1199 Effect of feeding fish oil on uterine secretion of $PGF_2\alpha$, milk composition, and metabolic status of periparturient Holstein cows. R.C. Mattos*[1], C.R. Staples[1], A.M. Arteche[1], M.C. Wiltbank[2], F.J. Diaz[2], T.C. Jenkins[3], and W.W. Thatcher[1], [1]*University of Florida,* [2]*University of Wisconsin,* [3]*Clemson University*.

Objectives were to determine the effect of dietary fish oil (FO) on secretion of $PGF_2\alpha$ by the uterus during the periparturient period as well as milk production and metabolic responses postpartum. Holstein cows were assigned randomly to diets containing FO (n = 13) or olive oil (OO, n = 13). Cows were fed prepartum and postpartum diets that provided approximately 200 g/d from 21 days before the expected parturition until 21 days after parturition. The FO used contained 36% eicosapentaenoic acid (EPA, C20:5, n-3) and 28% docosahexaenoic acid (DHA, C22:6, n-3). Blood samples were obtained once daily at 1730 h from 14 days before due date until parturition, and Days 15 to 21 postpartum. Samples were collected twice daily at 0800 and 1730 h from Day 0 to Day 14 postpartum. A total of 6 FO and 8 OO cows without periparturient disorders were used in the statistical analyses of PGFM ($PGF_2\alpha$-metabolite) and metabolite concentrations. Length of prepartum feeding with OO (22.5 ± 2.8) or FO (21.8 ± 3.3) did not differ. Proportions of individual and total n-3 fatty acids were increased in caruncular tissue and milk of cows fed FO. The combined concentrations of EPA and DHA in caruncular tissue were correlated positively with number of days supplemented with FO ($r^2 = 0.64$, $P < 0.01$). Feeding diets containing FO reduced dry matter intake (DMI) during the prepartum and postpartum periods by 30.3% and 18.1%, respectively. Production of 4% fat-corrected milk, milk protein and milk fat were less in cows fed FO than in cows fed OO and likely a consequence of their lower DMI ($P < 0.05$). Cows fed FO had reduced concentrations of plasma PGFM during the early postpartum period (0, 0.5, 2, and 2.5 days postpartum) compared to cows fed OO ($P < 0.05$). Steady-state levels of prostaglandin H synthase-2 in caruncular tissue was unaffected by diet. Feeding FO reduced plasma concentrations of glucose ($P = 0.03$). This effect could be due to the reduced DMI associated with the FO diet. Results suggest that fatty acids present in the periparturient diet can affect the uterine secretion of $PGF_2\alpha$ and milk fatty acid composition in lactating dairy cows.

Key Words: fish oil, prostaglandin, milk

1200 Characterization of uterine epidermal growth factor receptor expression during the estrous cycle and early pregnancy in pigs. J.G. Kim*, J.L. Vallet, and R.K. Christenson, *USDA, ARS, U.S. Meat Animal Research Center, Clay Center, Nebraska*.

Uterine capacity is a component contributing to litter size in swine. The epidermal growth factor (EGF) gene is located near a uterine capacity quantitative trait locus on chromosome 8. EGF has been reported to stimulate epithelial cell growth, and therefore may influence endometrial function. We have reported the cloning and sequencing of the cDNA for EGF along with changes in EGF mRNA levels in porcine endometrium. For porcine EGF receptor (EGFR), only partial cDNA sequences have been reported previously. Furthermore, the changes in mRNA levels in the endometrium have not been well characterized. The objectives of this study were to 1) clone and sequence the full coding region for EGFR, and 2) determine EGFR gene expression in endometrium during the estrous cycle and early pregnancy. Using iterative screening of a porcine reproductive tissue cDNA library, we obtained a 5037 bp cDNA clone containing the entire coding region for EGFR. The predicted protein sequence of the EGFR contains 1209 amino acids, similar to that of human EGFR (1210 amino acids, 88.3% identity). All the major domains of the EGFR, including three tyrosine residues in the cytoplasmic domain that are autophosphorylation sites in human EGFR, are conserved in porcine EGFR. Twenty μg of total RNA from endometrium of day 10, 13, and 15 cyclic gilts and day 10, 13, 15, 20, 30 and 40 pregnant gilts were used for Northern blotting. The probe used consisted of the entire EGFR clone. Bands corresponding to EGFR mRNA were determined by densitometry and results were analyzed by ANOVA. EGFR mRNA expression did not change during the estrous cycle and pregnancy between day 10 and 15, and decreased significantly ($P = 0.03$) from day 15 (102 ± 14) to 20 (72 ± 10 arbitrary units) of pregnancy. Endometrial EGFR gene expression coincides with a decrease in conceptus estrogen secretion. Endometrial EGFR gene expression, along with the decrease of EGF mRNA expression from day 13 to 15 of pregnancy, suggests that EGF and EGFR may play a role in the endometrial response to conceptus estrogen.

Key Words: Coding region, Endometrium

1201 Evidence for a intrafollicular role of alpha-2-macroglobulin in regulation of estradiol production. F Jimenez-Krassel*, M Winn, JLH Ireland, and JJ Ireland, *Michigan State University*.

