increase fertility in heifers. Primiparous crossbred beef heifers (n = 96) were divided into two breeding seasons, early breeding heifers (EBH, n = 48) and late breeding heifers (LBH, n = 48), and randomly assigned to one of two dietary supplement groups by weight and age for both EBH and LBH. Heifers were fed a control diet (CON) consisting of mixed alfalfa/grass hay and oat grain or a safflower seed diet (SAFF) consisting of the control diet plus safflower seed high in oleic (69.9 %) acid. Diets were fed 35 d prior to AI and continued 20 d post AI. Diets were formulated to be isocaloric and isonitrogenous for both groups. Heifers in each supplement group for both EBH and LBH received either an injection of hCG (3,300 IU) or saline 5 d post AI. All heifers were synchronized using 7-11 MGA Select Synch. Heifers were bred AI 12 h after the onset of estrus. Jugular samples were collected three different times prior to AI to determine changes in plasma fatty acid profiles. Body condition score, reproductive tract score, and weight did not differ (P > 0.05) between supplement groups for EBH and LBH, therefore, EBH and LBH were pooled together. There were no differences (P > 0.05) in conception rates for each supplement group or for hCG injected heifers (P > 0.05). More hCG injected heifers in both treatments conceived to AI (66.7 vs 59.5 %), but did not differ from saline treated heifers (P > 0.05). Plasma stearic acid increased (P < 0.01), while plasma linolenic acid decreased (P < 0.01) in both supplement groups. Plasma oleic acid increased for the SAFF group (P < 0.01) verses the CON group. Feeding 0.96 kg of whole safflower seed 35 d prior to AI did not improve fertility, yet conception rates appeared to increase when hCG was given

Key Words: Beef hiefers, Safflower, hCG

M31 Effect of exogenous progesterone before calf removal and prostaglandin  $F_2\alpha$  on estrous response and pregnancy rates in 3-year-old beef cows. J. L. Olson\*<sup>1</sup>, A. J. Roberts<sup>2</sup>, J. A. Paterson<sup>1</sup>, and R. N. Funston<sup>3</sup>, <sup>1</sup>Montana State University, Bozeman, <sup>2</sup>USDA-ARS, Miles City, Mt, <sup>3</sup>University of Nebraska, Lincoln.

Objectives for this experiment were to determine effects of a 7 d pretreatment with an intravaginal progesterone insert (CIDR) on estrous response and pregnancy rates in 3-year old postpartum beef cows synchronized with calf removal and prostaglandin  $F_2\alpha$ . Cows (BW = 488  $\pm$  7.4; body condition score = 3.8  $\pm$  .07; days postpartum = 58.7  $\pm$  1.2) were randomly allotted to either control (n=22; i.m. injection of 25 mg PGF $_2\alpha$  [Lutalyse  $^{\text{(B)}}$ ] on d 0) or CIDR treatment from -7 to 0 d preceding, PGF $_2\alpha$  injection on d 0 (CIDR; n=18). All calves were weaned on d 0. Cows were observed for estrus for 120 h after PGF $_2\alpha$  and inseminated by AI approximately 12 h after the onset of estrus. A bull was placed

with cows 12 d after  $PGF_2\alpha$  and removed 40 d after  $PGF_2\alpha$ . Circulating progesterone (P4) concentrations were determined in blood samples collected on d -7, 0, and 11. Pregnancy status was diagnosed by ultrasonography on d 54 and 145 after  $PGF_2\alpha$ . Synchronization rates were higher (P < 0.05) for CIDR (100%) compared to Control (77%) cows. Time of estrus did not differ (P > 0.10) between Control and CIDR cows (2.41  $\pm$  .15 d). Pregnancy rates by AI were not different (P = .28), between Control (18%) and CIDR (33%) cows. Overall pregnancy rates were higher (P < 0.10) in Control (97%) compared to CIDR (80%) cows. Concentrations of P4 on d -7, d 0, and d 11 did not influence (P > 0.10) overall pregnancy rates; however, progesterone concentrations were increased (P < 0.05) in CIDR cows on d 0 (5.6 vs. 2.9 ng/ml, for CIDR vs. Control) and d 11 (7.1 vs. 4.8 ng/ml, for CIDR vs. Control). Administration of a CIDR 7 d before calf removal and PGF<sub>2</sub>α increased concentrations of P4 on d 0 and 11, and increased the proportion of cows exhibiting estrus. However, CIDR treatment did not improve conception and AI pregnancy rates and reduced overall pregnancy rates in

Key Words: Postpartum, Beef cows, CIDR

M32 Effects of glucose concentration and presence of EGF and hormones on bovine oocyte maturation. D. J. Walker\*, J. F. De La Torre-Sanchez, and G. E. Seidel, Jr., *Colorado State University Fort Collins, CO 80523*.

