ABSTRACTS
AMERICAN DAIRY SCIENCE ASSOCIATION
AMERICAN SOCIETY OF ANIMAL SCIENCE

June 22–26, 2003
Phoenix, AZ

*** Author Presenting Paper**

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. Malone[1], F. N. Schrick[1], H. H. Dowlen[2], H. D. Moorehead[2], P. A. Lunn[2], and A. M. Saxton[1], [1]*The University of Tennessee, Knoxville,* [2]*Dairy 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 ± 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°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 visual detection 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 (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°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	59	37(62.7)	37(62.7)	19(51.4)
Aged	56	28(50.0)	28(50.0)	11(39.3)
P-value		0.19	0.19	0.45

Key Words: Frozen semen, Aging, Artificial insemination

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. Ambrose[2], [1]*Agriculture and Agri-Food Canada, Lethbridge, AB, Canada,* [2]*Alberta 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 (Fertiline; Vetoquinol) on Days -10 and -1 and 500 μg 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 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<0.6). 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

3 Pregnancy outcome in dairy cows fed diets supplemented with flaxseed or sunflowerseed. J. D. Ambrose*[1], J. P. Kastelic[2], R. Corbett[1], P. A. Day[1], J. A. Small[3], and H. V. Petit[4], [1]*Alberta Agriculture Food and Rural Development, Edmonton, AB,* [2]*Agriculture and Agri-Food Canada, Lethbridge, AB,* [3]*Brandon, MB,* [4]*Lennoxville, 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 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, 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. Tuggle*[1], J. A. Green[2], C. Fitzsimmons[1], R. Woods[2], R. S. Prather[2], S. Malchenko[3], M. B. Soares[3], T. Kucaba[3], K. Crouch[3], C. Smith[3], D. Tack[3], N. Robinson[3], B. O'Leary[3], T. Scheetz[3], T. Casavant[3], D. Pomp[4], J. B. Edeal[4], Y. Zhang[1], Z. Hu[1], M. F. Rothschild[1], K. Garwood[5], and W. Beavis[5], [1]*Iowa State University, Ames, IA,* [2]*University of Missouri-Columbia, Columbia, MO,* [3]*University of Iowa, Iowa City, IA,* [4]*University of Nebraska, Lincoln, NE,* [5]*National 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

5 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

6 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 ≥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 ≥5mm category. SW increased (P<0.05) plasma IGF-1 at weaning (105±3 vs. 87±3 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 to improve the fertility of weaned, first parity, sows.

Key Words: sow, lactation, ovary

7 Do calcium-mediated cellular signalling pathways, PGE_2, estrogen or progesterone receptor antagonists, or bacterial toxins affect bovine placental function in vitro? C. Weems*[1], Y. Weems[2], T. Welsh[3], G. Carsten[4], and R. Randel[5], [1,2]*Univ. of Hawaii,* [3,4,5]*Texas A&M Univ.*

The bovine placenta secretes little progesterone (P_4) when the CL is functional (Conley and Ford, J. Anim Sci 65:500, 1987), while the placenta secretes half of the circulating P_4 at day-90 of pregnancy in sheep (Weems et al Prostaglandins 46:277, 1992) and PGE_2 appears to regulate ovine placental secretion of P_4 (Bridges et al, Prostaglandins and Other Lipid Mediators 58:113, 1999). Calcium has been reported to regulate placental P_4 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_2 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; PGE_2; $CaCL_2$; cyclosporin A; lipopolysaccharide from Salmonella abortus, enteriditis, and typhimurium; monensin;, ionomycin; 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 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_2 and $PGF_2\alpha$ in the vehicle controls increased from 4 to 8 hr (P<0.05), but not P_4 (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) PGE_2 at 4 and 8 hr except treatment with PGE_2 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 receptor-mediated 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

8 Does estrous synchronization affect corpus luteum (CL) function? C. Weems*[1], Y. Weems[1], S. Tatman[2], A. Lewis[2], D. Neuendorff[2], and R. Randel[2], [1]*Univ Hawaii,* [2]*Texas 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_4; 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_2 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_2 or $PGF_2\alpha$ 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_2 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_2 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_2 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_2 secretion by luteal tissue.

Key Words: Estrous synchronization, Progesterone, Prostaglandins

9 Photoperiod and diet effects on heifer development. J. A. Small*[1], A. D. Kennedy[2], and D. R. Ward[1], [1]*Agriculture & Agri-Food Canada, Research Centre, Brandon, MB, Canada,* [2]*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±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 to 32 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 ±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 ±3.4 kg and 2.6 vs 3.3 ±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±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 ±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

10 Heat shock increases glutathione in bovine oocytes. R. R. Payton*[1], P. Coy[2], R. Romar[2], J. L. Lawrence[1], and J. L. Edwards[1], [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°C for 24 h (Control), 41°C for 6 h followed by 38.5°C for 18 h (HS 0-6), or 41°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