Alpha-2-macroglobulin inhibits protease activity, binds growth factors and cytokines that regulate estradiol production, and is in high concentrations (mg/ml) in follicular fluid. Although alterations in intrafollicular concentrations of alpha-2-macroglobulin could modulate local actions of growth factors and proteases, the physiological role of alpha-2-macroglobulin in dominant follicle development is unknown. Therefore, the objectives of our study were to: a) determine if bovine granulosa cells produce alpha-2-macroglobulin and have alpha-2-macroglobulin receptors, and b) test the effect of alpha-2-macroglobulin on capacity of bovine granulosa cells to produce estradiol. For our studies, ovaries were obtained from an abattoir and granulosa cells and follicular fluid were isolated from individual dominant or subordinate first wave follicles in cattle. Northern blot analyses of polyA+ RNA (5 ug) indicated the presence of a 4.8 kb mRNA for alpha-2-macroglobulin and a 15 kb mRNA for the alpha-2-macroglobulin receptor in granulosa cells. Immunoblot analysis of a commercial preparation of bovine alpha-2-macroglobulin (0.5 ug), follicular fluid from dominant or subordinate follicles (10 ug/well), and spent media (20 ug/well)following serum-free culture of bovine granulosa cells from dominant follicles demonstrated the presence of a major 720 kDa protein band in all samples. To test the effect of alpha-2-macroglobulin on estradiol production, granulosa cells (100,000 total cells/200 ul media) from dominant or subordinate follicles of three cows were treated with 19-0H androstenedione (1 uM) and with or without 1 mg bovine alpha-2-macroglobulin or bovine immunoglobulins (control). After 18 h serum-free culture, basal estradiol production was 5-fold greater in granulosa cells from dominant compared with subordinate follicles (30±6 pg/ml vs 6±2 pg/ml). However, alpha-2-macroglobulin enhanced the capacity of granulosa cells from dominant or subordinate follicles to produce estradiol 30- or 50-fold, respectively, compared with controls (Dominant = 984±353 vs 30±6 pg/ml; Subordinate = 309±30 pg/ml vs 6±2 pg/ml). We conclude that alpha-2-macroglobulin may have a intrafollicular role in stimulation of estradiol production by bovine granulosa cells.

Key Words: Alpha macroglobulin, Estradiol, Dominant follicle

1202 Placental weights are greater in gilts homozygous for a secreted folate binding protein (sFBP) gene polymorphism. J. L. Vallet*, R. K. Christenson, and B. A. Freking, *USDA, ARS, Roman L. Hruska U.S. Meat Animal Research Center*.

Secreted FBP likely plays a role in the transfer of folate to the conceptus during pregnancy in swine. A single nucleotide polymorphism (SNP) exists in the sFBP gene that encodes amino acid 175 as either a serine (C allele) or an arginine (A allele). Genomic DNAs from one-half Meishan, one-half white crossbred gilts were used as templates to amplify the region containing the SNP, and the products were sequenced for each gilt to determine the alleles present. The gilts were unilaterally hysterectomized-ovariectomized (UHO), mated, slaughtered on approximately d 105 of gestation, and litter size for each gilt and placental weights, fetal weights, fetal hematocrits, fetal plasma iron, and fetal plasma folates for each fetus were recorded. In addition, a subset of gilts were mated, laparotomized on d 11, the remaining uterine horn was flushed, and the gilts were remated to obtain the uterine capacity and fetal information described above at d 105. For these gilts, conceptus diameters and total intrauterine acid phosphatase (tAP), retinol binding protein (tRBP), and sFBP were measured at d 11. Based on sequence analysis, gilts were divided into 3 groups according to genotype: CC (n = 87 total; 37 d 11), CA (n = 11 total; 7 d 11), and AA (n = 6 total; 4 d 11). Data were analyzed using ANOVA and the following

orthogonal contrasts were used to compare means for each trait: (1) CC gilts vs CA gilts; (2) CC and CA gilts combined vs AA gilts. Litter size, fetal weights, fetal hematocrits, fetal plasma iron, fetal plasma folate, conceptus diameters, tAP, and tRBP did not differ between genotypes. Placental weights were greater ($P < 0.01$ using litter size as a covariate) in AA gilts (202 ± 20 g) compared to CC (153 ± 5 g), and CA (156 ± 16 g) gilts. Total sFBP was also greater ($P < 0.05$ using tRBP as a covariate) in AA gilts (13.5 ± 3.1 μg) compared to CC (6.3 ± 1.0 μg), and CA (7.0 ± 2.3 μg) gilts. Although animal numbers were small, the results suggest that sFBP containing arginine at amino acid 175 is associated with large placentas, which may have an influence on uterine capacity and litter size.

Key Words: Uterus, Folic Acid, Conceptus

1203 Cervical responses to a graded dose of genistein in postpubertal gilts. J.A. Ford, Jr.* and W.L. Hurley, *University of Illinois, Urbana, Illinois.*

Genistein, a soybean phytoestrogen, has a range of estrogenic actions. Little is known about the effects of genistein in swine. This project was designed to characterize the effects of administration of a graded dose of genistein on cervical wet and dry weights. Thirty postpubertal gilts were ovariectomized in order to remove endogenous estrogen. Gilts were randomly assigned to one of six treatment groups 15 d post ovariectomy. Treatment groups received either no hormone (negative control; NC), β-estradiol 3-benzoate at 2 mg/d (positive control; PC), or genistein at 50 mg/d (G50), 100 mg/d (G100), 200 mg/d (G200), or 400 mg/d (G400). Genistein and estradiol were solubilized in DMSO prior to mixing with peanut oil vehicle. Treatments were administered by IM injection at 12-h intervals for 10 d. Gilts were slaughtered after the 10 d of injections and cervixes collected at the time of slaughter, trimmed of all extraneous tissue, and weighed. Dry weights were determined by freeze-drying a portion of the sample. Cervical wet weights (g/100 kg bw) increased as the dosage of genistein increased ($p < .01$; NC = 25.3 ± 5.3, G50 = 33.3 ± 6.8, G100 = 39.8 ± 6.8, G200 = 46.5 ± 6.0, and G400 = 54.7 ± 5.0). Cervical wet weights from PC gilts (92.3 ± 8.0 g/100 kg bw) were greater than from either the NC or genistein treated gilts ($P < .01$). Cervical dry weights (g/100 kg bw) increased as the dosage of genistein increased as the dosage of genistein increased ($P < .01$; NC = 5.5 ± 1.0, G50 = 6.3 ± 1.3, G100 = 8.0 ± 1.3, G200 = 8.9 ± 1.1, and G400 = 10.2 ± 0.9). Cervical dry weights from the PC gilts (17.9 ± 1.5 g/100 kg bw) were greater than from either the NC or genistein treated gilts ($P<.01$). A graded dose of genistein causes an increase in cervical wet and dry weights, although estradiol treatment induces an even greater response. Administration of high doses of the soybean phytoestrogen, genistein, may affect reproductive tract development and function.