The purpose of this study was to examine effects of glucose concentration, epidermal growth factor (EGF), and hormones (FSH, LH, and estradiol 17  $\beta$ ) during bovine oocyte maturation on in vitro production of blastocysts. Oocytes from slaughterhouse ovaries were divided among the 12 factorial combinations of 3 glucose concentrations (0.5, 2.0, and 5.5 mM), presence or absence of 50 ng/ml of EGF, and presence or absence of LH, FSH, and E2 in CDM-1, a chemically defined medium similar to SOF. Oocytes were matured at  $38.5\mathrm{C}$  in 5% CO<sub>2</sub> in air for 231 h. After maturation, oocytes were fertilized at 1 X 10<sup>6</sup> sperm/ml in 6 replicates in F-CDM (0.5 mM glucose), and then cultured 2 days in CDM-1 (0.5 mM glucose) and 4 days in CDM-2 (2 mM glucose). Glucose concentration in maturation medium at 0.5, 2.0, and 5.5 mM had no effect on blastocyst rates per oocyte, 33%, 32%, and 31% respectively. However,  $0.5~\mathrm{mM}$  glucose resulted in a cleavage rate of 87%, higher than 81% seen for both 2 and 5.5 mM glucose (P=.004). EGF and hormones independently enhanced cumulus expansion, but there was no synergism between them, and they had no affect on cleavage or blastocyst rates. Both cleavage (P=.0003) and blastocyst rates (P=.02) were affected by which of 3 bulls was used for fertilization.

Key Words: Bovine, Embryo, Oocyte

## **Triennial Reproduction Symposium**

M33 Post-thaw fertility of bovine semen aged within an AI straw for 8.5 hours. J. L. Edwards\*1, M. N. Malone¹, F. N. Schrick¹, H. H. Dowlen², H. D. Moorehead², P. A. Lunn², and A. M. Saxton¹, ¹The University of Tennessee, Knoxville, ²Dairy Experiment Station, Lewisburg, TN, USA.

Objective was to evaluate fertility of frozen-thawed semen aged for 8.5 h. Estrus was visually assessed three times daily for at least 30 minutes each time. Jersey heifers (age:  $13.9 \pm 1.4$  mo; weight:  $272.8 \pm 19.2$  kg) observed standing to be mounted between 0700 and 1200 h were randomly assigned to be inseminated with a straw of frozen semen that had been thawed and maintained in a Cito Thaw Unit (34.4°C water bath) for 8.5  $\pm$  0.04 min (Control; range 3-14 min) or 8.5  $\pm$  0.68 h (Aged; range 6-10 h). Heifers observed in estrus after 1200 h were inseminated with control semen. All heifers were inseminated according to AM/PM rule. To age sperm, a straw of frozen semen was thawed immediately after visual detection of a heifer in estrus and then maintained in a Cito Thaw unit until insemination approximately  $8.5~\mathrm{h}$  later. Frozen semen was purchased from various AI organizations (n=6). Individual Jersev bulls (n=30) were randomly and evenly distributed across treatments. Establishment of pregnancy was determined by palpation per rectum at 45 to 65 d post-insemination. Animals were monitored throughout pregnancy and upon calving, sex of offspring was recorded. Data were analyzed using Chi-Square; variables of interest included proportion pregnant, calving, and sex of resulting offspring. Effects of inseminating Jersey heifers with sperm aged within an AI straw for 8.5 h post-thaw were minimal. Fifty percent of heifers inseminated with aged semen became pregnant and delivered a live calf at term (Table). Proportion of female offspring was similar. Ability to maintain frozen-thawed semen within an AI straw for 8.5 h in a  $34.4\,^{\circ}\mathrm{C}$  water bath without significant reductions in fertility demonstrates that sperm can be held post-thaw for extended time periods and suggests potential for manipulation post-thaw for sexing or performing diagnostics.

Treatment	No. Bred	Pregnant (%)	Calved (%)	Female (%)
Control Aged P-value	59 56	37(62.7) 28(50.0) 0.19	37(62.7) 28(50.0) 0.19	19(51.4) 11(39.3) 0.45

Key Words: Frozen semen, Aging, Artificial insemination

M34 Effects of presynchronization and/or postbreeding treatment with porcine LH or hCG on pregnancy rates in dairy cows. J. P. Kastelic\*<sup>1</sup> and J. D. Ambrose<sup>2</sup>, <sup>1</sup> Agriculture and Agri-Food Canada, Lethbridge, AB, Canada, <sup>2</sup> Alberta Agriculture Food and Rural Development, Edmonton, AB, Canada.

