in vivo expression of the Bovine Mammary IgG1 Receptor. G. M. Barrington*[1], T. E. Besser[2], C. C. Gay[2], T. B. McFadden[3], and R. M. Akers[4], [1]*Colorado State University, Ft. Collins, CO,* [2]*Washington State University, Pullman, WA,* [3]*AgResearch, Hamilton, New Zealand,* [4]*Virginia Polytechnic Institute, Blacksburg, VA*.

Induction of colostrogenesis in non-pregnant cows was used to evaluate the relationship between prolactin and mammary IgG1 receptor expression. Six of eleven non-pregnant, non-lactating Holstein cattle responded to a standard lactation induction protocol by development of elevated IgG1 concentrations in mammary secretions. In order to increase the diversity in prolactin concentrations, two of the six cattle were treated with bromocriptine, and two others were treated with recombinant bovine prolactin. Serum alpha-lactalbumin, serum prolactin, and mammary secretion IgG1 concentrations were measured throughout the experiment. Biopsies of mammary tissue were collected after induction of lactation, and after treatments to alter serum prolactin. Immunohistochemistry was used to evaluate IgG1 receptor expression. Administration of rbPRL was associated with increased lactogenic activity, decreased secretion IgG1 concentrations, and decreased IgG1 receptor expression. Decreased serum PRL, due to bromocriptine, was associated with decreased lactogenic activity and maintenance of IgG1 receptor expression. Results of this experiment are consistent with an effect of prolactin in decreasing the expression of the bovine mammary IgG1 receptor at the onset of lactogenesis.

Key Words: Prolactin, IGg1 Receptor, Bovine

817 Lactose synthase components in milk: Levels of α-lactalbumin and β1,4 galactosyltransferase in milk of cows from various breeds and at various stages of lactation. G. T. Bleck[1], M. B. Wheeler[1], L. B. Hansen[2], H. Chester-Jones[2], and D. J. Miller*[1], [1]*University of Illinois, Urbana,* [2]*University of Minnesota, St. Paul*.

The objective of this study was to determine if the concentration in milk of the two components of lactose synthase, α-lactalbumin and β1,4 galactosyltransferase, were related to genetic background, stage of lactation, breed or parity. α-Lactalbumin and β1,4 galactosyltransferase levels were measured from a single milk sample. A total of 279 cows were used in the analysis. The cows were from two herds; the University of Illinois herd and the University of Minnesota herd at Waseca. The University of Illinois herd contained Ayrshire, Brown Swiss, Holstein and Jersey cows, while the University of Minnesota herd contained Holsteins of both high and low genetic merit for milk production. $\alpha-$Lactalbumin concentration was higher in Jersey and Ayrshire milk than in the milk of Holsteins and Brown Swiss. However, no difference was observed between high and low genetic merit animals in the Minnesota herd or among different genetic backgrounds in the Illinois herd. β1,4 Galactosyltransferase levels were similar for all groups that were analyzed. α-Lactalbumin levels were positively correlated with milk protein percent, milk fat percent and lactose percent, while the β1,4 galactosyltransferase level in milk exhibited an extremely strong positive linear relationship with number of days in milk. Even though the levels of β1,4 galactosyltransferase were higher as lactation progressed, the values did not show any correlation with persistency of lactation or late lactation milk yield.

Key Words: Milk Proteins

818 Cloning and sequence analysis of a porcine β1,4 galactosyltransferase cDNA. E. A. Landers*, G. T. Bleck, L. A. Howell-Skalla, M. B. Wheeler, and D. J. Miller, *University of Illinois, Urbana*.

β1,4 Galactosyltransferase (GalTase) is a member of the glycosyltransferase family of enzymes that is commonly found in the trans-Golgi complex of the cell. These enzymes synthesize and extend complex oligosaccharides on glycoproteins and glycolipids. Glycosyltransferases are type II membrane proteins with short cytoplasmic domains, a transmembrane domain, a stem region and a catalytic domain. GalTase is an unconventional glycosyltransferase because it exits in two forms. The short form of the protein is found in the trans-Golgi where it galactosylates glycoproteins and in the lactating mammary gland where it binds to α-lactalbumin and synthesizes lactose. The longer form of GalTase is found on the plasma membrane and has approximately 13 extra N-terminal amino acids in the cytoplasmic domain. This form is involved in cell adhesion (i.e. sperm-egg binding) and migration. The objective of this study was to sequence the cDNA of porcine GalTase and examine the sequence for relationships to the bovine and human genes. The 1300 base pair coding region of porcine GalTase cDNA was sequenced. The porcine and the bovine gene exhibited 91.6 % similarity, while the porcine and the human gene showed a 87.8 % similarity. The strongest similarity was found in the catalytic domain. The two translation start sites used to create the long and short forms of the protein are conserved in the porcine sequence. The porcine protein contains one additional cytoplasmic amino acid compared to the bovine sequence and two amino acids compared to the human sequence. The close sequence homology suggests that GalTase may have comparable functions in swine.

Key Words: Glycosyltransferase, Cell Adhesion, Lactose Synthase

819 Gene expression in the bovine mammary gland. N. Mathialagan*, S. J. Wagner, P. M. Sullivan, and J. C. Byatt, *Monsanto Dairy Business, St. Louis, MO*.

Recombinant bST treatment enhances milk yield in cattle. To help define the mammary-specific mechanisms underlying this process we determined the relative expression levels of various genes during lactation and bST treatment in the bovine mammary gland (MG) by using a human DNA microarray system (UniGEM, Synteni, Inc., Palo Alto, CA). The DNA microarray system consisted of 10,000 genes (5000 known genes and 5000 expressed sequence tags, ESTs). We used mRNA prepared from mammary biopsy samples obtained at week 22 of lactation from control (n=7) and bST (n=7) treated cows. The microarray was simultaneously hybridized with two different fluorescent cDNA probes prepared from the two treatments. Hybridization signals from the two fluorophores was detected by a laser fluorescent scanner. A second microarray experiment was performed with mRNA isolated from lactating and involuted MG. In both experiments about 50% of the genes in the microarray produced positive signals under the stringent hybridization and washing conditions employed. Genes up-regulated by bST treatment included enzymes involved in lactose, fat and amino acid biosynthesis and proteins involved in vesicular transport and secretion. Genes down-regulated following bST treatment included transcriptional repressor(s), proteinases and several ESTs of unknown function. Expression profile of lactating vs involuted MG identified genes regulated during these two physiological conditions. Many of the genes up-regulated in lactating MG were the same as those up-regulated by bST, although to greater magnitude. Genes more highly expressed in the involuted MG are tumor suppressors, proteinase inhibitors, genes regulated by STAT-3/prolactin receptor pathway and IGF binding proteins. In conclusion, we have shown that the human microarray system can be used for studying gene expression in dairy cows. This approach is also useful in the identification of bovine homologues of human genes. Gene expression profile in the MG during lactating and dry periods will facilitate our understanding the biology of MG as well as provide candidate genes for milk production traits.

Key Words: Mammary Gland, Growth Hormone, Gene Expression

820 Pregnancy alters ICFBP appearance in milk of cows. C. A. Gibson, J. M. Fligger, M. D. Staley, M. A. Lykos, J. F. Herbein, and C. R. Baumrucker*, *The Pennsylvania State University, University Park*.

Insulin-like growth factor (IGF) system components are synthesized and secreted by mammary epithelial cells and IGF system components are known to affect mammary cell growth and differentiation. Our objective was to determine whether changes in milk IGF binding protein (IGFBP) concentrations during a lactation are associated with various biological parameters that impact milk production. Milk and blood samples from 33 cows (1st through the 4th lactation) were repetitively sampled at 2-week intervals. Eight cows did not become pregnant after multiple artificial insemination attempts (n=3.5). Electrophoresis and [^{125}I]IGF-II Western ligand blot analyses of the samples were normalized to internal controls. IGFBP were present at higher concentrations in serum than in milk and IGFBP-3 was the dominant IGFBP in both fluids. Our previous reports indicated changes in IGFBP in blood and milk of dairy cows are not highly correlated to milk production and lactation parity (Endocrine Meetings Abstracts P3-90, 1996). Analysis of Western blots showed that milk IGFBP concentrations were related to days in lactation prior to conception. Remarkably, conception had a drastic impact upon IGFBP profiles in milk, but not those in blood. It is known that pregnancy has an impact upon milk production. Previous work has shown that no pregnancy specific IGFBP-3 protease, as reported for rodents and humans, could be observed in the blood or milk of pregnant cows. Milk IGFBP concentrations, pre-conception, are not influenced by ovarian cycles. However, the pre-conception profiles of IGFBP in milk are different between cows that become pregnant and those that do not, indicative of fertility relationships. These data suggest that reproductive events (uterine/conceptus) are communicated to the mammary gland by factor(s) during lactation and are observable as alterations in IGFBP changes in milk. *Supported by USDA NRI 94-37206-0869 and the Pennsylvania State Agriculture Experiment Station*

821 Regulation of insulin-like growth factor (IGF) binding protein-3 gene expression by IGF-I and cyclic AMP in bovine mammary epithelial cells. W. S. Cohick*, P. Verma, B. Wang, and C. Grill, *Rutgers University, New Brunswick, NJ*.

IGF bioactivity is modulated by association with the IGF binding proteins (IGFBP). We have previously shown that the bovine mammary epithelial cell (MEC) line, MAC-T, synthesizes IGFBP-2, -3, -4 and -6. The synthesis of IGFBP-3 is stimulated by IGF-I and forskolin (FORSK; increases intracellular cAMP). The synthesis of the other forms of IGFBP is not altered by these agents. Exposure to either IGF-I (200 ng/ml) or FORSK (5 μg/ml) increased IGFBP-3 mRNA levels 3- to 5-fold above serum-free controls by 3 h, with maximal increases of 12- to 16-fold observed between 8 and 12 h. However, the mechanisms by which IGF-I and FORSK increase steady state levels of IGFBP-3 mRNA in MEC are not known. To determine if de novo protein synthesis is required, MAC-T cells were treated with FORSK, IGF-I or both factors $\pm$ 10 μg/ml cycloheximide (CYCLO) for 5 h. In the presence of CYCLO, the ability of IGF-I to increase IGFBP-3 mRNA levels was reduced by at least 50 % in each of 3 experiments. In contrast, FORSK + CYCLO and FORSK + IGF-I + CYCLO induced IGFBP-3 mRNA levels to an even greater extent than either treatment in the absence of CYCLO. The ability of IGF-I or forskolin to alter IGFBP-3 mRNA stability was examined by treating confluent cultures of MAC-T cells with IGF-I or FORSK $\pm$ 5,6 dichlorobenzimidazole riboside (DRB) for increasing periods of time. Neither IGF-I or FORSK were able to stimulate IGFBP-3 mRNA levels in the presence of DRB, suggesting that a transcriptional mechanism is involved. IGF-I and FORSK did not alter the degradation rate of IGFBP-3 mRNA in DRB treated cells compared to serum-free controls. The half-life of IGFBP-3 was estimated to be approximately 12 h. We conclude that IGF-I and FORSK do not stimulate IGFBP-3 synthesis by altering IGFBP-3 mRNA stability. Nuclear run-on assays are presently in progress to determine if IGF-I or FORSK stimulate IGFBP-3 gene transcription.

Key Words: Insulin-like Growth Factor Binding Protein, Mammary, Gene Expression

822 The expression and synthesis of IGF complex proteins by primary bovine mammary epithelial cells in culture. E. A. Matitashvili, D. A. Dwyer*, and D. E. Bauman, *Cornell University, Ithaca, NY.*

Bovine mammary epithelial cells have been cultured for 8 days on floating collagen gels in the presence of different combinations of insulin, prolactin, cortisol, IGF I and bovine somatotropin. Northern blot analysis revealed the stable expression of IGF I mRNA as combination of 4.4kb and a smaller band in all culture conditions. The expression of IGFBP 2 and, to a greater degree, IGFBP 3 varied in media with different hormonal combinations, but depended most strongly on the presence of IGF I. The amount of IGFBP 3 in media followed the pattern of its expression and appeared as a double band of 46–48KDa on membranes subjected to radioligand binding. The band of 1kb was detected by Northern blot after hybridization to human type I IGF receptor cDNA. The amount of this mRNA did not depend on culture conditions. The expression of GH receptor using cDNA clones for two alternative forms of GHR (GHR1A and GHR1B) has also been analysed. The bands of GHR1B mRNA, 3.4kb, 2.0kb and 0.5kb were detected in cells exposed to all treatments. The level of GHR1B mRNA was slightly elevated when IGF-I was included in culture media. In agreement with the findings of others that GHR1A form of GHR was peculiar to liver, we were unable to detect GHR1A mRNA in this studies.

Key Words: Mammary, Culture, IGF

823 Identification of extracellular matrix proteins in bovine mammary gland and in cultured epithelial cells: synthesis and secretion. E. A. Matitashvili* and D. E. Bauman, *Cornell University, Ithaca, NY.*

We have investigated the expression of extracellular matrix (ECM) proteins in lactating mammary gland and in bovine mammary epithelial cells cultured on plastic, floating collagen gel and a complex of ECM proteins isolated from lactating mammary tissue. The cells were cultured for 10 days in serum-free medium with insulin, prolactin and cortisol. Northern blot analysis utilized human cDNA probes for procollagen a2-chain, fibronectin 1 and B1 chain of laminin. Procollagen mRNA appeared as two bands of 6.5kb and 1.5kb long, fibronectin 1 had multiple mRNA bands 7.2kb–1kb long, laminin mRNA was presented as a 6kb band. The expression of procollagen and fibronectin by cells in all culture conditions was significantly higher than in vivo. Both messages declined 2- to 10-fold with increased level of cell differentiation assessed by the k-casein gene expression, from the highest level in cells cultured on plastic, to the lowest in the cells cultured on ECM. The expression of laminin B1 did not differ significantly in vivo and in vitro. On Western blot membranes anti-fibronectin antibody recognized a single broad 200KDa band in extracts from lactating mammary gland and epithelial cells cultured on plastic. The pattern of laminin bands was different in vivo and in vitro.

Key Words: Mammary, Culture, Extracellular Matrix

824 Enhancement of mammary tight junction formation by prolactin may be mediated by the α^- isoform of tight junction associated protein ZO-1. K. Stelwagen* and H. A. McFadden, *AgResearch, Hamilton, New Zealand.*

Mammary tight junctions (mTJ) are important for maintaining cells in a polarized state, and are a critical feature of differentiated mammary cells. mTJ are formed during lactogenesis, prior to milk secretion. Glucocorticoids and prolactin (PRL) are key lactogenic hormones, and the former are crucial for the formation of mTJ (Singer et al. Proc. Natl. Acad. Sci. USA 89:9069, 1992). The present study examined the role of PRL on mTJ formation in murine HC11 mammary epithelial cells. Cells were grown on permeable Millipore PCF inserts (0.6 cm^2) in RPMI 1640 supplemented with EGF and insulin. When confluent, cells were maintained in RPMI 1640 differentiation medium containing insulin (5 μg/ml) and dexamethasone (DEX, 1 μM). Treatments consisted of: A, 1, or B, 5 μg/ml PRL added at 0h; C, 1 μg/ml PRL added after 48h; D, no PRL. mTJ formation was measured by transepithelial electrical resistance (TER). After 24h DEX had significantly increased TER, but PRL caused a further increase (A, B, C, D: 9.3^{ab} vs 10.2^{a} vs 8.6^{bc} vs $8.1^{c} \pm 0.4$ $\Omega \cdot cm^2$, $^{abc}P < 0.05$). At 48h C and D did not differ (A, B, C, D: 15.3^{a} vs 14.8^{a} vs 12.9^{b} vs $12.8^{b} \pm 0.6$ $\Omega \cdot cm^2$, $^{ab}P < 0.05$), but at 72h , 24h after PRL, TER began to rise for C, reaching the same level as A and B at 96h (A, B, C, D: 23.7^{a} vs 23.2^{a} vs 22.7^{a} vs $19.2^{b} \pm 0.5$ $\Omega \cdot cm^2$, $^{ab}P < 0.01$). Treatments A and B did not differ at any time, suggesting that 1 μg/ml PRL is sufficient for mTJ formation. Western blotting, using a monoclonal antibody against mTJ-associated protein ZO-1, suggested that the effects of PRL may be mediated via the 214 kDa α^- isoform of ZO-1, as there was a stronger signal for this isoform in cell treated with PRL then in those treated with DEX only. In conclusion, PRL enhances mTJ formation, and its effects may be mediated via ZO-1-α^-.

Key Words: Polactin, Tight Junction, Mammary

825 α_{1c}-adrenergic receptor gene expression in the bovine mammary gland. O. Wellnitz*, R. R. Friis, A. Zurbriggen, J. W. Blum, and R. M. Bruckmaier, *University of Berne, Switzerland.*

Adrenergic receptors (α and β) are present in the bovine mammary gland and inhibit (α) or facilitate (β) milk ejection and milk flow. The distribution of α- and β-adrenergic receptors is part of the regulation of milk let down in dairy cows. The hypothesis tested was that α_{1c}-adrenergic receptor binding capacity in different regions of the mammary gland is regulated by gene expression. The gene expression of the α_{1c}-adrenergic receptor was measured by competitive reverse transcription polymerase chain reaction (RT-PCR) in 3 different regions of 1 front and 1 rear quarter of 5 lactating bovine mammary glands. A sequence of 299 bases of the α_{1c}-adrenergic receptor wild-type mRNA along with different concentrations of an internal RNA competitive reference standard (RNA-CRS)was transcribed to cDNA and amplified. The RNA-CRS was identical to the sequence of interest except for an insert of an additional 64 bases. RNA was extracted from (a) the muscular layer of the teat (teat tissue), from (b) tissue around the gland cistern including the large mammary ducts (cisternal region) and from (c) the proximal udder containing mammary parenchyma but no visible mammary ducts (parenchyma). The concentration of α_{1c}-adrenergic receptor mRNA in all quarters was highest in teat tissue (20–65 fmol/μg total RNA), lower in the cisternal region (7–20 fmol/μg total RNA), and lowest in the parenchyma region (< 5 fmol/μg total RNA). The distribution of α_{1c}-adrenergic receptor mRNA concentration was similar in front and rear quarters of all tested mammary glands. This distribution of α_{1c}-adrenergic receptor mRNA in the 3 udder regions is similar to the α_1-adrenergic receptor binding capacity described previously (J. Dairy Res. 61, 47–57, 1994). In conclusion, α_{1c}-adrenergic receptor protein density and binding capacity in the bovine mammary gland is regulated at the level of transcription.

Key Words: Bovine, α_1-adrenergic Receptor, mRNA

826 Milk ejection and milk removal in high-yielding dairy cows. R. M. Bruckmaier*, O. Wellnitz, and J. W. Blum, *University of Berne, Switzerland*.

Fifteen high-yielding cows (HC; >45 kg/d) with $\geq$ 2 lactations of the Swiss Braunvieh, Simmental x Red Holstein and Holstein Friesian breeds and corresponding control cows (CC; 25–30 kg/d; same age, breed, stage of lactation and farm as HC) were investigated during routine evening milking time. Intramammary pressure (IMP) was recorded in the teat cistern during a manual prestimulation until IMP maximum was reached. Immediately afterwards milking was started and single quarter milk flow curves were recorded. Quarter milk samples were taken during milking for determination of somatic cell counts (SCC). In addition, in 6 cows milk flow was recorded without any udder preparation to investigate the effect of missing prestimulation. IMP before stimulation was not significantly higher in HC than in CC cows (3.9 and 3.4 kPa, resp.) but IMP maximum after stimulation was higher in HC than in CC cows ($P<0.05$; 6.8 and 5.8, resp.). The time from start of prestimulation until reaching IMP maximum was similar in both groups (1.7 min). Milk yield was 23.4 kg in HC and 14.3 kg in CC. Milking time was longer in HC than in CC cows ($P<0.05$; 8.5 and 5.9 min, resp.), whereas average and peak flow rates were comparable in HC and in CC cows (2.8 and 2.5, resp.; 4.0 and 3.6 kg/min, resp.). Delayed milk ejection during milking without prestimulation was mirrored by bimodal milk flow curves in HC and CC. 43 % of the milk yield in both, HC and CC cows, was stored in the front quarters. Therefore, unavoidable overmilking due to uneven partitioning between the quarters was longer in front than in rear quarters in both groups (1.6 and 0.7 min in HC and 1.5 and 0.5 min in CC cows, resp.). Surprisingly, SCC were lowest in both groups in quarters with the the longest overmilking time (>3 min). In conclusion, the course of milk ejection in cows does not depend of the production level, but the IMP maximum after milk ejection does. However, the higher IMP maximum causes scarcely elevated milk flow rates resulting in prolonged milking time in high-yielding cows.

Key Words: High-yielding Cow, Milk Ejection, Milk Removal

827 The effect of morphine and naloxone on the release of oxytocin, cortisol and prolactin and on milk ejection in dairy cows. D. Schams*, V. Tancin, and W. Kraetzl, *Technical University Munchen-Weihenstephan, Germany*.

The aim of the study was to determine the role of opioids in the secretion pattern of oxytocin (OT), cortisol (CO), prolactin (PRL) and of milk let down (MLD) during milking in Brown-Swiss cows. Two experiments were carried out to find a dose-related effect of the specific mu-receptor agonist morphine and to find an inhibition of this effect by the opioid antagonist naloxone. On the day before starting the experiments, the cows were fitted with a permanent cannula in the jugular vein for intensive blood collections before, during and after milking. The milk flow was automatically recorded. In the first experiment, six cows were injected on three consecutive days after the control milking with 21, 70 and 210 mg morphine-HCl i.v. respectively 10 min before morning milking. Morphine did not influence the basal levels of OT, CO but increased significantly PRL basal levels (210 mg dose). After manual prestimulation and during milking morphine suppressed significantly OT and CO and potentiated PRL values with 70 mg and 210 mg dose. The MLD was inhibited with 70 mg (one cow), 210 mg (4 cows) and 350 mg (one cow). In the second part of experiment, naloxone (210 mg, -15 min before milking i.v., followed by inhibiting dose of morphine) clearly potentiated the OT and CO release during milking and prevented PRL basal release. In conclusion, there is strong evidence that dose dependent opioids affecting the release of OT, CO and PRL before, during and after milking in a naloxone reversible manner.

Key Words: Opioid, Milking, Hormones

828 Production of transgenic pigs and mice containing the gene encoding human insulin−like growth factor I (IGF−I) under control of the bovine α−lactalbumin promoter and regulatory regions. G.T. Bleck[1], M.H. Monaco[2], S.M. Donovan[2], and M.B. Wheeler*[1], [1]*Department of Animal Sciences,* [2]*Department of Food Science and Human Nutrition, University of Illinois, Urbana*.

In previous work from our lab, we found that artificially−reared piglets fed formula supplemented with 500 to 1000 μg/L of recombinant human IGF−I for the first 14 days postpartum had longer intestinal villi and higher lactase activity than piglets fed formula alone. Based on these results, our goal was to produce transgenic mice and pigs to analyze the effects that higher mammary and milk levels of insulin-like growth factor I (IGF-I) will have on neonatal growth, intestinal health and piglet survival as well as on sow mammary growth and milk synthesis. The gene construct is composed of the bovine α-lactalbumin gene and the gene sequence encoding the 70 amino acid mature human IGF-I peptide. Human IGF-I is identical to the porcine and bovine IGF-I peptides. The IGF-I gene sequence was inserted directly behind the α-lactalbumin signal peptide coding sequence in exon one of the gene to allow secretion of IGF-I into milk. To date, three lines of transgenic mice and four founder male transgenic boars have been generated using this gene construct. Seventy-one live piglets were born from recipients carrying injected embryos. Four of the animals were transgenic resulting in a 5.6 % transgenic efficiency. The oldest boar was mated and has passed the gene on to three female offspring.

Key Words: Mammary, Milk, Intestine

829 Expression of beta-galactosidase introduced by an adenoviral vector into the teat of goats. K. Plaut[1]*, D. Kerr[1], W. Fan[1], B. Trapnell[2], and A. J. Bramley[1], [1]*Northeast Dairy Foods Research Center, University of Vermont, Burlington and* [2]*Genetic Therapy Inc. Gaithersburg, MD*.

Introduction of anti-bacterial genes into the teat of a dairy animal may provide an appropriate defense against mastitic organisms that enter the mammary gland via the teat duct. This study was designed to examine the feasibility of expressing a gene in ductal epithelium of the teat. Two dry Toggenburg goats were infused twice with 10^{10} plaque forming units/ml of an adenoviral vector, Av1LacZ4, containing a nuclear targeted *E. coli lac Z* gene. The control udder half received an infusion of the diluent. Three days after the initial infusion animals were euthanized. Teat and mammary tissues were collected, fixed and exposed to an enzymatic reaction to detect expression of β-galactosidase. In both animals, there was bright blue staining along the length of the teat in the treated udder half. No staining was observed in the control halves. Fixed tissues were then embedded in paraffin and sectioned. The X-gal stain was localized to the nucleus of the epithelium lining the teat duct. Staining was also observed in mammary tissue from the treated half and blue cells could be observed after primary culture of mammary tissue. The staining in the teat supports the concept that adenoviral vectors can be used to introduce anti-bacterial genes into the teat canal.

Key Words: Mammary, Gene Therapy, Biotechnology

830 Expression of bovine beta-lactoglobulin in the milk of transgenic mice. A. Gutierrez-Adan, E. A. Maga, E. Behboodi, G. B. Anderson, and J. D. Murray*, *University of California, Davis*.

Transgenic mice were produced by microinjection of genomic sequences for the bovine beta-lactoglobulin (BLG) gene into pronuclear stage C57Bl/6 X CBA zygotes. The transgene contained the complete bovine BLG gene transcription unit as well as 1.2 Kb and 1 Kb of the 5′ and 3′ flanking regions, respectively. Nine lines of transgenic mice were produced, with six of them expressing bovine BLG in the milk during lactation. In seven lines the the transgene was stably integrated and and transmitted as a Mendelian locus. Line BLA 20 did not transmit the transgene and line BLA 26 was mosiac for two insertion sites. The level of bovine BLG expression on day 10 of lactation in hemizygous transgenic females ranged from 0.75 to 3.4 mg BLG per ml of milk. A position-dependent and copy number independent expression of the transgenic protein was observed.

Key Words: Beta-lactoglobulin, Transgenic, Mouse Milk

831 Effects of injection of β-carotene or vitamin E and selenium on reproductive function of lactating dairy cows. C. F. Aréchiga*[1], S. Vázquez-Flores[2], O. Ortiz[2], J. Hernández-Cerón[2], A. Porras[2], L. R. McDowell[1], and P. J. Hansen[1], [1]*University of Florida, Gainesville, FL and* [2]*Universidad Autónoma de México, Cd. Universitaria, México, D.F., Mexico*.

Experiments were conducted to test whether administration of antioxidants would improve fertility of lactating Holstein cows. First, β-carotene was injected in heat-stressed lactating cows in Florida. Cows were given prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) to synchronize estrus; they were also injected, i.m., with either 800 mg β-carotene or a control injection at days -6 and -3 before anticipated date of insemination and at the time of insemination. There were 37-41 inseminated cows in each group. Injection of β-carotene increased plasma concentrations of β-carotene for at least 13 d after last injection. Nonetheless, there was no effect of β-carotene on the proportion of cows detected in estrus following $PGF_{2\alpha}$, the timing of estrus after $PGF_{2\alpha}$ injection or the pregnancy rate in inseminated cows. In another study, cows in a temperate climate (Central Mexico) were either injected, i.m., with vitamin E (500 mg) and selenium (50 mg) at 30 d postpartum (n=97) or served as untreated controls (n=89). Treatment with vitamin E and selenium did not affect interval from calving to first breeding or the proportion of cows pregnant at first insemination but increased pregnancy rate at second service (69.8 vs 52.1%, P=0.07) and reduced services per conception (1.7 $\pm$ 0.10 vs 2.0 $\pm$ 0.11; P<0.05) and interval from calving to conception (84.6 $\pm$ 4.3 vs 98.1 $\pm$ 4.5 d; P<0.05). Thus, injection of vitamin E and selenium increased fertility in cattle not becoming pregnant at first service.

Key Words: Fertility, Cattle, Antioxidants

832 Immunization of heifers against a recombinant ovalbumin-LHRH. J. M. Sosa*, Y. Zhang, D. M. de Avila, K. P. Bertrand, and J. J. Reeves, *Washington State University, Pullman*.

A recombinant chimeric protein consisting of ovalbumin with seven LHRH peptides (ovalbumin-LHRH-7) has been developed. The plasmid for this protein is expressed in E.coli and is collected and purified as an insoluble protein. This protein has three LHRH sequences in tandem at the N-terminus bound to amino acid (aa.) 18 of ovalbumin, two in tandem at the C-terminus after aa. 381, and two at positions in the middle of the structure between aa. 65 and 66 and aa. 97 and 98. The protein was placed in an adjuvant for use with recombinant His-tag proteins (Z-Max, Zonagen, Inc., Woodlands, TX). Eight cycling crossbred heifers were randomly assigned to two treatment groups. Treatments consisted of recombinant ovalbumin (control) or ovalbumin-LHRH-7. One primary and two booster immunizations were administered (1 mg protein per injection) by intramammary injection at 5 wk intervals. Blood samples were collected weekly or biweekly to determine serum progesterone concentrations and LHRH antibody titers. On Day 111 from the primary immunization, a bull was placed with all heifers for 60 days to compare fertility of the two groups. All control heifers were cycling throughout the trial until becoming pregnant within 21 days of the time the bull was placed in the lot. LHRH antibody titers increased after the first immunization, peaked after the first booster and dropped. Surprisingly, no increase in titers was observed after the administration of the second booster. Serum progesterone concentrations (< 1ng/ml) indicate that the estrous cycle of the four ovalbumin-LHRH-7 treated heifers was suppressed for a time period of 60–123 days ($p < 0.05$). This preliminary study in heifers demonstrates suppression of ovulation and pregnancy ($p < 0.05$) for an effective period of 60 days.

Key Words: Recombinant, Sterilization, Heifers

833 Superovulation response of South African Indigenous and Boer goats to FSH-p. J. P. C. Greyling* and M. Van der Nest, *University of the Orange Free State, South Africa*.

8 Boer and 16 Indigenous goat does were superovulated using 20 mg FSH-p (Folltropin). Treatment started 2 days prior to CIDR (Hoechst) removal. The first two injections of 3.6 mg on day 14 were followed by twice daily doses of 3.2 mg and 2.4 mg on consecutive days and a single injection of 1.6 mg FSH on day 17. The intravaginal CIDR's were left in situ for 17 days. From CIDR removal does were monitored for oestrus at 8-hourly intervals. Donor animals were laparoscopically inseminated twice (36 and 48 hours after CIDR removal) with fresh semen. Surgical recovery of embryos was performed 6 days following AI. A high percentage (87.5%) of Indigenous does demonstrated oestrus, compared to 50% in the Boer goats. The interval from cessation of treatment to oestrus in Boer goats was 34.0 $\pm$ 7.2 h and 25.0 $\pm$ 1.6 h in the Indigenous goats. The mean duration of the induced oestrous period was significantly (P<0.05) shorter in the Boer goat does (30.0 $\pm$ 4.2 h vs 43.5 $\pm$ 3.9 h respectively). The ovulation rate (number of CL's/doe) was not influenced by breed (12.9 vs 12.8 for the Boer and Indigenous does respectively). Although not significantly different, the Indigenous does had a higher embryo recovery rate of 81% (10.4 $\pm$ 1.3 embryos/doe), compared to 64% (8.3 $\pm$ 3.3 embryos/doe) in the Boer goats. Boer goats tended to produce higher quality embryos when compared to Indigenous goats (96.9% vs 64.4% class I and II embryos). Of the total number of ova/embryos recovered only 2.9% and 1% were unfertilized in the Boer and Indigenous goats respectively. Folltropin (20 mg) proved efficient as superovulation agent, especially in the smaller Indigenous goat. The embryo recovery rate was also better in the Indigenous doe, compared to the Boer goat. Although the Indigenous does produced more embryos in total following superovulation, the quality of the Boer goat embryos was superior and more acceptable. A higher dose of FSH-p could have been more appropriate for the Boer goat.

Key Words: Goats, Superovulation, Ovulation Rate

834 Effect of body condition on reproductive efficiency of lactating dairy cows receiving a timed insemination. F. Moreira*, C. Risco, M. F. A. Pires, J. D. Ambrose, M. Drost, M. DeLorenzo, and W. W. Thatcher, *University of Florida, Gainesville*.

Objectives were to compare pregnancy rates using a timed insemination (TI) protocol for the first service of lactating dairy cows with body condition scores < 2.5 (1 to 5 scale; Low BCS group) versus ≥ 2.5 (Control group) and evaluate reproductive efficiency and milk production of both experimental groups throughout lactation. At 63±3 days postpartum (d PP), cows were assigned to experimental groups (n=81 for the Low BCS group and n=126 for the Control group), injected with Gonadotropin Releasing Hormone (GnRH, Cystorelin®, Rhone Merieux, GA; 100 μg, im), and injected 7 d later with Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, Lutalyse®, Pharmacia-Upjohn Co., MI; 25 mg, im). At 48 hours after injection of $PGF_{2\alpha}$, cows received an injection of GnRH and were inseminated 16 hours later. Cows returning to estrus were re-inseminated. Cows were examined by ultrasonography for pregnancy at 27 days after TI and again at 45 days by rectal palpation. Blood samples were collected prior to the injections of GnRH and $PGF_{2\alpha}$ for plasma progesterone analyses. Pregnancy rates to TI were less for the Low BCS group compared to the Control group at day 27 (18.11±6.10% < 33.83±4.55%; P<0.02) and at day 45 (11.14±5.49% < 25.64±4.10%; P<0.02). Rates of cumulative pregnancies through either 120 or 365 d PP were lower for the Low BCS cows (P<0.01). Days open by 120 d PP were greater for Low BCS cows compared to Control cows (90.30±3.16 d > 83.47±2.13 d; P<0.05). There were no differences (P>0.10) in milk production between experimental groups. A positive linear effect of milk production on total number of services (P<0.01), number of services per conception (P<0.03), and number of days open (P<0.07) were detected indicating that greater milk production was associated with poor reproductive performance. Economical analysis of the influence of Low BCS cows on fertility to a TI will be discussed. Low BCS cows have lower pregnancy rates at the first TI service compared to Control cows.

Key Words: Timed Insemination, Body Condition, Cattle

835 Estrous behavior and time of ovulation of beef cows in summer and winter. M. L. Looper*[1], R. P. Wettemann[1], T. Prado[2], and G. L. Morgan[2], [1]*Oklahoma Agricultural Experiment Station, Stillwater and* [2]*College of Veterinary Medicine,Oklahoma State University, Stillwater*.

Effect of season on estrous behavior and time of ovulation was evaluated in nonlactating Angus x Hereford cows during summer (n = 17) and winter (n = 20). Cows were maintained in pastures and average maximum daily temperatures were 31 ± 3 and 9 ± 3° C for summer and winter, respectively. The HeatWatch® system (an electronic sensor which records each time a cow is mounted) was used to determine duration of estrus, number of mounts, and the longest interval between two mounts during two estrous cycles. Blood samples were collected twice weekly and progesterone was quantified. Commencing 16 h after onset of the second estrus, ovaries were evaluated by transrectal ultrasound every 4 h to determine time of ovulation. Duration of estrus was shorter (P < .06) in summer (10.8 ± 2.9 h) than winter (14.8 ± 1.3 h). Cows tended to be mounted less times per estrus (P = .12) in summer than in winter (22.9 ± 4.1 and 34.3 ± 5.3, respectively). Periods during estrus when cows were not mounted were not influenced by season and averaged 3.6 ± .3 h. Interval from onset of estrus to ovulation was similar for cows in both seasons (31.9 ± 1.1 vs 32.7 ± .8 h, summer vs winter). Time of ovulation relative to the onset of estrus was not related to the duration of estrus. Concentrations of progesterone during the estrous cycle after ultrasonography were similar to concentrations during the previous cycle. Increased ambient temperatures decreased the duration of estrus in beef cows; however, the time of ovulation relative to the onset of estrus was similar in summer and winter.

Key Words: Cows, Estrus, Ovulation

836 Artificial insemination of superovulated heifers with 600,000 sexed sperm. Y.-G. Chung, J. L. Schenk*, L. A. Herickhoff, and G. E. Seidel, Jr., *Colorado State University Fort Collins and XY, Inc. Fort Collins, CO*.

Sperm transport is compromised in superovulated cattle, so they frequently are artificially inseminated on multiple occasions and/or with multiple doses of semen. Also, current procedures for sexing semen are relatively slow; therefore, it was of interest to determine fertilization rates after a single insemination of FSH-treated cattle with only 600,000 total sexed unfrozen sperm. Twelve Angus crossbred heifers were superovulated using standard procedures: 6, 6, 4, 4, 2, 2, 2, and 2 mg FSH were injected i.m. at half-day intervals beginning between days 9 and 12 of the estrous cycle; 25 and 12.5 mg prostaglandin F-2-alpha were injected i.m. with the 6th and 7th FSH injections. Sperm from bulls of unknown fertility were stained with Hoechst 33342 and then sorted using a MoFlo® flow cytometer/cell sorter yielding 700–800 live sperm of each sex/sec. Average sort purity was 89% of the desired sex. Sorted sperm were concentrated to 3.36 x 10^6 sperm/ml by centrifugation at 650 g for 10 min, cooled to 5°C, and stored 4 h. Then 184 μl were loaded in 0.25-ml plastic straws. Half the dose was inseminated into each uterine horn 20 to 24 h after onset of estrus using atraumatic side-opening embryo transfer sheaths. Embryos were collected by standard non-surgical procedures at 7 or 16 days post estrus. Results were similar between day 7 and 16 collections and between X- and Y-sorted sperm. Embryos were recovered from 9 heifers. There were 52 embryos (mean, 4.3±5.3/donor) at normal stages of development, 13 retarded embryos and 31 unfertilized ova. Forty-six embryos were sexed by PCR using primers for a Y-chromosome-specific DNA sequence; 43 (93%) were of the intended sex. Although this study involved few animals, insemination of superovulated heifers with only 600,000 total (live) sexed unfrozen sperm gave similar results to conventional procedures.

Key Words: Sexed Semen, Cattle, Embryo Transfer

837 Insemination of suckled beef cows after detected estrus and(or) at one fixed time in response to GnRH and $PGF_{2\alpha}$. K. E. Thompson, G. C. Lamb, D. M. Grieger, L. R. Corah, and J. S. Stevenson*, *Kansas State University, Manhattan*.

Estrus and ovulation in 536 suckled cows were programmed for artificial insemination (AI) using one system consisting of 100 μg of GnRH (d −7) and 25 mg of $PGF_{2\alpha}$ (d 0; body condition assessed) given prior to the onset of the spring breeding season (d 0). Breeds at three locations consisted of purebred Angus, Simmental, and Hereford cows at one location; and crossbred cows (Angus × Hereford and Hereford × Simmental × Angus) at the other two locations. Cows were assigned randomly to three breeding treatments in which inseminations occurred: 1) 8 to 10 h after detected estrus (twice daily detection) during a 5-d period after $PGF_{2\alpha}$ (estrus-AI); 2) 54 h after $PGF_{2\alpha}$ when a second, 100-μg injection of GnRH was given immediately after the insemination (TAI + GnRH); or 3) estrus-AI up to 54 h after $PGF_{2\alpha}$, then all remaining cows were inseminated and given GnRH at 54 h (estrus-AI or TAI + GnRH). Technicians and sires were nested within herd-location. Blood samples were collected once between 9 and 11 d before GnRH (d −7), and on d −7 and 0 for subsequent analysis of progesterone by RIA. Cows were classified as cycling or anestrus based on concentrations of progesterone in the first two serum samples. Only 45% of the cows were cycling at the onset of the breeding season. Pregnancy rates were reduced in both treatments in which the TAI was used regardless of body condition or days postpartum of cows at the onset of the breeding season.