Key Words: Gilt, Cervix, Genistein

1204 The use of a deslorelin implant during the late embryonic period to enhance embryo survival. JA Bartolome*[1], S Kamimura[1], FT Silvestre[1], ACM Arteche[1], TR Bilby[1], LF Archbald[1], TE Trigg[2], and WW Thatcher[1], [1]*University of Florida, Gainesville, Florida,* [2]*Peptech Animal Health, North Ryde, Australia.*

Late embryonic/fetal loss (PL) diminish reproductive efficiency. Follicular growth is suppressed in the ovary ipsilateral to the pregnant uterine horn and estradiol may be detrimental for embryo survival. The hypothesis was that administration of a Deslorelin (GnRH agonist) implant on d 27 of pregnancy would induce accessory corpora lutea (CL), suppress follicular growth and reduce PL. Objective was to evaluate PL between d 27 and d 45 and d 45 to d 90 in lactating dairy cows receiving a 2.1 mg Deslorelin implant on d 27 of pregnancy. The study included pregnant cows detected by ultrasonography (US; 5 MHz, Aloka 500®) on d 27 after insemination. A total of 179 pregnant cows were assigned randomly to receive a 2.1 mg, s.q. Deslorelin Implant (Implant; n=89; Ovuplant®, Peptech, Animal Health, North Ryde, Australia) on d27, or no treatment (Control; n=90). On d 27 and d 45, cows were diagnosed pregnant by US. The number of class 2 follicles (6-9 mm), class 3 follicles (≥10 mm), and CL were recorded at d 27 and 45. On d 90, pregnancy was determined per rectal palpation. PL was compared by logistic regression adjusted by parity, previous services and breeding type (Proc Genmod, SAS), and the number of follicles and CL were analyzed by the repeated measures and counted data procedures (Proc Mixed and Genmod, SAS). PL between d 27 and d 45 were 15.5% (14/90) for Control and 20.2% (18/89) for Implant (OR=0.8; 95%CI=0.34,1.75; P=0.54). PL between d 45 and d 90 were 10.5% (8/76) for Control and 7% (5/71) for Implant (OR=1.44; 95%CI=0.44,1.84; P=0.76). Overall PL (d 27 to d 90) was 24.4% (22/90) for Control and 25.8% (23/89) for Implant. On d 45, the number of class 2 (0.72±0.19) and class 3 (0.86±0.12) follicles for Implant were lower ($P<0.01$) than class 2 (1.90±0.18) and class 3 (1.92±0.12) follicles for Control. On d 45, the number of CL for Implant was higher than Control (1.80±0.07 > 1.31±0.07; $P<0.01$). In conclusion, a Deslorelin Implant administered on d 27 of pregnancy was able to increase the number of CL and reduce follicular growth, but did not increase embryo/fetal survival between d 27 to d 90 of gestation.

Key Words: Embryonic/fetal losses, GnRH agonist, Ovarian structures

1205 Nuclear transfer using nonquiescent bovine cumulus cells from primary cell populations. M. Murakami*, O. Perez, C.E. Ferguson, R.S. Denniston, and R.A. Godke, *Louisiana State University, Baton Rouge, LA, USA.*

Cumulus granulosa cells from oocytes were used to reconstruct bovine cytoplasts using nuclear transfer (NT) from primary cell (PC) populations and early cell passages (1-2); their development was compared with embryos derived from adult skin fibroblasts at later passages (7-9). Cumulus cells were obtained from in vitro matured oocytes collected by transvaginal ultrasound-guided aspiration from beef cows (n=4). The cells were used for NT 2 to 4 days after seeding onto a tissue culture plate (CUM; primary cells, PC) or after being subpassed once or twice (CUM; 1-2 passages). Fibroblasts obtained by skin biopsy from a mature cow were used for NT after being subpassed 7 to 9 times. The donor cumulus cells were continuously cultured with 10% fetal bovine serum (FBS). Oocytes (n=570) were enucleated after *in vitro* maturation (20 to 22 h). Couplets were induced to fuse with two DC pulses of 2.25 kV/cm for 15 μsec, delivered by ECM 200 in buffer. All the fused couplets were activated in 5 μM of ionomycin for 4 min followed by 2 mM DMAP for 3 h, and cultured in CR1aa supplemented with 5% FBS under 5% CO_2 in air. Number of fused couplets was greater ($P<.05$) in the CUM (PC) group than in the Fibroblast group. Number of cleaved embryos was greater ($P<.01$) in the CUM groups than in the Fibroblast group. Percentage of embryos developed to Grade-1 blastocysts was greater ($P<0.05$) in the CUM (PC) group (27%) than in the Fibroblast group (15%), there was no difference among the groups for the total number of blastocysts or both morulae and blastcysts. A total of 46 embryos were nonsurgically transferred to 23 cows on days 7 to 9 of their estrous cycle (2/female). Of those, 33.3%, 66.7% and 27.2% were diagnosed pregnant on day 40 by ultrasonography in CUM (PC), CUM (1-2) and Fibroblast groups, respectively. None of the females reached 90 days of gestation in Fibroblast group, while one female remained pregnant in each CUM group. A female in CUM (PC) group subsequently delivered a single heifer calf by C-section.