The objectives were to determine the effects of presynchronization with a prostaglandin  $F2\alpha$  analogue and/or post-breeding treatment with porcine LH (pLH) or hCG on pregnancy rates in dairy cows. In three experiments, an Ovsynch protocol was used to synchronize ovulation in

lactating Holstein cows (range, 50 to 125 d postpartum). Cows were given im injections of 100  $\mu\mathrm{g}$  GnRH (Fertiline; Vetoquinol) on Days -10 and -1 and 500  $\mu$ g cloprostenol (Estrumate; Schering Plough) on Day -3, with fixed-time AI on Day 0. Pregnancy was diagnosed by palpation per rectum between 45 and 60 d after AI. In Experiment 1, cows were randomly allocated to either a standard Ovsynch protocol (n=92) or to a presynchronization protocol (n=86; 500  $\mu g$  cloprostenol given twice, 14 d apart) followed by Ovsynch, with the first GnRH given 12 d after the second cloprostenol. Pregnancy rates were 35 and 49%, respectively (P<0.06). In Experiment 2, cows were given im injections of 2 mL saline, 12.5 mg pLH (Lutropin-V; Bioniche Animal Health), or 2500 IU hCG (Chorulon; Intervet) 5 d after timed-AI (41, 41 and 39 cows, respectively). Pregnancy rates were 22, 33 and 37% (P=0.32). Experiment 3 was a 2 x 3 factorial, with the factors being presynchronization and post-breeding treatment (as done in Experiments 1 and 2, respectively). Pregnancy data are presently available from 86 cows, with data collection ongoing on several farms. Pregnancy rates in the six treatment groups ranged from 40 to 55% (ns). Pregnancy rates were 47 and 50% without and with presynchronization, respectively, and were 42, 48 and 55% in cattle treated with saline, pLH and hCG, respectively (P<0.6). In conclusion, pregnancy rates to timed-AI were improved by presynchronization. Post-breeding treatment with pLH or hCG 5 d after timed AI numerically improved pregnancy rate.

Key Words: Ovsynch, Fertility, Dairy cows

M35 Pregnancy outcome in dairy cows fed diets supplemented with flaxseed or sunflowerseed. J. D. Ambrose\*1, J. P. Kastelic², R. Corbett¹, P. A. Day¹, J. A. Small³, and H. V. Petit⁴, ¹Alberta Agriculture Food and Rural Development, Edmonton, AB, ²Agriculture and Agri-Food Canada, Lethbridge, AB, ³Brandon, MB, ⁴Lennoxville, QC, Canada.

The objectives were to determine if a diet enriched in  $\alpha$ -linolenic acid (ALA; C18:3n-3) would enhance embryo survival and pregnancy rates in dairy cattle. Holstein cows were assigned to diets supplemented with about 2.35 kg of either rolled flaxseed (FS; 56.7% ALA, n=62) or rolled sunflowerseed (SS; 0.1% ALA, n=59) to provide approximately 750 g oil/cow/day beginning 4 wk before breeding (5522 d, meansd, postpartum). Diets continued for 32 d after timed-AI (Day 0) following a presynch/ovsynch protocol using Estrumate (cloprostenol, Schering Plough) and Fertiline (GnRH, Vetoquinol). Barley silage- and barley grain-based rations were formulated to meet or exceed NRC (2001) requirements. Metabolizable protein and  $NE_{l}$  concentrations were similar in diets. Based upon a mean DMI of 24.2 kg/d, cows fed FS or SS consumed >410 g or <1 g of ALA, respectively. Plasma progesterone concentrations determined on Days -10, -3, 0, 7, 21 and 24 were not affected by diet. Pregnancy was confirmed by ultrasound 32 d after AI and pregnant cows received no further oilseeds. Nonpregnant cows were placed on a second ovsynch regimen and rebred 42 d after first AI, and received oilseeds until 32 d after second AI. Relative to pre-diet levels. FS and SS diets increased the ALA content of milk by 187% and 21%, respectively. Presumptive pregnancy (plasma progesterone >1 ng/mL on Days 21 and 24) and confirmed pregnancy rates to first AI were higher in cows fed FS than in cows fed SS (72.6 vs 47.5%, P=0.01; and 48.4 vs 32.2%, P=0.07, respectively). Confirmed pregnancy rates (combined for both AI) were 67.7 vs 59.3% for FS vs SS (P>0.10). Apparent embryo survival rate was higher at Day 24 in cows fed FS, but it was not affected by diet between Days 24 and 32. Inclusion of rolled flaxseed in the diets of postpartum dairy cows improved fertility, apparently through enhanced early embryo survival.

Key Words: Pregnancy, Flaxseed,  $\alpha\text{-Linolenic}$ acid

M36 Completion of the Midwest Consortium Project: Sequencing of 21,499 reproduction ESTs and comparative mapping of 721 selected genes. C. K. Tuggle\*1, J. A. Green², C. Fitzsimmons¹, R. Woods², R. S. Prather², S. Malchenko³, M. B. Soares³, T. Kucaba³, K. Crouch³, C. Smith³, D. Tack³, N. Robinson³, B. O'Leary³, T. Scheetz³, T. Casavant³, D. Pomp⁴, J. B. Edeal⁴, Y. Zhang¹, Z. Hu¹, M. F. Rothschild¹, K. Garwood⁵, and W. Beavis⁵, ¹lowa State University, Ames, IA, ²University of Missouri-Columbia, Columbia, MO, ³University of lowa, lowa City, IA, ⁴University of Nebraska, Lincoln, NE, ⁵National Center for Genomic Resources, Sante Fe, NM.

