TH172 Identification of fertility markers in seminal plasma proteins of cryopreserved bull semen: A proteomic approach. J. F. Odhiambo*, L. Corum, S. Wolfe, E. E. Felton, and R. A. Dailey, *West Virginia University*, *Morgantown*.

Secretions from the accessory sex glands are mixed with sperm at ejaculation and contribute a majority of semen volume. However, during cryopreservation, most seminal plasma is replaced with semen extenders, mainly egg yolk or milk proteins. Experiments in rodents and pigs showed that seminal plasma was important for fertility and fetal survival (O et al, 1988). Previously, we observed consistent success of pregnancy after AI with adjunctive seminal plasma. The precise nature of the active constituents remains unknown, but four proteins (Osteopontin, Spermadhesin Z13, BSP 30 kDa and Phospholipase A_2) have been identified as markers of fertility in dairy bulls (Moura et al., 2006). Therefore, the present study used a proteomic analysis of expression patterns of seminal plasma proteins in cryopreserved semen of bulls of known low fertility ($1.8 \leq CFI \leq 2.9$; CFI: Composite Fertility Index™, Select Sires Inc.) Proteins were separated by 2-dimensional SDS-PAGE followed by staining with Coomassie blue and analysis of polypeptide maps using CQuest software. Proteins were identified by capillary liquid chromatography nanoelectrospray ionization tandem mass spectrometry (CapLC-MS/MS). Low fertility bulls had three expression patterns. Spot volume analysis and peptide identification of the patterns indicated higher expression (55%) of proteins from semen extender between 30 to 60 kDa, a *train* (35%) of spots within 20 to 30 kDa, while the remainder of the spot volume was expressed below 20 kDa. Three spots were identified as: 1) Seminal plasma protein PDC-109 precursor [BSP-A1/A2], MW 15.4 kDa, pI 4.76, 2) Spermadhesin-1 precursor [Acidic seminal fluid protein], MW 15.0 kDa, pI 4.93, and 3) Spermadhesin Z13, MW 13.4 kDa, pI 5.50. Low fertility of dairy bulls has been associated with higher expression of Spermadhesin Z13 isoforms in accessory gland fluid (Moura et al., 2006). Therefore, identification of that protein and its precursor in seminal plasma of low fertility bulls corroborates and strengthens its potential use as a marker of low fertility in bulls.

Key Words: Spermadhesin, BSP-A1/A2, Seminal Plasma

TH173 Effect of sex-sorted sperm dosage on conception rates of Holstein cows and heifers. J. M. DeJarnette*[1], R. L. Nebel[1], C. E. Marshall[1], J. F. Moreno[2], C. R. McCleary[2], and R. W. Lenz[2], [1]*Select Sires, Inc.*, *Plain City, OH*, [2]*Sexing Technologies, Inc.*, *Navasota, TX*.

Ejaculates were collected by artificial vagina from 3 Holstein sires and sorted to 90% purity for X-chromosome bearing spermatozoa (range 88 to 93%) using flow cytometry. Sorted sperm were diluted to 2.1, 3.5 or 5.0 x 10^6 sperm per dose in an egg-yolk (20%), TRIS, glycerol (7%) extender. Collections were repeated until a total of >600 straws per sperm dose per sire were obtained. Each sperm dose was loaded into color-coded 0.25 mL French straws with alternate colors used to define treatments across sires. Within sires, straws were packaged at 9 per cane (3 of each color) and strategically allocated to 75 Holstein herds with targets for 50% use in heifers and 50% in lactating cows. Straw color was recorded in the on-farm record keeping system at the time of insemination. Data were analyzed separately for cows and heifers. Among heifers, a total of 2,125 useable records were retrieved from 51 herds (238 ± 5.5 services/sperm dose/sire, range: 218 to 263). Conception rates in heifers were influenced by the sire by sperm dosage interaction. Within Sire A, conception rates of heifers were greater for the 5 x 10^6 (59.5%) than for the 2.1 x 10^6 (46.4%) sperm dose and intermediate for the 3.5 x 10^6 sperm dose (52.2%). However across sires, sperm dosage had no effect on heifer conception rates (46.7%, 51.2% and 52.5% for 2.1, 3.5 and 5.0 x 10^6 sperm dosages, respectively). Among cows, a total of 2,369 services were retrieved from 56 herds (263 ± 8.8 services/sperm dose/sire, range: 233 to 303). Conception rates of cows (29.4%) were not affected by sire or sperm dosage (27.0%, 29.1% and 30.3% for 2.1, 3.5 and 5.0 x 10^6 sperm dosages, respectively). In conclusion, these data indicate increased sperm dosage may enhance virgin heifer conception rates for some (but not all) sires, whereas neither sire nor sexed-sperm dosage impacted conception rates of lactating cows. Additional studies of sexed-sperm dosage across a larger sampling of bulls are warranted to determine if and how such a practice can be cost effectively implemented for the benefit of the dairy industry.