Key Words: Nuclear Transfer, Embryo, Bovine

1206 Large-scale generation and analysis of expressed sequence tags from porcine ovaries or ovarian follicles at different stages of development. H. Jiang*[1], K. M. Whitworth[1], N. Bivens[1], J. Ries[1], J. A. Green[1], L. J. Forrester[1], G. K. Springer[1], A. Guillen[1], B. A. Didion[2], and M. C. Lucy[1], [1]*University of Missouri-Columbia,* [2]*Monsanto Company.*

The factors and mechanisms controlling ovarian follicular development are not well understood. In the present study, cDNA libraries from fetal, neonatal, and prepubertal porcine ovaries as well as ovarian follicles (2, 4, 6, and 8 mm diameter) were constructed and sequenced. Clustering of cDNA sequences from the three ovary-only libraries and the four follicle-only libraries identified a set of 3,187 and 4,251 unigenes, respectively. Clustering of cDNA from all of the seven libraries identified a set of 6,443 unigenes. The rate of unigene discovery was 55% (6,433 unigenes from 11,634 clones). Thirty-five percent (2,266) of the clustered cDNA were not discovered previously in any species. The unigene set included genes associated with common cell functions such as protein synthesis and processing, cell structure, cell signaling and communication, energy metabolism, and cell division and differentiation, as well as genes associated with ovary-specific functions such as steroidogenesis. Analysis of the cDNA frequency across different libraries revealed that 47 ovary cDNAs and 73 follicle cDNAs were not uniformly represented ($P < 0.05$) in libraries from different stages of ovarian development (fetal, neonatal, or prepubertal) or different stages of follicular development (2 to 8 mm follicle). Twenty-seven genes decreased from the fetal to neonatal

or prepubertal stage, 14 genes increased from the fetal or neonatal to prepubertal stage, and 6 genes increased from the fetal to neonatal stage and then decreased from the neonatal to prepubertal stage. Within follicle libraries, 25 genes decreased and 48 genes increased as follicles grew from 2 mm to larger sizes. The expression of the selected genes (analyzed by ribonuclease protection assay) was generally consistent with the frequencies of their respective cDNA in the individual libraries. This 6,443 member porcine ovary/follicle unigene set and the information on changes in expression for individual clones should be useful in identifying factors and mechanisms controlling ovarian follicular development in a variety of species.

Key Words: Follicular Development, Expressed Sequence Tags, Porcine

1207 Transplantation of testicular explants from prepubertal bulls to nude mice and *ex situ* production of haploid germ cells over a 20-week period. Michael T. Kaproth*[1,2], Dong Ryul Lee[1], and John E. Parks[1], [1]*Cornell University, Ithaca, NY* , [2]*Genex Cooperative, Inc, Shawano, WI*.

Directed genetic modification of the bovine germline would be facilitated by routine methods for accessing premeiotic germ cells, promoting meiosis of committed cells, identifying and harvesting postmeiotic germ cells for use as gametes for embryo production by ICSI. Study objective was to develop procedures achieving germ cell progression through meiosis within explants of bovine prepubertal testicular tissue transplanted to sites in the athymic nude mouse. Testes from bulls of 19 weeks age (generally with only Sertoli cells and spermatogonia in seminiferous tubules) were dissected aseptically to recover 2-3 mm^3 cubes of testicular tissue. Under sterile conditions the tissue was surgically transplanted to the host mouse. Variables included transplantation site (subcutaneous flank, sites within the host mouse testis), number of sites per mouse, effect of exogenous testosterone, time to analysis post-surgery. Most flank explants successfully vascularized. Two but not six flank sites were tolerated. Seminiferous tubules in some explants contained only Sertoli cells. We observed fully developed sperm heads in the majority of flank explants carried out to 20 weeks. None were found in flank explants supplemented with testosterone or in sites within the host mouse testis. Round spermatids and elongated spermatids were first observed at 12 and 20 weeks respectfully. Round spermatids obtained at 15 weeks were injected into the cytoplasm of chemically activated oocytes. Sixty oocytes survived injection and 19 embryos developed to day 10 expanded blastocysts. Nested PCR analysis for a Y-chromosome specific DNA sequence identified 4 Y-chromosome positive embryos. A companion analysis of activated oocytes injected with somatic (XY) cells was not Y positive. These results demonstrate that access to premeiotic germ cells is possible and the resulting postmeiotic cells can be harvested and used as gametes in an ICSI procedure to produce embryos.

Key Words: Meiosis, Transplantation, Testis

1208 Mechanism by which high progesterone levels reduced diameter of dominant follicle during the growing phase of wave 1. L.F. Uribe-Velásquez*[1], E. Oba[2], H. Villa-Velásquez[2], M.I.L. Souza[2], L.C. Lara-Herrera[2], and L.D.S. Murgas[3], [1]*University of Caldas, Manizales, Caldas, Colombia,* [2]*UNESP, Botucatu, Sao Paulo, Brazil,* [3]*Federal University of Lavras, UFLA, Lavras, Minas Gerais, Brazil*.