To accelerate genetic improvement of reproductive traits, both molecular and comparative genomic data are required. We are developing extensive sequence and mapping data for cDNAs expressed in female reproductive tissues. We have produced 25 cDNA libraries from different stages of estrus or gestation for embryo, anterior pituitary, hypothalamus, ovary, uterus, and term placenta. A total of 21,499 EST sequences from random clones have been submitted to Genbank. The average read length across this dataset is >400 base pairs. As assessed by clustering analysis, these data represent 10,574 different genes. A BLAST analysis of these clusters indicates that 4,652 are unique relative to porcine Genbank genes/ESTs (BLAST score <200). To facilitate selection of genes for comparative mapping, we have developed software to predict the cytogenetic location of pig ESTs. We identified pig EST matches (BLAST score >200) to human loci that have consistent cytogenetic and RH mapping locations, and then predicted the pig location of high-scoring ESTs based on mapping data and human:pig chromosome painting information. A total of 721 loci have been mapped across all chromosomes, concentrating on pig chromosomes (1,4,6,7,8,15,X) where litter size or other reproductive QTL have been localized. More than 90% of these loci map to the chromosome predicted by comparative data. A WWW site (http://pigest.genome.iastate.edu) has been established for access to these sequences and the analysis data. This set of sequence and map data can be immediately used to study reproductive biology and look for genes controlling quantitative reproduction traits.

**Key Words:** Expressed sequence tags, Porcine reproduction, Comparative mapping

M37 Effect of semen packaged in 0.25 and 0.50 cc straws on conception rate of lactating dairy cows. N. Michael\*, C. Marti, E. Roberts, and M. Pace, ABS Global, Inc..

Cost and efficiency of semen storage can be dramatically improved by packaging semen in 1/4 cc straws. However, it is not clear if fertility of lactating dairy cows would be different by using 1/4 cc straws compared to 1/2 cc straws. This study evaluated the effect of straw packaging size (1/4 cc vs 1/2 cc straw) on conception rates in lactating dairy cows. At time of collection, semen from each A.I. sire (n = 8) was divided equally between 1/4 and 1/2 cc straws using a split collection technique. All straws were packaged and frozen using the ABS Global wind-tunnel freezing process. Numbers of sperm per straw were the same for 1/4 and 1/2 cc straws. Both straw types were equally divided by sire within each herd where herd owners chose the A.I. sires used in their herd; the number of sires used within the herds was one to four, for a total of 17 sire within herd comparisons. The fewest number of inseminations per herd x sire x straw type was 125. Cows (n = 6602) from eight herds located in Idaho and California were randomly inseminated by odd-even days of the month to receive A.I. in the uterine body from either (even day; n=3373) or 1/4 cc (odd day; n=3229) straws from seven professional A.I. technicians between September 2001 and October 2002. Straws were thawed in 35 # 37°C water baths for a minimum of 30 seconds and then held thermo-neutral until A.I. Pregnancy diagnoses were performed between 35 and 42 days following A.I. by the herd veterinarian in cows that had not returned to estrus during this period. Cows that were detected in estrus and re-inseminated between A.I. and pregnancy diagnosis were defined as not pregnant. All inseminations and pregnancy diagnoses information were entered into Dairy Comp 305. Data were retrieved from Dairy Comp 305 from each herd, summarized by sire comparison within herd and entered into Excel. Data were analyzed using a paired t-test on the conception rate means for each straw package type. Conception rates were similar (P > 0.05) between 1/2 (31.1 %) and 1/4 cc (31.3 %) straws. In summary, comparison of multiple A.I. sires in multiple locations indicated that

fertility was not different from semen in 1/4 vs 1/2 cc straws packaged using the ABS Global wind-tunnel freezing process.

Key Words: Semen packaging, Conception rate, Dairy cows

M38 Ovarian follicular development in first parity sows subject to varied split-weaning protocols. J. Barry\*, W. T. Dixon, and G. R. Foxcroft, Swine Research & Technology Centre, University of Alberta, Canada..