Item	Estrus-AI	TAI + GnRH	Estrus-AI/TAI + GnRH
No. of cows	177	176	183
Estrus, %	58.6	–	20.5
Anestrus	46.9	–	13.5
Cycling	80.4	–	27.4
Pregnancy rate, %	42.1	32.8^x	32.4^x
Anestrus	29.2	27.6	27.9
Cycling	56.3	40.6	38.9

^{x}Different ($P < .01$) from estrus-AI.

Key Words: Beef Cows, Pregnancy, GnRH

838 Synchrony of estrus and ovulation in crossbred Brahman heifers given an intravaginal progesterone releasing insert in combination with PG and estradiol benzoate (E). J. W. Lemaster*, J. R. Kempfer, and J. V. Yelich, *University of Florida, Gainesville*.

Cycling crossbred Brahman heifers (n=59) were utilized to determine the effect of a 7 d intravaginal progesterone releasing insert in combination with PG and E on time of ovulation and estrus behavior. In Phase I, heifers at random stages of the estrous cycle were assigned by BW and percentage *Bos Indicus* breeding to three treatments on day 0; 1) intravaginal insert for 7 d with PG on day 7(C; n=10), 2) intravaginal insert for 7 d with PG on day 7 and 0.5 mg E24 hr after insert removal (E24; n=9) or 3) intravaginal insert for 7 d with PG on day 7 with 0.5 mg E48 hr after insert removal (E48; n=10). Estrus was detected using HeatWatch®. Ultrasonography began 8 h after first mount and every 4 hr thereafter to detect ovulation. In Phase II, Phase I treatments (n=10/treatments) were replicated, but only behavioral estrus data were collected to minimize handling of heifers. Ovulatory follicle size was similar (P>.1) between C, E24 and E48 (14.4, 12.5, and 14.1 mm, respectively). Interval from insert removal to ovulation tended to differ (P<.06) between C, E24 and E48 (92.9, 76.3 and 79.6 hr, respectively). Interval from insert removal to first mount was decreased (P<.05) in E24 (45.7 hr) versus E48 (55.2 hr) and C (60.4 hr), which did not differ from each other. Frequent working did not influence (P>.1) the interval from insert removal to first mount. Duration of estrus was similar for C, E24 and E48 (12.2, 15.7 and 17.4 hr, respectively) and was not effected (P>.1) by frequent working. No difference (P>.1) was observed in number of mounts between C, E24 and E48 (26.8, 28.9 and 37.9 mounts, respectively), but number of mounts increased in the non-worked versus frequently-worked heifers (38.3 and 24.0 mounts, respectively; P<.05). In conclusion, frequent handling did not effect interval to first mount after insert removal or duration of estrus, but decreased estrus intensity. E hastened the interval from insert removal to ovulation without significantly altering behavioral estrus.

Key Words: HeatWatch®, Estrus, Ovulation

839 Use of radiotelemetry, visual observation and progesterone assay to determine true silent ovulation. M. P. Shipka*, C. B. Campbell, T. L. Wierenga, K. E. Panter, and R. C. Lamb, *Utah State University, Logan*.

This study was undertaken to determine the occurrence of silent ovulation (SO) in high-producing, postpartum (pp) Holstein cows. Nineteen high-producing, multiparous cows received transmitters (Heatwatch, DDX, Boulder, CO) for radiotelemetry (RT) and began visual estrus detection (VED) routine for 1/2 hr twice daily, on d 10 pp. Blood samples were obtained every other day commencing on d 10 pp, for RIA of progesterone (P_4). Sensitivity of the assay was approximately 0.2 ng/ml. Intra- and interassay CV were 3.9% and 6.9%, respectively. First pp ovulation was assumed to have occurred within 4 d prior to the first sustained increase in systemic P_4 above 1.0 ng/ml. Subsequent ovulations were assumed to have occurred during any period of nadir P_4 (< 1.0 ng/ml) following a period of sustained elevated P_4. Following P_4 assay, RT and VED data were evaluated at time periods coinciding with ovulation to determine whether standing estrus had occurred. First ovulation occurred at 29.1 ± 3.0 d pp (range 17 to 69 d) compared to first mount at 39.6 ± 4.0 d pp (range 17 to 69 d) and at 47.6 ± 3.6 d pp (range 24 to 77 d) for RT and VED, respectively. Visual estrus detection indicated 94.7% of first ovulations as SO while the inclusion of RT with VED indicated that only 42.1% of first pp ovulations were true SO. During second pp ovulations (n = 16), VED indicated 50% SO while the inclusion of RT indicated only 12.5% true SO. During third and fourth pp ovulations (n = 15), VED indicated 33.3% SO while the inclusion of RT indicated only 6.7% true SO. When RT was associated with ovulation, there were fewer mounts at first pp ovulations compared to subsequent ovulations (1.1 ± 0.1 vs. 3.8 ± 0.7, P < .0005) and lengths of individual mounts were shorter (2.6 ± 0.5 vs. 3.4 ± 0.3 sec., P < .05). Previous studies have indicated the majority of first postpartum ovulations to be silent ovulations, however, it appears more likely that, while a substantial number are silent ovulations, more are associated with a reduced expression of behavioral estrus.

840 Use of a slow-release estradiol-17β (SRE) and PGF2α to induce pseudopregnancy and control estrus in gilts. R. A. Cushman[1]*, P. E. Davis[1], U. Boonyaprakob[1], V. S. Hedgpeth[1], P. D. Burns[2], and J. H. Britt[1], [1]*North Carolina State University, Raleigh,* [2]*Thorn BioScience, Lexington, KY*.

The hypothesis was that a single injection of SRE would induce pseudopregnancy in gilts given gonadotropin 2 wk earlier to induce puberty, and that PGF2α would regress the CL of pseudopregnancy. Forty crossbred gilts were induced to ovulate by treatment with 400 IU hCG + 200 IU eCG (PG600) at 180 d of age (day 0). Gilts were injected (i.m.) with vehicle (n = 8) or one of four doses (n = 8/dose) of SRE (12.5, 25, 50 or 100 mg). Blood was collected prior to SRE and twice weekly until d 73 to monitor serum P4 and E2 concentrations. On d 18 to 26, an intact boar was used to determine return to estrus. On d 59, gilts received (i.m.) 10 mg PGF2α (Lutalyse) and were checked for estrus for 7 d. On d 62, mammary development was scored (0 = no development; 1 = some development; 2 = teat and gland development) by a neutral observer. Mammary score, peak E2 concentration and duration of function of CL (serum P4 > 1 ng/ mL) were analyzed by ANOVA. Percentage of gilts pseudopregnant at d 59 and percentage that had luteolysis after PGF2α were analyzed using Chi-square. Treatment with SRE increased duration (d) of function of the CL (P < .05; 0 mg = 25,12.5 mg = 46, 25 mg = 55, 50 mg = 51, and 100 mg = 55; SEM = 4), mammary gland score (P < .05; 0, .6, 1.1, 1.4, and 1.4, respectively; SEM = .4), and peak E2 concentrations (P < .05; 59, 1649, 3700, 4578, and 6635 pg/mL, respectively; SEM = 1175). Percentage pseudopregnant at d 59 was affected by treatment (P < .05; 0 mg = 0, 12.5 mg = 50, 25 mg = 88, 50 mg = 86, and 100 mg = 86). There was no difference among doses of SRE in the percentage of pseudopregnant gilts that showed luteolysis after PGF2α (P > .05; 12.5 mg = 50, 25 mg = 71, 50 mg = 83, and 100 mg = 100). We conclude that a single injection of SRE can induce pseudopregnancy and that the CL can be regressed with PGF2α, providing a simple method for controlling estrus in gilts.

Key Words: Gilts, Pseudopregnant, Estradiol

841 Effect of recipient breed (Angus and Senepol) on pregnancy and performance traits of Romosinuano embryo transfer calves of Colombian origin. C. C. Chase, Jr.*[1], A. C. Hammond[1], T. A. Olson[2], C. N. Murphy[3], and J. L. Griffin[4], [1]*USDA, ARS, Brooksville, FL,* [2]*University of Florida, Gainesville,* [3]*University of Missouri, Columbia,* [4]*Reproductive Technology International, Plant City, FL*.

To determine the effect of recipient breed on pregnancy and performance traits of Romosinuano embryo transfer calves, 140 embryos collected from 22 donor cows bred to 12 bulls in Venezuela were imported and transferred into Angus and Senepol recipients in Brooksville, Florida. All of the Romosinuano donor cows and bulls or their parents had been imported from Colombia. Estrous cycles were synchronized in Angus and Senepol recipients (nonlactating cows) using a progestogen treatment (Syncro-Mate-B®) and embryos were transferred 7 days after estrus. Breed of recipient did not affect (P > .10) the percentage of cows observed in estrus after progestogen treatment (75 of 88 = 85% Angus and 101 of 120 = 84% Senepol), the percentage of recipients that were pregnant after embryo transfer (33 of 67 = 49% Angus and 37 of 73 = 51% Senepol), or the percentage of recipients that calved after embryo transfer (32 of 67 = 48% Angus and 37 of 73 = 51% Senepol). As expected, sex of calf affected birth weight (P < .001) and 205-d adjusted weaning weight (P < .001), but not length of gestation (P > .10). Length of gestation was 4 days longer (P < .01) for Romosinuano calves from Senepol recipients (293 ± .8 d) than Angus recipients (289 ± 1.1 d). Birth weight was 2.9 kg heavier (P < .01) for Romosinuano calves from Senepol recipients (35.7 ± .55 kg) than Angus recipients (32.8 ± .76 kg). However, breed of recipient did not affect (P > .10) 205-d adjusted weaning weight of Romosinuano calves (205 ± 3.0 kg and 208 ± 2.8 kg for Angus and Senepol recipients, respectively). Breed of recipient (Angus and Senepol) did not affect the response to estrous synchronization or embryo transfer; however, recipient breed affected gestation length and birth weight but not 205-d adjusted weaning weight of Romosinuano embryo transfer calves.

Key Words: Cattle Breeds, Embryo Transfer, Recipents

842 An Evaluation of a Highly Interventionist Approach to Programmed Breeding of Dairy Cows. T. L. Kerbler*, J. S. Walton, W. H. Johnson, K. E. Leslie, K. G. Bateman, and C. J. S. Dunn, *University of Guelph, Canada*.

The objective of this study was to contrast a highly interventionist approach to managing reproduction in postpartum dairy cattle with more conventional management. The interventionist scheme required a logical sequence of regulators that serve to promote uterine involution, normal ovarian activity and maximize the chance of conception and success of early pregnancy. First-service pregnancy rates were determined for a highly interventionist programmed breeding protocol (treatment, T) in comparison with a protocol using only prostaglandin $F_{2\alpha}$ (control,C). In T, 73 cows were treated after calving with 100 μg Cystorelin^{TM} (GnRH) on d 14, 500 μg Estrumate^{TM} (PG) on d 28; GnRH on d 56, PG on d 63, GnRH on d 65, inseminated at a fixed time on d 66 (OVSYNC) and treated with 1500 I.U. Chorulon^{TM} (hCG) on d 70. In C, 76 cows were treated with PG on d 28 and 63. Cows observed in estrus after the d 63 PG were inseminated, the remainder received PG on d 77 and were bred either at estrus or 84 h after PG. Semen from a panel of AI sires was equally distributed across C and T by sire. Milk progesterone concentrations were determined twice weekly throughout. Pregnancy rates to first service were 43.8% for T and 38.2% for C. The efficacy of each injection was determined using milk progesterone profiles. GnRH on d 14 was effective in 65.8% of cases. PG on d 28 was effective in 56.2% of T and 39.5% of C. PG on d 63 reduced progesterone to basal levels in 63.0% of T and 57.9% of C. Pregnancy rate after PG on d 63 was 43.8% in T (69.5% when only those cows responding to PG were included) and 26.3% in C (45.5% when only those cows responding to PG were included). In C, 72.7% of cows responded to PG on d 77 with a pregnancy rate of 27.3%. Overall pregnancy rate in C increased to 38.2%. Failure of the programmed breeding protocol (T) to ensure a PG-sensitive CL on d 63 was a primary reason for reduced fertility at the fixed-time insemination (OVSYNC).

Key Words: Cows, Pregnancy Rate, OVSYNC

843 Factors affecting seasonal variation in non-return rate of lactating dairy cows. Y. M. Al-Katanani*, D. W. Webb, and P. J. Hansen, *University of Florida, Gainesville*.

The objective was to determine factors controlling seasonal variation in 90-day non-return rate (NRR) and to evaluate effects of weather variables on specific days before and after breeding. Dairy Herd Improvement records on first services from 8498 cows in South Georgia (GA, n=7 herds), North Florida (NF, n=5) and South Florida (SF, n=5) were used. In the first analysis, there was a location × month of breeding interaction (P<0.001). The summer drop in NRR was of lower magnitude in GA than in NF or SF. For example, least squares means for NRR in July was 23.8±8.8% (GA), 8.0±5.5% (NF) and 4.3±5.8% (SF). Moreover, the number of months where least squares means for NRR were $\leq$ 20% was 1 for GA (June), 5 for NF (May-Sept.), and 7 for SF (April–Oct.). When cows were grouped according to ME milk yield (1<4536 kg; 2= 4536–9072 kg and 3>9072 kg), there was a milk yield × month of breeding interaction (P<0.05). As milk yield class increased, summer depression in NRR was of greater magnitude (for example, NRR in July was 40.0, 13.2 and 4.4% for classes 1, 2 and 3) and lasted for more months [NRR$\leq$20% for 0 (1), 4 (2) and 6 mo (3)]. In the second analysis, weather data on the day of breeding from GA and NF were related to NRR. Using discriminant analysis, the average of the daily maximum and minimum dry bulb temperature (T) was as related to NRR as various combinations of temperature, humidity, wind speed and solar radiation. In the third analysis, effects of T at day −10, 0 and 10 relative to breeding were evaluated using subsets of cows in which T at d −9 to 0 (for analysis of d −10), d −10 to −1 (for d 0) or d 0-9 (d 10) were always less than 25 C. The NRR for cows having T>25 C on d −10 was less (P<0.001) than NRR for cows with T<25 C on d −10 (38.8 vs 62.8%). Similar results were found on day 0 (43.6 vs 61.9%, P<0.001) and day 10 (42.2 vs 59.6%, P<0.001). Thus, heat stress before and after breeding, as well as on the day of breeding, is associated with low NRR. (Support: Florida Milk Checkoff, USDA CBAG 95-34165-1860 and USDA NRICGP 96-35205-3728)

Key Words: Non-return Rate, Cattle, Heat Stress

844 The effect of reproductive tract scoring (RTS) on superovulation, embryo quality and quantity in beef cattle. G. J. Taylor*, F. J. C. Swanepoel, J. P. C. Greyling, and L. M. J. Schwalbach, *University of the Orange Free State, Bloemfontein, South Africa*.

The objective of the study was to use a RTS as a means of predicting ovarian response, number of embryos produced, fertilization rate and quality of embryos in first calving beef heifers following superovulation with a series of FSH injections. Santa Gertrudis heifers (n=50), ±60 days post partum were scored prior to superstimulation (SS). The RTS method used was as described by Anderson *et al.* (1991). Animals responding to SS were flushed 7 days after oestrus. Embryos recovered were graded according to IETS standards. Mean ovarian volume for the different RTS groups differed significantly (P<0.01) and was correlated to pre-superstimulation volumes (r=0.49). Cows responding to SS in RTS groups 1 to 5 were 0, 4, 21, 92 and 87% respectively. A significant difference (P<0.01) in ovulation rate was recorded between the RTS groups. Cows with a RTS of 4 and 5 responded better, with more corpus lutea and produced more embryos compared to RTS 2 and 3. No significant difference was found in the interval from prostaglandin administration to the onset of oestrus for animals responding to SS. The number of embryos recovered from the different RTS groups was significantly (P<0.01) higher in cows with a RTS of 2 and 3 (98% vs 86%) compared to cows with RTS of 4 and 5. Cows with RTS 5 produced more (P<0.05) unfertilized oocytes than RTS 2, 3 and 4. A positive correlation (r=0.62:P<0.01) between embryo recovery rate and number of viable embryos was found in all the RTS groups. The number of degenerated embryos recovered, was positively correlated with RTS (r=0.47:P<0.01) and number of embryos recovered (r=0.52:P<0.01). Although individual variation exists, SS results in unpredictable responses. Cows with a RTS of 4 and 5 respond better to SS and produce more embryos than cows with a RTS of 2 and 3. Cows with RTS 2 and 3 had the highest recovery rate and least unfertilized oocytes.

Key Words: Superovulation, Reproductive Tract Score, Cattle

845 In vitro fertilization and developmental competence of bovine oocytes relative to reproductive status. K. R. Chohan* and A. G. Hunter, *University of Minnesota, Saint Paul*.

Our objective was to compare the in vitro early embryonic development of bovine follicular oocytes recovered from ovaries during follicular, metestrus, diestrus, anestrus and pregnant statuses after maturation in a protein free medium. Bovine cumulus oocyte complexes were washed, transferred to 200μl droplets of medium M199 containing FSH, LH, Estradiol, Hepes and antibiotics under oil and incubated (24h, 39°C, 5%CO_2). The matured oocytes from each reproductive status were exposed to frozen-thawed TALP swim up, heparin capacitated sperm from two different bulls (20h, 39°C, 5% CO_2). Presumed zygotes (5–8) were placed in 200μl droplets of M199 containing 8mg/ml BSA-V, antibiotics, Hepes and cultured for 144 hours (39°C, 5% CO_2) without any medium freshening. Embryos/oocytes were fixed, stained and evaluated for fertilization and early development. Data were analyzed using chi square for bull effect and differences among various reproductive statuses. No difference (P>0.05) was observed for fertilization and subsequent embryonic development between bulls. Differences for oocyte maturation, fertilization and embryonic development among reproductive stages are presented below. Percentages within column with different letters, are different (P<0.05).

Reproductive Status	No Oocyte	Matur. Rate	Overall Fertil.	Cleav. Rate	2–8 Cells	9–16 Cells	17–64 Cells	Degeneration	Polyspermy
Follicular	292	93.2^{ab}	82.9^{a}	51.7^{a}	47.3^{bc}	3.1^{a}	1.4^{ab}	6.5^{ac}	1.0^{a}
Metestrus	307	90.6^{ac}	86.6^{a}	60.0^{b}	53.4^{bd}	4.6^{a}	2.0^{a}	9.5^{ac}	1.3^{ac}
Diestrus	304	95.4^{ab}	72.4^{b}	39.8^{c}	30.6^{ac}	5.9^{a}	3.3^{a}	4.6^{bc}	2.6^{ac}
Anestrus	245	92.2^{ab}	86.1^{a}	44.1^{ac}	43.3^{bc}	0.8^{b}	0.0^{b}	7.8^{ac}	3.7^{bc}
Pregnant	286	89.8^{ac}	81.1^{a}	46.9^{ac}	39.5^{bc}	4.6^{a}	2.8^{a}	10.5^{ad}	2.5^{ac}

In conclusion, oocytes, recovered during the metestrus phase of the estrous cycle resulted in the highest cleavage rate (P<0.05), while oocytes from the diestrus phase had the poorest fertilization and developmental rate (P<0.05). In addition, long term *in vitro* embryo culture without medium freshening or change is hypothesized to have caused the 8 cell stage block.

Key Words: Reproductive Status, Bovine, IVF

846 Morphology and taxol stabilization of microtubules of cooled porcine oocytes. E. M. Walters* and C. N. Graves, *University Of Illinois at Urbana-Champaign*.

The site of cooling damage for oocytes has been suggested to be microtubules (MT) of the meiotic spindle. Objectives of this study was (1) to determine effects of fast or slow cooling to either 15°C or 5°C for 45 min on MTs of porcine oocytes and (2) to determine if treatment with taxol, a MT stabilizer, could prevent depolymerization of MTs during cooling. Immunostaining with anti-α & β tubulin IgG and sheep anti-mouse IgG conjugated with Cy3 along with laser scanning confocal microscopy was used to visualize effects of cooling on MTs. For both germinal vesicle stage cooled oocytes and *in vitro* matured stage cooled oocytes cooled to 15°C or 5°C, there was a significant decrease ($P<0.05$) in the percentage of oocytes exhibiting a normal MT configuration compared to controls. However, there was a significant increase ($P<0.05$) in the percentage of oocytes exhibiting a normal MT pattern of the meiotic spindle for *in vitro* matured oocytes compared to germinal vesicle stage cooled oocytes. After determining cooling effects on MTs, we investigated whether a 5 min treatment of the oocytes at 39°C with either 1 or 10 μM taxol suspended in dimethyl sulfoxide would decrease depolymerization of MTs during cooling. Treatment with 1 μM taxol significantly ($P<0.05$) decreased both the percentage of oocytes exhibiting a normal MT configuration and also the percentage of oocytes completing fertilization compared to controls. When treated 10 μM taxol, there was no significant decrease ($P<0.05$) in the percentage of oocytes exhibiting a normal MT configuration compared to controls but there was a significant decrease ($P<0.05$) in percentage oocytes completing fertilization. Treatment with dimethyl sulfoxide alone had no effect on MTs or fertilization. This evidence suggests that MTs of pig oocytes are sensitive to cooling and that treatment with 10 μM taxol stabilizes MTs of the meiotic spindle but may subsequently decrease the percentage of oocytes completing fertilization.

Key Words: Pig, Cryopreservation, Meiotic Spindle

847 The effect of single or multiple doses of pFSH with or without pLH on the growth and ovulation of follicles in prepubertal gilts. D. J. Wright* and R. A. Dailey, *West Virginia University, Morgantown*.

Growth of medium and large follicles and ovulation after different regimens of pFSH with or without exogenous pLH were examined in prepubertal gilts. Gilts received either a single injection of 2 mg pFSH at 0 h (SUPER OV®; AUSA International, Tyler, TX) or injections every 8 h (18 mg over 64 h) and either 10 μg porcine LH (USDA-pLH-B2; Animal Hormone Program, Beltsville, MD) with the first injection of pFSH (n=4 and 3, respectively) or saline (n=3 each). Neither number of 3-6 mm (150.3±53.2, 71.3±15.6, 86.3±20.1 and 98±9.9) and ≥7 mm (6.7±4.7, 2.7±1.2, 0 and 0) diameter follicles nor ovarian weight (7.29±.6, 6.99±.1, 7.03±1.1 and 7.18±.8 g) differed at 72 h following initiation of single or multiple injections of pFSH with saline or single or multiple injections of pFSH with pLH, respectively. In a second experiment, injection of pFSH at time of hCG, the ovulatory stimulus, which would simulate the periovulatory FSH surge, was given to determine effects on ovulation rate. Initially, follicular growth was stimulated with pFSH (2 mg) and pLH (10 μg). Seventy-two h later, gilts received either additional pFSH (2 mg; n=10) or saline (n=9) at time of hCG (750 IU). At 72 h following hCG, injection of pFSH in conjunction with hCG reduced the number of gilts ovulating (4/10 vs 9/9, respectively; χ^2=7.89, P<.05) and the number of ovulations per gilt ovulating (5±1.9 vs 9.8±1.2, respectively; P<.05). More 3–6 mm (87.2±16.1 vs 40.6±6.8; P<.05) and ≥7 mm (14.1±3.7 vs 5.9±1.6; P<.05) follicles were present at 72 h following the second injection of pFSH than those treated with hCG alone. In conclusion, a single injection of pFSH was as effective as multiple injections. Fewer gilts ovulated following an injection of pFSH in conjunction with hCG.

Key Words: Prepubertal Gilt, FSH, Follicle

848 Effects of β-mercaptoethanol and oxygen tension on in vitro development of bovine embryos. H. Kato, M. Guler, and G. E. Seidel, Jr., *Colorado State University, Fort Collins*.

We examined effects of oxygen tension (5% and 20%) and addition of β-mercaptoethanol to culture medium on development of bovine embryos. Oocytes collected from slaughterhouse ovaries were matured 22 hr in TCM-199 + 10% estrous cow serum, 50 ng/ml FSH, 1 μg/ LH and 1 μg/ml E_2. Matured oocytes were co-incubated with frozen-thawed capacitated spermatozoa in Fert-TALP for 18 hr. After removal of cumulus cells, ova were cultured for 30 hr in a chemically-defined medium (Biol. Reprod. 50[Suppl. 1]:181) plus 10% FCS at 39°C in 5% CO_2, 5% O_2, 90% N_2. Embryos that developed to at least the 4-cell stage were assigned to 8 treatments and cultured in 4-well culture plates in 500 μl medium. Embryos were cultured for 6 days; cell numbers per blastocyst were counted after staining with Hoechst 33342. Results are in the table.

As has been shown by other studies, more blastocysts developed in 5% O_2 than air. For both oxygen tensions, development to the blastocyst stage was optimal with 10 or 50 μM β-mercaptoethanol ($P<0.05$). Irrespective of oxygen tension, no embryo developed to the blastocyst stage with 250 μM β-mercaptoethanol. Numbers of cells per blastocyst were not different for any treatment ($P>0.1$). Addition of 10 or 50 μM of β-mercaptoethanol to this bovine embryo culture system improved embryonic development.

Table 1. Percentage of blastocysts (four replicates of 17-22 embryos each).

	Concentration of β-Mercaptoethanol (μM)			
Atmosphere	0	10	50	250
5% CO_2 in air	0^a	8^b	11^b	0^a
5% O_2/5% CO_2/90% N_2	5^a	16^b	19^b	0^a

^{a,b} Means within rows with different superscripts differ ($P<0.05$), Chi-square.

Key Words: Antioxidant, Culture, Cattle

849 Effects of stage of lactation on hormonal profiles and recovered oocytes in Holstein cows. A. W. Pryor*, T. L. Bailey, S. Nadir, M. H. Irby, R. E. Bethard, M. L. McGilliard, and F. C. Gwazdauskas, *Virginia Tech, Blacksburg*.

Lactating cows were used to evaluate the effects of stage of lactation on serum IGF-1, LH, and progesterone (P4), follicular fluid estradiol (E2) and P4 (FFP4), and quality of oocytes recovered by ovum pick-up procedures. Cows in early lactation (EL; n=8) and mid-lactation (ML; n=7) were subjected to twice weekly ovum pick-up beginning at d 28 and d 118 postpartum, respectively, for 10 wk. Ova were aspirated and graded. Follicular fluid was collected from the largest follicle (>10 mm) at each session. The stage of lactation (TRT) by time interaction for IGF-1 showed decreasing IGF-1 in EL (34 ng/ml d 0 to 25 ng/ml d 100), but increasing IGF-1 in ML (38 ng/ml d 90 to 50 ng/ml d 190). LH changed by stage of lactation over time. LH in EL increased from .06 ng/ml (d 1) to .4 ng/ml (d 66 postpartum) and declined to .3 ng/ml (d 100), whereas in ML LH increased from .1 ng/ml (d 90) to .5 ng/ml (d 128) and declined to .4 ng/ml (d 190). Serum P4 was different between EL and ML cows (mean ± SE; EL .96 ± .10; ML 1.70 ± .11 ng/ml). There was a TRT by time interaction with EL having 1 ng/ml P4 at calving that increased to 2.4 ng/ml (d 42) and remained constant, but P4 in ML declined from 3.5 ng/ml (d 90) to .3 ng/ml (d 170 postpartum). The E2 did not change (254.5 ± 203.2 ng/ml). There was a significant TRT by time interaction for FFP4 with FFP4 in EL decreasing from 275 to 235 ng/ml, whereas in ML FFP4 increased from 65 to 550 ng/ml at aspiration wk 10. The percentage of grade 1 ova changed cubicly by TRT over time. EL cows began higher (11%) than ML (6%), but fell to 1% by aspiration wk 7 and increased to 10% by wk 10, whereas grade 1 ova in ML steadily declined to 2% by wk 10. Lower serum LH, IGF-1, and P4 and lower FFP4 may be positively related to a higher percentage of grade 1 ova.

Key Words: Ovum Pick-up, Hormones, Follicular Fluid

850 Response of 2-cell bovine embryos to heat shock: effect of magnitude and duration of heat shock and possible induced thermotolerance. R. M. Rivera*, Y. M. Al-Katanani, and P. J. Hansen, *University of Florida, Gainesville*.

We examined the effects of different heat shocks (HS) on blastocyst (BL) formation on day 8 of development. Also, an experiment tested whether a short HS would convey thermotolerance to 2-cell embryos later exposed to a more severe HS. The CO_2 percentage of the gas phase was adjusted to prevent pH changes caused by decreased solubility of CO_2 at increased temperatures. For all experiments, 2-cell embryos were selected and treated at 31-34 h after *in vitro* insemination. For exp. 1 and 2, treatments were 38.5 (control; 0 h of HS) or 40 (exp.1) or 41 C (exp.2) for 3, 6, 9 and 12 h. For exp. 1, there was a cubic effect ($P<0.1$) of time of HS; 9 h of HS caused a slight decrease in development while HS for 12 h did not (33.0, 38.6, 24.6, 15.8 and 28.1% for 0, 3, 6, 9 and 12 h of HS respectively; SEM=7.6%). For exp. 2, there was a linear effect ($P<0.001$) of time of HS. Least-squares means were 33.4 ±4.7 for 38.5 C (0 h HS), and 31.3±4.6, 22.3±4.5, 6.0±4.7 and 0.3±5.3% for 0, 3, 6, 9 and 12 h of 41 C, respectively. For exp. 3, embryos were first exposed for 80 min (trtA) to 38.5, 41, or 42 C. A temperature of 42 C has been shown previously to induce HS protein 70 synthesis. After 2 h of recovery 38.5 C, embryos were subjected to 12 h at either 38.5 or 41 C (trtB). While HS during trtB reduced %BL at day 8 ($P<0.001$), pretreatment with 41 or 42 C during trtA did not ameliorate this effect. In fact, 42 C during trtA reduced %BL. In conclusion, 2-cell embryos are sensitive to heat shocks of 41 and 42 C but less so to a HS of 40 C. Furthermore, thermotolerance to 41 C for 12 h was not achieved at this early stage of development.

	trtB	
trtA	**38.5**	**41**
38.5	37.4±3.4%	0.1±4.2%
41	31.4±3.5%	0.6±4.3%
42	12.2±3.4%	0.1±4.2%

Key Words: Bovine embryo, Heat shock, Induced thermotolerance

851 Potential applications of sexed semen in cattle. G. E. Seidel, Jr., *Colorado State University, Fort Collins*.

The congruence of methods of sexing sperm based on DNA content, high-speed flow cytometer/cell sorters, and procedures for inseminating heifers with fewer than 500,000 total sperm without compromising fertility has resulted in the possibility of a viable sexed semen industry in cattle within a few years. There will be a myriad of applications for sperm sexed at >85% accuracy. Perhaps the most obvious is inseminating one subset of cattle (both dairy and beef) for female herd replacements, and having the converse subset (both dairy and beef) bred to entirely different types of bulls to produced males for meat. A very important subset of the above is inseminating heifers with X-chromosome-bearing sperm to produce female calves, which have a lower incidence of dystocia than male calves, primarily due to smaller size. Furthermore, young dairy sires could be proved much more efficiently with a preponderance of heifer calves. Having more than 85% heifer calves also makes it feasible to lengthen lactations in dairy cows so that they average fewer than two surviving calves per lifetime, which would reduce problems associated with gestation and parturition. Single-sex systems of beef production also would become feasible, in which each female would replace herself and be slaughtered between 2 and 3 years of age, thus using a much higher percentage of nutrients in the system for growth, and a lower percentage for maintenance. Sexed semen would be useful for in vitro fertilization and insemination of cows superovulated for embryo transfer. Frequently one sex of calves is considerably more valuable than the other. Although accurate methods of sexing embryos are available, they are time-consuming, and half of the embryos produced are of the less valuable sex. It is surmised that accurately sexed semen would be widely adopted for artificial insemination of cattle if the sexing surcharge were low and fertility were only minimally compromised. The percentage of beef cattle inseminated artificially likely would increase substantially with sexed semen.

Key Words: Sex, Sperm, Flow Cytometer

852 Transgenic livestock: A decade in review. R. D. Bremel*, *University of Wisconsin, Madison*.

Transgenic plants are now becoming commonplace in agriculture. Although many thousands of lines of transgenic mice have been produced, application of the technology to livestock has lagged. The primary focus of transgenesis in livestock has been the development of mammary-specific expression to produce proteins of pharmaceutical value. In sheep a large fraction of the protein found in milk can be encoded by a transgene. Many technical challenges have been identified over the past decade. One of the main impediments to realization of the potential of transgenesis in livestock is the persistent low efficiency of transgenesis. In addition, issues of DNA configuration and stability of the transgene still prevent the routine application of transgenic technology. Many of these problems were recognized very early in the technology development and the pioneers in the field clearly had the impression that they would be shortly overcome. Recent advances have increased the prospects for use of nuclear transfer to accelerate the application of transgenics in livestock. Other technologies are in development that will also address some of the current limitations. In spite of the problems a number of notable successes have been achieved. The first of the protein therapeutic products are now in clinical trials and others are expected to enter trials shortly. Some of the prospects and on-going constraints for livestock transgenesis in the coming decade will be discussed.

853 Production of bovine α-lactalbumin in the milk of transgenic pigs. G. T. Bleck*, B. R. White, D. J. Miller, and M. B. Wheeler, *University of Illinois, Urbana*.

High production of milk and its components are necessary to allow maximal growth of developing piglets. In this study transgenic pigs were produced containing the gene for a potential limiting component in the production of milk. Two lines of transgenic pigs were produced to analyze the effects that overproduction of the milk protein α-lactalbumin may have on milk production and piglet growth. Transgenic pigs were produced through microinjection of the bovine α-lactalbumin gene. The gene construct contained 2.0 kb of $5'$ flanking region, the 1.7 kb coding region and 600 bp of $3'$ flanking region. Sows hemizygous for the transgene produced up to 0.9 g of bovine α-lactalbumin per liter of pig milk. The production of the bovine protein causes approximately a 50 % increase in the total α-lactalbumin concentration of pig milk. The concentration of bovine α-lactalbumin was highest in colostrum and gradually decreased until reaching a constant level about day 10 of lactation. No differences were observed in the level of milk protein and total solids between control and transgenic animals. The ratio of bovine α-lactalbumin to porcine α-lactalbumin was 4.3 to 1 on day 0 of lactation, but by day 20 of lactation the ratio was 0.43 to 1 suggesting that the bovine transgene and the endogenous porcine gene are under slightly different control mechanisms. The higher level of total α-lactalbumin present on day 0 of lactation was correlated with higher lactose percentage on day 0 in transgenic sows (3.8 %) as compared to controls (2.6 %) ($P<0.01$). This suggests that the transgene may be causing lactational performance to reach a maximum at an earlier time in transgenic sows.

Key Words: Milk Proteins, Milk Yield, Lactose

854 Development of transgenic livestock for biomedical applications. W. L. Fodor, *Alexion Pharmaceuticals, Inc., New haven, CT.*

The major barrier to clinically successful discordant xenotransplantation is the lack of effective therapies that eliminate antibody and complement-dependent hyperacute rejection. Genetic engineering of donor cells and animals to prevent hyperacute rejection has focused on reducing antibody deposition and/or complement activation. We have developed a strategy which utilizes a co-expression of transgenes that target complement inhibition and natural antibody reactivity. This combinatorial approach has lead to the development of transgenic mice and pigs that express human CD59, a species restricted complement inhibitor, and α1,2-fucosyltransferase (α1,2-FT), an enzyme that competitively inhibits expresssion of the predominant xenogeneic epitope, Gal α1,3-Gal. Double transgenic animals were were produced by intercrossing single transgenic independant lines and were identified by Southern Blot and flow cytometry analyses. Tissue analysis revealed RNA and protein expression of both transgenes in all tissues analyzed. Isolated splenocytes from control and transgenic mice containing one or both transgenes were used for complement-mediated cytolysis assays with human serum. α1,2-FT or hCD59 provided some protection from cell lysis at the different concentrations of serum assayed, however expression of both transgenes completely abolished lysis from 2.5 to 40% human serum. Thus, the combinatorial strategy, reduction of natural antibody binding by significantly reducing Gal α1,3-Gal epitope expression, and inhibition of human complement leads to the prevention of hyperacute rejection.

Key Words: Transgenic, Xenotransplantation

855 Expression of human growth hormone in the guinea pig mammary gland by *in vivo* transfection using DEAE dextran. J. R. Hens*[1], M. D. Amstutz[2], F. L. Schanbacher[2], and I. H. Mather[1], [1]*University of Maryland,* [2]*OARDC/Ohio State University.*

We have previously described a method for transfecting mammary glands *in vivo* with recombinant DNA expression vectors (Hens, J. R. *et al.*(1996) Mol. Bio. Cell 7, 141A).The objective of this study, was to improve and simplify the previous method for *in vivo* transfection. Guinea pig mammary glands were transfected by intramammary infusion via the streak canal of the nipple with a plasmid (pcDNAI/hGH) encoding human growth hormone (hGH) under the control of the CMV promoter. Expression was monitored in control and transfected glands by assaying milk samples for hGH with a radioisotopic assay (Nichols Institute, CA). In the previous procedure, mammary glands were transfected with DNA complexed to a mixture of DEAE dextran, histamine derivatized poly-L-glutamic acid, and poly-L-ornithine, and a maximum expression level of 40 ng/ml milk was achieved. In this study, we determined that only DEAE dextran was required for transfection and sustained expression in the guinea pig mammary gland. A maximum expression level of 551 ng hGH/ml milk was achieved using only DEAE dextran complexed to the DNA vector in the transfection mixture. Guinea pigs at different stages of mammary gland development (mid-pregnancy, prepartum, 3 d of lactation, and 3 d of involution) were transfected and assayed for hGH. Guinea pigs transfected prepartum (up to 5 d) had consistently higher levels than any other developmental time point which agrees with our previous study. Reverse transcriptase−PCR with primers specific to hGH verified that the RNA message was only present in the transfected glands. Using this transfection method, it should be feasible to study the role of proteins required in trace amounts, e.g., growth factor or cytokines on mammary gland development and/or function without the need to produce transgenic animals. Supported by Competitive Grant ANSC−95−53 from the Maryland Agriculture Experiment Station to I. H. M., and Natl. Dairy Prom. and Res. Board to F. L. S.