Pulsatile secretion of pituitary luteinizing hormone (LH) is the result of an interplay between a stimulatory input from the brain and an inhibitory feedback from the gonads. In the female, the inhibitory effect of the gonads is thought to result from the negative feedback action of two steroids, estradiol and progesterone. The aim of this study was determined how application of progesterone exogen affect the growth of the dominant follicle of wave 1. Estrous cycle of 14 ewes were synchronized using prostaglandin (PGF2α). Then, ewes were randomly in two groups (n=7/group); control Group (G1) and progesterone-treated Group (G2) with CIDR (AHI Plastic Moulding Company, Hamilton, New Zealand) after ovulation (day zero). From 1 d before PG injection until d 10 daily ultrasonic examinations were done to establish follicular growth. The pattern of ovarian follicle development was characterized using the definition of a follicle wave as the changes in the number of follicles among the days of the estrous cycle Blood samples by jugular venipuncture for progesterone (P4) plasma concentrations determinations were collected from 1 d before PG until day 10 postovulation. For LH pulse profiles, collection of blood was at 30-min intervals for a period of 8 h on the days 1 and 6. The mean emergence day of wave 1 was 0.7 $\pm$ 0.7 vs 0 $\pm$ 0.6, respectively, for G1 and G2. P4 treatment decreased the growing rate ($p<0.001$) with values of 0.7 $\pm$ 0.2 (G1) and 0.9 $\pm$ 0.7 mm/d (G2) and lenghtened the duration of its static phase ($p<0.05$). Mean maximum diameter size attained by the dominant follicle tended to be larger ($p<0.001$) in the G1 (5.5 $\pm$ 0.5 mm) group that in G2 (4 $\pm$ 0.5 mm). Mean concentrations of P4 ($p<0.001$) were differents among treatments. Number of LH pulses during 1 d in G2 (1.49 $\pm$ 0.11 pulses/8 h) was less ($p<0.01$) than that of G1 (2.55 $\pm$ 0.09 pulses/8 h). Number of LH pulses during 6 d was different ($p<0.05$) among treatments with a mean of 1.22 $\pm$ 0.11 and 2.20 $\pm$ 0.09 pulses/8h for G2 and G1, respectively. In conclusion, the inhibitory effects of exogen P4 on the diameter of dominant follicle was mediated by reduced LH pulse frequency. This work was supported by FAPESP - Sao Paulo - Brazil.

Key Words: Dominant follicle, LH, Sheep

1209 Differential expression of pyruvate carboxylase 5′UTR variants during transition to lactation. C. Agca* and S.S. Donkin, *Purdue University, West Lafayette, IN*.

Pyruvate carboxylase (PC) is regulated in some species through preferential translation of 5′ untranslated region (UTR) variant forms of PC mRNA. Bovine liver expresses six PC 5# UTR transcript variants: bPC5#A, bPC5#B, bPC5#C, bPC5#D, bPC5#E, and bPC5#F which are 68, 253, 363, 89, 274, and 178 bp respectively. Despite sequence differences in the 5# UTR regions of mRNA these variants contain an identical coding sequence. The objective of this experiment was to determine the relative abundance of PC transcripts in bovine liver during the transition to lactation. A ribonuclease protection assay (RPA) was developed to investigate the changes in abundance of each 5# UTR transcript and total PC mRNA. Liver biopsy samples collected from seven cows on -28, -14, +1, +28, and +56 days relative to parturition were pooled and analyzed by RPA to determine the change in PC 5# UTR variants during transition to lactation. Samples from seven cows collected one day after parturition were analyzed individually to determine variation among individual cows. Results show that the six bovine PC 5# UTR variants and the coding region can be determined using two related riboprobes in two parallel RPA analysis. Expression of all PC variants was greatest one day after parturition and followed changes in total PC mRNA. Transcript bPC5#A responded least and transcript bPC5#C increased the most during parturition. Transcripts bPC5#A, bPC5#B, bPC5#D, bPC5#E and bPC5#F increased 8, 58, 8, 49, 18 fold at calving compared to 28 days prepartum whereas the coding region increased 13.5 fold. Transcript bPC5#C could not be detected in samples obtained on #28 days relative to calving. Data indicate that the shortest PC transcripts, bPC5#A and bPC5#D are the most abundantly expressed, transcripts bPC5#E and bPC5#C are least abundant, and bPC5#F and bPC5#B are intermediate. The lack of uniformity in the pattern of PC 5# UTR variants across the transition to lactation suggests a complexity for control of PC expression during this period at either through transcriptional control, mRNA processing or both.

Key Words: Pyruvate carboxylase, 5' UTR, Bovine

1210 Manganese inhibits in vitro nuclear maturation in cumulus-enclosed bovine oocytes through the cAMP/protein kinase A pathway. S. Bilodeau-Goeseels*, *Agriculture and Agri-Food Canada Reserach Centre, Lethbridge, Alberta, Canada*.

It was recently discovered that manganese can inhibit *in vitro* nuclear maturation in bovine cumulus-enclosed oocytes (CEO) but not in denuded oocytes (DO). The goals of the present study were: (1) to determine if the effect of manganese on nuclear maturation is dependant upon protein kinase A (PKA) activity; (2) to compare the effects of manganese and activation of adenylate cyclase with forskolin (FSK) on nuclear maturation and cAMP levels in DO and cumulus-oocyte complexes (COC); and (3) to determine if protein kinase C (PKC) activation can reverse manganese-maintained meiotic arrest in bovine CEO. A total of 2052 oocytes were evaluated. The PKA inhibitor H-89 significantly decreased the percentage of CEO maintained at the germinal vesicle (GV) stage by manganese during a 9-h culture period (1, 82, 14, and 37% CEO at the GV stage for control, 0.05 mM $MnCl_2$, 125 μM H-89 and 0.05 mM $MnCl_2$ + 125 μM H-89 respectively). The adenylate cyclase activator FSK (20 and 100 μM) and $MnCl_2$ (0.5 mM) had the same inhibitory effect on nuclear maturation in COC, and $MnCl_2$

significanlty increased cAMP levels in COC but to a lesser extent than FSK (4.8, 28.7, 346.3, 631.0 fmol/COC for control, 0.5 mM $MnCl_2$, 20 μM FSK and 0.05 mM $MnCl_2$ + 20 μM FSK respectively). In DO, $MnCl_2$ decreased the percentage of oocytes at the GV stage compared to control medium and FSK (54, 26, 81 and 51% DO at the GV stage for control, 0.5 mM $MnCl_2$, 100 μM FSK and 0.5 mM $MnCl_2$ + 100 μM FSK respectively); however, neither $MnCl_2$ or FSK had a significant effect on cAMP levels compared to control. The PKC activator PDDβ reduced the percentage of CEO maintained in meiotic arrest by $MnCl_2$ (5, 5, 68 and 42% of CEO at the GV stage for control, 0.05 mM $MnCl_2$, 10 μM PDDβ and 0.05 mM $MnCl_2$ + 10 μM PDDβ, respectively). In conclusion, manganese activates the adenylate cyclase enzyme in cumulus cells to generate cAMP which in turns activates PKA and this would lead to maintenance of meiotic arrest.