Split-weaning (SW) of first parity sows decreases the weaning to estrous interval (WEI) and advances ovarian follicular development. However, follicles ovulating soon after weaning start development during lactation when sows are often in a catabolic state. We hypothesise that an extended interval between SW and final weaning will induce atresia in this wave of disadvantaged follicles, trigger a new wave of follicle development after weaning when sows will be less catabolic, marginally increase the WEI, but improve overall sow fertility. To test this hypothesis, first parity sows with standardized litters were randomly allocated to be either Control (C; n=45) or SW (all but the lightest 6 piglets removed) at d14 of lactation (n=45). Feed intake, litter growth and sow metabolic state were monitored during lactation. Ovarian follicular development was determined morphologically after euthanizing groups of C and SW sows (n=15) on d16, 18 and 20 of lactation. A baseline of follicular development was established in an additional group of 15 sows euthanized on d14 (C14). Fewer (5/15; P<0.05) C14 sows had follicles  $\geq$ 3mm diameter compared to all other groups, indicating a critical and possibly coordinated wave of follicular development between d14 and 16 of lactation. SW increased (P<0.05) the total number of follicles ≥3mm, mean size of the largest 10 follicles, maximum follicle size, mean FF volume, and the percentage of follicles in the  $\geq$ 5mm category. SW increased (P<0.05) plasma IGF-1 at weaning ( $105\pm3$  vs.  $87\pm3$  ng/mL) and decreased sow body mass loss during lactation  $(5.9\pm1.0 \text{ vs. } 9.1\pm1.0 \text{ })$ kg). Also, irrespective of treatment, plasma IGF-1 was lower (P<0.05) at d14 and weaning, and the decrease in loin muscle depth during lactation was greater (P<0.05), in sows with follicles <3mm diameter at slaughter. Increased catabolism during lactation can therefore critically limit follicle development. Refinements in SW protocols, based on a better understanding of ovarian follicular development in SW sows, have the potential to improve the fertility of weaned, first parity, sows.

Key Words: sow, lactation, ovary

M39 Do calcium-mediated cellular signalling pathways, PGE<sub>2</sub>, estrogen or progesterone receptor antagonists, or bacterial toxins affect bovine placental function in vitro? C. Weems\*<sup>1</sup>, Y. Weems\*<sup>2</sup>, T. Welsh³, G. Carsten⁴, and R. Randel⁵, 1,2 Univ. of Hawaii, 3,4,5 Texas A&M Univ.

The bovine placenta secretes little progesterone (P<sub>4</sub>) when the CL is functional (Conley and Ford, J. Anim Sci 65:500, 1987), while the placenta secretes half of the circulating P<sub>4</sub> at day-90 of pregnancy in sheep (Weems et al Prostaglandins 46:277, 1992) and PGE<sub>2</sub> appears to regulate ovine placental secretion of P4 (Bridges et al, Prostaglandins and Other Lipid Mediators 58:113, 1999). Calcium has been reported to regulate placental P<sub>4</sub> secretion in cattle (Shemesh et al, PNAS 81:6403, 1983). Diced placental slices from 193-243 day Brahman and Angus cows were incubated in vitro at 39.5 C under 95% air:5% CO<sub>2</sub> at PH 7.2 in 5 ml of M-199 for 1 hr in the absence of treatments and for 4 and 8 hr in the presence of treatments at a dose of 100ng/ml to determine regulation of placental function. Treatments were: vehicle; R24571; Compound 48/80; IP3; PGE2; CaCL2; cyclosporin A; lipopolysaccharide from Salmonella abortus, enteriditis, and typhimurium; monensin;, ionomycin; arachidonic acid, mimosine; palmitic acid; androstenedione, estradiol-17 $\beta$ ; A23187; RU-486; or MER-25. Jugular and uterine venous plasma and culture media were analyzed for  $P_4$ ,  $PGE_2$ , and  $PGF_2\alpha$  by RIA. Hormone data in blood were analyzed by a one way ANOVA and in culture media by a 2x21 Factorial Design for ANOVA since breeds did not differ (P>0.05) and were pooled.  $PGE_2$  in uterine venous blood was two fold greater (P< 0.05) in Angus than Brahman cows. PGE<sub>2</sub> and  $PGF_2\alpha$  in the vehicle controls increased from 4 to 8 hr (P<0.05), but not P<sub>4</sub> (P>0.05). Progesterone in culture media treated with RU-486 increased (P<0.05) at 4 and 8 hr compared to vehicle controls and was not affected by other treatments (P>0.05). All treatments decreased (P<0.05) PGE2 at 4 and 8 hr except treatment with PGE2 at 4 and 8 hr and RU-486 at 8 hr (P>0.05).  $PGF_2\alpha$  was increased (P< 0.05) by RU-486 at 8 hr and no other treatment affected  $PGF_2\alpha$  at 4 or 8 hr (P>0.05). In conclusion, modulators of cellular calcium signalling pathways given alone does not affect placental  $P_4$  secretion,  $P_4$  receptormediated events appear to suppress placental  $PGF_2\alpha$  secretion, and  $P_4$  receptors may play a role in placental secretion of  $P_4$  in cattle. In addition,  $PGE_2$  does not appear to regulate bovine placental  $P_4$  secretion.

Key Words: Placenta, Progesterone, Prostaglandins

M40 Does estrous synchronization affect corpus luteum (CL) function? C. Weems\*1, Y. Weems1, S. Tatman², A. Lewis², D. Neuendorff², and R. Randel², ¹Univ Hawaii, ²Texas A&M Univ.

