Objective was to evaluate fertility of frozen-thawed semen aged for 8.5 h. Estrus was visually assessed three daily times for at least 30 minutes each time. Jersey heifers (age: 13.9 ± 1.4 mo; weight: 272.8 ± 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 ± 1 °C water bath) for 8.5 ± 0.04 min (Control; range 3-14 min) or 8.5 ± 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 visualization of a heifer in estrus and then maintained in a Cito Thaw unit until insemination approximately 8.5 h later. Frozen semen was purchased from various AI organizations (n=6). Individual Jersey bulls 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 °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.

<table>
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<th>Treatment</th>
<th>No. Bred</th>
<th>Pregnant (%)</th>
<th>Calved (%)</th>
<th>Female (%)</th>
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<td>37(62.7)</td>
<td>37(62.7)</td>
<td>9(51.4)</td>
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<tr>
<td>Aged</td>
<td>56</td>
<td>28(50.0)</td>
<td>28(50.0)</td>
<td>11(39.3)</td>
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</table>

Key Words: Frozen semen, Aging, Artificial insemination

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Triennial Reproduction Symposium Poster Session

1. 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.

2. Effects of presynchronization and/or post-breeding treatment with porcine LH or hCG on pregnancy rates in dairy cows. J. P. Kastelic*1 and J. D. Ambrose2, 1Agriculture and Agri-Food Canada, Lethbridge, AB, Canada, 2Alberta Agriculture Food and Rural Development, Edmonton, AB, Canada.

The objectives were to determine the effects of presynchronization with a prostaglandin F2α 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 µg GnRH (Fertilin; Vetoquinol) on Days -1 and -6 or 500 µg cloprostenol (Estrumate; Schering Plough) 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 µg 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<0.06). 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
3 Pregnancy outcome in dairy cows fed diets supplemented with flaxseed or sunflowerseed. J. D. Ambrose1,*, J. P. Kastelic2, R. Corbett1, P. A. Day3, J. A. Small1, and H. V. Petri1,1,1,1Alberta Agriculture Food and Rural Development, Edmonton, AB, 2Agriculture and Agri-Food Canada, Lethbridge, AB, 3Brandon, MB, 4Lennonville, QC, Canada.

The objectives were to determine if a diet enriched in α-linolenic acid (ALA; C18:3n-3) would enhance embryo survival and pregnancy rates in dairy cows. Cows were fed 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, post-partum). Diets continued for 32 d after timed-AI (Day 0) following a presynch/ovsynch protocol using Estrumate (cloprostenol, Schering Plough) and Fertilone ( GnRH, Vetoquinol). Barley silage- and barley grain-based rations were formulated to meet or exceed NRC (2001) requirements. Metabolizable protein and NE3 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 ovxynch regimen and rebred 42 d after first AI, and received oilseeds until 32 d after second AI. Relative to pre-diets 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 at 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, α-Linolenic acid

4 Completion of the Midwest Consortium Project: Sequencing of 21,499 reproduction ESTs and comparative mapping of 721 selected genes. C. K. Tuggle1,*, J. A. Green2, C. Fitzsimmons2, R. Woods2, R. S. Prather2, S. Malchenko1, M. B. Soares1, T. Kucaba1, K. Crouch1, C. Smith1, D. Tack1, N. Robinson1, B. O’Leary2, T. Scheetz2, T. Casavant1, D. Pomp4, J. B. Edele1, Y. Zhang1, Z. Hu3, M. F. Rothschild1, K. Garwood5, and W. Beavis5, 1Iowa State University, Ames, IA, 2University of Missouri-Columbia, Columbia, MO, 3University of Iowa, Iowa City, 4University of Nebraska, Lincoln, NE, 5National Center for Genomic Resources, Sante Fe, NM.

To accelerate genetic improvement of 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, 7:15.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

5 Effect of semen packaged in 0.25 and 0.50 cc straws on conception rate of lactating dairy cows. N. Michael4, 1C. 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. Semen from the same bull (n=8) was divided equally between 1/4 and 1/2 cc straws using a split-colletion 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 °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


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 result 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 ≥5mm 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 ≥5mm, mean size of the largest 10 follicles, maximum follicle size, mean volume, and the percentage of follicles in the ≥5mm category. SW increased (P<0.05) plasma IGF-1 at weaning (105±±3 vs. 87±±4 ng/mL) and decreased sow body mass loss during lactation (5.9±±1.0 vs. 9.1±±1.0 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 improve the fertility of weaned, first parity, sows.

Key Words: sow, lactation, ovary
7 Do calcium-mediated cellular signalling pathways, PGE₂, estrogen or progesterone receptor antagonists, or bacterial toxins affect bovine placental function in vitro? C. Weems¹, Y. Weems⁴, T. Welsh¹, K. Carter¹, and R. Randal⁵, ¹Univ. of Hawaii, ³4,5 Texas A&M Univ.