Key Words: Oocyte, Meiosis, Cyclic AMP, Manganese

1211 Interferon tau does not regulate integrin αVβ3 expression in bovine endometrium. Sarah Kimmins and L.A. MacLaren*, *Nova Scotia Agricultural College, Truro, NS Canada*.

The integrin αVβ3 is a cell and extracellular matrix adhesion molecule present at the fetomaternal interface at implantation in several species. In cattle, this integrin is transiently downregulated on day 16 of the estrous cycle, but not on day 16 of pregnancy. Expression of integrin αVβ3 increases in the subepithelial stroma over the peri-implantation period (days 18-30). In other cell systems interferons have been shown to regulate this integrin. To test whether the pregnancy recognition factor interferon tau (IFNτ) was regulating integrin αVβ3 in bovine endometrium, primary cultures of intercaruncular stromal cells were treated in triplicate for 48 h with 50 ng/μl of recombinant ovine IFNτ. In addition, beef heifers (n=6) with estrous cycle lengths of 19-20 days were synchronized and treated via intrauterine infusion with IFNτ ($5x10^7$ antiviral units per day) or saline from days 14 -16. On day 16, endometrial samples were dissected and snap frozen in liquid nitrogen. Differences in β3 integrin subunit mRNA expression between treatments were determined by reverse transcriptase PCR and northern blotting. Immunohistochemistry was used to localize integrin αVβ3 protein expression. Data were analysed by analysis of variance to determine effects of IFNτ in comparison to controls. Cultured intercaruncular stromal cells treated with IFNτ expressed levels of β3 mRNA that were not different from untreated cells (P$\geq$0.10). Similarly, uterine infusion of IFNτ did not change β3 subunit mRNA expression (P$\geq$0.10) or affect immunohistochemical scores for integrin αVβ3 protein in vivo, despite influences on estrogen receptor expression. The results indicate that IFNτ is not an important regulator of integrin αVβ3 expression in bovine endometrium.

Key Words: Uterus, Ruminant, Cell adhesion molecule

1212 Suppression of basal and pulsatile LH with a GnRH antagonist is not sufficient to initiate ovulatory cycles in all cows with ovarian follicular cysts (cysts). MD Calder*, BE Salfen, M Manikkam, J Bader, RS Youngquist, and HA Garverick, *University of Missouri*.

Cows with cysts have follicular waves, but new dominant follicles often develop into cysts. Cows with cysts have high mean and pulsatile serum LH (Cook et al., 1991). Recently, use of intravaginal progesterone (P) decreased LH and restored ovulation in cows with cysts (Calder et al., 1999). In the current study, cows with cysts were given a GnRH antagonist (s.c.; SB-75; Asta Medica; Germany) to reduce LH in the absence of P. Cysts were induced hormonally and ovaries were monitored with ultrasonography. Cows were diagnosed with cysts when single (>20mm) or multiple (>15mm) follicles persisted for 7d when serum P concentrations were low. Three treatment groups were: cows with normal estrous cycles, [CYC; estrus (d 0) synchronized with prostaglandin; n=6]; cows with cysts that received carrier only from d 1-7; (CYST; n=7); and cows with cysts treated from d 1-7 with 10μg/kg SB-75 in 5% mannitol (SB-75; n=7). Blood samples (every 12 min for 8h) were collected for LH analysis in CYST and SB-75 cows on d 0, and from all cows on d 1, 4, 7, 10 and 13. CYC cows were injected with prostaglandin on d 11 following estrus to induce follicular phase LH patterns. Cows in all treatments initiated a new follicular wave about two days after study initiation. The mean diameter of the first wave dominant follicle was not different in SB-75 (10.90.8 mm) and CYC cows (13.50.8mm); both were smaller than in CYST cows (19.61.3mm, P < 0.05). A second follicular wave was initiated later in SB-75 (17.41.6d) and CYST (17.22.8d) cows than in CYC cows (10.80.2d, P < 0.05). Mean LH and LH pulse frequency was reduced in SB-75 cows from d 1 until at least d 13. Four SB-75 cows ovulated a second wave dominant follicle. In conclusion, SB-75 decreased LH concentrations, which restricted the growth of the first dominant follicle, but only 4/7 SB-75 cows ovulated within 30d compared to 2/7 CYST cows. Suppression of LH may not be sufficient to restore ovulatory cycles in all cows with cysts.

Key Words: Cysts, LH, follicular wave

1213 Assessment of the effects of flavonoids on the post-thaw motility of cryopreserved bovine spermatozoa. J. A. Pitchford*, S. A. Ericsson, K. K. Korth, L. L. Green, and W. T. Campbell, *Sul Ross State University, Alpine, Texas*.