Bovine CL secretes  $PGE_2$  and  $PGF_2\alpha$  in vitro, which increases with time (Weems et al, Prostaglandins 55:359, 1998). Synchronization with Synchromate B (SMB) in Brahman heifers decreases LH, conception rates, and circulating progesterone (P<sub>4</sub>; Rentfrow et al, Therio 28:355 1987). Nitric oxide (NO: Jaroszewski & Hansel, Proc Soc Expt Biol Med 224:50, 2000) and endothelin-1 (ET-1; Milvae, Rev Reprod 5:1, 2000) were reported to be luteolytic. In Expt 1, estrus in Brahman cows was synchronized with SMB and d-13 to 14 CL and caruncle slices were weighed, diced, and incubated in vitro. Treatments(100 ng/ml) were: vehicle A, L-NAME, L-NMMA, DETA, DETA-NONOate, sodium nitroprusside, or ET-1. In Expt 2, estrus was synchronized with Lutalyse, a CIDR, or natural; CL were collected and weighed on d-14; and CL slices were diced and incubated in vitro with treatments. Treatments (100 ng/ml) were: vehicle, L-NAME, L-NMMA, DETA, DETA-NONOate, sodium nitroprusside, SNAP, or ET-1. Tissues were incubated in M-199 for 1 hr without and for 4 and 8 hr with treatments. Media were analyzed for  $P_4$ ,  $PGE_2$ , and  $PGF_2\alpha$  by RIA. Hormone data in Expts 1 and 2, were analyzed by a 2x7 and a 3x2x8 Factorial Design for ANOVA, respectively, and CL weights in Expt 2 by a one way ANOVA. Concentrations of  $P_4$  were similar (P>0.05) among treatments within experiments. Concentrations of PGE<sub>2</sub> in CL samples in Expt 1 were undetectable in 90 and 57 % of the samples at 4 and 8 hr, respectively and  $PGF_2\alpha$ increased (P<0.05) with time but did not differ (P>0.05) among treatments. Secretion of PGE<sub>2</sub> or PGF<sub>2</sub> $\alpha$  by caruncles increased (P<0.05) with time and was not affected by treatment (P>0.05). In Expt 2, CL weights were decreased (P<0.05) by Lutalyse. Concentrations of PGE<sub>2</sub> and  $PGF_2\alpha$  increased (P<0.05) with time in controls of all three synchronization regimens. DETA-NONOate, SNAP, sodium nitroprusside (NO donors) and ET-1 increased (P<0.05) PGE<sub>2</sub> except in the CIDR group (P>0.05), and no treatment increased (P>0.05)  $PGF_2.\alpha$  in any synchronization regimen. It is concluded that SMB and a CIDR alters CL PGE<sub>2</sub> secretion, Lutalyse lowers CL weights in the next estrous cycle, and NO or ET-1 given alone are not luteolytic agents. It is possible that NO could have indirect luteotropic effects via increasing PGE<sub>2</sub> secretion by luteal tissue.

Key Words: Estrous synchronization, Progesterone, Prostaglandins

M41 Photoperiod and diet effects on heifer development. J. A. Small\*<sup>1</sup>, A. D. Kennedy<sup>2</sup>, and D. R. Ward<sup>1</sup>, <sup>1</sup>Agriculture & Agri-Food Canada, Research Centre, Brandon, MB, Canada, <sup>2</sup>University of Manitoba, Winnipeg, MB, Canada.

A 2\*2 factorial arrangement of photoperiod (A vs W) and diet (C vs S) treatments was applied to spring-born crossbred beef heifers (n = 144) assigned at weaning (Sep 21; 0 wk) by body weight (247 $\pm$ 19 kg) and age (191±12 d) to one of four pens in one of two similar open shed/drylot facilities. Supplemental light (350 lux, 1 m above ground) was used to extend photoperiod (natural + supplemental light) to 16 h for 12 wk starting on either Sep 27 (A), or Dec 20 (W). Diets were formulated to achieve 60% mature body weight at 32 wk, through either constant (C), or low then high (S) gain during the prepubescent (4 to 16 wk) and pubescent (16 to 24 wk) periods, respectively. One diet for moderate gain was provided to all groups from 0 to 4 and 24 to 32 wk. From 0 to 32 wk, observations of estrus were made twice daily, and estrus confirmed by progesterone in blood serum collected 8 to 12 d later, and body weight, backfat and serum prolactin were measured for each 4 wk period. Ambient temperatures averaged 3.4±12.1°C, -16.0±7.4°C and 0.3±8.0°C for the autumn (0 to 12 wk), winter (12 to 24 wk) and spring  $(24\ {\rm to}\ 32\ {\rm wk})$  periods. During the prepubescent period, weight gain was greater for C than S (0.74 vs 0.66 ±0.01; P<0.05), backfat greater for A than W (1.24 vs 0.87  $\pm$ 0.08; P<0.05), and only 9% of heifers attained puberty by 12 wk. During the pubescent period, diet influenced growth such that, as yearlings (24 wk), weight and backfat were lower for C

than S (392 vs 381  $\pm$ 3.4 kg and 2.6 vs 3.3  $\pm$ 0.12 mm; P<0.05), but the proportion of heifers that had two estruses was greater for A than W (48.6% vs 30.9%; P<0.05). Prolactin, initially 16.3 $\pm$ 1.6 ng/ml, was higher for A than W from 4 to 12 wk, and lower for A than W from 16 to 24 wk (12 wk; 10.1 vs 1.1 and 24 wk; 6.7 vs 20.0  $\pm$ 1.8 ng/ml, P<0.05). Extended photoperiod in autumn advanced puberty independently of the effects of diet on growth, and acute change in photoperiod influenced prolactin, in heifers housed outdoors.