Key Words: In Vivo Transfection, Mammary Gland

856 Effect of ergotamine on reproductive hormones in follicular phase heifers. R. Browning, Jr.[1]*, F. N. Thompson[2], F. N. Schrick[3], S. J. Gissendanner[1], and T. Wakefield, Jr.[1], [1]*Tennessee State University, Nashville,* [2]*University of Georgia, Athens,* [3]*University of Tennessee, Knoxville.*

Endocrine responses to ergotamine, an alkaloid associated with fescue toxicosis, were examined in four cycling Holstein F_1 heifers (397 kg; SD = 21). Luteolysis for paired heifers was induced synchronously with two $PGF_{2\alpha}$ injections given 11 d apart on two occasions. Two days after the second $PGF_{2\alpha}$ injection, plasma was sampled every 15 min for 1 h before a bolus i.v. treatment of saline (SAL) or ergotamine tartrate (19 μg/kg body weight; ET) and for 4 h after treatment. Heifers were then administered 100 USP units of oxytocin i.v. and bled every 30 min for another 3 h. Air temperature (31.4°C; SD = 1.3) and relative humidity (42%; SD = 11) was recorded hourly. Each heifer received one treatment per synchronized period and both treatments during the study. Progesterone decreased and estradiol increased during the 2 d from $PGF_{2\alpha}$ injection to plasma sampling in each instance. Treatment × time affected concentrations of prolactin (PRL; $P < .001$), FSH ($P < .1$), LH ($P < .01$), and 13,14-dihydro-15-keto-$PGF_{2\alpha}$ (PGFM; $P < .001$). Prolactin declined ($P < .001$) from 23.1 pg/mL before ET to 5.4 and 5.8 ± 2.1 pg/mL 3 and 4 h, respectively, after ET. Ergotamine increased ($P < .001$) PGFM from 30.7 pg/mL pretreatment to 68.9 ± 5.8 and 84.5 ± 8.2 pg/mL 4 and 5 h, respectively after treatment. Prolactin and PGFM returned to pre-ET concentrations 5 and 6 h, respectively, after ET. Prolactin and PGFM were unchanged after SAL, but LH and FSH were increased ($P < .001$) after SAL. Changes in LH and FSH were mainly observed on one day when both heifers were in estrus during sampling. The SAL heifer had a surge of LH (15.1 ng/mL pre-SAL to 50.7 ng/mL 2 h post-SAL) and FSH. The ET heifer had consistently declining LH (9.4 ng/mL pre-ET to 1.2 ng/mL 6 h post-ET) and stable FSH. Across the study, ET did not affect ($P > .1$) LH or FSH. Ergotamine altered PRL and PGFM, but not FSH or LH concentrations during the follicular phase of the bovine estrous cycle.

Key Words: Fescue Toxicosis, Ergotamine, Hormones

857 Follicular populations of beef heifers exposed to endophyte-infected (E+) tall fescue *in utero*. H. L. McLane*, N. R. Rohrbach, B. H. Erickson, and F. N. Schrick, *University of Tennessee, Knoxville.*

The objective of the study was to elucidate potential effects of exposure to E+ tall fescue from conception until weaning on follicle populations and anterior pituitary responsiveness in heifers. Eighty-five cows were bred and randomly placed on pastures of three endophyte-infection levels (control (C), <5%; moderate (M), 65%; high (H), >80%). Heifers (n=25) produced from these cows (C=6; M=12, and H=7; 7–9 mo; BW range=147–274 kg) were randomly selected 5 d prior to weaning, injected with GnRH agonist (0 h), and bled at 0, 1 and 2 h for LH, FSH, estradiol 17-β (E_2) and prolactin (PRL). At weaning, heifers were ovariectomized, ovaries measured, sectioned into quarters, fixed with Bouin's fixative and divided into eight 10 μm sections. Quantity of primordial/primary, growing and vesicular (small, medium and large; normal or atretic) follicular populations were identified via light microscopy. Concentrations of PRL were 150.0 ± 11.5, 12.8 ± 8.1, and 6.7 ± 10.6 ng/mL for C, M and H heifers, respectively (P=.0001). Concentrations of E_2 were greater for C (6.6 ± .2 pg/mL) than for M (5.6 ± .1 pg/mL; P=.0002) and H heifers (5.7 ± .2 pg/mL; P=.001). Peak concentrations of LH (36.8 ± 3.0 ng/mL) and FSH (2.9 ± .2 ng/mL) did not differ between C, M and H heifers. Numbers of primordial/primary follicles were greater for C (244,608 ± 40,928) compared to H heifers (129,805 ± 35,399; P=.04), while M heifers did not differ (176,378 ± 28,254). Growing follicle quantities were increased for C (15,198 ± 2,087) vs. H heifers (9,846 ± 1,805; P=.04); however, M heifers did not differ (11,545 ± 1,440). Total numbers of vesicular follicles, both normal and atretic, tended to differ among the three groups (P=.06); however, the variation in these data was due to the influence of age and BW. Exposure of heifers to E+ tall fescue from conception through weaning decreased the numbers of primordial/primary and growing follicles. Reduced reproductive efficiency associated with fescue toxicosis may in part be due to altered follicular populations within the ovary.

Key Words: Follicle, Fescue, Heifer

858 Effect of trace mineral supplementation on ovarian and luteal function in pubertal beef heifers. C. Story*, R. Rasby, D. Brink, and J. Kinder, *Dept. of Animal Science. University of Nebraska, Lincoln.*

Cross-bred, pubertal heifers were used to evaluate the effect of feeding trace minerals (Cu, Co, Mn, and Zn) on length of the luteal phase, progesterone production (P_4) during the luteal phase, size of the largest first dominant follicle, and number and size of ovarian follicles. Angus × MARC II heifers (n=19, 309 kg) were blocked by weight and age, and group fed grass hay daily. Heifers were individually fed 1.8 kg/hd/day dry-rolled corn and .6 kg/hd/day of either a control (C; Cu 13 ppm, Co .23 ppm, Mn 68 ppm, Zn 56 ppm; n=9) or treatment (Trt; Cu 521 ppm, Co 37.4 ppm, Mn 603 ppm, Zn 929 ppm; n=10) supplement for 109 days. Liver biopsies were taken on day −1 and 65 of the experiment. Beginning on day 66, blood samples were drawn twice daily (a.m. and p.m.) for the next 21 days. Ovarian follicular development was monitored daily from day 66 to day 73, then every other day through day 87 using ultrasonography. Average DMI and ADG were not different between C and Trt heifers and averaged 8.4 kg/hd/day and .7 kg, respectively. Liver biopsy mineral concentrations (ppm) on days −1 and 65 averaged: C; Cu 236 ± 124, 146 ± 124; Mn 7.8 ± .37, 7.5 ± .37; Zn 150 ± 7 , 94 ± 7; Trt; Cu 432 ± 116, 193 ± 110; Mn 7.2 ± .35, 7.0 ± .33; Zn 129 ± 7, 94 ± 7, respectively. The luteal phase began on day 5 (day 0 being the day of estrus) and lasted for 15.5 days for C heifers and was not different from Trt heifers in which the luteal phase began on day 5.5 and was 14 days in length. Luteal phase P_4 production averaged 3.36 ± .28 ng/ml and 2.91 ± .26 ng/ml for the C and Trt groups ($p > .10$), respectively. First follicular wave dominant follicle size averaged 13.4 ± .78 mm and 14.0 ± .82 mm ($p > .10$) for the C and Trt groups, respectively. Trt heifers had fewer (2.64 ± .23; $p < .07$) follicles greater than 8 mm compared to C (3.24 ± .24) heifers. Feeding Cu, Co, Mn, Zn at the levels fed in this experiment did not effect the length of the luteal phase or P_4 concentrations during the luteal phase, but Trt heifers had fewer large follicles during the first follicular wave.

Key Words: Beef Heifers, Trace Minerals, Ovary

859 Effects of intraovarian infusion of insulin-like growth factor-I (IGF-I) on ovarian follicular function in cattle. L. J. Spicer*, P. Alvarez, T. M. Prado, G. L. Morgan, and T. D. Hamilton, *Oklahoma State University, Stillwater.*

The objective of this study was to determine if increased secretion of intraovarian IGF-I, experimentally induced via minipumps, affects follicular function in cattle. Fourteen cycling Holstein cows were divided equally into two groups: Control, osmotic minipumps (containing 200 μL of .1 N acetic acid, pumping rate of 1.0 μL/h for 7 d) surgically inserted into each ovary, or IGF-I treated, osmotic minipumps as in Controls but containing 400 μg of recombinant human IGF-I. Thus, 2 μg of IGF-I were released per h into each ovary of the treated cows. All cows were synchronized with prostaglandin $F_{2\alpha}$ (2 injections, 11 d apart) and on the day of estrus, the cannulas from each pump were surgically inserted and secured into the center of the ovarian stroma, the pumps secured into the mesovarium, and all antral follicles > 4 mm were aspirated. Ovaries were assessed with rectal ultrasound on d 2, 3, 4, 5, and 6 post-surgery, and removed by left flank ovariectomy on d 7. Follicular fluid from small follicles (1 to 5 mm, pooled within cow) and individual follicles > 6 mm were collected at ovariectomy for progesterone, estradiol and IGF-I determinations. Data were analyzed by ANOVA. Intraovarian IGF-I infusion did not alter concentrations of IGF-I in follicular fluid on d 7 of treatment. Total ovarian weight (26.4 ± 2.6 g), and size of the largest (11.1 ± .3 mm) or second largest (9.1 ± .2 mm) follicles did not differ ($P>.10$) between control and IGF-I treated cows. Ultrasonography revealed that number of small (2 to 5 mm; 15.5 ± .6), medium (6 to 10 mm; 7.6 ± .4) and large (> 10 mm; 1.4 ± .1) follicles did not differ ($P>.10$) between Control and IGF-I treated cows. IGF-I treatment decreased ($P<.05$) estradiol concentrations in follicular fluid of large follicles, increased ($P<.05$) estradiol concentrations in follicular fluid of small follicles, but did not affect ($P>.10$) progesterone concentrations in follicular fluid of small or large follicles. We conclude that a 7-d infusion of IGF-I directly into the stroma of the ovary did not influence follicular growth but did alter follicular estradiol production.

Key Words: Insulin-like Growth Factor, Follicular Development

860 Ovarian follicular development following treatment with estradiol benzoate and progesterone (P4) in postpartum anestrous (AN) cows and cows which have resumed estrous cycles (CYC). F. M. Rhodes, C. R. Burke*, and K. L. Macmillan, *Dairying Research Corporation, Hamilton, New Zealand.*

During normal bovine estrous cycles, growth of a dominant ovarian follicle (DF) can be suppressed and synchronous development of a new follicular wave induced using a combination of P4 and estradiol. The aim of this study was to determine whether a strategic injection of 2 mg estradiol benzoate (E2) coincident with intra-vaginal P4 treatment (CIDR device, InterAg, New Zealand) had the same effect in postpartum AN cows as in postpartum CYC cows. On Day 3 after emergence of a new DF, all cows (n=31) were treated with a CIDR device for 6 days and CYC cows were injected with prostaglandin $F_{2\alpha}$. Half of the AN and CYC cows received E2 at the time of device insertion. Follicular turnover was monitored using transrectal ultrasonography and blood samples were collected daily for measurement of P4 and estradiol. Turnover of the DF during the period of CIDR device insertion was increased by E2 in CYC (5/5 vs 1/6; $p<0.05$), but not AN cows (5/9 vs 4/11). Mean (±SEM) duration of dominance of the DF present on Day 3 was reduced by E2 in both CYC and AN cows (7.9±1.1 vs 10.7±1.0 days; $p<0.001$). Maximum diameter of the DF was influenced by E2 and ovarian status ($p<0.05$); mean diameter was 18.3±1.1; 10.4±1.1; 12.3±1.1 and 10.0±1.1 mm for CYC; CYC+E2, AN; AN+E2 cows, respectively. Concentration of P4 following luteolysis was similar in all groups (Day 4 mean; 3.9±0.2 ng/ml). Concentration of estradiol during the treatment period was increased by E2 (Day 4 mean; 9.2±1.1 vs 1.2±1.1 pg/ml, $p<0.001$), but did not differ between CYC and AN cows. We conclude that treatment of AN cows with P4 and E2 on Day 3 after emergence does not alter the pattern of turnover of DF, but does reduce the duration of dominance and maximum diameter of the DF.

Key Words: Postpartum cows, Follicular turnover

861 Effects of fan and sprinkler cooling on the follicular chemistry of the ovulatory follicle in lactating Holsteins during the summer. S. C. Wilkinson*, J. W. Fuquay, B. L. Clark, R. B. Moore, and A. B. Moore, *Mississippi State University.*

In earlier research heat stress altered follicular chemistry and suppressed subsequent luteal progesterone concentrations whereas fan cooling resulted in increased luteal progesterone. The objective of this study was to determine the effect of fan and sprinkler cooling on steroid hormones and insulin-like growth factor-I (IGF-I) in the follicular fluid of lactating Holsteins after the onset of estrus. Twenty cows were randomly sorted into two groups. One group was placed in a shaded pen with fan and sprinkler cooling while the other group was assigned to a similar pen with no supplemental cooling. After 7d of acclimation, cows were synchronized with two injections of $PGF_{2\alpha}$, 10d apart. Starting 36h after the second injection, observations for estrus, rectal temperatures, and blood samples were obtained every 6h for 72h. Serum was separated and stored for luteinizing hormone and progesterone radioimmunoassays (RIA). Approximately 8h after observation of estrus the ovary with the ovulatory follicle was removed surgically via standing laparotomy. Follicular diameter was determined and follicular fluid was aspirated and frozen for RIA of estradiol, progesterone, testosterone, and IGF-I. Average daily temperature ranged from 21.7 to 31.1°C and relative humidity from 57.5 to 94.4%. Average rectal temperatures were 39.1 and 39.8°C for cooled and control cows, respectively ($p<0.0001$). Serum progesterone was 0.45±0.14 and 0.24±0.12 ng/ml for cooled and control cows, respectively, indicating complete luteolysis. Fan cooling lowered ($p<0.1$) testosterone in follicular fluid (3.1±6.9 vs. 20.7±7.6 ng/ml), but did not alter ($p>0.1$) estradiol, progesterone or IGFI, or the ratio of estradiol:testosterone and estradiol:IGF-I. Within treatment variation for these variables was high. Further study is needed to elucidate the cause of reduced luteal function during heat stress.

Key Words: Heat Stress, Follicle, Lactating Holsteins

862 A comparative study of ovarian function in American (US) Holstein and New Zealand (NZ) Friesian lactating dairy cows. C. R. Bilby[1], K. L. Macmillan[2], G. A. Verkerk[3], J. A. Peterson[4], A. T. Koenigsfeld[1], and M. C. Lucy[1], [1]*University of Missouri,* [2]*Universtiy of Melbourne,* [3]*Dairying Research Corporation, NZ,* [4]*AgResearch, NZ.*

First service conception rates are two-fold greater in NZ vs. US cows. A study was replicated in two locations [(LOC); US and NZ] to compare ovarian function in NZ and US cows. NZ cows (n=11) were pastured on rye grass and clover. US cows (n=11) were confinement-housed and fed a total mixed ration. An estrous cycle [day (d) 0=estrus] was synchronized beginning 50 to 80 d postpartum. Ovarian morphology was measured by ultrasound and blood samples were collected for progesterone (P4) analyses daily. P4 assays were the same for each LOC. US cows had greater body weight [(BW); 627 ± 17 kg; P<0.01] and milk yield (34 ± 1 kg/d; P<0.01), but similar body condition score (2.5 ± 0.1) compared to NZ cows (395 ± 17 kg; 17 ± 1 kg/d; 2.4 ± 0.1, respectively). There were more class 2 (6 to 9 mm; P<0.06) and class 3 (≥ 10 mm; P<0.01) follicles in the first follicular wave of US cows (LOC-by-d). The first wave dominant follicle (DF) was more persistent (P<0.01) in US cows. This persistency was associated with later emergence of the second wave DF in US vs. NZ cows (d 13.4 ± 0.4 vs. 11.6 ± 0.4, respectively; P<0.01). Nine NZ and 7 US cows had two follicular waves, but follicular turnover was sooner in NZ cows. Plasma P4 was greater in US cows (LOC-by-d, P<0.01) because of an extended luteal phase after d 15. On day 6 of the following estrous cycle, ovaries were removed. Ovarian weight (% BW) was similar, but CL weight (% BW) tended to be less for US vs. NZ cows (P=0.12). Cows were given one Controlled Internal Drug Release [(CIDR); 1.9 g of P4] device at ovariectomy. A second CIDR was inserted 4 to 7 d later, with both CIDRs being removed after a further 4 d. Plasma P4 was greater in CIDR-treated NZ vs. US cows (3.1 ± 0.1 vs. 1.8 ± 0.1 ng/ml, respectively; P<0.01). US cows had greater follicular growth, but slower follicular turnover than NZ cows. Plasma P4 differences after CIDR treatment were inversely associated with BW and milk yield in US and NZ cows.

863 Increased ovarian follicular development in cattle selected for twin births. S. E. Echternkamp* and K. E. Gregory, *USDA, ARS, Roman L. Hruska U.S. Meat Animal Research Center, Clay Center, NE.*

Selection of cattle using breeding values based on yearling ovulation rate and twinning rate of individuals and their relatives has increased twinning rate about 12% per generation. To determine associated responses in ovarian weight, follicular development, and corpus luteum (CL) number and weight, comparisons were made between ovaries of selected (S; n = 24) and unselected (US; n = 24) mature cows. Ovaries were collected immediately after slaughter at 0, 24, 48 or 72 h after a single injection of 30 mg prostaglandin F2alpha on d 17 (Estrus = d 0). Visible antral follicles were categorized by diameter as small (< 5 mm), medium (6 to 12 mm) or large (> 12 mm); CL (both active and regressing) were removed and weighed separately. Total CL weight decreased ($P > .01$) from 4.9 g at 0 h to 2.5 at 48 h and 1.8 at 72 h (SEM = .3). Ovaries of S cows were larger and contained more follicles and CL compared to US cows. Number of large follicles increased ($P > .01$) from 1.0 at 0 h to 1.8 at 48 h (SEM = .1), primarily due to a greater number in S cows (population x time; $P = .06$).

Trait	S	US	SEM	*P*
Ovarian wt, g	22.7	16.1	1.2	.01
Small follicle	63.0	43.6	5.2	.05
Medium follicle	7.9	3.6	1.3	.05
Large follicle	1.6	1.1	.1	.01
No. CL	1.5	1.1	.1	.01
Total CL wt, g	3.6	3.0	.2	.05

Results suggest that long-term selection for twin births in cattle has increased the size of the small follicle pool and the number of growing and ovulatory follicles.

Key Words: Cattle, Twins, Ovarian Follicles

864 Peripheral 13, 14-dihydro-15-keto-PGF$_{2\alpha}$ concentrations and corpus luteum development following metestrus administration of Syncro-Mate B. J. H. Hampton*, J. C. Spitzer, D. M. Henricks, G. L. Burns, S. L. L. Gray, B. S. Hix, and H. L. Higdon III, *Clemson University, Clemson, South Carolina.*

The objectives of this study were to examine the effects of metestrus administration of Syncro-Mate B ® (SMB) on PGF$_{2\alpha}$ secretion and corpus luteum (CL) development. In a study replicated over two years, 45 multiparous, postpartum beef cows were randomly allotted at estrus (d 1) to receive either the standard SMB regimen (n = 37) on d 3 of the estrous cycle or no treatment (n = 8). Twice daily serum samples were analyzed for progesterone (P_4) and 13, 14-dihydro-15-keto-PGF$_{2\alpha}$ (PGFM) from d 2 A. M. through d 10 A. M. of the estrous cycle. Prior to statistical analysis, SMB treated cows were sorted according to CL function at d 10 A. M. of the treated estrous cycle to either CL functional ($P_4 \geq 1$ ng/mL; n = 20) or CL non-functional ($P_4 < 1$ ng/mL; n = 17) groups. Fifty-four percent of SMB treated cows retained a functional CL through d 10 A. M. of the treated estrous cycle. Mean serum P_4 concentrations increased for cows in all treatment groups until d 7, after which P_4 concentrations increased in cows retaining functional CL and control cows and decreased in cows retaining non-functional CL. Mean serum PGFM concentrations tended to increase in cows with non-functional CL versus control cows on d 8 A. M. (P = .06) and was significantly higher than cows with functional CL on d 8 P. M. through d 9 P. M. (P < .05). These results indicate that retention of a functional versus a non-functional CL subsequent to metestrus administration of SMB is dependent upon premature release of uterine PGF$_{2\alpha}$.

Key Words: Corpus Luteum, Prostaglandin F$_{2\alpha}$, Syncro-Mate B

865 Hormonal regulation of the production of insulin-like growth factor binding proteins (IGFBPs) by bovine theca cells. C. C. Chamberlain* and L. J. Spicer, *Oklahoma State University, Stillwater.*

Insulin-like growth factors-I and -II (IGF-I and -II) and IGFBPs have been identified as potential regulators of ovarian function. In cattle, levels of the low molecular weight IGFBPs, including IGFBP-2, -4, and -5, decrease with follicular development and conversely, increase with follicular atresia. Thus, the objective of the present study was to determine the effects of insulin, LH, epidermal growth factor (EGF), and basic fibroblast growth factor (bFGF) on the production of IGFBPs by bovine thecal cells. Thecal cells from large (> 8 mm) follicles were isolated and cultured for 2 d in the presence of 10% fetal calf serum. Then cells were cultured for an additional 24 h in serum-free medium containing insulin (100 ng/mL), LH (100 ng/mL), insulin (100 ng/mL) plus LH (100 ng/mL), insulin (100 ng/mL) plus EGF (10 ng/mL), insulin (100 ng/mL) plus bFGF (10 ng/mL), or no additions (control). There were no significant (P > .05) treatment effects on IGFBP-2 production whereas a trend (P < .10) was observed with IGFBP-3 and a 24-kDa IGFBP. In contrast, a 27-kDa and 22-kDa IGFBP were affected (P < .05) by hormone treatment. Insulin increased (P < .05) production of the 27-kDa IGFBP, whereas LH had no effect on basal or insulin-stimulated IGFBP production. The addition of bFGF blocked the insulin-induced increase in the 27-kDa IGFBP, whereas EGF had no effect. Insulin plus LH increased (P < .05) the 22-kDa IGFBP, but the effects were not additive when compared to either insulin or LH. These results suggest that intrafollicular production of the IGFBPs are hormonally regulated in cattle. Insulin and bFGF, but not LH and EGF may play a role in modulating bioavailability of the IGFs during folliculogenesis via their effects on intrafollicular IGFBP production.

Key Words: Insulin-like Growth Factor, Binding Proteins, Thecal Cells

866 Intramuscular injection of recombinant ovine interferon-tau extends luteal life span in Angora goats. D. O. Kiesling*[1], A. D. Ealy[2], and A. N. V. Stewart[1], [1]*Lincoln University, Jefferson City, MO and* [2]*University of Missouri, Columbia*.

The objective of this experiment was to determine the effect of intramuscular injection of recombinant ovine interferon tau (roIFN-τ;p3 variant) on life-span of the corpus luteum, concentration of serum progesterone (P_4) and rectal temperature in non-pregnant Angora does (2–7 yrs of age). Estrus was synchronized by intramuscular injection of $PGF_{2\alpha}$ (15 mg). Does observed in estrus were assigned randomly to receive intramuscular injections of 1) PBS (control; n=11); 2)0.125 mg of roIFN-τ (n=11); or 3)0.5 mg of roIFN-τ (n=11) once daily from d 14–20 post-estrus. Concentrations of serum P_4 were quantified in blood collected on d 0 (estrus), d 7, d 14–21, then every 3 d until d 30 post-estrus when the study was terminated. Luteal life-span was determined as days from the synchronized estrus until the subsequent fall in serum P_4 (< 0.5 ng/ml). Does with elevated serum P_4 levels upon termination of the study (n=3) were considered to have a 30 d luteal life-span. Rectal temperatures were recorded daily preceding and at 3 h and 6 h (d 14 only) following treatment. Luteal life-span was of normal duration in controls (19.3±1.5 d) but was increased (P<.05) in does receiving 0.125 mg (24.0±2.7 d) and 0.5 mg (26.4± 2.6 d) roIFN-τ. Mean concentration of serum P_4 (d 14–17) was not affected by treatment with either dose of roIFN-τ. Rectal temperature was increased(P<.05) on d 14 at 3 and 6 h ($\overline{x}$= 40.2 ±.09°C) in does given 0.125 mg and returned to normal levels by 24 h (38.8 ±.09°C) when compared to controls (38.9 ± .04°C). Rectal temperatures of does given 0.5 mg on d 14 were greater than controls (P<.05) at 3 and 6 h ($\overline{x}$=40.1 ±.09°C) and remained elevated (P<.05) at 24 h (39.5±.09°C). Rectal temperatures were greater (P<.05) at 3h in the 0.125 mg roIFN-τ group on d 14–18 compared to controls, whereas, 0.5 mg roIFN-τ caused rectal temperatures to remain higher (P<.05) on d 14–20 compared to controls. In summary, as little as 0.125 mg/d of roIFN-τ was able to extend luteal life-span, did not affect serum P_4 concentrations but did induce a prolonged and repeated hyperthermia in goats.

Key Words: Goat, Ovine Interferon, Dosage

867 In vitro oxytocin (OT) and progesterone (P4) production by bovine luteal tissue incubated in a perifusion system and treated with prostaglandin $F_{2\alpha}$ (PGF). R. P. Del Vecchio*[2] and W. D. Sutherland[1], [1]*Agriculture & Agri-Food Canada, Brandon Research Centre, Brandon, MB,* [2]*Louisiana State University, Baton Rouge*.

To further study the discrepancy between in vivo and in vitro effects of PGF on luteal function at the time of expected luteal regression we examined the effects of varying doses of PGF on OT and P4 production by bovine luteal tissue collected on d16, 17 and 18 (n=6) of the estrous cycle and incubated in a perifusion system for 450 min at a flow rate of 100 μL per min. Treatments were control, 10^1, 10^2, 10^3, 10^4 and 10^5 nM PGF. Data were square-root transformed and expressed as pg per 100 mg of tissue per 10 min. A treatment x d interaction showed that (P<.01): in the control (135.5 ± 3.1) and 10^1 nM PGF (162.3 ± 4.7) groups, d16 tissue produced more P4 than d17 (128.7 ± 3.1 and 116.3 ± 4.7) or 18 (122.8 ± 3.1 and 125.2 ± 4.7); in the 10^2 nM treatment d18 tissue produced more P4 than d16 with d17 being intermediate; the 10^3 treatment was similar among d; and in the 10^4 and 10^5 PGF treatments d16 and 18 tissue produced more P4 than d17. Compared to controls (P<.05): on d16 10^1, 10^4 and 10^5 nM PGF increased P4, 10^2 decreased P4, and 10^3 had no affect on P4; on d17 10^1 decreased, 10^2 and 10^3 had no affect, and 10^4 and 10^5 increased P4; on d18 10^1 and 10^2 had no affect, 10^3, 10^4 and 10^5 increased P4. On d16 and 18 OT production was similar among treatments. On d17 10^4 nM PGF increased (P<.001) OT, all other treatments were similar. Production of OT on d16 in the control (18.7 ± .5) and 10^2 nM PGF (18.4 ± .6) treatment was similar to d18 (19.6 ± .5 and 18.0 ± .6), both were greater (P<.05) than d17 (17.3 ± .5 and 16.3 ± .6). In the 10^1, 10^3, 10^4 and 10^5 nM treatments OT production was greater (P<.05) on d18 than 16 or 17, with d16 greater than 17 in the 10^1 and 10^5 treatment, d17 greater than 16 in the 10^4 treatment and d16 and 17 were similar in the 10^3 nM PGF treatment. These data show that high doses of PGF can effectively stimulate in vitro P4 and OT production by late stage bovine luteal tissue.

Key Words: Corpus Luteum, Prostaglandin $F_{2\alpha}$, Oxytocin

868 Effect of short-term calf removal at three stages of a follicular wave on fate of a dominant follicle in postpartum beef cows. B. E. Salfen*, M. F. Smith, and H. A. Garverick, *University of Missouri, Columbia*.

In previous studies, short-term calf removal (CR) induced an ovulatory estrus in postpartum beef cows; however, the percentage of cows that responded to CR was variable. We hypothesized that response to short-term CR is dependent upon the stage of dominant follicle development at the time of CR. The objective was to characterize the fate of a dominant follicle following 48 hr CR on days 2, 4, or 8 of a follicular wave. Ovaries of sixty-one beef cows were examined, by transrectal ultrasonography, daily starting at day 20-21 postpartum. Blood was collected daily for subsequent analysis of serum progesterone (P_4) and LH. Treatments were: No CR (CON;n=14), CR at days 2 (DAY2;n=12), 4 (DAY4;n=16) or 8 (DAY8;n=10) of the first detected follicular wave. An additional group consisted of cows which had no detectable follicular waves (STATIC;n=9). The percentage of cows that ovulated a dominant follicle following treatment were 14.3, 33.3, 18.8, 30.0 and 0 for CON, DAY2, DAY4, DAY8, and STATIC, respectively (P=.62). Maximum size of a dominant follicle was larger in cows that ovulated (P=.002) across all treatments compared to cows that did not ovulate. Follicular growth rate (mm/day) was higher during days 0–2, 4–6 and 8–10 (P=.011, .012 and .038, respectively) for follicles destined to ovulate. The dominant follicle was larger (P<.05) on day 6 and days 8–13 of a follicular wave in cattle that ovulated compared to animals that did not ovulate. Serum P_4 was higher following calf return than before or during CR (P<.05). LH concentrations were not affected by treatment. In summary, follicular growth rate as well as maximum diameter of the dominant follicle may be factors that can predict the likelihood of ovulation and resumption of postpartum cyclicity. Stage of the dominant follicle development at the time of CR did not influence the likelihood of ovulation in this study.

Key Words: Postpartum, Calf-removal, Follicle

869 Acquisition of ovulatory capacity by ovarian follicles during growth of follicular waves in lactating dairy cows. R. Sartori, P. M. Fricke*, J. C. P. Ferreira, O. J. Ginther, and M. C. Wiltbank, *University of Wisconsin-Madison*.

Selection of dominant follicles in cattle is associated with deviation in growth rate between the dominant and largest subordinate follicle of a wave (Ginther et al., *Biol Reprod* 55:1187, 1996). To determine if acquisition of ovulatory capacity is temporally associated with deviation, follicular growth was synchronized in lactating dairy cows using gonadotropin-releasing hormone (G) and prostaglandin $F_{2\alpha}$ (P) as follows: Day −9, G (100 μg); Day −2, P (25 mg); Day 0, G (100 μg). Follicular dynamics were monitored using transrectal ultrasound, and ovulatory capacity was assessed by administration of LH (Lutogen, Ausa International, Inc., Tyler, TX). To determine when follicles acquire ovulatory capacity in relation to deviation, cows (n=27) received 4 mg LH on Day 3, 4, 5, or 6 (Experiment 1). Diameter of the dominant and largest subordinate follicles of the wave was 8.0 ± 0.4 vs. 7.1 ± 0.4 mm, respectively, at deviation onset and 10.5 ± 0.4 vs. 7.3 ± 0.3 mm, respectively, 24 h later. No follicles (0/16) ≤ 10 mm (range = 7–10 mm), 25% (1/4) of 11 mm follicles, and 100% (13/13) of follicles ≥ 12 mm (range = 12–15 mm) ovulated. To determine the effect of LH dose on ovulatory capacity, cows (n=26) received either 4 (LH4) or 24 (LH24) mg LH when the largest follicle of the wave first achieved 10 mm (Experiment 2). Ovulation occurred in 7.7% (1/13) of LH4 vs. 69.2% (9/13) of LH24 cows (p<0.05). To determine the effect of a pharmacological LH dose on ovulatory capacity, cows (n=22) received 40 mg LH when the largest follicle of the wave first achieved 7, 8.5, or 10 mm (Experiment 3). No 7-mm (0/7) or 8.5-mm (0/7) follicles ovulated compared with 75% (6/8) of 10-mm follicles. In summary, no follicles < 10 mm exhibited ovulatory capacity and, although follicles near deviation acquired ovulatory capacity, they required a higher LH dose to ovulate compared with larger follicles. Thus, acquisition of ovulatory capacity is temporally related to deviation and may occur due to granulosa cell LH receptor expression at deviation.

Key Words: Follicular Waves, Ovulation

870 Effect of a deslorelin implant on follicular dynamics and progesterone profiles of postpartum dairy cows. R. Mattos*, C. Orlandi, C. R. Staples, and W. W. Thatcher, *University of Florida, Gainesville, FL*.

Objective was to determine if an implant, containing the GnRH analogue deslorelin (DESL; 2100 μg, SC), would alter ovarian follicle dynamics and CL activity in postpartum dairy cows. Cows received DESL on day 7 postpartum (d7, n=8) or were untreated (Control, n=9). All cows were injected with gonadotropin releasing hormone (GnRH, 100 μg, im) on d14 and with $PGF_{2\alpha}$ (25 mg, im) on d21. Ultrasound scanning of the ovaries and collection of blood samples for progesterone (P_4) analysis were performed thrice weekly. Follicles were classified by size as class 1 (2-5 mm), class 2 (6-9 mm), or class 3 ($\geq$10 mm). A timed insemination (TI) protocol was initiated on d60 (GnRH [d60], $PGF_{2\alpha}$ [d67, 25 mg and d68, 15 mg], GnRH [d69], AI [d70]). Frequency of induced ovulations following GnRH injection on d14 did not differ between DESL (25.0%) and control groups (44%). Cumulative responses of follicle numbers in each class and plasma P_4 concentrations were evaluated for the periods of d7 to d21 and d22 to d60. Cumulative numbers of class 1 ($p<0.1$) and 2 ($p<0.001$) follicles were higher and class 3 ($p<0.001$) follicles were lower in DESL cows between d7 to d21. During the period of d22 to d60, DESL had a greater rate of cumulation of class 1 follicles and decreased rates of cumulation of class 2 ($p<0.001$) and class 3 ($p<0.001$) follicles, and plasma progesterone ($p<0.001$). DESL differentially arrested follicle development at the 10 mm (LH limited) and 6 mm (LH and FSH limited) sizes during periods d7 to d21 and d22 to d60, respectively. Restoration of follicle development (occurrence of class 3 follicle) was evident by d47.1 (range 28 to 67d) in the DESL group. All cows ovulated in response to the GnRH injection at d69 of the TI protocol, and subsequent luteal phase increases in plasma P_4 concentrations (d70 to d84) did not differ [$(0.61\ ng.ml^{-1})d^{-1}$]. DESL implant delays early ovarian activity and avoids high progesterone levels during the period of uterine involution.

Key Words: Cow, Deslorelin, Follicle

871 Reduction in size of the ovulatory follicle reduces subsequent luteal size and pregnancy rate. J. L. M. Vasconcelos[1], R. Sartori[2], H. N. Oliveira[1], J. N. Guenther[2], and M. C. Wiltbank*[2], [1]*FMVZ-UNESP-BOTUCATU, Brazil,* [2]*University of Wisconsin-Madison*.

Ovulation of a large persistent follicle reduces fertility compared to ovulation of a normal-sized follicle. We hypothesized that reducing the size of the ovulatory follicle would increase fertility. Lactating dairy cows (n = 53) had synchronized ovulation and artificial insemination (AI) by treating with gonadotropin releasing hormone (G) and prostaglandin F2a (P) as follows: Day -9, G (100μg); Day -2, P (25 mg); Day 0, G (100 μg); Day 1, AI. To vary the size of the ovulatory follicle, all follicles >4 mm diameter were aspirated on Day -5 (A-5; n = 15), on Day -6 (A-6; n = 15) or had no follicles aspirated (Con; n = 23). Size of follicles and corpora lutea (CL) were monitored by transrectal ultrasound. Some cows did not ovulate in response to the 2nd GnRH injection (A-5 = 5/15 - 33% ; A-6 = 3/15 - 20%; Con = 2/23 - 9%;) and some cows had double ovulations (A-5 = 4/15; A-6 = 0/15; Con = 2/23). These cows were not included in subsequent analyses. As expected, the ovulatory follicle on Day 9 was smaller ($P<0.01$) for cows that had follicles aspirated (A-5 = 11.0$\pm$0.8 mm; A-6 = 12.1$\pm$1.1) than controls (14.7$\pm$1.5). CL volume was smaller ($P<0.05$) for aspirated than control cows on Day 7 (A-5 = 3181$\pm$685 mm3; A-6 = 2829$\pm$828; Con = 5364$\pm$1625) or Day 14 (A-5 = 4299$\pm$1455; A-6 = 4881$\pm$770; Con = 6526$\pm$1602). Similarly serum progesterone concentrations tended to be lower ($P<0.1$) for aspirated than control cows. Pregnancy rate for synchronized cows (including cows with double ovulations) was lower ($P<0.05$) for aspirated (3/22 = 12.5%) than control (10/21 = 47.6%) cows. In contrast to our hypothesis, ovulation of smaller follicles produced lowered fertility apparently due to decreased serum progesterone concentrations due to development of a smaller CL.

Key Words: Reproduction, Follicle, Dairy Cows

872 Follicular selection and atresia in gilts selected for an index of high ovulation rate and high prenatal survival. H. W. Yen*, R. K. Johnson, and D. R. Zimmerman, *University of Nebraska, Lincoln*.

Our laboratory reported previously (J.Anim Sci 75:80, 1997)that White Line gilts selected for an index of high ovulation rate and high prenatal survival (WL-2) maintained a larger pool of medium follicles (3 to 6.9 mm) during the mid follicular phase than control line gilts (WL-1). The present study evaluated whether or not the medium follicles maintained by WL-2 gilts are healthier and capable of being selected as ovulatory follicles later in the follicular phase to achieve their ovulation rate advantage (6.6 ova). Sixty-seven 10^{th} generation WL-1 and WL-2 gilts with 2 or more estrous periods were assigned randomly within sire for ovary recovery on days 0, 2, 3, 4, and 5 following PGF2α induced luteolysis on day 13 (d0) of the estrous cycle. All surface follicles (F) above 3 mm were categorized into medium (M1F, 3 to 4.9 mm; M2F, 5 to 6.9 mm)and large (LF, $\geq$7 mm)follicles. Estradiol (E)concentration in follicular fluid was used to classify individual M2F and LF as healthy ($\geq$100ngE/mL) or atretic (<100ngE/mL). M1F were not yet estrogen active(<60ngE/mL)and could not be evaluated for atresia with this method. Mean E in M2F increased over time in both genetic lines (day, $p<.02$). Percentages of healthy M2F were greater numerically but not statistically in WL-1 gilts on days 2 and 3 (WL-1, 64.6 and 74.6% vs WL-2, 56.8 and 54.6%, $p>.1$). The percentage of healthy M2F increased rapidly in WL-2 gilts from day 3 to days 4 and 5 (54.6 to 90.3, $p<.05$ and 95.5%,$p<.07$)but was unchanged in WL-1 gilts after day 3 (74.5 to 68 and 77.8% on d4 and d5, respectively). All LF were estrogen active (>100ngE/mL)and classified as healthy in both lines. WL-2 gilts maintain a larger pool of healthy M2F during the mid to late follicular phase from which they select ovulatory follicles to achieve their ovulation rate advantage.

Key Words: Pig, Follicular Development, Genetic Selection

873 Cell turnover in the mammary gland. A. V. Capuco[1]* and J. Byatt[2], [1]*USDA-ARS, Beltsville, MD and* [2]*Monsanto Dairy Business, St. Louis, MO*.

The bovine mammary gland undergoes cyclical periods of growth and regression during the reproduction/lactation cycle. However, during a typical dry period, a net loss of mammary cells did not accompany the change to nonsecretory state. During the 60 d prepartum, mammary DNA content increased with advancing gestation and did not differ between lactating and dry cows at the same stage of gestation. Nevertheless, greater ^{3}H-thymidine labeling in mammary tissue from dry than lactating cows ($P \leq 0.05$) indicated that turnover of mammary epithelial cells was promoted by a dry period. Cows which had a dry period entered the next lactation with a proportionally larger ($P \leq 0.05$) and younger population of epithelial cells. During lactation, a gradual decrease in number of mammary epithelial cells occurs, but is accompanied by considerable cell replacement. One to 4% of mammary cells in lactating nonpregnant cows were labeled by a 24-h *in vivo* treatment with the thymidine analog, bromodeoxyuridine. Increasing cell renewal during lactation may provide a means to increase lactational persistency. Indeed, administration of bST to Holsteins during mid lactation increased the percentage of mammary epithelial cells that expressed the nuclear proliferation antigen, Ki-67, from 0.5 to 1.6% ($P \leq 0.05$). Influence of bST administration on gene expression profile was investigated using the gene expression microarray technology. Comparison of expression profiles for mammary tissue from control and bST-treated lactating cows revealed twofold or greater differential expression of several genes involved in cell proliferation and differentiation as well as many genes that remain to be identified. Data from expression array and from studies investigating the regulation of apoptosis and cell proliferation should provide leads to mechanisms that modulate cell turnover in the mammary gland. Increased cell turnover during the dry period may enhance milk yield in the next lactation, and a change in the ratio of epithelial proliferation to cell death during lactation may affect the persistency of lactation.