The objective of this study was to determine if the flavonoids catechin, epicatechin, and silibinin were capable of maintaining the post-thaw motility of cryopreserved bovine spermatozoa. Semen samples (n=30) were collected from 14 beef bulls using electro-ejaculation. Samples were extended in Triladyl extender (Minitube of America, Inc., Verona, WI) containing 0 (control), 10, 100, 1000, or 10000 μM catechin, epicatechin or silibinin. Extended samples were slowly cooled for 3 hours to 5°C, prior to packaging in 0.5 mL polyvinyl straws and freezing with liquid nitrogen. Microscopic analyses (250X) of the percentage of motile spermatozoa were obtained after 24 hr by thawing straws for 1 minute in a 37°C water bath. The percentage data were arcsine transformed and analyzed using ANOVA for differences among flavonoid levels. Samples extended with 10 μM (mean $\pm$ SD: 31.1% $\pm$ 14.6, P < 0.0001), 100 μM (27.7% $\pm$ 15.2, P < 0.0005), or 1000 μM (28.3% $\pm$ 14.5, P < 0.0001) catechin resulted in greater motility than the control (20.6% $\pm$ 11.4). Samples containing 10 μM (27.8% $\pm$ 14.3, P < 0.0001) and 100 μM (30.6% $\pm$ 13.3, P < 0.0001) epicatechin exhibited higher sperm motilities than the control. Similarly, extenders containing 10 μM (30.5% $\pm$ 14.5, P < 0.0001), 100 μM (32.6% $\pm$ 15.5, P < 0.0001), 1000 μM (31.4% $\pm$ 15.6, P < 0.0001) or 10000 μM (38.7% $\pm$ 17.1, P < 0.0001) silibinin retained greater motility than the control. The percentage of motile spermatozoa were significantly lower in samples containing 10000 μM catechin (7.0% $\pm$ 7.9, P < 0.0001) or epicatechin (3.1% $\pm$ 3.9, P < 0.0001) when compared to control. The samples containing 1000 μM (23.4% $\pm$ 11.9) epicatechin were not significantly different from control samples. These results suggest that catechin, epicatechin, and silibinin have a dose related effect on the post-thaw motility of cryopreserved bovine spermatozoa.

Key Words: Flavonoid, Spermatozoa, Bovine

1214 Stress during behavioral estrus delays the preovulatory surge of LH and ovulation in sheep. D. Wolfenson*[1], B.M. Adams[2], M.R. Dally[2], and T.E. Adams[2], [1]*Hebrew University, Rehovot, Israel,* [2]*University of California, Davis, CA*.

The effect of stress during behavioral estrus on ovulation in prolific sheep was examined in two studies. The estrous cycles of Finnish Landrace sheep were synchronized using intra-vaginal progesterone (P4) implants and the onset of estrus was determined using vasectomized rams. All ewes displayed estrus 20-36 h after implant removal. In studies I and II, bacterial endotoxin (LPS) was administered iv at 2 h intervals (200 ng/kg/injection) for 12 h beginning at onset of estrus (study I; n = 6) or 12 h thereafter (study II; n = 5). Control animals in studies I (n = 5) and II (n = 5) received saline. Blood samples were collected at 2-4 h intervals beginning at estrus and continuing for 3 d thereafter. Ovulation was assessed by laparoscopy 5 d after implant removal. A preovulatory surge of LH was evident in all control animals, with peak levels of LH (44.7 4.7 ng/ml) noted 22.6 1.9 h after the onset of estrus. In study I, stress at the onset of estrus delayed the LH surge in all ewes, with 2 LPS-treated ewes exhibiting an apparently normal LH surge 41 h after the onset of estrus. None of the 4 remaining LPS-treated ewes showed an LH surge during frequent blood collection. Although serum levels of P4 in these ewes increased after estrus, secretion of P4 was delayed 4 d relative to control animals (P<0.05). This is consistent with our view that ovulation was delayed in LPS-treated animals. Serum levels of P4 in LPS-treated ewes tended to be higher than in control ewes. In study II, administration of LPS beginning 12 h after onset of estrus also delayed ovulation in all ewes. Moreover, only 2 of the 5 LPS-treated ewes exhibited an LH surge (peak LH = 7.9 0.4 ng/ml) during the period of frequent blood collection. Taken together, these results indicate that

stress during behavioral estrus interrupts the terminal stages of follicle development and delays the preovulatory surge of LH and ovulation.

Key Words: Endotoxin stress, Delayed ovulation, Sheep

1215 The effects of a chronic elevation in plasma insulin during the early postpartum period on luteinizing hormone pulsatility and plasma estradiol in dairy cows. S.T. Butler* and W.R. Butler, *Cornell University, Ithaca, NY.*

Early lactation in dairy cattle is associated with a prolonged period of anestrous due to attenuated pituitary LH release and impaired ovarian LH responsiveness. Using the hyperinsulinemic-euglycemic clamp technique we previously demonstrated that an 8-fold increase in plasma insulin resulted in a marked decline in DMI in early lactation cows (2001; J. Dairy Sci. 84 (Suppl. 1): 34), thus negating any potential benefits on LH pulsatility and LH responsiveness. We have conducted another clamp experiment with a more moderate increase in plasma insulin to determine if alterations in LH pulsatility or responsiveness could be observed. Holstein cows (n=10) were subjected to either a hyperinsulinemic-euglycemic clamp (INS) or saline infusion (CTL) for 96-hours starting on day 10 postpartum. Insulin was infused continuously (0.3 μg/kg BW/hr) via a jugular catheter. Blood samples were collected hourly, and euglycemia was maintained by infusion of exogenous glucose. During infusion, insulin concentrations were increased 2.3-fold in INS cows over those in CTL cows (0.70 $\pm$ 0.05 vs. 0.30 $\pm$ 0.05 ng/ml; $P<0.001$), while blood glucose concentrations were not different between treatments. Blood samples were collected at 10 minute intervals for 8 hours immediately prior to commencement (PRE) and termination of infusions (END). In addition, 10 minute blood samples were collected from INS cows for a further 8 hours immediately following the commencement of the insulin infusion (START). Relative to values measured during PRE, the number of LH pulses, pulse amplitude and mean LH were not different ($P>0.05$) during END for either treatment or during START for the INS cows. Plasma estradiol levels declined in CTL cows during the infusion period, but increased in the INS cows following the onset of insulin infusion (treatment x time, $P<0.001$). The results indicate that insulin is an important metabolic hormone for determining ovarian responsiveness to LH, but do not implicate a role for determining LH pulsatility.