Key Words: Photoperiod, Puberty, Prolactin

M42 Heat shock increases glutathione in bovine oocytes. R. R. Payton\*1, P. Coy², R. Romar², J. L. Lawrence¹, and J. L. Edwards¹,  $^1$  The University of Tennessee, Knoxville, USA,  $^2$  The University of Murcia, Murcia, Spain.

Heat shock increases glutathione (GSH) content in a variety of cell types including embryos. Objective of this study was to examine GSH content in bovine oocytes cultured at an elevated temperature during maturation. Cumulus-oocyte complexes were randomly allocated to one of three treatments and then cultured in the following manner:  $38.5^{\circ}$ C for 24 h (Control),  $41^{\circ}$ C for 6 h followed by  $38.5^{\circ}$ C for 18 h (HS 0-6), or  $41^{\circ}$ C for

12 h followed by 38.5°C for 12 h (HS 0-12). After 24 hours, oocytes presumed mature were denuded of cumulus by vortexing. Pools of oocytes (25-32/treatment group) were solubilized in 0.63 M phosphoric acid and frozen at -20°C until further analysis. Glutathione content was determined using a 5,5'-dithiobis(2-nitrobenzoic acid)-glutathione disulfide reductase recycling assay and was expressed as per oocyte. Intra-assay coefficient of variation was 7.7%. Data were analyzed using an incomplete block design using mixed models of SAS after testing for normality. The experiment was replicated on 5 occasions and included a total of 8 to 11 pools of oocytes per treatment (236-330 total oocytes/treatment). Culture of oocytes at 41°C during the first 12 h of maturation increased GSH content (4.4 versus 2.7 and 1.6 pmol/oocyte for HS 0-12, Control and HS 0-6, respectively; SEM=0.57; P=0.02). Increases in an antioxidant such as GSH, suggest heat-induced increases in free radicals. Cytoplasmic perturbations involving increased free radical production may be one of several mechanisms contributing to reduced developmental competence of heat-stressed oocytes. Supported in part by USDA Initiative for Future Agricultural and Food Systems Program, "Improving Fertility of Heat-Stressed Dairy Cattle"; Grant #2001-52101-11318.

Key Words: Heat shock, Oocyte, Glutathione

## **Lactation Biology**

M43 Intramammary infusion of prostaglandin  $E_2$  (PGE) increases mammary development and milk yield of cows induced to lactate. J. M. Lukas\*<sup>1</sup>, W. J. Weber<sup>1</sup>, R. J. Collier<sup>2</sup>, J. L. Vicini<sup>3</sup>, M. F. McGrath<sup>3</sup>, and B. A. Crooker<sup>1</sup>, <sup>1</sup> University of Minnesota, St. Paul, <sup>2</sup> University of Arizona, <sup>3</sup> Monsanto Agricultural Group, St. Louis, MO.

Effects of intramammary infusions of PGE on mammary development and milk yield of cows induced to lactate were evaluated using a halfudder model. Multiparous, nonpregnant, healthy, reproductive cull Holstein cows (N = 11) were dry for 50 d prior to treatment initiation. Cows were induced to lactate by twice daily subcutaneous (SQ) injections of 17 $\beta$ -estradiol (0.05 mg/kg BW/injection) and progesterone (0.125  $\,$  mg/kg BW/injection) on d 1 through 7 and once daily SQ injections of POSILAC® (500 mg bST) on d 1, 11 and 21. On d 13, 16, 18 and 20, right and left quarters of each cow were infused with 10 ml of PGE (0.85 mg) or excipient and the quarters massaged to disperse infusate. Intramuscular injections of dexamethasone (0.05 mg/kg BW/d) were administered on d 21, 22 and 23 and 2X milking initiated on d 22. Cows received bST on d 31. Change in mammary gland development was assessed by photo documentation. Milk yield per quarter was determined for 14 d and half udder samples collected at 5 and 12 d in milk (DIM) for composition analyses by DHIA. Data from udder halves were analyzed as repeated measures using PROC MIXED of SAS with P < 0.05. Nine cows had visibly larger PGE treated half-udders by d 18. One cow was milked for only 2 d due to temperament. All cows were induced successfully as the untreated udder halves produced at least 4.5 kg/d by 14 DIM. Milk yields were greater in PGE half-udders in all cows on all days (5.1 vs.  $9.4 \pm 0.7$  kg milk; 6.5 vs.  $12.3 \pm 0.8$  kg FCM; 6.1 vs. 11.40.8 kg SCM). Milk composition did not differ between halves at 5 or 12 DIM. Milk fat content was unchanged  $(5.5 \pm 0.2\%)$  but protein (4.3 vs.) $3.7 \pm 0.1\%$ ) and log SCC (5.5 vs.  $5.0 \pm 0.1$ ) decreased and lactose (4.7 vs.  $5.0 \pm 0.05\%$ ) increased from 5 to 12 DIM. Results indicate PGE either enhanced mammary development or differentiation resulting in increased milk yield from cows induced to lactate.