Key Words: Proliferation, Apoptosis

874 Cell turnover in the ovary. L. P. Reynolds and D. A. Redmer, *North Dakota State University, Fargo*.

Ovarian tissues are critical for normal reproductive function, and also are some of the few adult tissues that exhibit regular cycles of growth and regression. Growth, development, and regression of ovarian follicles and corpora lutea (CL) is extremely rapid, equaling or exceeding that of even the most aggressive tumors. For example, during the CL's rapid growth phase (i.e., from ovulation until about day 10 after estrus) its tissue mass and number of cells double every 72 h. This rapid growth is reflected by a high rate of cell proliferation of the ovarian follicles and CL. In addition, for both follicles and CL, their rate of regression is even more rapid than their rate of growth. Recent work in cows and sheep has shown that gonadotropins (FSH and LH), which are the principle folliculotropic hormones, maintain the growth of tertiary (antral) follicles not only by promoting proliferation but also by inhibiting cell death (apoptosis) of granulosa and thecal cells. In CL of cows, pigs, and sheep, it recently has been shown that the rate of production of new cells remains high even after the CL has reached its mature size. These observations suggest that cell turnover plays a role not only during luteal regression but also in determining the final species-specific size of the CL. Thus, cell turnover, which includes both cell proliferation and cell death, is critical for the normal function of ovarian tissues, and therefore for the entire reproductive process. Our most recent studies have begun to unravel the importance of various cell types, including several vascular cell types, in normal growth and development of ovarian follicles and CL. In addition, we have identified locally-produced factors, namely vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) that are important regulators of these processes. These observations may lead to improved methods of regulating ovarian function in domestic livestock.

Key Words: Ovary, Cell Turnover, Livestock

875 Leptin mRNA expression, serum leptin concentrations and LH secretion in gilts as influenced by age, weight and estradiol. H. Qian*[1], C. R. Barb[2], M. M. Compton[1], J. Hausman[2], J. J. Azain[1], R. R. Kraeling[2], and C. A. Baile[1], [1]*University of Georgia, Athens,* [2]*USDA/ARS/R. B. Russell, Athens, GA*.

Effects of age, weight and estradiol on leptin gene expression and LH secretion was investigated. RNase protection assays were used to assess mRNA in adipose tissue of ovariectomized gilts at 90 (n=12), 150 (n=12) or 210 (n=12) days (d) of age. Six pigs from each age group received estradiol via (E) osmotic pump implants and the remaining animals received sham (S) implants (d 0). On d 6, blood samples were collected every 15 min for 8 h to assess LH secretion. On d 7, back fat and blood samples were collected to assess leptin gene expression, serum leptin, insulin, insulin-like growth factor-I (IGF-I), E and plasma glucose concentrations. Body weight increased ($P < 0.05$) with age and was 43 $\pm$ 1, 85 $\pm$ 3 and 115 2 $\pm$ kg for 90, 150, and 210 d old pigs, respectively. Plasma glucose levels were similar among animals. Serum insulin levels were similar among treatments for 150 and 210 d old pigs. However, insulin levels were greater ($P < 0.01$) in S than E pigs at 90 days. Serum IGF-I levels were similar between treatments within each age. Serum E levels were greater ($P < .05$) in E (9 $\pm$ 1 pg/mL) than S (3 $\pm$ 1 pg/mL) pigs. Serum leptin concentrations were not affected by age, but were greater ($P < .05$) in E (2.9 $\pm$ 0.1 ng/ml) than S (2.6 $\pm$ 0.1 ng/ml) pigs. Leptin mRNA expression was not affected by age in S pigs nor by E in 90 and 150 d old pigs. However, by 210 d of age, leptin mRNA expression was 2.5 fold greater ($P < 0.01$) in E treated pigs compared to S animals. Number of LH pulses was 2.5 fold greater ($P < .05$) in E-treated 210 d pigs compared to E-treated 150 d old pigs. Number of LH pulses were unaffected by age in S pigs. These results suggest that E-induced leptin mRNA expression is age and weight dependent and is associated with greater LH secretion in the 210 day old E gilts.

Key Words: Leptin, Pig, LH

876 Effects of lateral cerebroventricular infusion of leptin on ewe lambs. C. D. Morrison*, J. A. Daniel, B. J. Holmberg, O. U. Bolden, N. Raver[1], A. Gertler[1], and D. H. Keisler, *University of Missouri-Columbia,* [1]*The Hebrew Univ. of Jerusalem, Rehovot, Israel*.

The adipose derived hormone, leptin, has been implicated as a key component in the regulation of feed intake. Central and peripheral administration of leptin has been shown to suppress feed intake in mice, rats, and pigs. The objective of this study was to determine if centrally administered leptin would similarly suppress feed intake in ewe lambs. Ewe lambs were ovariectomized and fitted with two lateral cerebroventricular (LCV) cannula. Saline or saline with recombinant ovine leptin was continuously infused over an eight day period. Leptin was infused with a linearly increasing dose reaching a maximum dose of 50ug/hr (approximately 1.25ug/kg/hr) on day eight. Ewes were allowed access to feed for one hour each day, and feed intake was recorded on days 0–7. Data were analyzed using the general linear model (Model: feed intake = Treatment ewe(treatment) day treatment$*$day) and least square means for means separation procedures using SAS. A significant treatment$*$day interaction was detected. On days 4, 5, 6, and 7 feed intake was suppressed in leptin treated ewe lambs compared to control ewe lambs of the same day ($P<0.05$). These data support an inhibitory influence of leptin on feed intake in ewe lambs.

Key Words: Leptin, Feed Intake, Sheep

877 Ontogeny of circulating androstenedione and testosterone relative to estradiol-17β, luteinizing hormone, insulin-like growth factor-I, and somatotropin in beef heifers from 64 days of age through puberty. P. D. Schoppee[1]*, W. P. Washburn[1], and J. D. Armstrong[2], [1]*North Carolina State University, Raleigh,* [2]*Purdue University, West Lafayette*.

The contribution of ovarian androgens to regulation of prepubertal gonadotropin release in livestock has largely been ignored. This study characterized circulating androstenedione (A4) and testosterone (T) relative to estradiol-17β (E2), LH, IGF-I, and ST in Angus (n=7) and Simmental (n=11) heifers from 64 d of age through puberty. Samples were collected monthly through weaning then weekly through puberty (serum progesterone $\geq$ 1 ng/mL for 2 consecutive wk). Breed and age effects were analyzed within 3 growth phases: nursing (N), 64 to 231 d of age; growth restriction (GR), 256 to 300 d of age; and peripuberty (PP), 9 wk before to 1 wk after the week of first ovulation (wk 0). Average daily gains (kg/d) were: 0.89 (N), 0.15 (GR) and 0.84 (PP). Throughout the study, A4 and T were greater and IGF-I was less in Simmental than in Angus while E2, LH and ST were similar between breeds. During N, A4 decreased from 64 to 144 d then stabilized, whereas T increased from 64 to 231 d and E2 increased from 64 to 88 d and from 202 to 231 d of age. Thus, BW was correlated to A4 (r=−0.36), T (r=0.46) and E2 (r=0.27). LH was less at 244 d and 258 d than at weaning but had increased by 265 d of age. From 244 d to 258 d of age, A4 and T increased, E2 and IGF-I decreased, and ST tended to increase. During GR, both A4 (r=−0.59) and T (r=−0.55) were correlated to IGF-I; T was also correlated to ST (r=0.40). During PP, A4 and T were lowest at wk −1 and E2, LH and IGF-I were greatest at wk 0. During wk −1 and wk 0, E2 was positively correlated to LH (r=0.82, r=0.57) and T was negatively correlated to IGF-I (r=−0.34, r=−0.41). The change in androgen concentrations with age during N and their relationship to IGF-I and ST, particularly during growth restriction, suggests a potential role for ovarian androgens in gonadotropin regulation.

Key Words: Androstenedione, Testosterone, Heifer

878 Influence of early lactation energy balance and weight change on neuropeptide-Y concentrations in primiparous beef heifers. C. A. Lents*[1], D. L. Lalman[1], D. H. Keisler[2], and J. E. Williams[2], [1]*Oklahoma State University, Stillwater,* [2]*University of Missouri, Columbia.*

Thirty-Six primiparous Angus and Angus sired heifers, calving in thin body condition score (BCS), were used to evaluate the influence of early lactation energy balance as well as prepartum and postpartum weight change on neuropeptide Y (NPY) concentrations. Animals were fed low quality hay beginning 90 d prepartum until parturition to develop animals in thin condition at calving (weight, 370 ± 31 kg; BCS, 4.0 ± .53). Weight and BCS was determined 90 d prepartum, and beginning at parturition, every 14 d. The postpartum BCS change was defined as the change in BCS from calving to 90 d postpartum. At parturition, cows were blocked by calving date and BCS and randomly assigned to one of four dietary treatments: 1.8 (L), 2.1 (M), 2.4 (MH), and 2.7 (H) Mcal ME/kg DM . Cows were maintained in drylots and fed in groups of three at 2.5% of body weight. Cerebrospinal fluid was collected at parturition, 30 d, 60 d, and 90 d postpartum. Mean concentrations of NPY were not different between the four sampling periods and were 56 ± 6, 64 ± 6, 67 ± 6, and 68 ± 6 ng/mL at calving, 30 d, 60 d, and 90 d postpartum respectively. Neuropeptide Y concentrations tended to be greater for MH and H heifers than L or M heifers ($P < .05$). Regression analyses were conducted for prepartum weight change with NPY at parturition, as well as for postpartum weight change with NPY concentrations 90 d postpartum. There was a positive regression coefficient ($\beta = .944$; $P < .03$) for prepartum weight change on NPY at parturition. The relationship of NPY at 90 d postpartum tended ($P < .07$) to be associated with postpartum weight change ($\beta = .589$). It is important to note that although variation between individual animals could result in statistical significance, this does not necessarily indicate a significant physiological difference within an animal. These data indicate that NPY concentrations were sensitive to prepartum and postpartum weight change, as well as postpartum energy content of the diet.

Key Words: NPY, Energy Balance, Heifers

879 Progesterone and estrogen effects on adipose tissue lipolysis. C. Cadorniga*, S. J. Bertics, M. Vazquez-Anon, M. C. Lopez-Diaz, and R. R. Grummer, *University of Wisconsin, Madison.*

The in vivo effects of progesterone (P_4) and in vitro effects of P_4, estradiol (E_2), and 2OH-E_2 on adipose tissue lipolysis were studied in tissue biopsies taken from six ovariectomized Holstein heifers before and after in vivo P_4 administration (75 mg/head/d; S. C.) for 14 d. Tissue slices were incubated for 48 h in the presence of E_2 (500 or 50 pg/ml), 2OH-E_2 (1 or .1 ng/ml) or P_4 (20 ng/ml), or no hormone. After a 2 h wash, lipolysis rates were measured in the presence of isoproterenol (ISO, 10 uM), 2OH-E_2 (1 ng/ml), or no addition from 50 to 52 h. In vivo P_4 administration reduced lipolysis (see Table). Lipolysis was reduced when 2OH-E_2 (1 ng/ml) was present during the 2 h lipolysis measurement (see Table) or when applied for 48 h (data not shown). In contrast, exposure to .1 ng/ml 2OH-E_2 for 48 h increased lipolysis rates in tissue from animals not receiving P_4 (265 vs 311 ug glycerol released/g tissue/h), but had no effect after P_4 injection (164 vs 160 ug glycerol released/g tissue/h; interaction, $P < 0.05$). Treatment with E_2 did not affect lipolysis. Data support the hypothesis that decreasing P_4 concentrations in blood during late gestation may increase basal or catecholamine stimulated lipolysis. Estrogen effects may be mediated by catechol metabolites. Further study is needed to understand 2OH-E_2 x P_4 interactions.

Effects of ISO or 2OH-E_2 treatment during 50–52 h of incubation on rates of lipolysis (ug glycerol released/g tissue/h).

In vivo treatment[a]	Basal	ISO[d]	2OH-E_2
$-P_4$	265	691	234
$+P_4^d$	164	307	142
mean	214	499[b]	188[c]

[a]in vivo P_4 effect ($P < 0.001$).
[b]ISO effect ($P < 0.001$).
[c]2OH-E_2 effect ($P < 0.05$).
[d]ISO x in vivo P_4 interaction ($P < 0.001$).

Key Words: Adipose Tissue, Lipolysis, Progesterone

880 Acute reduction in serum progesterone concentrations due to feed intake in pregnant lactating dairy cows. J. L. M. Vasconcelos*[2], K. A. Bungert[1], S. J. Tsai[1], F. S. Wechsler[2], and M. C. Wiltbank[1], [1]*University of Wisconsin-Madison,* [2]*FMVZ-UNESP-Botucatu, Brazil.*

In lactating dairy cows, high milk production is associated with a reduction in fertility. We hypothesized that the high dry matter intake associated with high milk production reduces serum concentrations of progesterone possibly by increased clearance of progesterone by the liver. The design of this trial consisted of three 4 X 4 balanced Latin squares with cows (n = 12 cows at 85 ± 5 days pregnant) receiving either: 100% of total mixed ration (TMR) at 0h (100%); 50% of TMR at 0h and 12h (50%); 25% of TMR at 0h, 6h, 12h, and 18h (25%); or 100% of TMR at 12h (0/100%). All feed was removed 12h before the start of the experiment. Blood samples for measurement of serum progesterone concentrations were collected every h for 25 h beginning just before first feeding (0h). Feeding management, time after feeding, and the interaction had effects ($P<0.05$) on serum progesterone (see Table). Feeding 100% or 50% of TMR reduced progesterone concentration about 15% between 1 and 9h after feeding. Cows that were not fed had an increase in progesterone concentration (0/100% group at 4 to 12h) but a reduction was observed after feeding at 12h. The group receiving 25% of TMR at 4 times/day maintained serum progesterone at or above control progesterone concentration throughout the 24h time period. Thus, abruptly feeding 50–100% of TMR can acutely reduce serum progesterone concentrations possibly by increasing progesterone clearance. Feeding cows 4 times per day maintained high serum progesterone and may improve reproduction.

Treatment	Changes in Serum Progesterone (% of P4 at 0h)								
Group	0h	1–3	4–6	7–9	10–12	13–15	16–18	19–21	22–24
100%	100a	85b	86b	87b	97a	95a	94b	100a	94b
50%	100a	89b	82b	88b	100a	88b	91b	92b	101a
25%	100a	104c	99a	99a	103a	103a	107c	102a	104c
0/100%	100a	100a	105c	109c	105c	99a	98a	94b	89b

Different letters indicate differences from 0h within a row ($P<0.01$) b indicates lower than 0h, c indicates greater than 0h. 100% averaged 6.61 ng/ml.

Key Words: Reproduction, Progesterone, Dairy Cows

881 Progestagen treatment and dietary restriction affecting ovarian activity of postpartum Retinta cow. F. I. Hernández García*[1] and S. P. Ford[2], [1]*SIA-Junta de Extremadura, Aptdo.22, Badajoz-06080, Spain,* [2]*Iowa State University, Ames.*

Retinta beef cows are adapted to the seasonal pasture scarcity of southwestern Spain but exhibit a prolonged calving interval. This study characterized the ovarian activity and response to progestagen treatment (Synchromate-B type) in postpartum (PP) Retinta cows that were feed-restricted to simulate their typical ranging conditions. Plasma samples (daily/weekly; 2nd through 9th PP months) from 39 cows (20 Control and 19 Treated) were assayed for progesterone (P4) and estradiol by RIA. Norgestomet was implanted for 9 days (starting on PP day 90) in the Treated cows, followed by 48-h calf withdrawal. Calves were weaned at 7–7.5 months of age. Estrus and conception rates (ER, CR) were 27/39 and 24/39, respectively. The ranges of PP intervals to first estrus (PPE) and conception (PPC) were 16–35 and 19–35 weeks, respectively. Treatment did not affect ER, CR, PPE, or PPC. Higher body condition score (BCS) cows had greater CR and shorter PPE and PPC than Lower BCS cows (1–9 scale; mean score 3.6–6 and 2–3.5; 19 and 20 animals, respectively). Cows remaining nonpregnant until weaning had a lower and more rapidly decreasing BCS than cows conceiving before weaning. Peak P4 values reached within the first 3 weeks of pregnancy (range 7–15, mean 10.4 ng/ml) were considerably higher than those usually reported for other beef cattle breeds. The weekly mean of cumulative P4 release up to the 3rd week of pregnancy was not affected by treatment but it was greater and increased more rapidly in Higher- versus Lower-BCS cows. In view of their refractoriness to treatment and their prolonged PP anestrus regardless of BCS level, cows seemed hypersensitive to the calf's inhibitory influence on the resumption of estrous cyclicity. However, for Higher- or Lower-BCS cows, most conceptions (14/14 or 8/10, respectively) occurred at first estrus, suggesting a low embryo mortality and an inherent resistance to the effects of undernutrition on rebreeding.

Key Words: Postpartum, Estrous Synchronization, Retinta Cattle

882 Effect of feeding regimen and supplemental progesterone on intrauterine concentrations of progesterone-dependent uterine proteins in gilts. J. Liu*[1], W. G. Zollers[2], D. C. Kenison[2], and W. E. Trout[1], [1]*University of Missouri, Columbia,* [2]*Ivy Laboratories, Overland Park, KS.*

Studies were conducted to determine the effects of pre-mating feeding regimen and post-mating progesterone (P4) treatment on serum P4 and uterine expression and secretion of retinol-binding protein (RBP), uteroferrin (UF), and uterine plasmin/trypsin inhibitor (UPTI). In Exp. 1, 40 gilts were allotted to a 2×2 factorial. From 10 d before estrus, gilts were fed either 5.5 (restricted) or 11.0 Mcal (flushed) DE/d. Gilts were artificially inseminated at 8 and 24 h after onset of estrus (D0). From D2, all gilts were fed at 5.5 Mcal DE/d. Gilts were given 400mg of P4 in sesame oil or sesame oil in a single injection on D2. Blood was collected every 4 h from D2 to D5 and then every 48 h until hysterectomy on D13 of pregnancy. Uterine secretions were recovered by flushing each horn with 30ml of physiological saline and endometrium was collected. Serum P4 concentrations were elevated in P4-treated gilts on D2 - D4 of pregnancy ($P<.05$). In P4-treated gilts, P4 concentrations were higher ($P< .01$) in restricted fed vs flush fed gilts. Flush feeding increased ($P< .001$) ovulation rate by 2.2 (14.8 vs 12.6). Neither feeding regimen nor P4 supplementation affected intrauterine concentrations or endometrial expression of RBP, UF, or UPTI. P4 concentrations on D2 were correlated with intrauterine concentrations of RBP (r = .61, $P< .001$) and UF (r = .43, $P< .01$) at D13. In Exp. 2, 20 gilts were flush fed 11.0 Mcal DE/d from 10 d before estrus until D1. Gilts were given 5 injections of P4 (80mg every 12 h) or sesame oil starting at 8 am on D2. Multiple injections of P4 increased ($P< .001$) serum P4 concentrations on D2 - D5 of pregnancy but had no effect on endometrial expression or intrauterine concentrations of RBP, UF, or UPTI. In conclusion, P4 administration on D2 - D5 of pregnancy did not affect endometrial expression or uterine secretion of RBP, UF, or UPTI on D13 of pregnancy.

Key Words: Progesterone, Uterus, Gilts

883 Effect of plasma cholesterol concentration on progesterone clearance in Holstein cows. C. M. Cook*, T. H. Herdt, and N. K. Ames, *Michigan State University.*

Concentrations of cholesterol and progesterone in diestrual bovine plasma are correlated positively, but a mechanism for this relationship has not been established. Changes in plasma progesterone concentrations imply changes in progesterone kinetics. The objective of our study was to determine the effect of plasma cholesterol concentration on progesterone clearance in cows. Four multiparous, non-lactating Holstein cows were ovariectomized and fitted with cannulas to allow for regulated removal of bile from the body. Plasma cholesterol concentrations in cows can be regulated in this manner by influencing bile acid pool size, as we have previously described (Chen et al., J. Lipid Res., 1995). Treatments consisted of either supplemental fat feeding without bile removal, or removal of 50% of bile flow volume with no supplemental dietary fat. Each cow experienced each treatment in a switch-back design. Cows were maintained on each treatment for approximately three weeks before progesterone administration. At the time of progesterone infusion, the mean (±SEM) value for plasma cholesterol was 191.5±28.6mg/dl for cows receiving fat and 14.2±8.1 mg/dl for cows undergoing bile removal. Progesterone was administered intravenously as a primed (24mg), continuous-dose infusion at the rate 14mg/cow/h. Blood was sampled every 30 m for 10 h to determine steady-state plasma progesterone concentrations. Progesterone was measured by direct RIA. Mean progesterone clearance (±SEM) was 3.51±.08 and 2.66±.06 L/min ($p<.01$) during the high and low cholesterol periods, respectively. Diversion of bile during a portion of the progesterone infusion period showed no direct effect of bile diversion on progesterone clearance. We conclude that progesterone clearance in cows is increased in association with increasing plasma cholesterol concentrations.

Key Words: Progesterone, Pharmacokinetics, Cholesterol

884 Effects of diet and exogenous bST on reproductive performance of dairy heifers. R. P. Radcliff*, M. J. VandeHaar, L. T. Chapin, T. E. Pilbeam, R. W. Ashley, S. M. Puffenbarger, E. P. Stanisiewski, D. K. Beede, and H. A. Tucker, *Michigan State University, East Lansing and Pharmacia and Upjohn Company, Kalamazoo, MI.*

Holstein heifers (initial BW = 135 ± 2 kg) at two research farms were assigned to one of three treatments. Thirty-five heifers were fed a control diet to gain 0.8 kg/d (LC). Thirty-five heifers were fed a diet higher in protein and energy to gain 1.2 kg/d (HC). Thirty-five heifers were fed the high protein, high energy diet and injected daily with bST (Trobest® sterile powder; 25 μg/kg BW; intramuscularly; HB). Heifers were eligible to be artificially inseminated (AI) when BW reached 363 kg. Heifers were fed their respective diets and HB heifers were injected with bST until confirmed pregnant. All pregnant heifers were commingled and fed a common diet until parturition. One heifer from the HB group was removed before she was eligible for AI for reasons unrelated to treatment. Three heifers from the HB group and 1 heifer from the HC group failed to conceive. Neither location nor treatment affected pregnancy rates or calving rates. Inseminations per heifer were not different among treatments. Heifers in HB and HC groups were younger than heifers in the LC group at first AI (336 and 334 vs 425 ± 6 d, respectively). The interval from 363 kg BW to first AI was greater for HB than HC or LC groups (25 vs 13 and 11 ± 4 d, respectively). There was a location by treatment interaction for age when confirmed pregnant; specifically, heifers in HB and LC groups were older at one farm than their respective groups at the second farm (HB 423 ± 14 vs 363 ± 13 and LC 512 ± 14 vs 438 ± 12 d), but ages at confirmed pregnant for the HC groups were not different between farms (400 ± 14 vs 387 ± 12 d). Number of days from the time a heifer was eligible for AI until confirmed pregnant were not different among treatments. The high protein, high energy diet decreased age at breeding without detrimental effects on reproductive performance. Injection of bST from 135 kg BW until confirmed pregnant was not detrimental to reproductive performance.

885 Effects of diet and exogenous bST on growth and lactation of dairy heifers. R. P. Radcliff, M. J. VandeHaar, L. T. Chapin*, T. E. Pilbeam, R. W. Ashley, S. M. Puffenbarger, E. P. Stanisiewski, D. K. Beede, and H. A. Tucker, *Michigan State University, East Lansing and Pharmacia and Upjohn Company, Kalamazoo, MI.*

Sixty Holstein heifers (initial BW = 133 ± 2 kg) from the campus dairy and 45 heifers (initial BW = 135 ± 2) from Kellogg Biological Station Dairy (KBS) at Michigan State University were assigned to one of three treatments. Heifers were fed a control diet to gain 0.8 kg/d (n = 35; LC) or a diet higher in protein and energy to gain 1.2 kg/d (n = 35; HC). Thirty-five heifers were fed the high protein, high energy diet and injected daily with bST (Trobest® sterile powder; 25 μg/kg BW; intramuscularly; HB). Heifers were fed their respective diet and HB heifers were injected with bST until confirmed pregnant. All pregnant heifers were commingled and fed a common diet until parturition. Before confirmation of pregnancy heifers in HC and HB groups had greater average daily gains (ADG) than ADG of the LC group (1.13 and 1.15 vs 0.78 ± 0.02 kg/d). During gestation ADG of heifers in the LC group was greater than ADG of HC or HB groups (0.93 vs 0.72 and 0.74 ± 0.03 kg/d). At breeding, heifers in the HC group had a greater body condition score (BCS) than that of the HB group, and BCS of the HB group was greater than that of the LC group (4.2, 4.0, and 3.5 ± 0.1). At parturition, BCS was not different among treatments. Withers height was not different among treatments at breeding or calving. There was a treatment by location interaction for age at first calving (AFC); specifically, heifers in HB and LC groups at KBS were older at calving than HB and LC groups at the campus dairy (659 vs 583 ± 12, HB and 749 ± 12 vs 688 ± 10 d, LC), but the HC groups were not different between locations (633 ± 12 at KBS, vs 628 ± 10 at campus, d). Cows in the LC group had greater 270-d average daily milk production than HC cows (29.1 vs 25.6 ± 0.8 kg), while HB cows were intermediate (26.9 ± 0.9 kg). High protein, high energy diet reduced AFC as well as 270-d average daily milk production. The high protein, high energy diet plus bST reduced AFC without affecting milk production

886 Influence of somatotropin, limit feeding and oocyte retrieval on follicular dynamics and breeding in yearling beef heifers. M. W. Tripp*, T. A. Hoagland, J. C. Ju, X. Yang, J. W. Riesen, and S. A. Zinn, *Unversity of Connecticut, Storrs.*

The objective of this study was to determine the effects of supplemental bovine somatotropin (ST) and limit feeding on follicular growth and oocyte competence in yearling beef heifers. Sixteen growing heifers (403 ± 2 kg) were randomly assigned to one of four treatment groups arranged as a 2 x 2 factorial, with main effects of ST (0 or 33 $\mu g \cdot kg^{-1} \cdot BW^{-1}$) and limit feeding (.75 ad libitum intake). Animals were treated for 100 days before follicular aspiration, and treatments continued for the 42 d period that follicles were aspirated. In addition, six non-aspirated animals of similar weight and age were maintained with the same management as the experimental animals (but fed ad libitum without ST during the aspiration period) for determination of possible effects of aspiration on future breeding. Follicles of treated animals were observed ultrasonically, then aspirated. Recovered oocytes were matured, fertilized and developed to the blastocyst stage in an in vitro culture system. The number of follicles observed ultrasonically was greater in ST-treated animals (9.7 vs 6.8 ± 2/heifer; $P < .01$), but was unchanged by plane of nutrition. Number and quality of recovered cumulus-oocyte-complexes were similar among all treatments (3 ± 1.2/heifer), as were number of fertilized oocytes resulting in cleavage and subsequent growth to blastocyst. Following aspiration, response to estrus synchronization was not different among treatment groups, but heifers that were aspirated required a greater length of time and number of inseminations to achieve pregnancy than the group of heifers that were not aspirated.

Key Words: Somatotropin, Beef Cattle, Oocyte

887 Expression of growth hormone (GH) receptor (r) mRNA variants in lactating dairy cows. Y. Kobayashi*[1], M. C. Lucy[1], M. J. VandeHaar[2], H. A. Tucker[2], B. Sharma[2], and M. Mosely[3], [1]*University of Missouri-Columbia,* [2]*Michigan State University, and* [3]*The Upjohn Conpany, Kalamazoo, MI.*

The GHr gene contains two different promoters that transcribe the RNA with different exon 1 sequence (GHr 1A and GHr 1B). Three studies were conducted to characterize expression of GHr 1A and GHr 1B mRNA before and during lactation in dairy cattle. In Study 1, liver samples were collected from 6 multiparous cows in early (E, 17–42 days in milk [DIM]) and mid (M, 113–155 DIM) lactation, and from 12 cows in late lactation (L, 162–215 DIM). Six of L were treated with recombinant bovine GH (rbGH, 25 mg/d) for 7 d. Samples were also collected from dry cows (D, 247–266 d in gestation, n=6). In Study 2, liver samples were collected from primiparous cows (n=6) on d −14, 0 (parturition), 15, 30, 60, and 90 of lactation. In Study 3, liver samples were collected from primiparous lactating cows that either received no infusion (n=10) or rbGH continuously for 63 d (29 mg/d, n=10). Amount of GHr 1A, GHr 1B, and GAPDH (control) mRNA was measured by ribonuclease protection assay. There was an effect of lactation stage on GHr 1A amount in Studies 1 ($P < .1$) and 2 ($P < .01$). Amount (arbitrary units) of GHr 1A was least in E (39.1 ± 10.1) and M (30.0 ± 9.2). Greater GHr 1A amount was found in D (48.4 ± 9.2), L (54.8 ± 10.1), and L treated with rbGH (73.1 ± 10.1). Amount of GHr 1B and GAPDH were similar for cows at different stages of lactation. Amount of GHr 1A was decreased at calving (34.8) compared to dry (59.3 on d −14) or early lactation period (54.5, 54.0, 59.8, and 57.3 [SE ± 3.5] for 15, 30, 60, and 90 d, respectively), but GHr 1B and GAPDH did not change. Cows treated with rbGH (Study 3) had a greater GHr 1A mRNA (352.5 ± 3.4) than those without (157.8 ± 3.6, $P < .01$), but amount of GHr 1B and GAPDH mRNA were similar. We conclude that GHr 1A promoter primarily regulates changes in GHr mRNA in liver of lactating dairy cattle.

Key Words: GHr, Promoter, Lactation

888 Influence of insulin administration and feeding level between weaning and remating on follicular development and ovulation rate in primiparous sows. N. C. Whitley* and N. M. Cox, *Mississippi State University, Mississippi State.*

Sixty-three primiparous crossbred sows lactating 21.3 ± 0.1 d and weaning 9.1 ± 0.2 pigs were used to evaluate possible interactions of insulin and feeding level on follicular development and ovulation rate. Beginning the d after weaning (= d 0), sows were injected with 0.4 IU/kg BW insulin (Eli Lilly Lente Iletin II) and fed either 2.3 (I2.3; n = 17) or 4.5 kg/d (I4.5; n = 18) or an equivalent volume of saline with similar feeding levels (S2.3 and S4.5; n = 14/treatment) for 5 d. In a subset of sows (n = 3/treatment except S4.5 for which n = 4), ovaries were collected at slaughter on d 5 to assess follicular development. Fluid from the 15 largest follicles ≥ 2 mm in diameter was collected for evaluation of IGF-I and estradiol by radioimmunoassay. The remaining sows were mated, and ovaries were collected at slaughter between d 30 and 35 of gestation for determination of ovulation rate. There was a treatment by feeding level interaction ($P < 0.02$) for follicular fluid IGF-I concentrations with means of 302 (S2.3), 378 (I2.3), 380 (S4.5) and 333 ng/mL (I4.5; SEM = 29). Insulin increased ($P < 0.04$) IGF-I in the lower feeding level only, producing concentrations equivalent to saline treatment with the higher feeding level. In addition, IGF-I increased ($P < 0.03$) with feeding level for saline treatment. Follicular fluid estradiol was influenced only by a treatment by size class interaction ($P < 0.01$) in which insulin treatment increased ($P < 0.01$)follicular fluid estradiol in large follicles (diameter ≥ 7 mm) compared to saline treatment (18.6 ± 9.8 and 81.56 ± 10.6 ng/mL). For mated sows, ovulation rate (16.7 ± 0.5) was not influenced by treatment or feeding level. In conclusion, during postweaning follicular development insulin treatment produced similar effects on follicular IGF-I as elevating feeding level and enhanced estradiol independently of feeding level. However, an effect on ovulation rate was not identified.

Key Words: Primiparous Sow, IGF-I, Insulin

889 Effects of dietary lasalocid and sex of calf on reproductive hormone concentrations, follicular populations, body weights and body condition scores in postpartum Brahman cows. F. J. Padilla-Ramirez*, D. A. Neuendorff, A. W. Lewis, S. M. Webb, and R. D. Randel, *Texas Agricultural Experiment Station, Overton.*

Seventy-three Brahman cows were allotted within body condition score (BCS) and sex of calf (SC) to two groups: control (C, n=35), or lasalocid (L, n=38). The C diet consisted of 3.6 kg x hd-1 x d-1 of 6:1 corn:soybean meal concentrate, plus ad-libitum intake of rye-ryegrass pasture, water, salt and minerals. The L diet was identical to the C diet with the addition of 200 mg L x hd-1 x d-1. Groups were fed once daily from d 1 after calving through the first normal estrous cycle. Blood samples were taken on alternate days from d 1 through d 13, and then weekly from d 14 through estrus, and thereafter on d 6 and 12 after observed estrus. Plasma PGF metabolite (PGFM), P4, and E2 concentrations were quantified using RIA. BCS and BW were recorded at calving and at 14 d intervals through first estrus. Follicular populations were monitored weekly from d 14 after calving until d 90 or formation of corpus luteum (CL). Plasma PGFM, E2, and P4 concentrations from d 1 to d 13 after calving were not influenced ($P > .10$) by diet or SC. Plasma P4 tended to be higher ($P < .10$) in L than in C cows on d 6 and 12 after estrus. Changes in BW and BCS were not affected ($P > .10$) by diet or SC. Numbers of medium follicles (4.0 to 7.9 mm) were greater ($P < .05$) in L compared with C cows during the early postpartum period. Follicular populations were not affected ($P > .10$) by diet or SC during the wk prior to return to estrus. Intervals from calving to: behavioral estrus, formation of a CL, and estrus with a CL were shorter ($P < .01$) in L than in C cows. The cumulative frequency of return to estrus by d 55 was higher ($P < .05$) in L than in C cows. In postpartum Brahman cows dietary lasalocid enhanced follicular growth and shortened the intervals to reproductively important end points without altering reproductive hormone concentrations before estrus, BW, BCS, or pregnancy rates (L=92%, C=94%).

Key Words: Lasalocid, Follicular Populations, Postpartum Cows

890 Plasma fatty acids, prostaglandin $F_{2\alpha}$, and fertility of postpartum heifers fed rumen by-pass fat. S. J. Filley[1]*, H. Turner[1,2], and F. Stormshak[1], [1]*Oregon State University, Corvallis, OR,* [2]*Eastern Oregon Agricultural Research Center, Burns, OR.*

The objectives of this experiment were to determine whether feeding rumen-protected fatty acids (FA) to postpartum (PP) heifers would increase linoleic acid and $PGF_{2\alpha}$ in plasma, shorten the interval from calving to first rise in plasma progesterone (P_4), and increase pregnancy rate relative to controls. Forty Hereford x Angus heifers (346 kg) were assigned randomly to supplement treatments of .23 kg·head^{-1}·d^{-1} calcium salts of FA (CSFA; n = 20) or an isocaloric amount of barley (control; n = 19) for the first 30 d PP. Supplements, with .23 kg barley as a vehicle, and a basal diet of meadow and alfalfa hays were pen fed to heifers (5/pen). Heifers were bled on alternate d (d 1 to 30) and 2× weekly (d 30 to 2 wk after first estrus) for $PGF_{2\alpha}$ metabolite (PGFM) and P_4 assay, respectively. Weight percent of major FA in d 1 and 7 plasma was determined by gas chromatography. First behavioral estrus was detected by use of intact bulls, and confirmed by an increase in plasma P_4. Data were analyzed using χ^2 and ANOVA (repeated measures and one-way). On d 7, but not d 1, plasma from heifers fed CSFA had altered ($P < .01$) proportions of major FA, including an increase ($P = .003$) in linoleic acid compared with that of controls (29.1 vs 25.6 weight %, SE = .75). There was no difference ($P > .32$) in PGFM between treatments on d 1, 3, 5, 11, 13 and 15 PP. Analysis of variance of contrast variables revealed an effect of treatment on change in PGFM from d 3 to 5 ($P = .004$). By d 7 and on d 9, plasma concentrations of PGFM were greater in heifers fed CSFA compared to those of controls ($P = .02$ and $P = .06$, respectively). Days to first estrus with ovulation, pregnancy rate, and calving interval were not affected by treatments ($P > .10$). Although supplemental lipid fed to primiparous beef heifers altered plasma FA profile to favor increased linoleic acid, and increased production of $PGF_{2\alpha}$ in the early PP period, it did not improve the fertility of these heifers in the subsequent breeding season.

Key Words: Prostaglandin, Fatty Acids, Cattle

891 Effect of excess degradable intake protein on blood urea nitrogen, ovarian steroids, and early embryonic development in ewes. J. G. Berardinelli*, J. Weng, P. J. Burfening, and R. Adair, *Montana State University, Bozeman.*

The objective of this study was to determine if feeding a diet high in degradable intake protein during a synchronized estrous cycle alters blood urea nitrogen (BUN), estradiol-17β (E2), progesterone (P4), or early embryonic development in ewes. Ewes were fed either 100 (C; n = 15) or 200% (HP; n = 16) of the NRC protein recommendation for maintenance. Estrous cycles were synchronized with intravaginal sponges containing a progestogen. Ampullae (AMP), isthmi (I), and uterine horns (UT) of HP and C ewes were removed on either Day 2 or 4 after the next estrus and breeding to fertile rams. Jugular blood samples were collected daily from each ewe starting on Day 2 of the synchronized cycle until surgery. AMP, I, UT were flushed with Delbecco's PBS. Flushings were examined microscopically for embryos. Samples were assayed for BUN, E2, and P4. BUN concentrations were higher ($P < .05$) in HP ewes than in C ewes during the synchronized cycle and the first 4 d of the next cycle. P4 concentrations during the synchronized cycle did not differ ($P > .10$) between HP and C ewes. E2 concentrations were higher ($P < .05$) in C ewes than in HP ewes during the periovulatory period. During the first 4 d of the next cycle E2 concentrations were lower ($P < .05$) in HP ewes than in C ewes and P4 increased ($P < .05$) to higher concentrations by Day 4 in HP ewes than in C ewes. Ovulation rates did not differ ($P > 10$) between HP and C ewes. More ($P < .05$) embryos were found in AMP of HP ewes than in AMP of C ewes on Day 4. Fewer ($P = .06$) embryos were found in UT of HP ewes than in OT of C ewes on Day 4. Feeding mature ewes excess digestible intake protein during a synchronized estrous cycle and during the first 4 d after breeding delayed embryo transport through the oviduct, possibly caused by an alteration in the ovarian steroid environment.