Key Words: Luteinizing hormone, Estradiol, Insulin

1216 Effects of GnRH administered at onset of estrus on endocrine responses and conception in lactating cows. M. Kaim[1], A. Bloch[2], D. Wolfenson*[2], M. Rosenberg[1], H. Voet[2], and Y. Folman[1], [1]*Agricultural Research Organization, Bet-Dagan, Israel,* [2]*Hebrew University, Rehovot, Israel.*

Two studies examined the effects of GnRH injection at onset of estrus on LH surge, progesterone concentrations, interval to ovulation, and conception rates, in Holstein cows. In study I, cows were monitored for estrus, blood samples were taken, and ovulation was checked by ultrasound. Treated (n=24) and control (n=25) cows were injected with GnRH analogue (Buserelin, 10 mg) or saline at onset of estrus. 19 control cows had a normal estrus-ovulation (E-O) interval (<30 h) and 6 (24%) had a long interval (>30 h). All GnRH-treated cows had a normal E-O interval (26.10.5 h). GnRH-treated cows showed a higher LH surge than control cows ($P<0.05$). Control cows with a long E-O interval had lower preovulatory estradiol ($P<0.05$), and progesterone levels in the subsequent mid-luteal phase were about 2 ng/ml lower ($P<0.05$) than in control cows with a normal E-O interval and GnRH-treated cows. In study II, in summer and winter, 152 primiparous and 211 multiparous synchronized cows at 60-100 days post-partum were used. Estrus was monitored frequently, and randomly assigned GnRH-treated cows were injected, as above, within 2.5 h of onset of estrus. Overall, in both seasons, conception rates (first 2 inseminations) of multiparous cows did not differ between groups, whereas that of GnRH-treated primiparous cows was higher than that of control cows (42.2 and 63.2%; $P<0.05$). GnRH increased conception rates in cows with low body condition (BCS; 36 vs 62%, $P<0.05$). In summer, conception rates of 31% of all control primiparous cows and of multiparous cows with low BCS, were increased to 56% by GnRH ($P<0.05$). In winter, GnRH was less effective. Results suggest that administration of GnRH at estrus is likely to shorten long E-O intervals and to increase low P4 post-ovulation in cows exhibiting a low preovulatory LH surge; these changes could be associated with fertility improvement by GnRH, mainly in cows with low BCS and in the summer season.

Key Words: GnRH at estrus, LH surge, Fertility

Production, Management, and the Environment
Dairy Management

1217 Non-nutritional factors that influence milk urea nitrogen concentration. P.M. Meyer*[1], P.F. Machado[1], A. Coldebella[1], C.H. Corassin[1], L.D. Cassoli[1], and P.H.M. Rodrigues[2], [1]*Clinica do Leite. Escola Superior de Agricultura Luiz de Queiroz/University of Sao Paulo, Brazil,* [2]*Faculdade de Medicina Veterinaria e Zootecnia, University of Sao Paulo, Brazil.*

The purpose of this study was to determine which non-nutritional factors have most influence on milk urea nitrogen (MUN) and further to establish targets concentration. Data from approximately 500 Holstein cows were collected for 10 months (n=5082) from a farm in Sao Paulo state (Brazil). Factors studied were: milk production (MP), days in milk (DIM), lactation number (LN), somatic cells count (SCC) and milk fat (F), protein (P), lactose (L) and total solids (TS) percentage. The association of MUN concentration (dependent variable) and the other variables studied (independent variables) was estimated using the multiple linear regression analysis. To identify among independent variables those that could best explain variability in MUN concentration, coefficients of determination (R^2) and adjusted coefficients of determination were estimated for the several equations. Maximum R^2 obtained was 0.1285, when all independent variables were included in the model, which can be considered low. The highest R^2 value found was for MP (R^2= 0.0987), which indicates that MP explains 9.9% of the total variability of MUN. The other variables studied are responsible for the remaining 2.9% of the variability. It was concluded that the best factor to correct MUN target, among the ones studied, is MP. Financial support: FAPESP and CNPq (Brazil). Table. Coefficient of determination (R^2) of multiple linear regression analysis for maximum R^2 in the model, using MUN as dependent variable.

Variables included	R^2	Adj. R^2
MP	0.0987	0.0985
MP, P	0.1113	0.1109
MP, P, L	0.1139	0.1134
MP, P, L, CCS	0.1160	0.1153
MP, P, L, CCS, ST	0.1180	0.1171
MP, P, L, CCS, ST, F	0.1259	0.1249
MP, P, L, CCS, ST, F, DIM	0.1282	0.1270
MP, P, L, CCS, ST, F, DIM, LN	0.1285	0.1271

Key Words: MUN, dairy cows, target concentration

1218 Relationship among having mud in milking-cow barns, somatic cell counts and decreased milk yield in Thai dairy herds. W. Suriyasathaporn*[1], P. Maneeratanarungroj[1], S. Sangmaneedej[1], P. Tungtanatanich[1], S. Takong[1], U. Parinyasutinun[2], and S. Pangjuntuk[2], [1]*Faculty of Veterinary Medicine, Khonkean University, Thailand,* [2]*Dairy Farming Promotion Organization of Thailand.*

The objective of this study was to evaluate relationship among having mud in milking-cow barns, somatic cell count, and milk yield in Thai dairy herds. Milk samples were collected from 78 dairy cows from 6 small dairy holders in the Northeast Thailand, a tropical country where a difference of temperature among seasons is relatively small. Farm environment, individual milk yield, calving date, and parity of the cows were recorded. Characteristics of the barns in this study were loose housing with ground floor, partial roof, and no wall. Each farm was