 $\textbf{Key Words:} \ \operatorname{Induced} \ \operatorname{lactation}, \ \operatorname{Mammary} \ \operatorname{development}, \ \operatorname{Milk} \ \operatorname{yield}$ 

M44 Effects of induced lactation on milk fatty acid profiles in multiparous Holstein cows. H. C. Hafliger, III\*1, L. H. Baumgard¹, W. J. Weber², M. Chahine², G. C. Lamb², T. H. Klusmeyer³, M. F. McGrath³, J. L. Vicini³, and B. A. Crooker², ¹University of Arizona, ²University of Minnesota, ³Monsanto Animal Agriculture Group, St. Louis, MO.

Fatty acid profiles of milk from cows (previous 305 ME > 8,400 kg) that calved or were induced to lactate were compared to determine effects of induced lactation. Nonpregnant, reproductive culls were induced to lactate after a 50-d dry period by 2X/d subcutaneous (SQ) injection of  $17\beta$ -estradiol (0.05 mg/kg BW/injection) and progesterone (0.125

mg/kg BW/injection) for 7 d (-13 to -7 DIM) and 0 or 1 SQ injection of POSILAC® (500 mg bST) at -13 and -3 DIM. An intramuscular injection of dexamethasone (0.05 mg/kg BW/d) was administered at 0 DIM and 3X milking initiated at 1 DIM. Induced cows received bST at 7 and 17 DIM and at 14-d intervals thereafter. Calved cows received bST at 14-d intervals after 63 DIM. Milk samples collected at 2 and 12 weeks of lactation (WOL) from a subset of successfully induced (10 of 34) and calved (10 of 19) cows were analyzed for fatty acid content. Method (calved, induced) and WOL effects were assessed by PROC MIXED of SAS with P < 0.05. Milk yield and composition of the subsets did not differ from their respective groups. During 2 and 12 WOL, milk yield of calved and induced cows averaged 40.6 and 22.4 kg/d. Milk from calved cows contained more protein (3.5 vs. 3.0%) and other solids (5.9 vs. 5.6%) but fat (4.3%) and log SSC (5.6) did not differ from induced cows. On a weight (mg/g) and molar (% of total moles) basis,  $de\ novo$ fatty acid synthesis and incorporation of preformed fatty acids into milk fat were similar for calved and induced cows. Substrate to product ratios (a proxy of  $\Delta^9$ -desaturase capability) of  $C_{14:0}/C_{14:1}$ ,  $C_{16:0}/C_{16:1}$ ,  $C_{18:0}/C_{18:1}$  and trans-11  $C_{18:1}/cis$ -9, trans-11 CLA were 16 to 28% less for induced cows. Total percentage of cis-9, trans-11 CLA did not differ but contents of trans- $C_{18:1}$  isomers were 17 to 20% less in induced cows. Although induced cows produced less milk and had an enhanced  $\Delta^9$ -desaturase system, overall milk fatty acid profiles were similar.

Key Words: Milk fat, Induced lactation

M45 Effects of different milking intervals on composition of cisternal and alveolar milk in dairy cows. M.A. Ayadi, G. Caja\*, X. Such, and E. Albanell, *Universitat Autonoma de Barcelona, Spain.* 

Milk composition change in cisternal and alveolar compartments at different milking intervals has been rarely studied. Interest is higher as a consequence of robotic milking and milking omission routines. Four Holstein cows (20.5 kg/d, 215 DIM) regularly milked daily at 0800 and 1800 were used to study the effects of different milking intervals on cisternal milk (CIS) and alveolar milk (ALV) in a 5 wk experiment. Experimental milkings were made at random and in duplicate at 4, 8, 12, 16, 20 and 24 h after a regular milking. A wash-out period of 2 d with regular milkings was allowed between experimental milkings. A teat cannula was used to drain CIS after an i.v. injection of an oxytocin receptor blocking agent (Atosiban; 10  $\mu$ g/kg). Oxytocin was then injected to remove ALV. Samples of each milk fraction per quarter were analyzed for composition. Ratio of CIS:ALV varied according to milking interval and averaged 30:70. Milk fat content decreased in CIS and increased in ALV as milking interval increased (P<0.001). Minimum fat percentage in front and rear CIS (0.93%) was reached at the same milking interval (16 h). Milk fat content in ALV was constant during the first 16 h, increasing rapidly thereafter. Final fat content in ALV (6.95%) was higher than CIS initial (5.62%; P<0.05) and final (0.96%; P<0.001) values. Total