Key Words: Protein Nutrition, Embryo Transport, Oviduct

892 Effect of diurnal high ambient temperature and supplemental lysine on puberty onset and reproductive performance in gilts. D. J. Mandey-Kumajas, M. L. Connor, and A. H. Fallah-Rad, *University of Manitoba, Winnipeg, MB, Canada.*

To study the influence of prolonged high ambient temperature and supplementary lysine on puberty onset and reproductive performance, 48 prepubertal gilts averaging 105d of age were assigned to either 20°C (RA) or diurnal 12h at 32°C and 12h at 26°C (RB) and one of the two levels of lysine: the NRC standard or 50% above standard. The experiment ended at 45d of gestation following breeding at second estrus. High ambient temperatures had no effect on rectal temperature, feed intake, backfat, BW gain, duration of first proestrus but increased respiration rate, water usage and tended to increase age at puberty and duration of first estrous ($P<0.1$). Supplemental lysine had no effect on the physiological responses and growth performance. Although, high ambient temperature decreased duration of second estrus ($P=0.0002$) and ovulation rate, it did not affect length of the estrous-cycle, ovarian weight, uterine weight or fetal numbers, weight and length at d45 post-mating ($P>0.1$). Supplemental lysine had no effect on BW, age and backfat thickness at puberty, nor on estrous-cycle length, second ovulation rate, ovarian weight or uterine weight ($P>0.1$). There was a trend ($P<0.10$) towards supplemental lysine decreasing proestrus duration and increasing first ovulation rate. However, supplemental lysine decreased fetal numbers and slowed fetal development at d45 ($P<0.05$). There was no interaction between lysine and high ambient temperature on growth performance, puberty onset or second estrus characteristics. However, there was a tendency ($P<0.1$) for empty uterine weight to be greater in pigs with supplemental lysine in RA but lower in pigs with supplemental lysine in RB. In conclusion, growth and reproductive performance indicated that the gilts adjusted to the diurnal high ambient temperature. Although supplemental lysine had a beneficial effect on first ovulation rate and no adverse effect on the overall gilt performance, it had a detrimental effect on fetal growth and development to d45 of gestation.

Key Words: Sow, High ambient temperature, Lysine, Estrus, Puberty

893 Effect of feeding endophyte-infected fescue seed on conception rate and early embryonic development in beef heifers. R. W. Rorie*, W. D. Hazlett, D. L Kreider, and E. L. Piper, *University of Arkansas, Fayetteville.*

A study evaluated the effects of feeding endophyte-infected fescue seed on conception rate and embryonic development through the second week of gestation. Crossbred beef heifers (n = 20) were placed in drylot and fed bermudagrass hay and supplements containing either oats or endophyte-infected fescue seed for 100 days. The fescue seed supplement provided 10 mg of ergovaline daily to treatment heifers. Heifers were weighed at 30, 60 and 90 d of the study. The heifers were synchronized with Lutalyse, inseminated and non-surgical embryo collections performed on D 15 to 16 (D 0 = onset of estrus). After each embryo collection, the heifers were injected with Lutalyse and inseminated at estrus. The procedures above were repeated, allowing for embryo recoveries to be performed on the heifers at approximately 20 d intervals for the duration of the study (potential of 5 embryo recoveries per heifer). Blood samples were collected at the start of the study and at each embryo collection for analysis of serum prolactin. Throughout the study, serum prolactin was reduced (P = .008) in the heifers fed the fescue seed supplement compared to the control heifers (19.6 vs. 98.2 ng/ml). Heifers fed fescue seed gained less (.74 vs. .98 kg/hd/d) but this difference was not significant (P = .255). Across replicates (embryo collections), 78 and 84% of the heifers fed fescue seed or oat supplements, respectively, were observed in estrus after Lutalyse treatment and were inseminated. Overall conception rates (based on embryo recovery rates) of heifers observed in estrus and bred were similar (P = .697) for the heifers fed oat or fescue seed supplements (74 and 78%, respectively). Mean protein content of intact embryos recovered from heifers fed oat or fescue seed supplements was also similar (518 vs. 444 μg, respectively; P = .509). These results suggest that fertilization and early embryonic developmental are not significantly affected in heifers consuming by endophyte-infected fescue.

Key Words: Fescue, Embryo Development, Reproduction

894 Influence of endophyte-infected fescue on serum and intra-uterine insulin growth factor I and II in beef heifers. W. D. Hazlett*, T. L. Lester, and R. W. Rorie, *University of Arkansas, Fayetteville*.

A study was conducted to investigate whether consumption of endophyte-infected fescue effects serum or intra-uterine levels of insulin growth factor (IGF) I and II in cattle. Twenty beef heifers were individually fed supplements containing either oats or endophyte-infected fescue seed for 100 days. At the initiation of the study, the heifers were synchronized and artificially inseminated at estrus. Non-surgical embryo collections were performed on D 15 to 16, (D 0 = onset of estrus) using 25 to 30 ml of physiological saline as the flush medium. Ultrasonography was used to confirm the presence of a corpus luteum and only the uterine horn adjacent to the corpus luteum was flushed. Blood samples were collected at each embryo collection for analysis of serum prolactin and IGF I and II. Uterine flushes (flush medium) were frozen until analysis for total protein, IGF I and II. After each embryo collection, the heifers were synchronized, inseminated at estrus and the embryo recovery procedures repeated (five replicates) at approximately 20 day intervals for the duration of the study. The heifers fed fescue seed had reduced (P = .008) prolactin (19.6 vs 98.2 ng/ml) compared to control heifers. There was no treatment by replicate effect (P $\geq$ .644) for either serum or intra-uterine IGF I or II levels. Overall, serum IGF I levels (100.7 vs. 84.9 ng/ml) were reduced (P = .059) in heifers fed fescue seed as compared with control animals, but IGF II levels were not affected (P = .917) by treatment (57.1 vs. 57.3 ng/ml, respectively). Intra-uterine IGF I and II were compared on the basis of ng/mg of protein in the flush medium. Both uterine IGF I and II were similar (P $\geq$ .263) between heifers fed fescue seed or oat supplements (.38 vs .50 and 1.07 vs. 1.41 ng/mg protein, respectively). These results indicate serum IGF I is reduced in heifers consuming endophyte-infected fescue but intra-uterine levels of IGF I and II are not affected.

Key Words: Fescue, Insulin Growth Factor, Reproduction

895 Leptin as a metabolic regulator of reproduction: Effects at the brain. D. H. Keisler*, J. M. Simmons, and C. J. Dyer, *University of Missouri-Columbia*.

Infertility associated with suboptimal nutrition is a major concern among livestock producers. Undernourished prepuberal animals will not enter puberty until they are well fed; similarly, adult, normally cycling females will stop cycling when faced with extreme undernutrition. Our lab has focused on how body fat (or adiposity) of an animal can communicate to the brain and regulate reproductive competence. In 1994, the discovery in rodents of the obese (ob) gene product leptin, secreted as a hormone from adipocytes, provided a unique opportunity to understand and hence regulate whole body compositional changes. There is now evidence that similar mechanisms are likely functioning in livestock species where food intake, body composition, and reproductive performance are of considerable economic importance. Leptin has been reported to be a potent regulator of food intake and reproduction in rodents. Evidence exists to suggest that at least part of the effects of leptin occur through receptor mediated regulation of the hypothalamic protein neuropeptide Y (NPY). NPY is a potent stimulator of food intake, is elevated in feed restricted cattle and ewes, and is an inhibitor of LH secretion in these livestock species. In our investigations in sheep, we have cloned a partial cDNA corresponding to the ovine long-form leptin receptor, presumably the only fully active form, and have localized the long-form leptin receptor in the ventromedial and arcuate nuclei of the hypothalamus. Leptin receptor mRNA expression was colocalized with NPY mRNA-containing cell bodies in those regions. We have also determined that hypothalamic leptin receptor expression was greater in feed -restricted ewes than in well fed ewes. These observations provide a foundation for future investigations into the nutritional modulators of reproduction in livestock.

Key Words: Reproduction, Nutrition, Neuroendocrinology

896 Leptin as a metabolic regulator of reproduction: Effect at the ovary. L. J. Spicer*, *Department of Animal Science, Oklahoma State University, Stillwater*.

Leptin, a hormonal product of the obese (ob) gene, circulates in the blood at levels paralleling those of fat reserves in humans, and regulates satiety in rats. Since its discovery in 1994, a plethora of information has accumulated in the scientific literature regarding the physiologic actions of leptin, particularly in the brain. One more recently described site of action of leptin is the ovary. Evidence indicates that leptin receptors and leptin receptor mRNA are present in ovarian tissue of several species including granulosa and thecal cells of cattle. Moreover, both granulosa and thecal cells respond to leptin in vitro. Specifically, leptin inhibits insulin-induced estradiol production by cultured bovine granulosa cells, and inhibits insulin-induced androstenedione production by bovine thecal cells. The inhibitory effects of leptin are not due to leptin inhibiting insulin binding to granulosa and thecal cells. Additional studies from our laboratory indicate that the effect of leptin is specific to insulin action since leptin does not influence basal or IGF-I-induced steroidogenesis of bovine granulosa or thecal cells. However, a definitive role of leptin in bovine reproduction awaits development of a valid RIA for bovine leptin so that in vivo associations can be made among leptin, body condition score, dietary intake and ovarian function in cattle.

Key Words: Leptin, Ovaries, Follicles

897 Effects of metabolic fuel restriction on patterns of LH and GH secretion and serum leptin concentrations in the prepuberal gilt. C. R. Barb*[1], J. B. Barrett[1], G. B. Rampacek[2], and R. R. Kraeling[1], [1]*USDA/ARS, Athens, GA,* [2]*University of Georgia, Athens*.

Previous reports demonstrated that nutrient deprivation suppressed pulsatile LH secretion and enhanced GH secretion in the pig. We recently demonstrated that acute feed deprivation suppressed serum leptin concentrations in gilts. The objective of the present study was to determine if the effect of metabolic fuel restriction on LH and GH secretion is associated with changes in serum leptin concentrations. Gilts, averaging 140 days of age (n=15) and which had been ovariectomized, were individually penned in an environmentally controlled building and exposed to a constant ambient temperature of 22 C and 12:12 hr light: dark photoperiod. Pigs were fed daily at 0700 hr. Gilts were randomly assigned to the following treatments: saline (S, n=7),100 (n=4), or 300 (n=4) mg/kg BW of 2-deoxy-D-glucose (2DG), a competitive inhibitor of glycolysis, in saline iv. Blood samples were collected every 15 min for 2 hr before and 5 hr after treatment. All samples were assayed for LH, GH and leptin by RIA. Selected samples were quantified for insulin. Treatment did not alter serum insulin levels. Serum leptin concentrations were 4.0 $\pm$.1, 2.8 $\pm$ .2, and 4.9 $\pm$ .2 ng/mL for S, 100 and 300 mg 2DG pigs, respectively, prior to treatment and remained unchanged following treatment. The 300 mg dose of 2DG increased (P<.0001) mean GH concentrations (2.0 $\pm$ .2 ng/mL) compared to S (.8 $\pm$ .2 ng/mL) and 100 mg 2DG (.7 $\pm$. 2 ng/mL). Frequency and amplitude of GH pulses were unaffected. However, number of LH pulses /5 h were decreased (P<.01) by the 300 mg dose of 2DG (1.8 $\pm$ .5) compared to S (4 $\pm$ .4) and the 100 mg dose of 2DG (4.5 $\pm$ .5). Mean serum LH concentrations and amplitude of LH pulses were unaffected. These results suggest that acute effects of energy deprivation on LH and GH secretion are independent of changes in serum leptin concentrations.

Key Words: Leptin, Nutrition, Hormone Secretion

898 Plasma leptin concentrations and first postpartum ovulation in dairy cows differing in energy balance. M. Frajblat*[1], S. W. Beam[2], and W. R. Butler[1], [1]*Cornell University, Ithaca, NY and* [2]*University of California-Davis.*

This study examined leptin concentrations in dairy cows differing widely in energy balance (EB) during the early postpartum (PP) period. Multiparous Holstein cows (n=32) were assigned to one of 3 energy balance groups consisting of NLAC cows (n=8, not milked after calving), LAC2 cows (n=13, milked 2x/day) and LAC3 cows (n=11, milked 3x/day). LAC3 cows were fed a less energy dense ration. Daily EB was calculated for all cows and ovarian follicular development monitored by ultrasonography. Blood was collected daily for analysis of leptin (Linco Research). Body weight (BW) and body condition score (BCS) were monitored weekly. Energy balance (P<0.001), BW loss (-5 ± 22, -25 ± 22 and -48 ± 27 kg, P<0.004) and BCS loss ($+0.1 \pm 0.2$, -0.6 ± 0.4 and -0.7 ± 0.4, P<0.001) for NLAC, LAC2 and LAC3, respectively, were significantly different among groups during the first 3 weeks PP. Days to first ovulation PP did not differ (P=0.15) among EB groups (18 ± 5, 29 ± 4 and 31 ± 4 for NLAC, LAC2 and LAC3, respectively. The dominant follicle (DF) from the first follicular wave PP ovulated in 75% (6/8) of NLAC, 54% (6/13) of LAC2 and 36% (4/11) of LAC3 cows (P>.2). Leptin concentrations were similar (P>0.9) among EB groups from days 5 to 21 PP (4.5 ± 2.3, 4.3 ± 2.2 and 4.1 ± 1.8 ng/ml for NLAC, LAC2 and LAC3, respectively). A small, but significant increase in leptin concentrations was observed from day 5 (3.9 ± 1.9 ng/ml) to day 21 (4.4 ± 2.2 ng/ml, P<0.01). Leptin levels for cows that lost less than or more than the mean BCS loss (-0.5 ± 0.5) were 4.9 $\pm$ 2.7 and 3.7 $\pm$ 1.1 ng/ml, respectively (P=0.13). There was a trend (P<0.06) for cows that ovulated the first DF to have higher concentrations of leptin (4.8 ± 2.3 ng/ml) compared to non-ovulating cows (3.5 $\pm$ 0.7 ng/ml). In summary, leptin concentrations did not differ between NLAC, LAC2 and LAC3 cows during days 5 to 21 PP, despite significant differences in EB, BCS and BW during this period. In PP cows, higher leptin concentrations may contribute to ovulation of the first DF.

Key Words: Leptin, Follicle, Cows

899 Serum leptin is associated with carcass traits in finishing cattle. J. E. Minton*, D. J. Bindel, J. S. Drouillard, E. C. Titgemeyer, D. M. Grieger, and C. M. Hill, *Kansas State University, Manhattan.*

Leptin, a recently discovered hormone of adipose tissue origin, is associated with measures of body fat content in rodents and humans. The major physiological action of leptin is that it exerts a negative effect on food intake, although it also increases metabolic rate. We hypothesized that cattle in the final phase of the finishing period might have increased leptin in circulation as ribeye fat thickness and intramuscular fat (marbling) also increased. Although leptin mRNA was found in bovine adipose tissue, to date, no quantitative assay was available to measure circulating leptin in cattle. We validated a commercially available leptin assay (Linco Research, Inc.; cat. XL-85K) for use in bovine serum. Human leptin was used as standard in the assay. Varying volumes of a pooled sample of bovine serum produced a binding curve that paralleled the standard curve. When concentration of leptin measured in the assay from bovine serum was regressed on volume assayed, the 95% confidence interval (CI) about the slope of the regression line included 0. Leptin (human) also could be quantitatively recovered from bovine serum. Regression of leptin concentration measured by the assay on concentration expected in bovine serum samples to which leptin had been added produced a regression line with a slope and CI that included 1.0. The assay was sensitive to 1 ng/ml human leptin equivalent. This assay was used to measure leptin in serum from finishing heifers. In brief, these animals were part of a 120-d finishing study designed to evaluate the effect of added dietary choline and fat on growth performance and carcass traits. Samples of serum were obtained from all animals on the study (n=318) 30 d prior to slaughter for leptin determination. At slaughter, ribeye fat thickness, marbling score, yield grade, and kidney, pelvic, and heart fat (KPH) were determined. Leptin ranged from 2.8 to 15.3 ng/ml. Serum leptin was positively correlated (P<.001) with ribeye fat thickness (r=.32), KPH (r=.18), marbling score (r=.28), and yield grade (r=.28). The data suggest that circulating leptin is positively associated with measures of carcass fatness in cattle.

Key Words: Leptin, Carcass Traits, Cattle

900 Feeding protected and unprotected fish oil to dairy cows: I Effect on animal performances. P. Lacasse* and C. E. Ahnadi, *Dairy and Swine R&D Centre, Lennoxville, Qc, Canada.*

Thirty Holstein cows in mid-lactation (158 ± 20 days in lactation) were fed with a total mix ration based on haylage, corn silage, high moisture corn grain and a supplement. Cows were paired into 4 groups, according to the DMI and milk yield. After one preliminary week, cows' ration have been mix with nothing (control), unprotected "dry" fish oil (UFO; 4% of DM; Vaculift, Ca) or glutheraldehyde-protected microcapsules of fish oil (PFO; Ocean Nutrition Ltd, NS), at 2% or 4 % of DM, for four weeks. The UFO and PFO supplements contained 42 an 40% of lipid; of which, 28.7 % and 25.5 % were polyunsaturated fatty acids. Cows fed with UFO reduced their feed intake by more than 25 % (P<0.01). Consequently, UFO fed cows loose body weight (P<0.01) and reduced their milk production (P<0.01). Feed intake, milk production and ADG were unaffected by feeding PFO at 2 or 4% (P>0.1). Feeding UFO or PFO reduced (P<0.01) milk fat content. During the treatment period, milk fat content averaged 3.14, 2.41, 2.18 and 2.47 for control, 2%PFO, 4%PFO and UFO, respectively. Milk protein content was lower in cows fed UFO (3.16%; P<0.01), 4%PFO (3,15%; P<0.01) or 2%PFO (3.25%; P<0.1) than in cows fed the control diet (3.39%). Feeding unprotected fish oil to lactating dairy cows reduces animal performances. Fish oil, unprotected or protected from ruminal biohydrogenation by glutheraldehyde treatment, reduces protein and fat content of milk. Project supported by Ralston Purina International.

Key Words: Milk Fat, Fish Oil

901 Feeding protected and unprotected fish oil to dairy cows: II Effect on milk fat composition. P. Lacasse[1], J. J. Kennelly[2], and C. E. Ahnadi*[1], [1]*Dairy and Swine R&D Centre, Lennoxville, Qc, Canada* [2]*University of Alberta, Edmonton, Canada.*

Thirty Holstein cows in mid-lactation were fed with a total mix ration (control), supplemented with unprotected "dry" fish oil (UFO; 4% of DM) or glutheraldehyde-protected microcapsules of fish oil (PFO) ,at 2% or 4 % of DM, for four weeks. The UFO and PFO supplements contained 42 an 40% of lipid; of which, 28.7 % and 25.5 % were polyunsaturated fatty acids. Feeding fish oil decreased (P<0.01) the proportion of short chain fatty acids (less than 14 carbons) in milk fat. At the end of the treatment period, these fatty acids represented 11.7, 10, 7.6 and 8.3 % of the total fatty acids for control, 2%PFO, 4%PFO and UFO, respectively. Similarly, the proportion of stearic and oleic acids were reduced (P<0.01) in fish oil fed cows. Milk fat trans C:18:1 fatty acids at the end of the experiment was 2.9% for control cows, but, increased in both UFO (9.7%; P<0.01) and 4%PFO (8%; P<0.01). Therefore, it is likely that the protection against rumen biohydrogenation was incomplete. Content of very long chain fatty acids, including arachidonic acid (C20:4), eicosapentaenoic acid (C20:5) and docosahexaenoic acid (C22:6) increased (P<0.01) with fish oil feeding. Polyunsaturated fatty acids (PUFA) content was higher in 2%PFO (3.8%; P<0.1), 4%PFO (4.2%; P<0.01) and UFO (5.7%; P<0.01) than in control (3.0%). Accordingly, the peroxide index increased (P<0.05) and a taste panel was able to detect unusual taste in 4%PFO milk and disliked UFO milk. In conclusion, feeding fish oil to lactating cows decreased de novo synthesis of fatty acids, increased long chain PUFA and susceptibility of milk to oxidation and negatively affected taste of milk. Project supported by Ralston Purina International and Alberta Agriculture Research Institute.

Key Words: Milk Fat, Fish Oil, Fatty Acids

902 Feeding protected and unprotected fish oil to dairy cows: III Effect on mammary lipid metabolism. C. E. Ahnadi[1], N. Beswick*[2], J. J. Kennelly[2], and P. Lacasse[1], [1]*Dairy and Swine R&D Centre, Lennoxville, Quebec, Canada,* [2]*University of Alberta, Edmonton, Canada.*

Sixteen Holstein cows in mid-lactation were fed with total mix ration supplemented with nothing (control), unprotected "dry" fish oil (UFO; 4% of DM) or glutaraldeyde-protected microcapsules of fish oil (PFO; at 2% or 4% of DM) for four weeks. At the end of the treatment period, milk fat content was lower in cows fed UFO (2.56%; P<0.05), 2%PFO (2.36%; P<0.01) or 4%PFO (2.08%; P<0.002) than in cows fed control diet (3.69%). This is due mainly to a decrease of the proportion of short chain fatty acids. To explain this decrease, we examined the expression of the genes encoding acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), lipoprotein lipase (LPL) and stearoyl-CoA desaturase (SCD) in mammary gland biopsies taken at the end of treatment period. Feeding PFO decreased ACC, FAS and SCD mRNA abundance in mammary gland. In cows fed 4% PFO the amount of ACC, FAS and SCD mRNA averaged only 30% (P<0.01), 25% (P<0.01) and 25% (P<0.001) of control values, respectively. Feeding 4% UFO to cows decreased ACC (P<0.05), FAS (P<0.01) and SCD (P<0.2) mRNA abundance slightly but markedly reduced the amount of LPL mRNA (about 5-fold decrease, P<0.001). Milk content of short chain fatty acids and medium chain fatty acids were correlated with ACC (R^2=0.61; P<0.01 and R^2=0.72; P<0.002, respectively) and FAS (R^2=0.58; P=0.06 and R^2=0.53; P=0.09, respectively). In conclusion, these results suggest that fish oil reduce milk fat percentage by inhibiting gene expression of mammary lipogenic enzymes. Project supported by Dairy Farmers of Canada.

Key Words: Milk Fat, Lipogenic Enzymes, Fish Oil

903 Role of blood insulin and trans fatty acids in the occurrence of low fat syndrome. F. Piccioli-Cappelli, M. G. Maianti, S. Franzini, and G. Bertoni*, *Facoltà di Agraria, Piacenza, Italy.*

The role of insulin in the occurrence of low fat syndrome has been recently reconsidered; milk fat depression in dairy cows fed low forage diet has been attributed to a higher level of absorbed trans fatty acids. With the aim to verify the relative role of these factors 44 dairy cows ranging 80–120 DIM and with a low SCC (less then 300/μl) have been selected from two very high yielding commercial herds. The cows were free and fed a TMR characterized to have 1.63 Mcal NE_L/kg, 15.4% CP, 33% NDF and 5.3% EE (mainly whole cotton seeds) on DM basis. At evening milking yield was measured and samples collected for the analysis of milk traits and particularly for the investigation of the fatty acids composition of fat. The following morning, before the TMR distribution, blood samples were withdrawn from the jugular vein for the determination of the metabolic profile and insulin content. Of 44 dairy cows checked, only 32 resulted suitable for statistical evaluation. At the regression analysis, milk fat content and plasma insulin level resulted negatively and significantly correlated (r = −0.48; P<0.01). Sorting the data on milk fat content, dairy cows were divided into 3 groups: having fat content < 3.0% (LFC; 8 obs.), fat content ranging between 3 and 3.9% (MFC; 11 obs.) and fat content > 4.0% (HFC; 13 obs). The ANOVA have shown that insulin is higher in LFC, i.e. 12.5 *vs* 10.3 (P<0.1) and *vs* 9.9 (P<0.05) μU/ml respectively for MFC and HFC groups. On the contrary, the content of short-medium chain fatty acids and of trans fatty acids, measured only on 7 samples from the LFC and 7 from the HFC groups, have not shown significant differences; namely trans-6 C18:1 was not detectable, while the sum of trans-9 C18:1 and trans-11 C18:1 resulted of 1.8 and 1.5% in LFC and HFC groups respectively. Therefore, considering that insulin was negatively correlated to milk fat content, while the mammary synthesized fatty acids were similar, it can be suggested that under our farm conditions the insulin level is the most important factor in determining low fat syndrome.

Key Words: Milk Fat, Insulin, Trans Fatty Acids

904 Dietary fish oil effects on milk fatty acid composition. D. F. Jones[1], W. P. Weiss[1], D. L. Palmquist*[1], and T. C. Jenkins[2], [1] *Ohio Agricultural Research and Development Center, The Ohio State University.* [2] *Clemson University.*

Diets with no supplemental fat, or supplemented with fatty acids from tallow (3.2% of DM), fish oil (2.6% of DM), or fish oil treated with ethanolamine to form the ethylamide (1.9% of DM) were fed to 8 blocks of Holstein cows in a randomized design. Cows were fed the basal diet (alfalfa silage, 23; corn silage 20; concentrate, 47% of DM) once daily for 4 wk. Fish oil fatty acids decreased DMI (21.0, 19.3, 16.1 and 17.1 kg/d, respectively, $P<.05$); milk and milk component yields were decreased by all fat supplements. There were no treatment effects on fat and protein concentrations; however, milk fat percentage was low for all. Fat supplements decreased proportions of milk fatty acids 6:0-14:0 ($P<.001$); proportions of these were higher for fish oil than for tallow ($P<.05$). Fat supplements increased 16:1, 17:0, 18:1t, CLA and all long-chain PUFA ($P<.001$). Compared with tallow, fish oil increased all unsaturated fatty acids except 18:1c; which was lower ($P<.01$). Treated fish oil fatty acids decreased 18:1t, 18:2, 20:4n6, 20:5n3, and 22:5n3 compared with fish oil ($P<.05$). Compared with control, fish oil treatments remarkably decreased 18:0 and 18:1c and increased 18:1t; long chain n-3 fatty acids were 0.2-0.5% of total milk fatty acids ($P<.001$). Although fish oils increased CLA (2.7 vs 0.8% for controls; ($P<.001$), this must be viewed cautiously because we could not separate CLA from 20:1. Treating fish oil with ethanolamine did not increase concentration of polyunsaturated n-3 fatty acids above concentrations observed with fish oil alone.

Key Words: Milk Fat Composition, Fish Oil, Protected Lipid

905 A mechanistic model to predict nutritional effects on milk fatty acid profile in dairy cows. M. K. Bargh* and J. P. Cant, *University of Guelph, Canada.*

Refereed scientific journal research was compiled into a database to identify major metabolic influences on individual fatty acids in milk. Only seven metabolic considerations were needed to explain the 163 significant treatment effects. These considerations were described mathematically to create a dynamic, mechanistic model. Previously published rumen submodels computed acetate and butyrate production rates. Calculations were derived for small intestine absorption of fatty acids directly from the ration and following ruminal biohydrogenation. Mobilization of fatty acids from adipose tissue also contributed to blood long-chain fatty acid pools. Once in the bloodstream, long-chain fatty acids were transferred into mammary state variable pools. The mammary glands had a pivotal role in the final proportions of individual fatty acids within milk fat. Firstly, desaturase activity elevated unsaturated fatty acid levels. Secondly, increased long-chain fatty acid concentrations inhibited acetyl-coenzyme A carboxylase activity and, consequently, short-chain fatty acid production. Parameter values for first-order and Michaelis-Menten equations were defined with the aforementioned database. Sensitivity of model predictions to a change in parameter values was evaluated. Finally, accuracy was determined by comparing model predictions with observed results from a switch-back between high- and low-fat rations fed to 20 lactating Holsteins over a three-month period.

Key Words: Fatty Acid, Mechanistic Model, Nutrition

906 Conjugated linoleic acid content of milk from cows fed different sources of dietary fat. P. Y. Chouinard[1], L. Corneau[1], D. E. Bauman*[1], W. R. Butler[1], Y. Chilliard[2], and J. K. Drackley[3], [1]*Cornell University, Ithaca, NY,* [2]*INRA, Theix, France,* [3]*University of Illinois, Urbana.*

Conjugated linoleic acid (CLA) is a naturally occurring anticarcinogen found in dairy products. Our objective was to determine the effect of dietary fat sources on milk fat content of CLA. In trial 1, 24 cows were used in a complete randomized block design. A control (CTL) diet was compared with diets supplemented with 4% (DM basis) Ca salts of fatty acids from canola (C), soybean (SB), or linseed (L) oil. Milk CLA contents were 3.5^c, 13.0^b, 22.0^a, and 19.0^a mg/g fat ($P < 0.01$) for CTL, C, SB, and L, respectively. In trial 2, 24 cows were used in a complete randomized block design. Treatments consisted of a TMR supplemented with ground raw (RA), extruded (E), micronized (M), or roasted (RO) soybeans included at 17.5% of the dietary DM. Milk CLA contents were 3.0^c, 8.9^a, 7.0^b, and 6.6^b mg/g fat ($P < 0.01$) for RA, E, M, and RO, respectively. In trial 3, 8 cows were used in a replicated 4 x 4 Latin square. Diets contained 35% alfalfa haylage, 20% corn silage, and 45% concentrate (DM basis); corn grain was 25.5% of DM. Diets contained normal (N) or high oil (H) corn grain (G) and corn silage (S) in a 2 x 2 factorial. Milk CLA contents were 2.8, 3.1, 3.9, and 4.6 mg/g fat for NS+NG, HS+NG, NS+HG, and HS+HG, respectively (S effect, $P < 0.05$; G effect, $P < 0.01$; S x G, NS). In trial 4, 9 cows were used in a replicated 3 x 3 Latin square. A CTL diet was compared to diets supplemented with 200 (FO1) or 400 (FO2) ml/d fish oil of Menhaden type. Milk CLA contents were 6.2^b, 17.4^a, and 16.5^a mg/g fat ($P < 0.01$) for CTL, FO1, and FO2, respectively. In trial 5, 45 cows were used in a randomized design to test the effect of a mixture of tallow/yellow grease (7:1) TG. A CTL diet was compared to diets supplemented with 2.2% (TG1) or 4.4% (TG2) of the fat mixture (DM basis). Milk CLA contents were 2.2^c, 3.8^b, and 4.7^a mg/g fat ($P < 0.01$) for CTL, TG1, and TG2, respectively. Results demonstrate that milk CLA content can be manipulated by the addition of different fat sources in the diet.

Key Words: Conjugated Linoleic Acid, Milk Fat

907 Effect of dietary manipulation on milk conjugated linoleic acid concentrations. P. Y. Chouinard, L. Corneau*, M. L. Kelly, J. M. Griinari, and D. E. Bauman, *Cornell University, Ithaca, NY.*

Conjugated linoleic acid (CLA), a potent naturally occurring anticarcinogen, is formed by rumen microorganisms as an intermediate in the biohydrogenation. We examined milk content of CLA over a wide range of dietary situations in dairy cows. In trial 1, Timothy (*Phleum pratense* L) grass silage cut at three different growth stages and supplemented with a concentrate at three levels, was fed to 18 cows. Growth stages were early heading (EH), flowering (FL), and second cutting (SC). Concentrate was offered at 0% (0C), 19% (LC) and 38% (HC) of TMR DM. Milk CLA contents were 11.4^b, 4.8^a, and 8.1^a mg/g fat ($P < 0.01$) for EH, FL, and SC, and 8.8, 8.6, and 6.8 mg/g fat (Linear, $P < 0.01$) for 0C, LC, and HC, respectively. In trial 2, 10 cows were used in a double 5 x 5 Latin square design to determine the effect of dietary buffers. Treatments were: 1) no buffer; 2) 1.1% $NaHCO_3$ + 1.1% $KHCO_3$; 3) 1.9% $NaHCO_3$; 4) 0.5% MgO, and 5) 2% Na sesquicarbonate (DM basis). Cows were fed TMR containing 53% grass silage and 42% concentrate including 4% Ca salts of canola oil fatty acids. Milk CLA content averaged 11.1 mg/g fat and was not affected by dietary treatments ($P = 0.7$). In trial 3, 15 cows were use in a cross-over design to test the effect of dietary forage to concentrate ratio (F:C). A high forage (HF) diet (F:C, 80:20) was compared to low forage (LF) diet (F:C, 20:80). Milk CLA contents were 4.5 and 7.3 mg/g fat ($P > 0.1$) for HF and LF, respectively. In trial 4, 19 cows were fed either control diet (CTL, $n = 9$) or a diet supplemented with 20 mg monensin/kg DM (M, $n = 10$). Milk CLA contents were 3.9 and 4.2 mg/g fat ($P = 0.6$) for CTL and M, respectively. Results indicate that some, but not all, dietary situations known to modify rumen environment affect milk content of CLA.

Key Words: Conjugated Linoleic Acid, Milk Fat

908 Conjugated linoleic acid in milk fat of dairy cows originates in part by endogenous synthesis from trans-11 octadecenoic acid. B. A. Corl*[1], P. Y. Chouinard[1], D.E. Bauman[1], D. A. Dwyer[1], J. M. Griinari[2], and K. V. Nurmela[2], [1]*Cornell University, Ithaca, NY,* [2]*Valio, Ltd., Helsinki, Finland.*

The recent discovery that conjugated linoleic acid (CLA) is a potent anti-carcinogen found in the milk of lactating dairy cows has led us to elucidate its origins. It is known that CLA is produced in the rumen as an intermediate in the biohydrogenation of linoleic acid. However, results from studies comparing diets suggested that rumen production may not be the only source of CLA. Knowing that stearoyl-CoA desaturase is capable of adding a c9 double bond to stearic acid, we hypothesized that it may also add the necessary double bond to vaccenic acid (t11 C18:1) producing c9, t11 CLA. An emulsified mixture of t11 and t12 C18:1 fatty acids (25 g/d) was continuously infused into the abomasum of 3 lactating cows. The treatment period was 3 days in length and was preceded by a 3-day pre-treatment period and followed by a 5-day post-treatment period in which the vehicle was abomasally infused. During the pre-treatment period, t11 C18:1, t12 C18:1 and c9, t11 CLA were evident in milk fat, but no c9, t12 C18:2 was present. During the treatment period t12 C18:1, c9, t11 CLA and c9, t12 C18:2 gradually increased in milk fat whereas minimal changes occurred in t11 C18:1. On day 3 of the infusion c9, t11 CLA was increased by 55% over the pre-treatment value. Fatty acid composition of the milk fat returned to baseline during the post-treatment period. Results demonstrate that the lactating cow can endogenously produce c9, t11 CLA from t11 octadecenoic acid.

Key Words: CLA, Desaturase, Milk Fat

909 Fatty acid distribution in blood plasma lipid fractions of Jersey cows fed canola oil and(or) soybean oil. J. J. Loor*, L. E. Quinlan, A. B. P. A. Bandara, and J. H. Herbein, *Virginia Polytechnic Institute and State University.*

To determine fatty acid distribution in lipid fractions of bovine blood plasma in response to dietary unsaturated fatty acids, 24 lactating Jersey cows were fed a control diet or the control diet supplemented at 3.5% of DM with high-oleic canola oil (CO), soybean oil (SO), or a mixture of equal amounts of CO and SO for 4 wk. Diets contained 35.1 (control), 68.6 (CO), 69.2 (mixture), or 69.7 (SO) g fatty acids/kg DM. Plasma lipids were extracted with chloroform/methanol (2:1, vol/vol), and individual lipid fractions were isolated with aminopropyl columns (500 mg). Oil supplementation did not affect DM intake, but daily intakes of individual (except myristic) and total fatty acids were increased. Intake of oleic acid was 361 g/d greater for cows fed CO compared with those fed SO. In contrast, linoleic acid intake was 279 g/d greater for cows fed SO compared with CO-fed cows. Overall, fatty acid concentration in the phospholipid, cholesterol ester, and triglyceride fractions of plasma from cows fed supplemental oil was 141, 144, and 26 μg/mL greater than those of the control group. Oleic acid increased from 12 to 17, 10 to 15, 4 to 5, and 6 to 11% of total fatty acids in the free fatty acid, phospholipid, cholesterol ester, and triglyceride fractions, respectively, when CO-fed cows were compared with the control. In contrast, SO intake increased linoleic acid in the free fatty acid, phospholipid, and cholesterol ester fractions from 11 to 18, 33 to 37, and 68 to 75%, respectively. In addition, *trans*-vaccenic acid was 1 to 7% of total fatty acids in the phospholipid and triglyceride fractions, and conjugated linoleic acid was 0.03 to 0.3% of total fatty acids in the phospholipid and cholesterol ester fractions. Results indicated a linear relationship between CLA and *trans*-vaccenic acid concentration in plasma($r^2 = 0.6$; $P < 0.01$)and milk fat ($r^2 = 0.7$; $P < 0.01$). Concentration of *trans*-isomers in blood plasma and milk fat ranked by treatment reflected linoleic acid intake and was greater in SO-fed cows compared with controls or CO-fed cows. Plasma lipid fractions can be used to appraise incomplete biohydrogenation of unsaturated fatty acids derived from fermentation in the rumen.

Key Words: *Trans*-vaccenic Acid, Conjugated Linoleic Acid, Oleic Acid

910 Effects of amylin on mineral metabolism in lactating goats. S. H. Min*[1], V. C. Farr[1], J. Lee[1], G. J. S. Cooper[2], and S. R. Davis[1], [1]*AgResearch, Ruakura Research Centre, Hamilton* [2]*School of School of Biological Sciences, University of Auckland (New Zealand)*.

Amylin is a 37-amino acid peptide secreted by the pancreas, structurally related to calcitonin-gene-related peptide (CGRP), adrenomedullin and calcitonin (CT). Although one of the main actions of this peptide is to alter carbohydrate metabolism, there is increasing evidence that amylin may play a potent role in the regulation of mineral metabolism, particularly calcium (Ca) and phosphate (PO_4). To examine effects of amylin in mineral metabolism in ruminants, rat amylin or saline was administered for 6 h by close (external pudic)-arterial infusion (320 pmol/kg LW/h) to six lactating goats in a cross-over design experiment. Circulating concentrations of Ca, measured at hourly intervals, were significantly reduced from 86.6±0.8 ug/ml at 0 h to 71.4±2.8 ug/ml at 6h. Similarly, circulating concentrations of PO_4 (ug/ml) were also decreased during infusion from 61.0±6.2 at 0h to 44.3±.4.3 at 6h. In contrast, amylin had no effect on concentrations of Ca and PO_4 in milk, measured at two-hourly intervals over the same period. Similarly, amylin infusion failed to affect milk concentrations of other minerals (Mg, K, Na, Fe, Cu, S, Sr) except Zn, where amylin significantly decreased Zn contents (ug/ml) in milk from 3.70±0.36 at 0h to 2.82±0.11 at 2h of post-infusion, the effect lasting beyond 6h post infusion. In conclusion, amylin is a potent regulator of Ca and PO_4 in plasma although these changes were not paralleled by corresponding changes in milk. Alteration of Zn concentrations in milk warrants a further study.

Key Words: Amylin, Mineral Metabolism, Lactating Goats

911 Milking five times daily in the presence of a cow's own calf was insufficient to prolong postpartum anovulation similar to cows suckled ad libitum. G. C. Lamb*, K. E. Thompson, J. S. Heldt, C. A. Loest, and J. S. Stevenson, *Kansas State University, Manhattan*.