The bovine placenta secretes little progesterone (Pg) when the CL is functional (Conley and Ford, J. Anim Sci 65:500, 1987), while the placenta secretes half of the circulating Pg at day-90 of pregnancy in sheep (Weems et al, Prostaglandins 49:277, 1992) and PGE₂ appears to regulate ovine placental secretion of Pg (Brooks et al, Prostaglandins and Other Lipid Mediators 58:113, 1999). Calcium has been reported to regulate placental Pg 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% CO₂ in M-199 for 1 h in the absence of treatments and for 4 and 8 h 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; cyclopisinol A; lipopolysaccharide from Salmonella abortus, enteritidis, and typhimurium; mensenin; iononycin; arachidonic acid, mimosine; palmitic acid; androstenedione, estradiol-17β; A23187; RU-486; or MER-25. Jugular and uterine venous plasma and culture media were analyzed for Pg₁, Pg₂, and Pg₂ in RIA. Hormone data in Expts 1 and 2 were analyzed for Pg₁ using a 2x21 Factorial Design for ANOVA since breeds did not differ (P>0.05) and were pooled. Pg₂ in uterine venous blood was two fold greater (P<0.05) in Angus than Brahman cows. Pg₁ and Pg₂ in the vehicle controls increased from 4 to 8 hr (P>0.05), but not Pg₂. 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) Pg₂ at 4 and 8 hr except treatment with Pg₂ at 4 and 8 hr and Ru-486 at 8 hr (P>0.05). Pg₂α was increased (P<0.05) by Ru-486 at 8 hr and no other treatment affected Pg₂α at 4 or 8 hr (P>0.05). In conclusion, modulators of cellular calcium signalling pathways given alone do not affect placental Pg₂ secretion. P₄ receptors-mediated events appear to suppress placental Pg₂α secretion, and Pg₂ receptors may play a role in placental secretion of Pg₁ in cattle. In addition, Pg₂α does not appear to regulate bovine placental Pg₂ secretion.

Key Words: Placenta, Progesterone, Prostaglandins

8 Does estrous synchronization affect corpus luteum (CL) function? C. Weems¹, Y. Weems¹, S. Tamagni², A. Lewis², D. Neendorff², and R. Randal², ¹Univ Hawaii, ²Texas A&M Univ.

Bovine CL secretes PGE₂ and PGF₂α in vitro, which increases with time and was not affected by treatment (P>0.05). In Expt 1, estrus were made to estrus confirmed by progesterone in blood collected 8 to 12 h later, and body weight, backfat and serum prolactin were measured for each 4 wk period. Ambient temperatures averaged 3.4±1.1°C, -16.0±4.7°C and 5.5±8.9°C for the autumn (0 to 12 wk), winter (12 to 24 wk) and spring (24 to 32 wk) periods. During the prepubertal period, weight gain was greater for S than C (0.74±0.01; P<0.05), backfat greater for A than W (1.24±0.08; P<0.05), and only 9% of heifers attained puberty by 12 wk. During the pubertal period, diet influenced growth such that, as yearlings (24 wk), weight and backfat were lower for C than S (392±381 ±3.4 kg and 2.6±3.3±10.1 mm; P<0.05), but the proportion of heifers that had two estruses was greater for A than W (48.6% vs 39.9%; P<0.05). Prolactin, initially 16.3±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 11.4; 24 wk; 6.7 vs 20.0 ±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

9 Photoperiod and diet effects on heifer development. J. A. Small¹, A. D. Kennedy², and D. R. Ward¹, ¹Agriculture & Agri-Food Canada, Research Centre, Brandon, MB, Canada, ²University 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±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 prepubescence (4 to 16 wk) and pubescence (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 4 wk, observations of estruses were made twice daily, and estrus confirmed by progesterone in blood sampled collected 8 to 12 h later, and body weight, backfat and serum prolactin were measured for each 4 wk period. Heat stress increases glutathione in bovine oocytes. R. R. Payton¹, P. Coy², R. Romar³, J. L. Lawrence¹, and J. L. Edwards¹, ¹The University of Tennessee, Knoxville, USA, ²University of the 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°C for 24 h (Control), 41°C for 6 h followed by 38.5°C for 18 h (HS-6), or 41°C for 12 h followed by 38.5°C for 12 h (HS-0-12). After 24 h, oocytes pre- matured 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 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 by 4.4 versus 4.4 ng/oocyte for HS-12. Control and HS-0-12, 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 (1911000197) to control and Food Systems Program, “Improving Fertility of Heat-Stressed Dairy Cattle”; Grant #2001-52101-11318.

Key Words: Heat shock, Oocyte, Glutathione