The objective was to determine whether milking a beef cow five times daily in the presence or absence of her own nonsuckling calf would alter postpartum interval to first ovulation. Thirty multiparous Angus × Hereford cow-calf pairs were assigned randomly between 13 and 18 d postpartum to three treatments for 4 wk: 1) calf was present continuously with its dam (calf present [CP]; n = 10); 2) cows were milked twice daily in the continuous (24 h/d) presence of their calf, but udder-contact was prohibited (calf restricted + 2× milked [CR+2×M]; n = 10); or 3) same as CR+2×M but cows were milked five times daily (calf restricted + 5× milked [CR+5×M]; n = 10). Blood was collected daily to estimate first postpartum ovulation based on the first increase in serum progesterone (>.5 ng/mL for two consecutive days) that was followed by a progesterone profile typical of an estrous cycle. A 24-h milk yield and percentage milk components (fat, protein, lactose, solids-not-fat, and somatic cell count) were determined at the onset and termination of all treatments. During treatments, daily milk yield and percentage milk components were determined for CR+2×M and CR+5×M treatments. Interval to first increase in progesterone from onset of treatments was shorter ($P < .05$) in the CR+2×M (23.6 ± 3.5 d) and CR+5×M (26.1 ± 3.7 d) treatments than in the CP (37.7 ± 3.7 d) treatment. Average daily milk yield during treatment of CR+5×M cows (7.7 ± .6 kg) exceeded ($P < .05$) that of CR+2×M cows (6.4 ± .6 kg). During the first week of treatment, percentage protein was greater ($P < .05$) for CR+5×M cows (3.9 ± .1%) than CR+2×M cows (3.6 ± .1%), whereas percentage lactose for CR+2×M cows (5.0 ± .1%) exceeded that of CR+5×M cows (4.8 ± .1%). We concluded that milking cows either 2× or 5× times daily, in the presence of the cow's own nonsuckling calf, is insufficient inhibition to mimick ad libitum suckling and thereby prolong postpartum anovulation.

Key Words: Milking, Suckling, Anovulation

912 Effects of once-daily milking (ODM) on yield and composition of milk and metabolic status of dairy cows with high or low pasture intake. M. J. Auldist[1], C. G. Prosser[2], and T. R. Mackle[1]*, [1]*Dairying Research Corporation Ltd., Hamilton New Zealand and* [2]*AgResearch Dairy Science, Hamilton New Zealand*.

In dairy cattle, ODM can alleviate nutritional stress during underfeeding, and may possibly be used to treat anoestrus. This study evaluated effects of short-term ODM on milk yield and composition in cows grazing an *ad libitum* pasture allowance (>45 kgDM/cow/day) and cows in which milk yield was low due to a restricted pasture allowance (<18 kgDM/cow/day). 48 Friesian cows were subjected to each treatment for 10 days in a cross-over experiment during spring, following which milk yields averaged 22 and 16 l/day for *ad libitum* and restricted cows, respectively. All cows were then subjected to ODM for 2 days. ODM reduced milk yield, lactose concentration and casein:whey ratios, but increased concentrations of albumin, immunoglobulin and total whey protein. Effects were generally greater in higher-producing cows. In blood, ODM decreased concentrations of non-esterified fatty acids (NEFA), previously elevated by restricting pasture, but increased concentrations of glucose. Short-term ODM has detrimental effects on milk yield and composition. These effects may be minimised by restricting the practice to cows producing below their peak due to underfeeding. The use of ODM appears to alleviate nutritional stress in underfed cows.

	Effect of ODM (% change)		Significance (P<)	
	Ad libitum	Restricted	ODM	Int.[1]
Milk yield	−19.0	−13.3	0.01	0.01
Fat %	+10.5	+14.0	0.01	NS
Protein %	−0.1	+0.5	NS	NS
Lactose %	−3.0	−1.8	0.01	0.01
Casein %	−1.3	+0.3	NS	NS
Whey %	+11.9	+10.2	0.01	NS
Casein:whey	−10.5	−7.5	0.01	NS
Serum albumin	+30.0	+13.3	0.01	0.01
Immunoglobulin	+26.1	+19.2	0.01	0.01
NEFA in blood	−13.0	−51.7	0.01	0.01
Glucose in blood	+7.6	+7.9	0.01	NS

[1]Significance of interaction between ODM and nutrition; NS, not significant (P>0.05).

Key Words: Milk Composition, Once-daily Milking, Pasture Intake

913 Compensatory nutrition regimen affects mammary development and lactation. C. S. Park*, W. L. Keller, and Y. S. Moon, *North Dakota State University, Fargo*.

A nutritionally regulated compensatory growth regimen imposed during a growing period from prepuberty to gestation can significantly affect mammary development and subsequent lactation performance. The objectives of this study were 1) to determine how a compensatory nutrition regimen affects lactation performance, and 2) to determine the effect of a compensatory nutrition regimen on cell proliferation, differentiation, and apoptosis in mammary tissues of female rats during lactation. One hundred twenty-two female Sprague Dawley rats (35 d of age) were randomly assigned to either a control or a stair-step compensatory nutrition (SSCN) feeding regimen, an alternating 2-2-3-3-wk schedule beginning with 40% energy restriction for 2 wk followed by re-alimentation (free access to control diet) for 2 wk; this step was then repeated at 3 wk intervals. Mammary tissues were obtained from early, mid-, and late lactating rats. Pups of dams from the SSCN group gained more during mid-lactation than control group pups. Mammary tissue from the SSCN group exhibited increased cell proliferation and greater gamma-glutamyltranspeptidase gene expression than did tissue from the control group during early lactation. Mammary tissue from the SSCN group also had less apoptotic cells than tissue from the control group throughout the lactation cycle. These results suggest that the stair-step compensatory nutrition regimen affects lactation performance by modulation of cell proliferation and differentiation.

Key Words: Compensatory Growth, Lactation Performance, Mammary Cell

914 Effect of dopamine antagonist on serum growth hormone and prolactin concentrations in primi- and multiparous Holstein cows during the early postpartum. A. Ahmadzadeh, M. A. Barnes*, R. M. Akers, and M. L. McGilliard, *Virginia Polytechnic Institute and State University.*

Two experiments investigated the effect of i.v. injection of dopamine antagonist, fluphenazine (FLU) on blood concentrations of GH, and prolactin (PRL) in primi- and multiparous Holstein cows during the early postpartum. In experiment 1, in wk 2 postpartum, 12 primiparous cows received either saline (n=6) or .3 mg/kg BW FLU (n=6). Jugular blood samples were collected every 15 min for 4 hr before (pre-FLU) and 4 hr after FLU or saline (SAL). Immediately thereafter, all cows received 90 ug growth hormone releasing hormone (GHR) and blood collection continued for an additional 1.5 hr. Mean serum GH and PRL concentrations did not differ between the groups prior to treatments. FLU caused a transient decrease ($P < .05$) in mean serum GH concentration (from $5.8 \pm .3$ to $3.9 \pm .3$ ng/ml) and an increase ($P < .01$) in serum PRL concentration (from 14.4 ± 3.3 to 118.1 ± 3.3 ng/ml). Mean serum GH and PRL remained unchanged in SAL-treated cows. In experiment 2, 6 multiparous cows (wk 2 postpartum) were used and all cows received FLU (.3 mg/kg BW). Experimental procedures were the same as those used with primiparous cows, however the GRH dose used was increased to 110 ug. Mean serum GH concentration decreased ($P < .05$) in response to FLU (from $5.7 \pm .3$ to $3.2 \pm .3$ ng/ml) and mean PRL concentration increased ($P < .01$; from 8.5 ± 8.9 to 75.6 ± 8.9 ng/ml) in all cows. GHR increased ($P < .05$) serum GH concentration in both experiments, however, GH increase in response to GHR was delayed for 30 min in FLU-treated groups. These results suggest involvement of endogenous dopamine in modulation of both GH and PRL secretion in primi- and multiparous lactating cows during the early postpartum period.

Key Words: Dopamine Antagonist, Growth Hormone, Dairy Cattle

915 Effects of long day photoperiod and bST (Trobest®) on milk yield in lactating cows. A. R. E. Miller[1]*, E. P. Stanisiewski[2], R. A. Erdman[1], L. W. Douglass[1], and G. E. Dahl[1], [1]*University of Maryland, College Park* [2]*Pharmacia & Upjohn Co., Kalamazoo, MI.*

When used independently, bST and exposure to long day photoperiod (LDPP) increase milk yield in dairy cattle. Our objective was to test the hypothesis that LDPP and bST increase milk yield in an additive manner in lactating cattle. Starting at the winter solstice, forty lactating cows >70 d in milk (DIM), were blocked by parity, DIM and production then randomly assigned to one of four treatments (n=10/treatment): 1) ambient photoperiod (ADPP), 2) ADPP+bST (14 mg/d Trobest® i.m.), 3) LDPP (18 h light/d), 4) LDPP+bST. The experiment consisted of ten 14 d periods, including one pretreatment and one posttreatment period. Cows were milked twice daily. Cows were fed, to appetite, a total mixed ration formulated to meet the nutritional demands of lactation (45.3 kg milk/d). Feed intake was recorded daily and feed samples collected weekly. Milk samples were collected every 28 d for composition analysis. Blood was collected every 14 d by tail vessel puncture for somatotropin (ST) and IGF-1 assay. BW was recorded every 14 d. Treatment with LDPP (29.2±1.6) tended to increase FCM (kg/d) relative to ADPP (27.2±1.7). BST (32.7±2.04) increased FCM relative to ADPP ($p<.02$), and LDPP+bST (34.9±1.1) increased FCM relative to LDPP ($p<.01$). Treatment with LDPP+bST produced the highest yields of FCM. An increase in serum ST (ng/ml; $p<.01$) was observed with bST, but not photoperiod treatment (ADPP=1.3±1, ADPP+bST=6.8±1, LDPP=1.5±1, LDPP+bST=7.2±1). Also, bST increased serum IGF-1(ng/ml; $p<.01$) (ADPP=58.3±5.4, ADPP+bST=115.9±5.7, LDPP=61.4±5.4, LDPP+bST=105.6±5.4). LDPP+bST increased DMI relative to ADPP and ADPP+bST ($p<.05$); LDPP treatment increased DMI relative to ADPP+bST($p<.05$). BW and milk composition did not differ among treatments. In conclusion, bST increases serum ST, serum IGF-1 and milk yield in lactating cattle. Combination of bST with LDPP tended to amplify the increases in milk production.

Key Words: Photoperiod, bST, Lactation

916 Exogenous porcine prolactin in lactating sows: Effects on mammary gland development and prolactin receptors. D. Petitclerc[1], M. T. Sorensen[2], S. Robert*[1], and C. Farmer[1], [1]*Agriculture and Agri-Food Canada Lennoxville Research Centre, Canada* [2] *Danish Institute of Agricultural Sciences, Foulum Research Centre, Denmark.*

The goal of the present project was to determine the effects of exogenous porcine prolactin on prolactin receptors as well as development of the mammary gland in lactating sows. Twenty-four third-parity sows received s.c. injections of either water (CTL) or recombinant porcine prolactin (PRL; 15 mg/injection) at 0730, 1530 and 2330 from d 2 to 23 of lactation. Within 48 h of birth, litters were standardized to 10 ±1 1 pigs. Sows were slaughtered immediately after weaning (d 24). In order to empty the glands, piglets were separated from their dam for 90 min prior to a last suckling before slaughter. One row of mammary glands was used for dissection of parenchymal and extraparenchymal tissues, and for determination of DNA, RNA, dry matter, protein, fat, and lactose contents. Tissue from the other row was used for measures of prolactin receptor number and affinity. Weights of parenchymal (4441.6 vs 4415.5 g for PRL vs CTL, SEM=179.0) and extraparenchymal (6.45 vs 6.30 g for PRL vs CTL, SEM=.16) tissues were not altered by PRL treatment ($P>.1$) and none of the biochemical measurements (expressed on a dry matter basis) were affected by treatment ($P>.1$). Residual milk (based on lactose content) was also not affected by PRL injections ($P>.1$). Number of prolactin receptors in parenchymal tissue (48.6 vs 57.2 fmol/mg protein for CTL vs PRL, SEM=.27) as well as receptor affinity (.76 vs .81 ng/mL for CTL vs PRL, SEM=.41) were similar in both groups ($P>.1$). Plasma prolactin levels in CTL sows (overall mean in lactation 72.6 ng/mL) were approximately 100-fold higher than prolactin receptor affinity, indicating that virtually all prolactin receptors are saturated in lactating sows. This is probably the reason that additional exogenous prolactin had negligible effects (Porcine prolactin kindly provided by Monsanto, St-Louis, MO).

Key Words: Sows, Prolactin, Mammary gland

917 Growth of mammary gland during lactation in the sow. S. W. Kim[1], W. L. Hurley[1], I. K. Han[2], and R. A. Easter*[1], [1]*University of Illinois at Urbana-Champaign* [2]*Seoul National University.*

A total of 16 primiparous sows was used for this study to determine the extent of mammary gland growth during lactation in the sow. After birth, litter size was standardized to 9 or 10 pigs. Sows were slaughtered in groups representing days 5, 10, 14, 21 and 28 of lactation. During lactation, sows were provided 17.5 Mcal ME and 65 g of lysine per day. Mammary glands were collected at slaughter and trimmed of skin and the extraneous fat pad. Each gland was weighed, ground and stored at −20°C for chemical analysis. Frozen ground tissue was used to determine dry matter content (105°C for 4 hr), dry-fat-free tissue content (DFFT; ether extraction of dried tissue), total tissue protein (Kjeldahl), and DNA content (Fluorometric assay). Only glands known to have been suckled were included in this data. Hyperplasia resulted when there is an increase in the number of cells in the tissue and is reflexed in the DNA content. There was a linear increase in the amount of DNA , DNA percentage, and DNA/DFFT during lactation ($P<0.05$). In addition, there was also a quadratic increase in protein/DNA ($P<0.05$) with the highest on day 21 of lactation. This indicates that hyperplasia occurs during lactation.

	Day of Lactation				
Variables	5	10	14	21	28
DNA, mg*	729±99	894±133	1113±94	1489±100	1412±114
DNA, %*	.21±.01	.22±.02	.24±.01	.26±.01	.24±.02
Protein/DNA**	52.0±2.7	46.4±3.6	54.1±2.6	54.5±2.7	47.4±3.1
DNA/DFFT*	14.3±1.6	13.3±2.1	20.0±1.5	24.1±1.6	23.0±1.8

* Linear effect of day ($P<0.05$); **Quadratic effect of day ($P<0.05$)

Key Words: Sow, Mammary Gland, Lactation

918 Exogenous porcine prolactin in lactating sows: Lactational and hormonal responses. C. Farmer[1]*, S. Robert[1], M. T. Sorensen[2], and D. Petitclerc[1], [1]*Agriculture and Agri-Food Canada, Lennoxville Research Centre, Canada* [2] *Danish Institute of Agricultural Sciences, Foulum Research Centre, Denmark.*

This project was done to determine the effects of exogenous porcine prolactin on the lactational performance and hormonal responses of lactating sows. Twenty-four third-parity sows received s.c. injections of water (controls) or recombinant porcine prolactin (PRL; 15 mg/injection) at 0730, 1530 and 2330 from d 2 to 23 of lactation. Litters were standardized to 10 ±1 pigs. Weights of pigs were recorded weekly until d 56. Backfat thicknesses and weights of sows were recorded at 110 d of gestation, and d 2 and 23 postpartum. The interval between sucklings, duration of teat massage and duration of milk ejection were determined over four consecutive sucklings on d 20 of lactation. On d 22, milk yield was estimated and a milk sample was obtained the next day. Jugular blood samples were collected from the sows on d 2, 7, 14 and 21 of lactation to measure concentrations of prolactin, IGF-I, insulin, glucose, and FFA. Daily feed and water intakes of sows were recorded in lactation. Injections of PRL doubled the concentrations of prolactin (P<.001) on d 7 (162.4 vs 84.1 ng/mL, SEM=7.9), 14 (148.9 vs 72.8 ng/mL, SEM=10.9) and 21 (116.4 vs 60.8 ng/mL, SEM=8.3) of lactation and decreased IGF-I on d 14 (155.3 vs 189.9 ng/mL, SEM=12.6, P=.07) and 21 (146.8 vs 191.1 ng/mL, SEM=10.7, P<.01). Weights, backfat of sows, mean piglet weights and milk yield (12.24 ± .02 kg/d) were not affected by PRL (P>.1). Feed intake of sows was unaltered by PRL (P>.1), but there was a tendency (P=.07) for the increase in water intake between wk 2 and 3 to be greater in PRL sows (4.1 vs 2.1 L/d, SEM=.41). Protein and lactose contents of milk were not affected by treatment (P>.1), while dry matter and fat percents were lower in PRL sows (P<.01). Suckling behavior was also unaffected (P>.1) by PRL. In conclusion, exogenous porcine prolactin did not improve lactational performances of sows even though plasma prolactin levels doubled (Porcine prolactin kindly provided by Monsanto, St-Louis, MO).

Key Words: Sows, Prolactin, Lactation

919 The anticarcinogenic agents of bovine milk fat. P. W. Parodi., *Dairy Research and Development Corporation, Melbourne, Australia.*

Prevention is an important strategy for conquering cancer. Milk fat contains a number of components such as conjugated linoleic acid (CLA), sphingomyelin (SM), butyric acid (BA), ether lipids, β-carotene, vitamin A and vitamin D that help prevent the development of cancer. Cell culture studies show CLA suppresses growth of human malignant melanoma, colorectal, breast, lung, prostate, ovarian, leukemia, hepatoma, glioblastoma and mesothelioma cancer cell lines. In animals, CLA reduced the incidence of chemically-induced mouse epidermal tumors, forestomach neoplasia and precursors of colon tumors. CLA protects against DNA-adduct formation in a number of rodent organs. Notably, a number of studies show diets supplemented with as little as 0.1% CLA inhibit mammary cancer independent of the amount or type of fat in the diet. The milk phospholipid SM, through its biologically active hydrolysis products, ceramide and sphingosine (now referred to as tumor suppressor lipids) play a major role in cellular signal transduction influencing three major antiproliferative pathways associated with carcinogenesis; inhibition of cell growth, induction of differentiation and cell death (apoptosis). Mice fed diets supplemented with SM had fewer chemically-induced colon tumors than controls. About one third of all milk triacylglycerols contain one molecule of BA. BA is a potent inhibitor of proliferation and inducer of differentiation and apoptosis in a wide range of cancer cell lines. In addition, synergy with other micronutrients, such as vitamins A and D, can enhance the antiproliferative potential of BA. BA produced by colonic fermentation of fiber is thought to protect against colon cancer. However, dietary supplementation with BA reduced the incidence of chemically-induced rat mammary carcinomas and adenocarcinomas. Ether lipids, β-carotene and vitamins A and D, present in milk fat, may exert cancer chemopreventive effects at a number of sites. Finally, the cow has the abilit y to extract anticarcinogenic components from pasture and feed and transfer them to milk. Examples are β-ionine from alfalfa and gossypol from cottonseed meal.

Key Words: Milk Fat, Cancer Prevention, Anticarcinogenic Agents

920 *Trans* fatty acids in milk: health risk or a benefit. J. M. Griinari, *Valio Ltd., Helsinki, Finland.*

Trans fatty acids (TFA) in partially hydrogenated oils (PHO) and the proposed relationship between TFA intake and coronary heart disease have received substantial media attention. The issue has been widely portrayed as a butter versus margarine challenge. Several expert panels have summarized the available experimental and epidemiological data and concluded that the risk attributable to the TFA at current intake levels appears to be small. In spite of that, the European oil industry has already taken the challenge and implemented new technologies to produce margarines with very low levels of TFA. This change in the manufacture of PHO is reflected in the changing estimates of TFA consumption in Europe. The proportion of TFA coming from PHO has decreased to roughly half of the total TFA intake. In comparison, PHO still contribute 80-90% of the TFA in the American diet. Based on epidemiological data a hypothesis which suggests that the negative health effects are attributable to the TFA from PHO not to the naturally occurring TFA from ruminant fats has been put forward. Variable physiological effects of TFA could arise from the differences in their chemical composition. Milk fat TFA composition is unique as it is characterized by two predominant isomers which account for approximately 50% of the total TFA in milk fat: 11t-18:1 (vaccenic acid) and 9c,11t-18:2 (isomer of conjugated linoleic acid or CLA). CLA is a potent anticarcinogen and vaccenic acid is its closely associated metabolite in the rumen and in the body tissues. Milk fat TFA-isomer profile is known to be influenced by feeding, but the regulatory steps in the rumen or in the animal metabolism are not well described.

Key Words: Milk fat, Vaccenic acid, Conjugated linoleic acid

921 Testicular biopsy in rams: semen, ultrasonographic, gross and histological examinations and study with fibrin glue derived from snake venom. R. Sartori*, N. C. Prestes, A. M. O. Canavessi, W. G. Kempinas, P. R. Curi, I. A. Thomazini, M. J. S. M. Giannini, and B. Barraviera, *University of São Paulo State, Botucatu, Brazil.*

Testicular biopsy (TB) has been a useful technique in reproductive examination. However, hemorrhage, adhesion and fibrosis might be limiting factors. This study evaluated if TB with Tru-Cut needle in rams is executable in order to obtain material for histology and studied possible reproductive complications resulting from the use of TB. The applicability of the fibrin glue (FG) derived from snake venom was also evaluated in regard to its hemostatic and sealing properties, prevention of testicular adhesion formations and its healing effect on the testicle, tunica vaginalis and skin. Mature rams were divided into 3 groups of 10. G1: animals not subjected to TB, G2: fibrin glue on puncture sites and skin incisions after bilateral TB, and G3: hemostasis by compression with a swab on puncture sites and skin suturing with nylon after TB. Semen and testicular size evaluations were carried out before biopsy and 20, 40, 60, 80 and 100 days after. Ultrasonography (US) was performed before biopsy and 4, 8, 14, 24, 50, 70 and 100 days after. At the time of orchiectomy (100 days after TB) the material was evaluated for gross lesions, presence of subcutaneous (SC) and/or tunica vaginalis adhesions and used for histological examination. G1 did not present abnormalities. G2 and G3 presented 4 testicles (20%) with adhesion between the tunics at biopsy site. Adhesions in SC were observed once (5%) in G2 and 3 times (15%) in G3. FG was easy to apply and provided fast and good-quality skin healing. This biopsy technique did not compromise semen characteristics or testicular size in the animals of G2 and G3. US allowed mapping of alterations (mainly calcifications) and analysis of the evolution of the lesions. TB with Tru-Cut needle in rams provided enough material for histology and was found to be a simple, safe and efficient technique.

Key Words: Testicular Biopsy, Fibrin Glue, Ram

922 Effect of breed on testicular traits, spermatogenesis, and spermatogenic apoptosis in crossbred bulls in the subtropics. V. H. Monterroso*[1], C. C. Chase, Jr.[2], R. E. Larsen[1], and P. J. Chenoweth[3], [1]*University of Florida, Gainesville,* [2]*USDA, ARS, Brooksville, FL,* [3]*Kansas State University, Manhattan.*

Breed effects on bull testicular traits, spermatogenesis, and spermatogenic apoptosis were studied over 2 yr. In yr 1, Brahman × Angus (BA; n = 18), Senepol × Angus (SA; n = 18), and Tuli × Angus (TA; n = 22) bulls were used; in yr 2, BA (n = 14), SA (n = 22), and TA (n = 18) bulls were used. Bulls were sacrificed in June (17 mo of age) and testes were processed to assess total daily sperm production (DSP), DSP/g of testicular parenchyma (DSPG), and spermatogenic apoptosis (using ELISA). Breeds did not differ (P > .10) in age at sacrifice, although BA bulls were heavier (P < .05) than SA and TA bulls (485 ± 8.7, 428 ± 7.7, and 428 ± 7.7 kg, respectively) and had smaller (P < .05) individual testicular circumference than SA and TA bulls (17 ± .2, 18 ± .2, and 18 ± .2 cm, respectively). Despite this, breed did not affect (P > .10) paired testicular volume (720 ± 27.0, 734 ± 21.8, and 777 ± 21.8 cm^3 for BA, SA, and TA, respectively), paired testicular weight (447 ± 16.6, 473 ± 13.7, and 489 ± 13.6 g, respectively), or paired epididymal weight (52 ± 1.7, 53 ± 1.4, and 53 ± 1.4 g, respectively). When a *Bos taurus* time divisor (TD = 5.32) was used, total DSP and DSPG were lower (P < .05) for BA (1 ± .1 × 10^9 and 5 ± .4 × 10^6) than SA (2 ± .1 × 10^9 and 6 ± .3 × 10^6) and TA (2 ± .1 × 10^9 and 7 ± .3 × 10^6). However, when a *Bos indicus* TD (5.11) was used for BA only, total DSP was lower (P < .05) in BA than SA and TA (1 ± .1, 2 ± .1, and 2 ± .1 × 10^9), but no breed difference was observed for DSPG (6 ± .4, 6 ± .3, and 7 ± .3 × 10^6 for BA, SA, and TA, respectively). Spermatogenic apoptosis was not different (P > .10) among breeds (1,264 ± 179.3, 1,040 ± 184.2, and 918 ± 179.3 mU/mg for BA, SA, and TA, respectively). In conclusion, BA bulls had smaller individual testicular circumference and lower spermatogenesis (total DSP and perhaps DSPG) than SA and TA bulls; however, breeds did not differ in either testicular or epididymal weights or spermatogenic apoptosis.

Key Words: Cattle Breeds, Bulls, Spermatogenesis

923 Reproductive performance of spring- and fall-born bulls divergently selected on the basis of blood serum insulin-like growth factor-I concentration. A. Yilmaz*[1], M. E. Davis[1], and R. C. M. Simmen[2], [1]*The Ohio State University, Columbus and* [2]*University of Florida, Gainesville.*

The objectives of this study were to examine season effects and differences in scrotal circumference, sperm motility and percentage of normal sperm cells between two lines of Angus beef cattle divergently selected for blood serum IGF-I concentration. Data were obtained from an ongoing experiment initiated in 1989 involving divergent selection for IGF-I in 100 spring-calving (50 high and 50 low line) and 100 fall-calving (50 high and 50 low line) purebred Angus cows. Divergent selection of bulls was based on blood serum IGF-I concentration measured at d 28, 42, and 56 of the postweaning test. Numbers of observations for scrotal circumference, and for percentage of motile and normal sperm cells were 311, 219, and 288, respectively. Scrotal circumference, and percentage of motile and normal sperm cells did not differ between high and low line bulls (P=.98, .28 and .84, respectively). Scrotal circumference was significantly larger in spring-born bulls (P<.01), whereas percentage of motile and normal sperm cells was significantly higher in fall-born bulls (P<.01 and P.<01, respectively). Coefficients for quadratic regressions of scrotal circumference and percentage of normal sperm cells on IGF-I at d 42 were significant (P=.03 and .03, respectively). Coefficients for quadratic regressions of scrotal circumference and percentage of motile and normal sperm cells on IGF-I at d 56 were either significant or approached significance (P=.15, .09, and .09, respectively). Coefficients for quadratic regressions of scrotal circumference, and percentage of motile and normal sperm cells on mean IGF-I were either significant or approached significance (P=.03, .07, and .11, respectively). Therefore, scrotal circumference and percentage of motile and normal sperm cells have important quadratic relationships with mean blood serum IGF-I concentration.

Key Words: Beef Cattle, Insulin-like Growth Factor, Male Reproductive Traits

924 The effect of 2-bromo-ergocriptine on sexual behavior, seminal characteristics and plasma LH in light horse stallions. K. Bennett-Wimbush, *Ohio State University Agricultural Technical Institute, Wooster, Ohio.*

Four mature stallions (ages 4–20) were used in a paired t-test experiment to determine the effects of 2-bromo-ergocriptine, a D_2 dopamine receptor agonist, on sexual behavior, seminal characteristics and plasma LH. Stallions were teased daily using an ovariectomized, estrogen treated mare, starting July 5, 1996. The time from first exposure to the mare until the stallion got an erection was recorded. Semen was collected daily and the number of mounts for ejaculation, total gel-free volume, concentration and motility were recorded and daily sperm output was calculated. Pre-treatment values (week 0) for each of the above variables were averaged using day 7, 8, 9 and 10 data. In addition, blood was collected daily into heparinized Vacu-tainer tubes, centrifuged and the plasma was decanted off and stored at −10° C until it was analyzed by radioimmunoassay for equine LH. After this initial collection period, stallions were treated with .08 mg/kg metabolic body weight ($kg^{.75}$) of 2-bromo-ergocriptine, im, twice daily for 63 consecutive days. In addition, semen and plasma were collected at 2 and 8 weeks post-treatment as described for the pre-treatment period. Data were categorized into week of treatment and each week (1–9, 11, 18) was tested for statistical differences between week 0 using paired t-tests. There were no differences in time until erection, number of mounts, motility or mean plasma LH between week 0 and weeks 1 through 9, 11 and 18. Mean daily sperm production was 3.1 billion sperm in week 0 which did not differ from the daily sperm production observed during weeks 1–8. However, daily sperm production decreased (P < .05) to 2.1 billion during week 9 of treatment and returned to pre-treatment values both 2 and 8 weeks post-treatment. Mean plasma LH was 8.7 ng/ml for week 0 and decreased numerically, but not statistically, to 6.9, 5.3 and 6.2 ng/ml for weeks 1, 5 and 9 respectively. This data suggests that long-term administration of a dopamine agonist may decrease daily sperm production in light-horse stallions.

925 Effects of immunizing ram lambs against inhibin on puberty and testicular growth and function. R. W. Godfrey[1], J. R. Collins[1], E. L. Hensley[1], B. M. Pannagl[1]*, and J. E. Wheaton[2], [1]*University of the Virgin Islands, Agricultural Experiment Station, St Croix,* [2]*University of Minnesota, Department of Animal Science, St Paul.*

Hair sheep ram lambs were used to evaluate the effects of immunization against the α-subunit of ovine inhibin (oα-I) conjugated to human α-globulin (hα-G; Meyer, et al., 1991, J. Anim. Sci. 69:747–754). Immunized lambs (IMM; n = 7) received antigen and control lambs (CON; n = 7) received hα-G at 3, 7, 13, 19, 25, 31, 37 and 43 wk of age. All immunizations were emulsified in Freund's adjuvant (complete initially, incomplete for boosters) and administered s.c. over four sites. Blood samples were collected on the day of injection and 1 wk later. Plasma was harvested and stored at −20°C until assayed for antibody titer. Antibody titers were estimated by incubating plasma diluted 1:4,000 with ^{125}I-O-αI (24,000 cpm) and expressed as percent bound. Beginning at 5 mo of age semen was collected by electroejaculation and testes were measured. Puberty was defined as the time when a ram produced and ejaculate containing 50 × 10^6 sperm. At 57 wk of age testes were harvested and daily sperm production (DSP) was determined. The antibody titer of IMM rams during wk 4 through 52 was 8.6 ± 1.3 %. Antibody titer was positively correlated with body weight (R = .346, P < .002) and scrotal circumference (R = .281, P < .04) of IMM rams. Control rams reached puberty sooner (P < .02) than IMM rams (206 ± 4 vs 223 ± 5 d, respectively). Paired testes volume at puberty was greater (P < .08) in IMM than in CON rams (183 ±13 vs 149 ± 12 cm^3, respectively). Testicular parenchyma weight at 57 wk of age was greater (P < .03) in CON than in IMM rams (119 ± 8 vs 92 ± 7 g, respectively). There was no difference (P > .10) in DSP (10^6 sperm/g) between CON and IMM rams (13.1 ± 2.9 vs 14.6 ± 2.4, respectively). These data indicate that immunoneutralization of inhibin during the early postnatal period in ram lambs does not decrease days to puberty or increase sperm production.

Key Words: Ram, Inhibin, Immunization

926 Monoamine neurotransmitter antagonists alter LH, GH, and Prl secretion in prepubertal bull calves. D. L. Fernandez, D. B. Imwalle*, and K. K. Schillo, *University of Kentucky, Lexington.*

Development of pulsatile LH secretion is a physiologically important prepubertal event in cattle. Data from bull calves support a role for monoamine neurotransmitters in the control of LH secretion. The objectives of this study were to determine whether monoamine antagonists alter LH release in prepubertal bull calves. Angus calves (109 ± 11 kg; n=28) were subjected to sequential blood sampling at 8, 12 and 14 wk of age. Samples were collected every 15 min between 1 h before and 4 h after injections of ethanol (Con), 4 mg phenoxybenzamine (Phe), 1.5 mg cyproheptadine (Cyp) or .5 mg fluphenazine (Flu) per kg body weight. Mean concentrations of LH and LH pulse frequency increased (P<.05) between 8 and 12 wk and remained elevated at 14 wk. Mean concentrations of GH decreased (P<.05), but GH pulse frequency did not change with age. Prolactin concentrations were higher (P<.05) at 12 and 14 wk than at 8 wk of age. The norepinephrine antagonist (Phe), decreased (P<.05) mean LH at 12 and 14 wk, increased (P<.05) mean Prl at 8 and 14 wk, and decreased (P<.05) mean GH at 14 wk. The serotonin antagonist (Cyp) increased (P<.05) mean Prl at 8 and 14 wk, decreased (P<.05) mean GH at 8, 12 and 14 wk, decreased (P<.05) GH pulse frequency at 12 and 14 wk, but had no effect on LH. The dopamine antagonist (Flu) decreased (P<.05) mean LH and LH pulse frequency at 12 and 14 wk, increased (P<.05) mean Prl at all ages, but did not influence GH patterns. Our results support the hypothesis that dopamine and norepinephrine stimulate, whereas serotonin does not regulate LH secretion in prepubertal bull calves.

Key Words: Neurotransmitters, Luteinizing Hormone, Bull Calves

927 N-methyl-d,l-aspartate (NMA) increases concentrations of LH in serum of mature boars. M. J. Estienne[1]*, T. G. Hartsock[2], and C. R. Barb[3], [1]*University of Maryland Eastern Shore, Princess Anne,* [2]*University of Maryland, College Park,* [3]*USDA-ARS, Athens, GA.*

Glutamate is an important neurotransmitter and has been implicated in the neuroendocrine control of LH secretion in several domestic animal species. We previously reported, however, that NMA, an agonist of glutamate, at i.v. doses of 1.25 to 10 mg/kg BW, failed to alter circulating LH concentrations in immature boars that were approximately 180 d of age (Broughton et al., 1996; J. Anim. Sci. 74[Suppl. 1]:91). The objective of this experiment was to determine the effect of NMA on circulating concentrations of LH in mature boars. Five Yorkshire × Poland China boars (401 d of age and 179.1 ± 6.6 kg BW [mean ± SE] were fitted with jugular vein catheters 24 h prior to the start of the experiment. On Day 1, blood was sampled every 15 min for 2 h. One h after the initiation of blood sampling, boars received i.v. injections of NMA (10 mg/kg BW; n = 3) or .9% saline (n = 2). The experiment was repeated on Day 2 except that boars that received NMA on Day 1 received .9% saline and vice versa. All serum samples were assayed for LH using RIA. Serum concentrations of LH prior to i.v. injections were similar for NMA- and saline-treated boars and were .30 ± .05 ng/ml and .25 ± .04 ng/ml, respectively. Injection of NMA (P < .01), but not .9% saline (P > .1), increased serum LH concentrations. Serum concentrations of LH for the one hour period after injections were .60 ± .06 ng/ml for NMA-treated boars and .25 ± .02 ng/ml for .9% saline-treated individuals. The results of our previous study, and the current experiment, are consistent with the notion that in boars, there is an age-related change in responsiveness to NMA, with regard to the ability of the compound to evoke LH secretion. This hypothesis will be tested in future experiments.

Key Words: Luteinizing Hormone, N-Methyl-d,l-Aspartate, Boars

928 Changes in peripheral plasma LH and testosterone in male goats from 1 to 6 months of age. E. S. Coleman*[1], T. D. Braden[2], D. A. Coleman[2], and H. O. Goyal[1], [1]*Tuskegee University, AL,* [2]*Auburn University, AL.*

Previous studies reported the onset of puberty in the Nubian buck to be 141 ± 4 days of age. In this study, serial peripheral plasma profiles for LH and testosterone were monitored monthly in 3 male Nubian goat kids from 1 to 6 months of age. Blood samples were collected every 15 minutes for six hours from an indwelling jugular catheter placed the day prior to collection. Plasma samples were evaluated by radioimmunoassay for LH and testosterone concentrations. Mean and baseline plasma LH concentrations were highest at 2 months of age (0.72±0.04 ng/ml and 0.29±0.02 ng/ml) with a decrease (P<.05) thereafter to 6 months (0.22±0.04 ng/ml and 0.07±0.02 ng/ml). LH pulse frequency was highest at 2 months of age with 4 ± 0.3 peaks/6 hrs, decreasing to 1.3 ± 0.3 peaks/6 hrs at 6 months (P=.1). The interval between LH pulses increased over time (P<.05). Testosterone mean (P<.01) and baseline (P<.05) concentrations increased over time from 0.37±0.18 ng/ml and 0.02±0.11 ng/ml at 1 month to 2.21±0.18 ng/ml and 0.82±0.11 ng/ml at 6 months of age. Testosterone peak amplitude increased over time (P<.001) from a mean at 1 month of 0.95±0.27 ng/ml to 4.71±0.27 ng/ml at 6 months of age. The interval between testosterone pulses increased over time (P<.05). There was a strong correlation between the number of LH and testosterone pulses (r=.89, P<.001) with a testosterone pulse occurring approximately 15–30 minutes after each LH pulse. This correlation was evident during all months studied including month 1, supporting early activity of the hypothalamo-pituitary-gonadal axis prior to the onset of puberty. The increase in LH concentrations at 2 months of age is consistent with a decrease in steroid-responsive negative feedback typically seen in male domestic animals at 8-10 weeks of age, and as in other species, likely plays a role in gonadal development and onset of puberty in the buck. The results of the present study suggest that the hypothalamo-pituitary-gonadal axis organizes as early as 1 month of age in the Nubian buck.

Key Words: Goat, LH, Testosterone

929 In vitro capacitation of equine spermatozoa. J. J. Parrish*[1], J. L. Susko-Parrish[1], E. L. Squires[2], and J. K. Graham[2], [1]*University of Wisconsin,* [2]*Colorado State University, Ft. Collins.*

The objective was to develop a repeatable method to capacitate equine sperm by manipulating sperm cAMP, calcium and pH. Sperm from 5 stallions were incubated with 0 or 0.5 mM 8-bromo-cAMP for 0–5 hr at 39°C. At 15 min before evaluation, 100 nM ionomycin (IO) in DMSO was added. Next, 0 or 100 ug/ml lysophosphatidylcholine (LC) was added for a further 15 min to induce acrosome reactions (AR) in capacitated sperm. Sperm AR were determined by staining with FITC labeled lectin, PNA, and propidium iodide. The percentage of AR sperm did not change in response to LC for sperm incubated in medium alone, with or without the addition of IO (17 ± 2 vs 19±2 and 15±2 vs 16±1, p>0.05). Similarly, sperm incubated with 8-bromo-cAMP but not exposed to IO did not AR in response to LC (18±3 vs 22±3; p>0.05). However, there was a time dependent increase (p<0.05) in the ability of sperm to AR in response to LC when sperm were incubated with 8-bromo-cAMP and exposed to IO (15 min). At 0.25, 1, 2, 3, 4 and 5 hr of incubation, the percent AR sperm was 12±3, 12±3, 18±4, 20±4, 26±2, and 29±4 before LC and 12±3, 15±2, 26±3, 38±6, 47±3, and 57±4 after LC. In the next experiment, the effect of intracellular acidification was investigated by incubating sperm from 4 stallions for 0.25–5 hr in media with either 0 or 5 mM glucose. The addition of glucose inhibited the time dependent ability of sperm to AR in response to LC after incubation with 8-bromo-cAMP and exposure to IO (p>0.05). In a third experiment, when sperm from 4 stallions were incubated with 8-bromo-cAMP for less than 4 hours, an increase in the length of IO exposure from .25 to 1 or 2 hr resulted in more sperm undergoing AR upon exposure to LC (p<0.05). For example, at 2 hr, ionomycin exposures of .25, 1 or 2 hr resulted in 17±1, 14±3 and 15±1 percent AR before LC and 51±6, 46±7 and 29±2 after LC. In conclusion, these results demonstrate that changes in equine sperm indicative of capacitation can be induced when equine sperm are incubated in the absence of glucose, with 8-bromo-cAMP and a brief exposure to a low dose of IO. Work was supported by the College of Ag and Life Sci, UW and the College of Vet Med, CSU.

Key Words: Capacitation, Equine, Sperm

930 Bovine and porcine spermatozoal motility as it relates to measures of mitochondrial efficiency. A. M. Fontenot*, J. E. Chandler, A. M. Canal, A. G. Marquette, and R. W. Adkinson, *LSU Agricultural Center, Louisiana Agricultural Experiment Station, Department of Dairy Science, Baton Rouge*.

Maternally inherited mitochondria produce ATP for energy demanding cellular processes such as milk production and protein synthesis. Mitochondrial function can be measured by methylene blue reduction rate (MeBRR) under anaerobic conditions or aerobically by ATP usage rate. The objective of two studies, one using bulls and the other boars, was to examine the relationship between sperm motility and mitochondrial function in different populations. Males were selected to maximize differences in mitochondrial populations by requiring different maternal lineages and contrasting breeding values for high energy production traits. Two ejaculates from two Brahman and two Holstein bulls were used. Spermatozoal motility and ATP concentration, using the luciferin/luciferase assay, were measured in duplicate on each ejaculate. Measurements were taken at 0h and again after 3h-37 C incubation. ATP usage was calculated as the ATP concentration decline during 3 hours. Mitochondrial function efficiency was defined as the slope of motility regressed on ATP usage. In the second study, boars were selected based on maternal EPD for milk production (EPD 21 day litter weight, EPD21). Semen was obtained from 2 males representing each of 4 full sibling EPD groups equally divided across Landrace and Yorkshire breeds. Landrace dam EPD's averaged 1.59 and Yorkshire dam EPD's averaged 8.15. Motility and MeBRR were measured in duplicate on each ejaculate. Efficiency was defined as the slope of motility regressed on MeBRR. Mitochondrial efficiency in the Brahman was significantly (P<.06) different from that in Holsteins. The Brahman efficiency was -224.6(b=0, P<.28). The Holstein mitochondrial efficient was 778.9(b>0, P<.001). Yorkshire mitochondrial efficiency was (P<.05) greater than Landrace. The Landrace efficiency was -.001(b=0, P<.88). The Yorkshire efficiency was .04(b>0, P<.11). The correlation between dam EPD21 and porcine sperm motility was .35(P—r—<>0,.04). Since mitochondria are of maternal origin and differences in sperm mitochondrial efficiencies were discernible, selection of males for spermatozoal motility should include a strong influence of the dam.

Key Words: ATP, Motility, Mitochondrial Function

931 Effect of artificial insemination of superovulated cows at 0, 12, or 24 h post onset of estrus on fertilization status, embryo quality and accessory sperm number per embryo. J. C. Dalton*, S. Nadir, J. H. Bame, M. Noftsinger, A. Robertson, and R. G. Saacke, *Department of Dairy Science, Virginia Polytechnic Institute and State University, Blacksburg*.

Thirty nonlactating Holstein cows were superovulated to determine the effect of time of artificial insemination on fertilization status, embryo quality, and accessory sperm number per embryo. A single ejaculate from one bull (> 70% normal morphology and > 60% estimated progressive motility) was cryopreserved using clarified egg yolk-citrate-glycerol extender. Beginning on day 8, 9, 10 or 11 of the estrous cycle, cows were administered 38mg FSH-P^{TM} in a four-day descending dose regimen. Luteolysis was induced with two injections of prostaglandin on the last day of FSH-P^{TM} treatment. All cows were continuously monitored for behavioral estrus by HeatWatch® and inseminated once with one .5-mL straw (50×10^6 sperm) at either 0 h (n=10), 12 h (n=10), or 24 h (n=10) after the first standing event. Five hundred twenty-nine embryos (ova) were recovered nonsurgically 6 d post insemination. Fertilization rates were 29% (0 h); 60% (12 h); and 81% (24 h)(P < .01). Percentages of degenerate, fair to poor and good to excellent embryos were: 21, 35, 44 (0 h); 18, 43, 39 (12 h); and 25, 33, 42 (24 h)(P > .05). Mean ± S.D. and median values for accessory sperm per embryo were: .07 ± .31 and 0 (0 h); .24 ± 1.3 and 0 (12 h); and 2.9 ± 8.3 and 0 (24 h)(P < .01). Percentages of embryos with accessory sperm were: 5 (0 h); 8 (12 h); and 41(24 h)(P < .01). Artificial insemination of superovulated nonlactating Holstein cattle 24 h post onset of estrus as determined by HeatWatch® increased fertilization rate and percentage of embryos with accessory sperm when compared to AI at 0 or 12 h post onset of estrus. Embryo quality was not affected by time of insemination using this semen.

Key Words: Artificial Insemination, Superovulated Cattle, Embryo

932 Effect of artificial insemination time and natural service on accessory sperm number, fertilization status and embryo quality in cattle. J. C. Dalton, S. Nadir, J. H. Bame, M. Noftsinger, R. L. Nebel, and R. G. Saacke*, *Virginia Polytechnic Institute and State University, Blacksburg*.

Two experiments were conducted to determine the effect of insemination time on accessory sperm number per embryo(ovum), fertilization status and embryo quality. From each of three bulls, semen was collected, frozen in egg yolk-citrate-glycerol, and used during three periods of the year. All cows were continuously monitored for behavioral estrus by HeatWatch®, and were artificially inseminated with one .5-mL straw (25×10^6 sperm) at heat onset, 0 h (n=39), 12 h post onset (n=39), or received natural service at 0 h (Nat 0 h) from one of the three bulls (n=37)(Exp. 1). In Exp. 2, cows were artificially inseminated at 0 h (n=39), 12 h (n=39), or 24 h(n=39) after the first standing event. One hundred fifteen embryos(ova)(Exp. 1) and 117 embryos(ova)(Exp. 2) were recovered nonsurgically 6 d post insemination from nonlactating Holstein cows. For Exp. 1, median accessory sperm values were 1 (0 h), 10 (12 h), 27 (Nat 0 h) and differed between 12 h and Nat 0 h (P = .05), and 0 h and Nat 0 h (P < .01). For Exp. 2, median accessory sperm values were 1 (0 h), 2 (12 h), 4 (24 h) and differed between 0 h and 24 h (P < .05). Fertilization rates were: 67% (0 h), 79% (12 h) 98% (Nat 0 h)(P < .05); and 66% (0 h), 74% (12 h), 82% (24 h)(P > .05) for Exp. 1 and 2 respectively. Percentages of degenerate, fair to poor, and good to excellent embryos were: 8, 31, 61 (0 h), 10, 29, 61 (12 h), 11, 11, 78 (Nat 0 h) (P > .05)(Exp. 1); and 8, 15, 77 (0 h), 10, 38, 52 (12 h), 34, 19, 47 (24 h) (P < .05)(Exp. 2). In Exp. 1, natural service increased median accessory sperm number and fertilization rate when compared to AI at 0 or 12 h post onset of estrus; however, embryo quality was unaffected. In Exp. 2, AI at 24 h increased median accessory sperm number when compared to 0 h. Embryo quality was affected by time of AI, whereas fertilization rate was not different among the times of insemination.

Key Words: Artificial Insemination, Accessory Sperm, Embryo

933 Prostacyclin production by lipopolysaccharide-treated arteries from luteal versus follicular phase ewes. M. A. Janowiak*, G. R. Holyoak, P. H. Martin, and K. E. Vagnoni, *Utah State University*.

Prostacyclin (PGI_2) is a potent vasodilator and is a prostaglandin produced by various tissues and arteries including the uterine, renal, omental, and mammary arteries (UA, RA, OA, MA, respectively). Increases in PGI_2 can be indicative of immune response to immunogens such as lipopolysaccharide (LPS), a component of the cell wall of gram-negative bacteria. Previous studies show that *in vivo* estrogen treatment to ovariectomized ewes increases the ability of uterine arteries to respond to *in vitro* LPS treatment as indicated by increased PGI_2 production. The objective of this study is to identify the effect of reproductive phase (follicular or luteal) on PGI_2 production by LPS treated UA, OA, RA, and MA in culture. Each artery was removed and dissected from ewes in either luteal, day 10, (day 0 is estrus, n=4) or follicular, day -1 to 0, phase (n=4). Two to 2.5 cm segments of each artery were cultured for 24 hours at 37°C in media treated in duplicate with either 0, 1, or 10 μg/ml LPS. The amount of the stable metabolite of PGI_2, 6-keto prostaglandin $F_{1\alpha}$, was measured in each supernatant using an enzyme immunoassay and then divided by the wet weight of the corresponding vessel. All four arteries from both phases exhibited an increase in PGI_2 upon addition of 1 μg/ml LPS (p<0.05) except OA from follicular ewes (p>0.68) and RA from luteal animals (p>0.09). Addition of 10 μg/ml LPS to the media did not increase production over the 1 μg/ml treatment (p>0.06) from arteries from both animal groups. There was no difference in basal PGI_2 (0 LPS) production by arteries from luteal and follicular ewes. But upon addition of 1 and 10 μg/ml LPS, UA from luteal phase ewes produced more PGI_2 than those harvested from follicular phase ewes(p<0.05). No other arteries exhibited a statistically significant phase effect (p<0.06). These data suggest that a combination of estrogen and progesterone, as seen in the luteal phase, optimizes PGI2 production following LPS exposure in UA, but not in RA, OA, and MA.

Key Words: Prostacyclin, Lipopolysaccharide, Estrous Cycle

934 Elevations of cyclooxygenase-2 (COX-2) in uterine arteries following exposure to lipopolysaccharide (LPS). K. E. Vagnoni*[1] and R. R. Magness[2], [1]*Utah State University,* [2]*University of Wisconsin-Madison.*

Estradiol enhances cellular responses to LPS, an immunogen which initiates activation of phospholipase-A2 and the release of arachidonate from membrane phospholipids. Released arachidonic acid is metabolized by cyclooxygenase to yield prostacyclin and other prostaglandins. COX-2 is inducible in response to LPS. The objectives of this study were to determine if LPS stimulates uterine artery prostacyclin and COX-2 production and if the phase of the estrous cycle (follicular vs luteal) effects the ability of uterine arteries to respond to LPS. Uterine arteries from follicular (Day −1 to 0, Day 0=estrus, n=4) and luteal (Day 10, n=4) phase ewes were collected and placed in RPMI with 0, 1 or 10μg/ml LPS. After 24 hours, supernatants were collected, arteries were placed into lysis buffer, and a segment of each artery was collected for immunohistochemical analysis. Artery supernatants were analyzed for the prostacyclin metabolite 6 keto-$PGF_{1\alpha}$ by EIA. Artery homogenates were analyzed for COX-2 protein by Western analysis and scanning densitometry. Uterine artery sections were stained for COX-2 using immunohistochemistry; cells which stained for COX-2 were counted. Data were analyzed by ANOVA. The phase (follicular vs luteal) of the estrous cycle had no effect on either the amount of 6-keto- $PGF_{1\alpha}$ in supernatants or the amount of COX-2 protein in arteries. However, LPS increased the amount of 6-keto-$PGF_{1\alpha}$ (P=0.004) in supernatants and the amount of COX-2 protein (P=0.0002) in arteries. Immunohistochemical analysis showed COX-2 to be primarily associated with the vascular smooth muscle (VSM) cells of uterine arteries. Treatment of arteries with LPS significantly enhanced (P<0.05) the number of VSM cells staining positively for COX-2. These data demonstrate that LPS stimulates uterine artery production of prostacyclin and COX-2 and that increases in COX-2 are associated with the VSM and not the endothelium. The phase of the estrous cycle, however, does not appear to influence uterine artery response to LPS. Supported by USDA 95372932647, 97352044912 and NIH HD33255, HL49210.

935 Regulation of prostaglandin $F_{2\alpha}$ secretion in bovine endometrial explants. D. R. Arnold[1]*, J. M. Burke[2], and W. W. Thatcher[1], [1]*University of Florida, Gainesville,* [2]*Springfield, OR.*

Two *in vitro* experiments were conducted to validate a system to study regulation of prostaglandin $F_{2\alpha}$($PGF_{2\alpha}$) secretion by bovine endometrial explants. In experiment 1, nonlactating dairy cows (n=10) were injected with $PGF_{2\alpha}$(25 mg) on days 15 or 16 of the estrous cycle to regress the CL and slaughtered 24 h later. Endometrial explants from intercaruncular and caruncular tissues (50-80 mg) were collected, preincubated in Krebs-Hensleit buffer (KHB) for 120 min, and randomly assigned to receive either KHB (control) or oxytocin (OT,10^{-6} M) in the medium (2x2 factorial design). Media were removed at 0, 20, 40, and 60 min of incubation and analyzed for $PGF_{2\alpha}$. $PGF_{2\alpha}$ secretion rate for caruncular explants was greater than for intercaruncular explants (0.69 vs. 0.44 ng/g/min; P<0.01). Oxytocin failed to stimulate $PGF_{2\alpha}$ secretion compared to KHB (0.62 vs. 0.51 ng/g/min). In experiment 2, intercaruncular endometrial explants were collected on days 17 to 18 of the estrous cycle from nonlactating dairy cows (n=6). Explants were incubated with KHB, phorbol 12,13 dibuytrate (PE, 10^{-6} M), Ca^{++} ionophore A23187 (10^{-5} M), melittin (10^{-4} M), A23187+melittin, or OT (10^{-6} M). The incubation protocol was the same as experiment 1. The $PGF_{2\alpha}$ secretion rate for KHB treatment was 0.58 ng/g/min. $PGF_{2\alpha}$ secretion rates were increased for melittin (1.51 ng/g/min; P<0.01) and A23187 (1.04 ng/g/min; P<0.05). An interaction of A23187 x melittin (P<0.01) indicated no additive or synergistic effect of A23187+melittin (1.64 ng/g/min). Secretion rates for PE (1.10 ng/g/min; P<0.01) and OT (0.71 ng/g/min; P<0.01) treatments were increased compared to KHB. Comparisons between treatments indicated that melittin (P<0.01), PE (P<0.01) and A23187+melittin (P<0.025) increased secretion rates of $PGF_{2\alpha}$ greater than OT. Thus OT did not stimulate $PGF_{2\alpha}$ secretion 24 h after induction of CL regression on days 15 to 16. Intracellular regulators of PLA_2, PKC, and Ca^{++} stimulated a greater $PGF_{2\alpha}$ secretion response than OT.

Key Words: $PGF_{2\alpha}$, Endometrium, Cattle

936 Structure of the gene for porcine endometrial folate binding protein. J. L. Vallet*, T. P. Smith, T. S. Sonstegard, and M. P. Heaton, *USDA, ARS, Roman L. Hruska, U.S. Meat Animal Research Center, Clay Center, NE.*

Previous work suggests that a uterine folate binding protein (FBP) is involved in transport of folate to the developing swine conceptus during pregnancy. Two different cDNAs have been isolated from porcine endometrium, corresponding to putative secreted (binding protein) and membrane (receptor) forms of this protein. In the current study, the gene for the putative secreted FBP was characterized. A porcine genomic yeast artificial chromosome (YAC) library was screened by PCR using a primer pair that does not distinguish between the two forms of FBP. A positive YAC clone was then subcloned into the SuperCOS cosmid vector, and subclones were screened by PCR using primer pairs which were specific to the 3 prime end of each cDNA. A cosmid containing the putative secreted FBP gene was obtained and sequenced. The putative secreted FBP gene spanned 6221 bp. Comparison of this sequence to the cDNA sequence identified six exons (exon 1, base 1409–1434; exon 2, 1447–1462; exon 3, 2275–2697; exon 4, 4682–4870; exon 5, 5067–5202; and exon 6, 5335–5762), demonstrating that the gene structure is similar to folate receptor genes described for other species. The sequence was analyzed using Bioinformatics and Molecular Analysis Section (BIMAS) signal scan, to search for possible controlling elements within the FBP gene. Three CCAAT binding factor sites, two c-Myb sites, two hepatocyte nuclear factor 3 beta sites, and a LyF-1 (lymphocyte specific transcription factor) site were all located in the region upstream from exon 1. Other noteworthy possible controlling sites within the rest of the gene included two AP-1 sites, 13 AP-2 sites and multiple SP1 sites. These results suggest several possible regulatory factors which could be involved in controlling expression of this gene. Elucidation of the controlling factors may allow improved transport of folate to the developing conceptus.

Key Words: Uterus, Pregnancy, Folic Acid

937 The effect of uterine environment on Meishan (M) and Yorkshire (Y) fetal development and placental size and vascularity at term. M. E. Wilson*, N. J. Biensen, and S. P. Ford, *Iowa State University, Ames.*

When M and Y embryos were co-transferred to Y recipients which were then allowed to farrow, it was observed that the birth wts of M and Y littermates were similar, averaging 1.15±.06 kg. In contrast, placentae matched to M piglets were markedly lighter (∼70% smaller) and more vascular (∼2-fold) than Y placentae [Wilson et al., (1998); Biol Reprod 58: In press]. To investigate the effect of uterine environment on conceptus development to term, M and Y embryos were co-transferred to M recipients (n=7) which were slaughtered one day before expected parturition (d 113). Fetal wt, placental wt, and placental surface area were recorded. Additionally, a section of the intact maternal placental interface was excised, fixed, embedded, sectioned and stained to allow quantitation of the placental vascular density (PVD). As observed when M and Y fetuses were co-gestated by Y recipients, littermate M and Y fetuses gestated in M uteri were similar in wt (1.04±.03 vs 1.03±.05 kg) at term. Further, M conceptuses exhibited markedly reduced (P<.03) placental wts (170±19 vs 249±10 g) and suface areas (1017±70 vs 1506±96 cm^2) when compared to their Y littermates. As was the case for conceptuses gestated in Y uteri, the similarity in fetal wt between the two breeds with very different placental sizes appears to result from an increased (P<.09) M PVD when compared to littermate Y fetuses (2.5±.3 vs 1.4±.4%). These data indicate that in both the M and Y uterine environment, the reduced size of the M as compared to Y placenta is compensated for by an increase in PVD. Currently it is unknown whether M placentae contain a greater amount of vasculature (total volume) or a similar amount of vasculature simply squeezed into a smaller space. To investigate breed differences in total placental vasculature we have also perfused placentae of M and Y conceptuses co-gestated in M uteri on d 113 of gestation with vascular casting material and will compare the volume of the corrosion casts.

Key Words: Pig, Placenta, Vascularity

938 Plasma cortisol and aldosterone during early porcine pregnancy. H. G. Klemcke*, P. L. Pearson, J. L. Vallet, and R. K. Christenson, *USDA-ARS, U.S. Meat Animal Research Center, Clay Center, NE.*

Cortisol and aldosterone content and concentration in swine uterine flushings increased two- to sixfold between d 10 and 19 of porcine pregnancy with the greatest changes noted after d 13 (Klemcke et al., Biol. Reprod. 58:240, 1998). The current study was conducted to determine if these intrauterine increases in corticosteroids result from similar plasma increases. Four white crossbred gilts were catheterized on d 7–8 of pregnancy. On d 12 through 18, blood samples from these jugular catheters were obtained at 0700, 1100, and 1500 h. Using radioimmunoassay procedures, cortisol was directly measured in plasma samples, whereas aldosterone was measured after extraction of plasma with ethyl acetate and using ^{3}H-aldosterone to measure procedural losses. Cortisol concentrations decreased daily between 0700 h and 1500 h ($P = .056$) reflecting the known diurnal rhythm of cortisol in pigs. Cortisol concentrations did not, however, change between d 12 (19.22 ± 2.5 ng/ml) and d 18 (32.6 ± 6.3; $P = .48$) of pregnancy. Plasma aldosterone concentrations did not change with time of day ($P = .07$), nor between d 12 (91.1 ± 22.8 pg/ml) and d 18 (37.2 ± 6.3 pg/ml; $P = .37$). These data strongly suggest that previously measured uterine increases in these corticosteroids represent intrauterine mechanisms rather than simply reflecting increases in plasma corticosteroids and subsequent diffusion of these steroids into the uterine lumen.

Key Words: Pregnancy, Pig, Corticosteroids

939 The effects of exogenous progesterone on pregnant ewe performance. T. F. Crosby*, A. P. O'Donnell, and J. V. O'Doherty, *University College Dublin, Lyons Research Farm, Newcastle, Co. Dublin, Ireland.*

The objective was to examine the effects of exogenous progesterone administration to ewes in late pregnancy on ewe serum progesterone concentrations, gestation length and colostrum yield and quality. Twin bearing ewes (n=40) were offered grass silage or hay ad libitum supplemented with concentrates. Half the ewes was given daily injections of 100 mg progesterone in oil from day 143 of pregnancy until lambing while the other half remained untreated. In addition, a control treatment of eight non pregnant ewes were treated concurrently with the same dose of progesterone. Average intakes of dry matter (1.03 kg v. and 1.31 kg), metabolisable energy (2550 calories v. 3107 calories) and crude protein (171 g v. 205 g) were higher in the hay than in the silage fed treatments. Ewes supplemented with progesterone had longer gestation lengths than unsupplemented ewes (150.4d v. 147.8d; S.E.M ± 0.57; P<0.05). Progesterone supplementation increased serum progesterone (ng/ml) by 4.3; S.E.M. 2.41, 10.1; S.E.M. 1.42 ($P < 0.05$) and 9.5; S.E.M. 0.32 ($P < 0.05$) in the pregnant, freshly lambed and non pregnant ewes respectively. Ewes which received progesterone supplementation had significantly higher serum P4 values on days 144 (23.22 v. 19.05 ng/ml; S.E.M. 1.50; P<0.05), 147 (23.8 v. 18.1 ng/ml; S.E.M. 2.40; $P = 0.07$) and 149 (17.9 v. 10.9 ng/ml; S.E.M. 2.26; P<0.05) of gestation. Progesterone supplementation (i) increased ($P < 0.05$) colostrum yields at 1 h post lambing (746g v. 439g; S.E.M. 89.3) but lowered the yield at 10 h (525g v. 674 g; S.E.M. 51.2) and (ii) lowered the concentration (g/l) of colostral IgG at 1 h (103 v 139; S.E.M. 10.0; $P < 0.05$). The data indicate the relatively high efficiency with which the ewe in late pregnancy clears progesterone and that high progesterone has significant effects on gestation length, colostrum yield and composition.

Key Words: Ewe, Progesterone, Colostrum

940 Effect of administration of PGF2α on embryonic development and quality in cows supplemented with exogenous progestogen. M. E. Hockett*, N. R. Rohrbach, and F. N. Schrick, *University of Tennessee, Knoxville.*

An experiment was performed to examine the effect of prostaglandin $F_{2\alpha}$ on embryonic development in cows supplemented with exogenous progestogen. Cows were monitored for estrous behavior and artificially inseminated at estrus (d=0) and 12 h after the onset of estrus. A synthetic progestogen (melengestrol acetate; 4 mg/cow·d) was supplemented in feed from days 3 through 8. Cows were randomly allotted to receive either 15 mg $PGF_{2\alpha}$ (TRT; n=14) or 3 ml saline (CON; n=10) at 0600, 1400 and 2200 from days 5 through 8. Blood samples were collected at 0600 and 2200 from days 5 through day 8 for determination of progesterone and 13, 14-dihydro-15-keto- $PGF_{2\alpha}$ (PGFM). Single embryos were recovered on day 8 by flushing the uterine horn ipsilateral to the corpus luteum (CL) as determined by ultrasonography on day 5. Each embryo was assigned a quality score ranging from 1 (excellent) to 4 (poor), and stage of development was recorded. Analyses of data were performed using Proc Mixed of SAS. Concentrations of PGFM were elevated in TRT animals (375.7 ± 55.8 pg/mL) compared to CON (53 ± 63.1 pg/mL; P =.0003) during the day−5 through −8 period. Quality score of embryos was decreased by administration of $PGF_{2\alpha}$ (Fisher's Exact Test, P =.003[a]). Furthermore, stages of embryonic development differed between CON and TRT embryos (Fisher's Exact Test, P =.003[b]). None of the morula stage embryos in the TRT group continued development when placed into culture for 48 h.

Quality score (%)[a]	**1**	**2**	**3**	**4**
CON	60	30	10	0
TRT	28.6	7.1	0	64.3

Development (%)[b]	**Morula**	**Blastocyst**	**Expanded Bastocyst**
CON	0	20	80
TRT	64.3	7.1	28.6

In conclusion, $PGF_{2\alpha}$ had a negative effect on embryonic development in progestogen-supplemented beef cows resulting in premature death of the embryo prior to blastocyst development.

Key Words: Embryo, $PGF_{2\alpha}$, Progestogen

941 Selection of piglets with a reduced placental size does not hinder production traits. N. J. Biensen*, L. L. Christian, and S. P. Ford, *Iowa State University, Ames.*

Using a purebred population of Yorkshire (Y) females, our laboratory recently reported [Wilson et al., (1998), Midwest ASAS Abstr.] that the ratio of a piglet's wt to that of its placenta (RATIO) varies ∼3-fold within a litter, due predominantly to variations in placental wt which are known to be a limiting factor in litter size. It was further determined that selection of boars and gilts with a higher than average RATIO for breeding resulted in an increased litter size and reduced placental wts. The objective of this experiment was to examine the effects of RATIO on economically important production traits (piglet survivability, 21-d wt) in a purebred breeding stock herd of Y and Landrace (L) pigs. Sows were monitored throughout farrowing, and as each piglet appeared its umbilical cord was clamped close to the dam's vulva and again near the neonate's body, then cut between the clamps. A number designating birth order was then tied around the exposed umbilical cord with surgical silk and the tagged umbilical cord was allowed to retract back into the birth canal. Each piglet was then notched to match its numbered placenta. Following expulsion, placentae were separated, piglets and placentae weighed and RATIO determined for each. Because piglet wt, placental wt, RATIO and litter size were similar for Y (n=20) and L (n=10) females, data was pooled and analyzed across breeds. Neither piglet wt, placental wt nor RATIO was associated with neonatal gender or birth order. Placental wt and piglet wt exhibited significant (P<.001) correlations with RATIO (r=.-80 and .52, respectively). As in our previous study, placental size was more highly correlated to RATIO than piglet wt. Further, neither neonatal survival nor 21-d piglet wt were associated with RATIO. These data indicate that piglet selection based on a high RATIO (increased placental efficiency) is not detrimental during the first 21 d of life. Data will continue to be compiled on these piglets as they grow, reach puberty and reproduce.

Key Words: Piglet, Placental Size, Production Traits

942 Isolation, purification and partial characterization of pregnancy-specific protein B from elk and moose placenta. F. Huang*, D. C. Cockrell, and R. G. Sasser, *Department of Animal and Veterinary Science, University of Idaho, Moscow*.

This study was conducted to isolate and develop an assay for elk and moose pregnancy-specific protein B (PSPB). Cotyledons were collected from the placentas of elk and moose. After mincing and homogenizing cotyledonary tissues, the supernatants were treated with ammonium sulfate. The most immunoreactive PSPB was in the 40–75% ammonium sulfate fractions. After dialyzing and lyophilizing, DEAE-cellulose ion exchange chromatography and Sephadex G-75 gel filtration chromatography were performed for further purification. The final purification step was to subject the proteins to granulated gel-bed isoelectric focusing. At each step in the isolation and purification, presence of PSPB was determined by Ouchterlony immunodiffusion and RIA using antibodies to bovine PSPB. Elk and moose PSPB were viewed for purity by SDS-PAGE and staining with Coomassie blue. Specific bands for PSPB were identified by Western blotting using antibodies to bovine PSPB. There were three bands of PSPB immunoreactivity. The molecular weights ranged from 30 to 60 kDa and isoelectric points ranged from 4.0 to 6.0. A homologous double antibody radioimmunoassay for each of elk and moose PSPB has been developed. The PSPB from each species was radioiodinated (^{125}I) or used as a protein standard. Anti-PSPB sera for each species were developed in sheep while anti-sheep IgG was developed in goats. These results provided a quantitative assay of elk and moose PSPB in sera of pregnant animals.

Key Words: PSPB, Elk, Moose

943 Recombinant porcine somatotropin effects on placental characteristics in gilts with reduced uterine capacity. J. A. Sterle*, T. C. Cantley, J. A. Carroll, R. L. Matteri, M. C. Lucy, and W. R. Lamberson, *University of Missouri and Animal Physiology Unit, USDA-ARS*.

Crowded uterine conditions were induced by unilateral hysterectomy-ovariectomy in 42 gilts to determine the effect of recombinant porcine somatotropin on fetal and placental growth. Gilts were randomly assigned across three replicates to one of three treatments: Control (C), daily injections of 1 mL saline d 0 to 64 of gestation; and Early (E), 5 mg of rpST/d from d 0 to 30, followed by 1 mL saline from d 31 to 64; Late (L), 1 mL saline/d from d 0 to 29, followed by 5 mg of rpST/d from d 30 to 64 of gestation. Gilts were hysterectomized on d 65 of gestation. There was a tendency (P = .06) for L rpST treatment to increase fetal weight (165.14 ± 5.06, 153.70 ± 5.66 vs 174.76 ± 4.25 g, C, E vs L, respectively). Late rpST treatment numerically increased placental dry weight. Both rpST treatments increased the percentage of placental protein (58.99 ± 1.30, 63.68 ± .98 and 62.22 ± 1.08%; P = .01) and placental dry matter (5.23 ± .33 vs 6.85 ± .23, 6.56 ± .28%; C, E and L; P = .10). Contact area of uterine/placental interface (based on pixel density of a standard area) was increased with both treatments of rpST (.37 ± .04 vs .56 ± .10, .55 ± .03 mm^2; C vs E, L; P = .01). Treatment with rpST did not affect placenta wet weight (105.42 ± 21.26, 79.45 ± 14.83, 124.57 ± 17.17 g; C, E, L; P = .33), or DNA content (P = .30). Fetal weight was positively correlated with both placental wet and dry weight (r = .73; P < .0001 and r = .73; P < .0001) but negatively correlated with dry matter percentage (r = −.45; P < .0001), protein (r = −.20; P < .02), and DNA content (r = −.73; P < .0001) of the placenta. Late rpST treatment numerically increased uterine gland histological variables (total area, lumen area, and nuclei/gland). Based on these results and results from our previous studies, increased placental growth resulting from rpST administration was not maintained after cessation of treatment.

Key Words: Fetal Growth, Placenta, Somatotropin

944 Development of a competitive binding assay to measure the ability of bovine sperm to bind to oocytes. D. J. Miller, J. M. Demers*, A. G. Braundmeier, and M. L. Behrens, *University of Illinois at Urbana-Champaign*.

Current laboratory assays of fertility are often not able to identify less fertile males. Assays that measure traits necessary for fertility should be the most accurate predictors of the fertility of a semen sample. There is evidence that more fertile sperm are able to fertilize oocytes more quickly, perhaps by binding to the zona pellucida more effectively. To test this hypothesis, it was necessary to develop an accurate assay of egg binding ability. More accurate estimates of egg binding ability should be derived using a competitive assay, mixing sperm from two males. A group of males can be ranked from a series of pairwise comparisons. This assay requires that sperm be stained so that the donor can be identified. We developed such an assay. To have a ready supply of oocytes, we fixed mature oocytes in 1.5% formaldehyde. Fixed oocytes bound the same number of sperm as unfixed oocytes. Frozen bovine semen was thawed. Sperm were washed, capacitated and stained for 60 min with one of two fluorescent dyes; either DiQ [4-(4-(dihexadecylamino)styryl)-N-methylquinolinium iodide; a red fluorochrome] or $DiOC_{16}$ [3,3'-dihexadecyloxacarbocyanine perchlorate; a green fluorochrome]. Dye concentrations of <100 μM had a negligible effect on motility. DiQ and $DiOC_{16}$ concentrations of 75 μM stained 61% and 71% of the sperm respectively. Lower concentrations stained a lower percentage of sperm. To test the effect of each dye on egg binding ability, sperm from the same collection were separately stained with 75 μM DiQ or $DiOC_{16}$ and mixed together at ratios of 25:75, 50:50 and 75:25 stained sperm. Fixed oocytes were added to these mixtures of sperm and after 15 min, oocytes were washed to remove free and loosely adherent sperm. The percentage of bound sperm stained by each fluorochrome was directly proportional to the percentage of sperm labeled with each dye (36:64, 55:45, 76:24). Therefore, the dyes do not have differing effects on sperm binding to oocytes. Approximately 82% of the bound sperm were identifiable, since they were labeled with either of the two dyes. This assay may prove useful to measure egg binding ability of sperm more accurately.

Key Words: Fertility, Zona Pellucida, Fluorochrome

945 Porcine semen quality estimates and their relationship to monospermic fertilization in vitro. J. M. Popwell* and W. L. Flowers, *North Carolina State University, Raleigh*.

Our objective was to determine if estimates of semen quality could be used as accurate indicators of monospermic fertilization rates for boars used in in vitro systems. Semen from 3 boars was collected every other week for 18 weeks. Each ejaculate was evaluated for: % motile sperm, % normal head and tail morphology, % normal acrosome integrity, and acrosin activity. In addition, $1x10^9$ cells of each ejaculate were placed on percoll gradients to determine their distribution characteristics. Finally, the remainder of the ejaculate was used at four different sperm to oocyte ratios ($2x10^7$, $2x10^4$, $2x10^3$, $2x10^1$) to evaluate monospermic (MS) and polyspermic (PS) fertilization rates. No differences in motility, acrosome, and acrosin were found (p=.80) among boars. However, morphology (p<.05) was different. Percoll gradients separate normal motile and presumably fertile sperm from their less fertile counterparts. No differences in motility/gradient were found (p=.21). Within the 70% fraction, the % cell number and motility were different among boars. There were no differences in overall MS (p=.98) and PS (p=.45) rates, however, sperm/oocyte ratios influenced MS and PS rates. The $2x10^1$ and $2x10^3$ ratios achieved the highest MS rates and $2x10^4$ and $2x10^7$ ratios the highest PS rates. Regression procedures were used to determine the relative importance of the following factors on MS fertilization: acrosome, motility, boar, morphology, acrosin, cell number in the 90%, 70%, and 50% fractions. The largest R^2 value occurred when acrosome was present in the model. Only .30 of the variation in fertility was accounted for when all parameters were included in the model. In a stepwise regression, acrosome entered the model (p≤.15) for MS and PS fertilization. These results suggest that common semen quality factors have limited predictable value for MS fertilization rates in vitro. Of factors examined, the % of sperm with normal acrosomes appears to be the most useful.

Key Words: Semen, Fertility, IVF

946 Bull fertility: Our changing perspectives. R. G. Saacke*, J. Dalton, S. Nadir, J. Bame, and R. L. Nebel, *Virginia Polytechnic Institute and State University, Blacksburg, Virginia*.

From an andrological viewpoint, success of a mating is dependent upon both quality and quantity of semen delivered to the female. Differences exist among males in the efficiency of establishing pregnancies regardless of sperm dosage and also with respect to the minimum number of sperm per insemination required to reach maximum fertility. Therefore, deficiencies in seminal quality among males could be expected to fall into components which may be considered "compensable" or "uncompensable". A compensable component would be a seminal deficiency that could be minimized or eliminated with respect to its impact on fertility by increasing sperm dosage to the female, whereas fertility would not be improved by increased dosage if the seminal deficiency were uncompensable. Compensable semen traits are those believed to be associated with the inability of sperm to traverse barriers in the female tract and reach the site of fertilization under sustained transport or to functionally compete for fertilization by traversing the vestments of the oocyte or initiate the block to polyspermy. Uncompensable deficiencies are associated with spermatozoa capable of initiating the block to polyspermy, the fertilization process and (or) embryogenesis, but incapable of sustaining any one or more of these events. It is now important in evaluation of semen and the development of semen quality tests that these two aspects of male reproductive deficiency be considered independent, i.e., single tests should not be expected to address both deficiencies.

Key Words: Fertility, Embryo Quality, Sperm Quality and Transport

947 New developments in sex preselection: Improved efficiency for artificial insemination using an orienting nozzle and high speed sperm sorting. L. A. Johnson, *U.S. Department of Agriculture, Beltsville, MD*.

The USDA-Beltsville Sperm Sexing Technology has been proven conclusively as the only effective means of altering the sex ratio of offspring in livestock. Progress in methods development has extended the usefulness of the technology for use in broader management situations. The method is based on the flow cytometric separation of X and Y chromosome bearing sperm based on X/Y DNA difference and is an effective means of producing progeny of predetermined sex in cattle, swine, sheep and laboratory animals (Fertil. Reprod. Dev. 7:893-903, 1995). Sperm are treated with a DNA binding fluorochrome, Hoechst 33342 and flow cytometrically sorted into separate X and Y populations that can be used for surgical insemination, deep-uterine insemination and AI in cattle, IVF to produce sexed embryos for transfer, or ICSI of ova. Skewed sex ratios of 90 to 95% of one sex or the other have been repeatably achieved. The method has been used worldwide to produce several hundred normal offspring of the predicted sex. The method represents the only fully validated sexing method for man and animals. Recent improvements to the technology take two forms. Firstly, the development of a new orienting nozzle that we have fitted to standard and high speed cell sorters that have been modified for sperm. The addition of the nozzle to Coulter Epics sperm sorters has increased the utilization of the starting population of sperm by two to three fold (Rens et al., J. Anim. Sci. 75:suppl. 1, 215,1997). We have also adapted the orienting nozzle to a Cytomation MoFlo high speed cell sorter modified for sperm. This adaptation has increased the overall production rate of sorted X and Y sperm from about 0.4 million per hour to 4 to 5 million per hour. The Sperm Sexing Technology continues to show significant promise for application to the livestock industry.

948 Components of reproduction in Large White, Yorkshire and reciprocal cross sows. S. M. Neal* and K. M. Irvin, *The Ohio State University, Columbus*.

Purebred Yorkshire (Y, n=11), purebred Large White (LW, n=14), and reciprocal cross Y sire x LW dam (YLW, n=12), and LW sire x Y dam (LWY, n=14) sows were mated to Hampshire boars. Females were slaughtered at a commercial slaughter facility at an average of 74.2 d of gestation. Reproductive tracts were removed and evaluated for uterine weight (UTWT), uterine horn length (UTLN), ovulation rate (OR), number of fully formed fetuses (NF), number of mummified fetuses (NM), percentage of fetal survival (FS = NF / OR), fetal space (FSPACE = UTLN / (NF + NM), and total fetus weight (TFWT). Females of varying parities were included in the evaluation, therefore, line, parity and d of gestation were included in the statistical model. Ovulation rate was greater on the left side (10 ± .4 left vs 8.9 ± .4 right, P<.06), however OR did not differ significantly (P>.10) between lines (Y = 18.6 ± 1.3, LW = 19.9 ± 1.02, YLW = 18.2 ± 1.1, LWY = 18.9 ± .97). Total NF did not differ significantly among lines (P>.10)(Y = 11.4 ± 1.2, LW = 11.8 ± .9, YLW = 9.96 ± .95, LWY = 9.91 ± .86). However, when the analysis was done within uterine horn LW sows had significantly greater NF (5.9 ± .35) than LWY (4.95 ± .34, P<.06) and YLW (4.98 ± .37, P<.08), but did not differ from Y (5.7 ± .44). Percentage fetal survival was greater for the right uterine horn (P<.05) (.69 ± .04, right horn vs .56 ± .04, left horn), however FS was not different among the lines (Y = .62 ± .07, LW = .62 ± .05, YLW = .55 ± .06, LWY = .53 ± .05). Fetal space, UTWT and UTLN did not differ among lines. Total fetus weight within uterine horn, adjusted for number of fetuses, was greater for LW compared to LWY (1926 ± 53.5 g vs 1782 ± 51.1 g, P<.06), but did not differ from YLW (1904 ± 55.9 g) or Y (1789 ± 66.1 g). Differences for reproductive components exist between pure and reciprocal cross Large White and Yorkshire sows. Additional data are needed to estimate heterosis and confirm line differences.

Key Words: Pigs, Reproduction, Uterus

949 Effect of rismorelin on ovulation rate and early embryo survival in high prolificacy gilts. J. L. Roth[1]*, J. D. Muegge[1], K. D. Cross[1], A. J. Wuethrich[1], A. E. Pusateri[3], J. T. Symanowski[1], N. M. Cox[2], and D. B. Anderson[1], [1]*Elanco Animal Health, Greenfield, IN,* [2]*Mississippi State University, Mississippi State, MS,* [3]*United States Army Institute of Surgical Research, Ft. Sam Houston, TX*.

Rismorelin porcine (RIS) is an analog of porcine GHRH. The objective of this study was to further evaluate the effects of RIS on ovulation rate and early embryonic survival in high prolificacy Camborough 15 maternal line PIC gilts. Eighty-five gilts were assigned to the following treatments: non-injected control (CON; n=34), RIS (n=35), and a sham injected treatment (n=16) using a replicated randomized complete block design. The sham was not used in the second of two replications since the sham treatment was not significantly different from CON treatment in the first replication. Gilts were checked for estrus twice daily and were blocked by date of pubertal estrus. All gilts were fed a 16% crude protein diet at 2.72 kg/day. All treatment injections were administered on days 12–18 after pubertal estrus at 30 μg/kg/d i.m. once daily. Gilts were bred twice daily at second estrus. All gilts were euthanized between days 32–42 after breeding and the following data were collected: ovulation rate, number of embryos, uterine length, embryo spacing , and embryo weights. There were no differences in any of the reproductive parameters measured between the CON and sham injected groups (P>.05). During the treatment period, RIS-treated gilts gained 3.4 kg (P<.001) more body weight than CON group. The RIS treatment increased ovulation rate by 2.9 (18.6 vs. 15.7; P<.001), total embryos by 1.6 (P=.020), and tended to increase live embryos by 1.2 (P=.087) compared to CON. Uterine length and embryo weight were not affected in this study (P>.05). However, individual embryo spacing and embryo survival tended to be decreased (P=.191 and P=.153, respectively) in RIS treated gilts. In conclusion, RIS administered to gilts prior to ovulation increased ovulation rate and total embryo number at 32-42 d after breeding.

Key Words: GHRH, Swine, Reproduction

950 Effect of rismorelin on ovulation rate and 105 day fetal survival in gilts. N. M. Cox[1]*, J. L. Roth[2], J. D. Muegge[2], K. D. Cross[2], A. J. Wuethrich[2], B. R. Higgason[1], A. B. Moore[1], J. T. Symanowski[2], and D. B. Anderson[2], [1]*Mississippi State University, Mississippi State, MS,* [2]*Elanco Animal Health, Greenfield, IN.*

The objective of this study was to evaluate effects of rismorelin porcine (RIS), an analog of porcine GHRH, on ovulation rate and fetal survival to 105 days of gestation in two different herds with high (Exp 1) and average (Exp 2) prolificacy gilts. Exp 1 used 183 Camborough 15 maternal line gilts: non-injected control (CON; n=97) and RIS (n=86). Exp 2 used 103 Euroswine or similar crossbred gilts: CON (n=54), and RIS (n=49). Both experiments utilized a randomized complete block design using date of pubertal estrus as the blocking factor. Gilts were checked for estrus twice daily. All gilts were fed a 16% crude protein diet at 2.72 kg/d. All treatment injections were administered daily on d 12–18 after pubertal estrus at 30 mg/kg/d i.m. Gilts were bred twice daily at second estrus. All gilts were euthanized between d 102–108 after breeding and the following variables were collected: ovulation rate, number and weight of fetuses, uterine length, and fetal scoring. In Exp 1 RIS-treated gilts gained 2.1 kg (P=.005) more body weight than the CON group during the treatment period. Backfat depth at necropsy tended to be lower in the RIS group than the CON group (26.5 vs 28.6 mm; P=.068). The RIS treatment increased ovulation rate by 1.8 (18.2 vs. 16.4; P<.001), total fetuses by 1.3 (P=.002) and live fetuses by 0.9 (P=.022). Uterine length and embryo weight were not affected (P>.05). However, fetal survival was decreased (P=.022) with RIS (67.7% vs. 69.4%). In Exp 2, neither gain nor backfat depth were affected by treatment. RIS increased ovulation rate by 2.1 (15.8 vs. 13.7; P<.001), but did not affect numbers of total or live fetuses (P>.05). RIS tended to decreased (P=.199) fetal weight (.930 vs. 1.04 kg for RIS and CON groups, respectively). Furthermore, fetal survival was decreased (P=.015) in RIS-treated gilts (66.4% vs. 76.8%). In conclusion, RIS increased ovulation rate in both experiments but increased litter size in only the high prolificacy gilts. These results illustrate that genetic as well as other factors interface to affect fetal numbers when ovulation rate is altered.

Key Words: GHRH, Swine, Reproduction

951 Changes in reproductive performance of Holstein dairy cows in Kentucky from 1972 to 1996. W. J. Silvia*, *University of Kentucky, Lexington.*

DHI records from Kentucky Holstein dairy herds were used to study changes in reproductive performance that have occurred from 1972 to 1996. Data were obtained from the Dairy Records Processing Center, Raleigh, NC. To be included in the data set, herds had to have records from at least 17 of the 25 years examined. The data set consisted of the herd summary data from the 12 months prior to each year's September DHI test. If a test was not conducted in September then data from the test conducted closest to September (either August or October) was used instead. The variables examined were milk production (rolling yearly herd average), calving interval, days open and services per pregnancy (beginning in 1975). Yearly mean values were weighted by herd size. The number of herds included in this data set ranged from 49 in 1996 to 73 in 1980–84 and 1986–91. The number of cows ranged from 4606 in 1972 to 7370 in 1992. During the period of study, average annual milk production per cow increased from 12,906 to 16,849 lbs (164 lbs/year). During this same period, calving interval increased from 13.5 to 14.4 months and days open increased from 132 to 159. Services per pregnancy also increased from 1.62 in 1975 to 2.91 in 1996. These data clearly indicate that reproductive performance has declined during this 25 year period.

Key Words: Reproduction, Calving Interval

952 Dose titration of estradiol benzoate (EB) in heifers and cows after treatment with an intravaginal progesterone releasing (IVP4) insert and $PGF_{2\alpha}$. M. A. Lammoglia*[1], R. E. Short[1], S. E. Bellows[1], R. A. Bellows[1], M. D. MacNeil[1], and H. D. Hafs[2], [1]*ARS, USDA, Miles City, MT and* [2]*Rutgers University, New Brunswick, NJ.*

Beef heifers (n=57) and cows (n=52) received an IVP4 (EAZI-BREED™ CIDR®) for 7 d (d 0 = insert day) with a 25 mg injection of $PGF_{2\alpha}$ (Lutalyse®) on d 6. At 24 to 30 h after IVP4 removal, EB was injected at the following doses: heifers 0, .2, .38, or .75 mg and cows 0, .25, .5, or 1 mg. Seven heifers and 7 cows from each dose group were bled every 4 h for 76 h starting at EB injection. Serum was assayed for LH and estradiol-17β (E2). Percentage of females showing estrous behavior was increased by EB (P<.04) with greatest response at .38 mg in heifers (86%) and 1 mg in cows (100%). Dose x time interaction affected (P<.01) E2 concentrations in heifers and cows with animals receiving the higher doses of EB having greater E2 concentrations in a shorter time than those receiving the smaller doses. Percentage of cows and heifers with an acute preovulatory LH release (peak LH) was affected by dose with a linear (P<.01) and quadratic (P<.01, increase was less as dose increased) response. Highest concentrations of LH during peak LH were affected by dose with a linear (P<.01) response in heifers and linear (P<.01) and quadratic (P<.08, increase was less as dose increased) response in cows. Heifers receiving .38 mg and cows receiving .5 and 1 mg of EB had the highest peak LH. Time to LH peak had a linear (P<.03) response in heifers and linear (P<.04) and quadratic (P<.05) response in cows. Pregnancy rate was affected (P<.02) in heifers by anestrus vs cycling before IVP4 treatment (52% vs 22%) and in cows by dose of EB (P<.01; 8, 23, 21, and 67% for 0, .25, .5, and 1 mg, resp.). In conclusion, in females treated with IVP4 and $PGF_{2\alpha}$ to induce and synchronize estrus, an injection of EB increased concentrations of E2 and LH and increased number of animals showing estrus. Also, EB increased pregnancy rates in cows. Optimal responses were at .38 mg EB for heifers and at 1 mg EB for cows.

Key Words: Estrous Synchronization, Estrogen Dose, CIDR

953 Does timing of insemination effect gender of the resultant calf? S. M. Jobst*, R. L. Nebel, and M. B. G. Dransfield, *Virginia Polytechnic Institute and State University, Blacksburg.*

Cervical mucus conductivity changes have been used to determine the onset of estrus and, recently, gender selection by altering timing of insemination (Anim. Reprod. Sci. 1997.46:27–34.). Heifer calves were predominately obtained from early inseminations (20 ± 3 h preovulation). Inseminations 10 ± 2 h preovulation, resulted in predominately male offspring. The objective of this investigation was to determine if timing of insemination using a radiotelemetric estrus detection system, HeatWatch®, to determine the onset of standing activity, could influence gender of the resultant offspring. Eleven dairy farms representing diverse management styles (total confinement feeding and housing systems to a primarily grazing system) and herd sizes (56 to 556 lactating cows) participated in the study. HeatWatch® was utilized to monitor behavioral events associated with estrus and to determine the onset of standing estrus. Each farm selected a 3-h interval to inseminate animals identified in estrus during the previous 24 h. Calving data were merged with breeding data if the calving date was within 275 to 290 days after breeding date. A total of 822 calvings with gender identification were obtained. The mean percentage of male calves was 53.5% with 36 sets of twins (4.4%). Intervals from initial standing event of estrus to AI were divided into three categories by 8-h increments. Parity was grouped by heifers, first, second and third and greater lactations. No gender frequency differences for parity, herd or interval from onset of estrus to AI were revealed by Chi-square analysis. Results from this study would suggest that gender could not be pre-selected for by timing of insemination.

Key Words: Gender Selection, HeatWatch®, Artificial Insemination

954 Synchronization of estrus in postpartum beef cows using GnRH and prostaglandin $F_{2\alpha}$. M. L. Borger*[1], L. H. Anderson[2], and W. A. Greene[1], [1]*The Ohio State University, Wooster,* [2]*University of Kentucky, Lexington.*

Seventy-eight beef cows were allotted to three equal groups, based on breed, age, postpartum interval, and ultrasonography of ovarian activity to compare pregnancy rates among three synchronization regimens using GnRH and prostaglandin $F_{2\alpha}$ (PG). All cows received 100μg GnRH i.m. on d −7 and 25 mg PG i.m. on d −1, were observed for estrus at 0700 and 1900 from d −1 to d 3, and artificially inseminated (AI) approximately 13 h after detected estrus. Cows allotted to group C continued to be observed for estrus from d 4 to d 7 and bred as previously described. Cows in groups T0 and T8, not observed in estrus by d 3, received 100μg GnRH 72 h after PG. T0 cows were AI at the time of the second GnRH injection and T8 cows were AI 8 h later. All cows were observed for estrus and AI from d 8 to d 26. Cows were pregnancy diagnosed by rectal palpation on d 89. Estrus was observed in 61.5%, 57.7%, and 61.5% of cows in C, T0, and T8 groups, respectively, from d −1 to d 3. The percent of cows pregnant to AI from d −1 to d 3 for C, T0, and T8 cows was 38.5, 46.2, and 53.9%, respectively (P>.05). The non-return rate (%) from d 3 to d 26 was similar (P>.05) for C (81.0), T0 (65.4), and T8 (65.4). The percent of cows pregnant to AI from d −1 to d 26 was lower (P<.05) for C (50.0) than T0 (76.9) and T8 (73.1). Based upon rectal palpation, pregnancy rate, following a 27 d breeding period, was significantly higher for cows receiving a second injection of GnRH 72 h following the PG injection, whether cows were bred at the time of GnRH injection or 8 h after the second GnRH injection.

955 Estrous response and fertility of postpartum suckled beef cows after treatment with $PGF_{2\alpha}$ or GnRH and $PGF_{2\alpha}$. D. J. Patterson*, F. N. Kojima, R. F. Randle, and B. L. Larson, *University of Missouri, Columbia.*

Field studies were designed to compare estrous response and fertility of postpartum suckled beef cows after treatment with two injections of $PGF_{2\alpha}$ (PG; 25 mg) compared with cows treated with GnRH (G; 100μg) and PG. Cows at two locations (n=243) were assigned by age, days postpartum and body condition to one of two treatments. Cows assigned to the PG treatment received two injections of PG 14 d apart. Cows administered G and PG received one injection of G 7 d prior to PG. Blood samples were collected from all cows on the last day PG was administered. This single sample was used to determine how treatments compared in synchronizing cows based on concentrations of P_4 in serum. Cows were inseminated 12 hr after detected estrus and exposed to fertile bulls 14 d after the last AI breeding date. Pregnancy was diagnosed by rectal palpation within 110 d of the first AI date. Proportions of cows detected in estrus and resulting conception and pregnancy rates are listed below. Neither treatment resulted in significant improvement in percent of cows synchronized or resulting fertility. Potential to shorten the synchronization schedule with G + PG may offset increased cost of this treatment compared to PG. We conclude that G + PG and PG perform similarly when used to synchronize estrus in postpartum suckled beef cows.

Treatment	No.	% In Heat[a]	% Conception[a]	% Pregnant[a]
PG	119	55	51	28
G + PG	124	60	45	27

[a]NS

Key Words: Postpartum Cow, GnRH, $PGF_{2\alpha}$

956 Use of a synthetic progestogen in combination with a superovulatory treatment for induction of synchronized estrus in seasonally anovular ewes. L. P Reynolds*, J. D. Kirsch, K. C. Kraft, A. T. Grazul-Bilska, and D. A. Redmer, *North Dakota State University.*

To determine if a simple, efficacious technique we have developed for inducing multiple ovulations (superovulation) in cyclic ewes (Jablonka-Shariff et al., Biol. Reprod. 51:531-540, 1994) would effectively induce superovulation in anestrous ewes,+/- non-lactating seasonally anestrous ewes were randomly assigned to progestogen (Synchro-Mate-B [SMB], Merial Limited, Athens, GA) alone (SMB, n=10 ewes) or SMB in combination with an FSH superovulation regimen (SMB/FSH, n=10 ewes). One-half of an SMB implant was implanted into the left ear of each ewe, left in place for 10 d and then removed. Beginning on the day of SMB removal, ewes received twice daily intramuscular injections of saline (salt water) or FSH (FSH-P, a pituitary extract; Sioux Biochemical, Sioux Center, IA) for three days as follows; Day 1, 5 mg/injection; Day 2, 4 mg/injection; Day 3, 3 mg/injection (total dose = 24 mg). Rams were penned with the ewes at the time of the first saline or FSH-P injection, brisket painted daily to aid in estrous detection, and left with the ewes for 25 d. On days 7–9 after the synchronized estrus, all ewes were subjected to a laparoscopy to determine ovulation rates. Nine of 10 ewes in each treatment group expressed out-of-season estrus. Furthermore, estrus in all ewes was synchronized, occurring between 1 and 3 days after removal of the SMB implant. However, ewes receiving the superovulation treatment exhibited estrus earlier (P<0.01) than ewes receiving SMB alone (1.2 +/- 0.1 vs. 2.2 +/- 0.2 d after SMB removal). Ovulation rates also were greater (P<0.01) for SMB/FSH compared with SMB ewes (9.9 +/- 1.5 vs. 1.7 +/- 0.4 ovulations; range 3–17 vs. 1–4 follicles ovulated). Moreover, all ewes receiving the superovulation treatment (SMB/FSH) were superovulated (more than two follicles ovulated), whereas only two of the SMB ewes ovulated more than 2 follicles. Thus, our superovulation regimen does effectively induce superovulation in anestrous ewes.

Key Words: Superovulation, Anestrous, Ewes

957 Melengestrol acetate blocks the preovulatory surge of LH in beef heifers. D. B. Imwalle*, D. L. Fernandez, J. M. Reinhart, and K. K. Schillo, *University of Kentucky, Lexington.*

It is unclear whether all progestins inhibit the preovulatory surge of LH in cattle. Therefore, we examined the effects of an orally active progestin, melengestrol acetate (MGA), on the LH surge in post-pubertal heifers. Twelve Angus heifers were randomly assigned to two treatment groups; control (CON; n=6) and MGA (n=6). Prior to the onset of the experiment, all heifers were injected with prostaglandin $F_{2\alpha}$ to induce regression of corpora lutea and thus synchronize estrous cycles. Each MGA-treated animal received .5 mg MGA/day in a wheat middlings-corn carrier, whereas CON animals received only the carrier for 7 days beginning on day 0 (1 day after prostaglandin). Animals were observed for estrus and blood samples were collected every four hours between days 0 and 5. Concentrations of LH were determined in each sample. None of the MGA-treated heifers and 5/6 CON heifers exhibited estrus during this period. Analysis of variance revealed that treatment and time interacted (P = .01) to affect LH concentrations. LH concentrations were greater (P = .001 to .11) in CON heifers than MGA -treated heifers between hours 32 and 48. This was due to the fact that LH surges were present in 5/6 CON heifers and 0/6 MGA-treated heifers (P = .005). We conclude that MGA blocks the preovulatory surge of LH in beef heifers.

Key Words: Melengesterol Acetate, Beef Heifers, LH Surge

958 Secretion of LH in response to challenge with a GnRH agonist in heifers with ovarian follicular cysts. J. Gong*, B. Campbell[1], and R. Webb[2], *Roslin Institute, Roslin, Midlothian, [1]University of Edinburgh, Edinburgh, and [2]University of Nottingham, Sutton Bonington Campus, Loughborough, Leics, UK.*

Ovarian follicular cysts are one of the major causes of infertility in postpartum dairy cows, and therefore a significant source of economic loss to the dairy industry. Despite intensive efforts, the mechanism underlying the development of this condition remains poorly understood. It has been suggested that a failure in the generation of a normal preovulatory LH surge may be responsible. In this study we investigated whether the LH responsiveness to GnRH is altered in animals with follicular cysts, using an experimental model we have recently developed.
Twelve heifers were continuously infused with a GnRH agonist (buserelin, Hoechst) for 8 weeks as described previously (Gong *et al.*, Biol Reprod 1996, **55**: 68–74). After the termination of treatment, 7 heifers developed follicular cysts. Plasma samples were collected every 15 min for 8 h from these 7 animals (FC), and from a further 12 normal heifers [6 at the mid-luteal (L) and 6 at the follicular phase (F) of the oestrous cycle], for the characterization of LH pulsatile profiles. The animals were then challenged i.m. with 30 μg of buserelin and hourly plasma samples were collected for the measurement of LH concentrations. The LH pulse frequency in FC (7.25 $\pm$ 0.9) and F (7.50 $\pm$ 1.13) was higher than that in L (3.80 $\pm$ 0.47 pulses/8h) ($P<0.01$), with no differences in pulse amplitude observed among the 3 groups. The basal and mean LH concentrations were also higher ($P<0.01$) in FC than in L. Following challenge with buserelin, LH increased significantly both in heifers with follicular cysts and normal animals. However, the maximum LH concentrations were nearly 2-fold higher ($P<0.001$) in animals with follicular cysts when compared to the controls.
We conclude that the responsiveness of the pituitary gland to GnRH in terms of LH secretion appears to be increased in animals with follicular cysts. In addition, LH pulsatile profiles in heifers with ovarian follicular cysts are similar to those of animals at the follicular phase of the oestrous cycle. (Supported by the SOAEFD)

Key Words: Ovarian Follicular Cysts, LH, Cattle

959 Anterior pituitary responsiveness to exogenous GnRH during pregnancy and postpartum in goats. O. S. Gazal*, E. A. Amoah, B. Kouakou, and S. Gelaye, *Fort Valley State University, Fort Valley, GA.*

The objective of this study was to determine the contribution of pregnancy-induced suppression of luteinizing hormone (LH) secretion as a factor in postpartum anestrus in dairy goats. Eighteen dairy goats were injected with 5 μg gonadotropin-releasing hormone (GnRH), i.v. on either day 140 of gestation (d -10; n = 6), day 3 (d 3, n = 5) or day 20 (d 20, n = 7) postpartum. Blood samples were obtained at 15-min intervals for 1 h followed by GnRH injection and sampling continued for 3 h at frequent intervals. Injection of GnRH on d -10, d 3 and d 20 induced LH release with mean peak concentrations of 1.5 $\pm$ 0.4 , 1.8 $\pm$ 0.5, and 2.2 $\pm$ 0.2 ng/mL, respectively. Baseline LH concentrations were similar across all days. Total LH secretion tended ($P = .09$) to increase from 6 $\pm$ 2 ng/mL on d -10 to 9 $\pm$ 2 ng/mL on d 3 and reached 13 $\pm$ 2 ng/mL on d 20 postpartum. The injection $-$ first LH peak interval decreased ($P < .01$) with days postpartum averaging 55 $\pm$ 7, 39 $\pm$ 8 and 20 $\pm$ 7 min on d -10, d 3 and d 20, respectively. On d -10, GnRH injection stimulated a spike-like LH release while a distinctly bimodal pattern of release was observed on d 20. Area under the curve (AUC, ng/mL/h) for plasma LH increased ($P < .04$) with days postpartum from 1.6 $\pm$ 0.6 on d -10 to 3.7 $\pm$ 0.5 on d 20. These results indicate a progressive increase in the responsiveness of the anterior pituitary with time postpartum suggesting that although the suppression of anterior pituitary function during late pregnancy may be reversed as early as d 3 postpartum, a marked increase in anterior pituitary responsiveness occurs between d 3 and d 20 in the doe.

Key Words: Goat, Postpartum, GnRH

960 Effects of supradiaphragmatic vagotomy on LH release in ovariectomized ewe lambs. B. J. Holmberg*, R. S. Marion*[2], and D. H. Keisler, *University of Missouri-Columbia.*

Nutritionally deficient animals display a decrease in LH which can be reversed with ad libitum feeding. The mechanism by which the gut relays nutritional status to the brain is not clear, but contains hormonal and neural components. Neural afferent and efferent fibers within the vagus nerve form a physical pathway between the gut and brain. Our objective was to determine the effect of vagotomy on LH release in full-fed (F) and feed-restricted (R) ewe lambs. The left vagus nerve and the right vagal dorsal branch were dissected in the thoracic cavity and isolated by nylon suture which extended outside the cavity. On experimental day 0 (d0), lambs were bled for 4h, the suture pulled to transect the vagus, and bled an additional 4h (d1). On d3 and d6, blood sampling was repeated and samples were assayed by RIA for LH. Feed intake decreased in F lambs from d3 to d6. R lambs consumed all feed offered until d6, when feed intake decreased. Fecal output in both R and F lambs appeared to decrease (not quantitated). On d6, transection of the left vagus nerve was confirmed at necropsy. Three lambs (1R and 2F) retained an intact right dorsal vagal branch, although there was no response difference ($p>0.05$) between intact or right-branch-transected lambs. Lambs also had distended rumens suggesting impaired rumenal motility due to loss of parasympathetic vagal input. LH pulse frequency and mean LH were lower ($p<0.001$) in R vs F lambs. Within both R and F lambs, LH pulse frequency differed ($p<0.05$) between d0, d1, d3, and d6. R lambs exhibited a decrease ($p<0.05$) in LH pulse frequency on d1 and d3 vs d0. Vagotomy did not alter mean LH in R lambs. F lambs had a difference ($p<0.05$) in mean LH among days, and demonstrated an acute decrease in LH on d1 ($p<0.09$) and d3 ($p<0.05$) vs d0. The responses may have been dampened due to the presence of the right ventral vagal branch innervating the GI tract. These results suggest an acute neuromodulatory role of afferent vagal input on mean LH in F ewes and LH pulsatility in R ewes.

Key Words: Vagotomy, Sheep, LH

961 Reproductive endocrinology of lactating dairy cows selected for increased milk production. M. C. Lucy*[1], W. J. Weber[2], L. H. Baumgard[2], B. S. Seguin[2], A. T. Koenigsfeld[1], L. B. Hansen[2], H. Chester-Jones[2], and B. A. Crooker[2], [1]*University of Missouri, Columbia and* [2]*University of Minnesota, St. Paul.*

Increased milk yield is associated with decreased reproductive performance in postpartum (PP) dairy cows. Multiparous cows from control (C; n=9) and select (S; n=9) lines of Holsteins were used to determine effects of selection for milk yield on reproductive hormones. Lines were established in 1964 and currently differ by more than 4,000 kg milk/305 d lactation. Blood was collected every Monday, Wednesday, and Friday from calving to about 100 d PP and analyzed for plasma progesterone (P4), follicle stimulating hormone (FSH) and insulin-like growth factor-I (IGF-I). Daily plasma estradiol (E2) was determined on the last 6 d of an estrous cycle after 45 d PP. Luteinizing hormone (LH) window bleeds (15 min sampling for 7 h) were done on d 7 PP and after luteolysis of an estrous cycle (>45 d PP). Select cows produced more milk (34.5, 25.6 kg FCM/d; SEM=1.9; P=.01), consumed more feed (18.5, 14.0 kg DMI/d; SEM=1.4; P=.06), and had an energy status similar (-1.76, -2.94 Mcal/d; SEM=1.3; P=.55) to C cows. Compared to C cows, S cows had greater IGF-I from 2 to 14 d PP but lower IGF-I after 21 d PP (line-by-day; P<.01) . Intervals (d PP) to first (35.6$\pm$1.2), second (57.3$\pm$1.4), and third (79.1$\pm$1.8) luteal phases were similar for S and C cows. Plasma P4 concentrations were less (3.2$\pm$.3 vs. 4.4$\pm$.4 ng/ml; P<.05) and luteal phase length tended to be shorter (16.6$\pm$1.2 vs. 20.0$\pm$1.4 d; P<.10) in S vs. C cows. Plasma E2 (preceding estrus) as well as mean and pulsatility for FSH (0 to 100 d PP) and LH (d 7 and after luteolysis) were similar for S and C cows. Select cows had lower IGF-I (after 21 d PP), lower plasma P4, and tended to have shorter luteal phases. Other hormones (E2, FSH, and LH) were similar for S and C cows. Decreased plasma P4 in S cows suggests that reduced luteal function associated with low IGF-I may partially explain poorer reproduction in cows selected for milk production.

Key Words: Reproduction, Cow, Ovary

962 Luteinizing hormone (LH) receptor and follicle-stimulating hormone (FSH) receptor gene expression in neonatal pig thymus and spleen. R. L. Matteri*, J. A. Carroll, and C. J. Dyer, *Animal Physiology Research Unit, Agricultural Research Service, USDA, Columbia, MO.*

Administration of human chorionic gonadotropin suppresses thymosin $\beta 4$ levels in young castrated pigs (Wise, Biol. Reprod. 46:892, 1992). Direct effects of FSH on human immune tissues have also been reported. The presence of LH receptors has been demonstrated in immune tissues; however, no corresponding information exists with regard to the FSH receptor. The objective of the present study was to evaluate the presence and developmental regulation of LH and FSH receptor mRNAs in neonatal pig thymus and spleen. PCR primers were designed for amplification of LH and FSH receptor cDNA produced from testicular RNA. The resulting PCR products were cloned for purposes of cRNA probe synthesis. The identities of the cloned cDNAs were confirmed by dideoxy termination sequencing. Specific LH and FSH receptor mRNA were readily detectable by northern blot analysis of pooled RNA samples from testis, thymus, and spleen (8 young pigs per tissue). LH and FSH receptor transcripts of sizes similar to those previously reported were observed in all tissues. Male piglets were sacrificed for tissue collection at 1, 7, 14, 21, 28, 35, and 42 days of age (n=8/age group). Slot-blot hybridization assays were performed on total RNA from thymus and spleen. Data were quantitated by densitometry, followed by normalization to 28S ribosomal RNA levels. LH receptor mRNA levels were higher in thymus than in spleen ($3.24 \pm .49$ vs $1.96 \pm .1$ relative units (RU), respectively; $P < .0001$), but did not change with age in either tissue ($P > .2$). Similarly, expression of FSH receptor was elevated in thymus, relative to spleen ($.68 \pm .08$ vs $.38 \pm .03$ RU, $P = .003$), but did not vary among age groups ($P > .5$). These data support the hypothesis that gonadotropins may exert direct effects on immune tissues through gonadotropin-specific receptors. To our knowledge, this is the first evidence for FSH receptor gene expression in immune tissues of any species.

Key Words: Gonadotropin, Receptor, Immunology

963 Establishment of continuous bovine intestinal non-epithelial cell culture system. B. Zavizion*, A. J. Bramley, J. H. White, and J. R. Knapp, *University of Vermont, Burlington.*

In order to study interactions between different types of bovine intestinal cells, non-epithelial cells were isolated in conjuction with epithelial cells. Fresh intestinal tissue was obtained at slaughter of crossbred beef cattle. A 50 cm section of duodenum was obtained 25 cm distal to the pylorus, filled with ice-cold HEPES-buffered saline, and transported on ice to the laboratory. An enzymatic digestion system (collagenase, 0.5 mg/ml; elastase, 50 U/ml; dispase, 0.5 mg/ml in medium 199 supplemented with antibiotics) was used to isolate cells. The digestion was conducted at RT for 1h with 3 changes of medium. First isolate contained mainly epithelial-like cells, while second was enriched with non-epithelial cells The yields of cells were estimated by cell counting using a hemocytometer. Cell viability was estimated by trypan blue exclusion. The cells were plated in DM/F12: RPMI 1640:NCTC 135 (1:1:1) supplemented with ascorbic acid, hydrocortisone, bovine transferrin, 10% FBS, 2.5 new-born CS, 2.5 CS iron-supplemented at two densities, 0.5-1x 10^4 and 0.5-1 x $10^6/cm^2$. Cells began to attach to the plates within 4 hours. After colonies began to form, cells with non-epithelial morphology were collected and propagated for two weeks. These cells were transfected with a plasmid encoding neo-resistance and the large ts-T SV-40 antigen. Resistant colonies were selected after 3 weeks of G418 treatment (0.25 mg/ml). Despite the temperature sensitivity of the oncogene, cells incubated at 37C grew more rapidly than those at 32C. Five distinct colonies with different morphology were identified and selected. Between 34–36 doublings, cells entered crisis, with only 4 colony types surviving. These have been propagated as individual cell lines for approximately 50 doublings. The cells display non-epithelial morphology but differ from fibroblast-like morphology. Population doubling time is approximately 25–30 hours depending on cell line. Cells secreted significant amounts of extracellular matrix (ECM) proteins, which are not trypsin-digestible. When cells were plated on their own ECM, the rate of proliferation decreased two-fold and their morphology changed.

Key Words: Intestine, Epithelium, Bovine

964 Development of continuous bovine intestinal epithelial cell culture system. B. Zavizion, A. J. Bramley, J. H. White, and J. R. Knapp*, *University of Vermont, Burlington.*

In order to establish a continuous cell line representative bovine intestinal epithelium, fresh intestinal tissue was obtained at slaughter of crossbred beef cattle. A 50 cm section of duodenum was obtained 25 cm distal to the pylorus, filled with ice-cold HEPES-buffered saline, and transported on ice to the laboratory. Two methods were utilized to disperse cells, a Ca++free buffer system and enzymatic digestion system (collagenase, 0.5 mg/ml; elastase, 50 U/ml; dispase, 0.5 mg/ml). The yields of cells were estimated by cell counting using a hemocytometer. Cell viability was estimated by trypan blue exclusion. The cells were plated in DM/F12: RPMI 1640:NCTC 135 (1:1:1) supplemented with ascorbic acid, hydrocortisone, bovine transferrin, 10% FBS, 2.5% newborn CS, 2.5% CS iron-supplemented at two densities, 0.5-1x 10^4 and 0.5-1 x $10^6/cm^2$. The Ca++ free buffer system of isolating cells resulted in large quantities of largely epithelial, viable cells. However, these cells did not survive more than 48 hours in culture. Cells isolated using the enzymatic digestion system began to attach to the plates within 4 hours. After colonies began to form, cells with epithelial morphology were collected and propagated for two weeks. These cells were transfected with a plasmid encoding neo-resistance and the large ts-T SV-40 antigen. Resistant colonies were selected after 3 weeks of G418 treatment (0.25 mg/ml). Despite the temperature sensitivity of the oncogene, cells incubated at 37C grew more rapidly than those at 32C. Seven distinct colonies with different morphology were identified and selected. Between 27–30 doublings, cells entered crisis, with only 5 colony types surviving. These have been propagated as individual cell lines for more than 80 doublings. The cells display typical epithelial morphology with a single, large, distinct nucleus and 2–3 nucleoli per cell. When grown at low densities, colonies are formed with the cells in the center forming a characteristic "cobblestone" and the cells at the edges an irregular monolayer. Cells were further characterized as epithelial by immunocytochemical staining with antibodies to cytokeratins.

Key Words: Intestine, Epithelium, Bovine

965 Effects of increasing dietary grain on viscosity of duodenal digesta and plasma hormone and glucose concentrations in steers. P. S. Mir*, G. J. Mears, Z. Mir, and S. D. Morgan Jones, *Research Centre, Agriculture and Agri-Food Canada, Lethbridge, AB, Canada.*

Effects of increasing the proportions of dietary barley grain on viscosity of duodenal digesta supernatant and concentrations of cholecystokinin (CCK), insulin, and glucose in plasma from portal and jugular veins were determined. Four steers were surgically fitted with ruminal and duodenal cannulae and indwelling portal vein catheters. The steers were fed diets containing 20, 40, 60, and 80% rolled barley (DM basis) in a 4×4 Latin square design. After adjustment to the diets, digesta samples were collected from the duodenum, and blood samples were collected from the portal and jugular veins. Viscosity of duodenal digesta supernatant, portal and jugular CCK and glucose concentrations, and jugular insulin concentration were determined. With increasing proportion of dietary grain, viscosity of duodenal supernatant increased (1.42 to 2.01cP, $P < .05$). Portal plasma CCK concentration increased (1.85 to 6.03pmol, $P < .05$) as the proportion of dietary grain increased from 20 to 60%, but no significant change was observed as dietary grain was increased to 80%. Changes in jugular plasma CCK concentration, and in portal and jugular plasma glucose concentrations were not significant. Jugular plasma insulin concentration increased linearly (2.92 to 3.97ng/ml, $P < .05$) with increasing dietary grain, however, insulin concentrations in steers fed 60 and 80% barley were similar ($P > .05$). These data suggest that feeding cattle barley grain beyond 60% DM resulted in increased duodenal viscosity without a corresponding increase in secretion of metabolic hormones which control nutrient uptake and partition.

Key Words: Cholecystokinin, Duodenal Viscosity, Portal Vein

966 Correlations of the metabolites concentrations in plasma among the various ages and conditions. O. Sasaki*, N. Yamamoto, and K. Togashi, *Hokkaido National Agricultural Experiment Station, Japan.*

The correlations of the metabolites concentrations in plasma among the four different experiments were estimated by using canonical correlation analysis. The metabolites concentrations of 29 calves were measured at 30, 60 and 90 days of age (Ex1). The metabolites changes during 12 and 24 hours of fasting were measured in 16 calves at 40 days of age (Ex2). These calves were weaned at 75 days of age in Ex1 and Ex2. The metabolites changes during 24 and 48 hours of fasting were measured in 32 calves at 5 months of age (Ex3). The metabolites concentrations were measured in 16 cows at 1, 4, 8 and 12 weeks of primipara milking period (Ex4). Each animals were allotted over two experiments. The concentrations of urea nitrogen (UN), glucose (Glu), non-esterified fatty acids (NEFA), triglyceride (TG) and total ketone (Ket) were analyzed in all experiments. The first canonical correlation coefficients were 0.95 to 0.99 ($P<0.01$) between the sampling times within each experiment. Ket explained mostly the first canonical variate by canonical correlation between the sampling times within Ex1. UN explained mostly the first canonical variate by canonical correlation between the sampling times within Ex2. Glu explained mostly the first canonical variate by canonical correlation between the sampling times within Ex3 and Ex4. The first canonical correlation coefficients were 0.86 to 0.99 ($P<0.05$ or $P<0.01$) among the experiments at some experiment times, except Ex4. The animals had high Ket in Ex1 largely decreased TG in Ex2 and largely changed the all metabolites concentrations in Ex3. The animals largely increased UN in Ex2 were largely decreased TG and small increased Ket in Ex2.

Key Words: Physiology, Cow, Metabolites

967 Effects of prenatal dietary copper on thermometabolism and brown fat norepinephrine turnover in newborn lambs. G. E. Carstens*[1], G. E. Eckert[1], J. C. Branum[1], and L. W. Greene[2], [1]*Texas A&M University, College Station, and* [2]*Amarillo.*

Copper deficiency has been shown to impair triiodothyronine (T_3) and norepinephrine (NE) synthesis in rodents. To determine the effects of prenatal dietary Cu level on thermometabolism in lambs, 12 twin-bearing ewes were assigned to low- or high-Cu treatments 3 mo prior to lambing. A basal diet containing 7 ppm Cu, 2 ppm Mo and .2% S was fed with 0 (low-Cu ewes) or 20 ppm supplemental Cu as Cu-lysine (high-Cu ewes). An oral drench of tetrathiomolybdate was also administered to low-Cu ewes biweekly (avg 43 mg/d) beginning 2 mo prior to lambing. At birth, lambs were fed pooled bovine colostrum at 30 mL/kg BW and placed in a chamber at 20°C. To determine NE turnover rates in brown adipose tissue (BAT), one lamb within each twin pair was administered i.v. a tyrosine hydroxylase inhibitor (α-methyl-DL-tyrosine; AMPT) at 6 (250 mg/kg BW) and 9 h of age (125 mg/kg BW), while the control twin lamb received saline. Lambs were killed at 12 h of age and perirenal BAT samples collected for NE analysis using HPLC. Reductions in BAT NE concentrations between AMPT- and saline-infused lambs were used to estimate NE turnover rates. Lamb rectal temperatures and blood samples were obtained at 2, 6 and 12 h of age and plasma analyzed for T_3 and thyroxine (T_4). Birth and BAT weights were not affected by prenatal Cu treatment and averaged 4.1 $\pm$ .2 kg and 20.5 $\pm$ 1.2 g, respectively. Low-Cu lambs had lower ($P < .01$) liver Cu levels than high-Cu lambs (128 vs 308 $\pm$ 34 ppm DM). Rectal temperatures were lower ($P < .05$) in low-Cu lambs at 2 h of age (37.3 vs 39.2 $\pm$ .5 °C), but not at 6 and 12 h of age. Plasma T_4 concentrations were not affected by prenatal Cu treatment and averaged 7 $\pm$ .6 μg/dL. However, T_3 concentrations were lower ($P < .01$; 271 vs 175 $\pm$ 30 ng/mL) and BAT NE turnover rates were lower ($P < .05$; .3 vs .16 $\pm$ .04 ng/mg/h) in low-Cu compared to high-Cu lambs. These data suggest that prenatal Cu deficiency may impair thermometabolism of newborn lambs by altering endocrine control of brown fat thermogenesis.

Key Words: Brown Fat, Copper, Lambs