Key Words: Sexed Semen, Flow Cytometry, Conception Rate

TH174 An update on the commercial application of sex-sorted semen in Holstein heifers. J. M. DeJarnette*, R. L. Nebel, and C. E. Marshall, *Select Sires, Inc.*, *Plain City, OH*.

Insemination and (or) calving data were retrieved from herds that used sex-sorted semen (SS) in Holstein heifers from Jan, 2005 to Jan 2008. The unadjusted conception rate (CR) to SS across 132 herds and all service numbers was 45.2% (n=41,398). Across all herds, 74% of SS was used at 1st service, 18% at 2nd service, and 8% at ≥3 service. The unadjusted CR was 47%, 40%, and 34% for service numbers 1, 2 and ≥3, respectively. Among 49 herds reporting ≥ 50 services to both sexed (724 ± 132) and conventional semen (CS; 961 ± 110), CR was influenced by herd and the service number x semen type interaction (See table). Among unsuccessful services in these 49 herds, heifers bred to SS were more likely to be re-inseminated in a normal 21 day interval than were CS bred heifers. Among all single births the percentage of female calves born to SS was 89.3% (n=13,515) and increased to 90.1% for validated gestation lengths of 265 to 295 d (n=7,768). Among twin births, a greater percentage ($P<0.01$) of female-female pairs were observed for SS (74%, n=68) than CS (26%, n=197). Among 27 herds reporting ≥ 50 females calves born to SS (340 ± 59) and CS (592 ± 79), the percentage of still births was influenced by herd, age at freshening, month of freshening, and sire of conception but was not influenced by semen source ($P = 0.68$). Due to the known bias in use and preferential treatment of SS vs. CS, these results must be interpreted with caution. However, these data suggest SS averages ~45% CR in Holsteins heifers resulting in ~90% female offspring with no evidence of a negative effect on incidence of still births nor repeat intervals to estrus.

Table 1. Conception rates by service number and distribution of repeat AI intervals in 49 herds reporting ≥ 50 services to both sexed and conventional semen % (n)

	1st Service	2nd Service	≥ 3rd Service		
Conventional	56.4 (21,864)	53.8 (13,389)	45.4 (11,772)		
Sexed	47.0 (26,465)	43.1 (6,375)	38.1 (2,567)		
Sexed as % of conventional	83.3%	80.1%	83.9%		
Repeat AI interval					
	<17 d	18-24 d	25-35 d	36-48 d	49-90 d
Conventional (n=17,184)	10.7%	65.4%	9.6%	9.6%	4.7%
Sexed (n=16,413)	7.4%	69.7%	8.8%	9.5%	4.6%

Key Words: Sexed Semen, Flow Cytometry, Conception Rate

TH175 Use of commercially available oocytes and sexed sperm for IVF/ET and AI in dairy cattle. S. Rasmussen*, Z. Brink, K. McSweeney, and G. E. Seidel, *Colorado State University*, *Fort Collins*.

Assisted reproduction technologies allow options for dairy farmers to improve herd genetics, provide greater biosecurity, and possibly increase profit in other ways. Transfer of embryos (ET) produced with sexed semen, and artificial insemination (AI) with sexed semen are examples. Sexed and non sexed semen from 3 bulls was used for either AI or to produce embryos in vitro. Holstein oocytes, matured in a portable incubator during shipment, were purchased commercially and fertilized 23 h after collection with standard IVF procedures using $1x10^6$ sperm/mL. There was no difference in blastocyst rate per oocyte due to bulls: A (11.1%; n = 893), B (13.5%; n = 1531), or C (11.8%; n = 1362); however, sexed sperm resulted in fewer blastocysts (8.1%; n = 3018) than nonsexed sperm (15.4%; n = 768) ($P<0.01$). Blastocysts were transferred 7.5 d after onset of IVF. Estrous cycles of cows at least 63 d post partum were synchronized as follows: reproductive tracts were scanned using ultrasonography, and cows with a normal-appearing uterus were injected with 100 μg GnRH i.m. Seven d later cows were scanned again and given 25 mg of $PGF_2\alpha$ i.m. only if a CL and follicle between 13-19 mm were present; cows not meeting these criteria were not used for this project; 2.5 d later cows received 100 μg GnRH i.m. Cows were bred 16-18 h later with control ($>10x10^6$ sperm/dose) or sexed semen ($2x10^6$ sperm/dose), or a fresh embryo was transferred one wk later. Pregnancy status was determined at ≥d 35 of pregnancy by ultrasound. Cows with dead or dying fetuses were considered non pregnant. There was no difference ($P>0.1$) between sexed (36.9%; 31/84) and nonsexed AI (47.2%; 42/89) pregnancy rates, which were higher than sexed (19.4%; 14/72) or non sexed (23.2%; 16/69) ET pregnancy rates ($P<0.05$). The pregnancy rate with sexed semen was 78.2% of control semen (mainly due to one bull which was 44.8% of control). Pregnancy rates were unsatisfactory with embryos produced in vitro from shipped oocytes under the conditions of this experiment. Supported by NRI grant no. 2006-55203-17390 from USDA.

Key Words: Sexed Semen, AI, IVF

TH176 Effects of heterospermic insemination on conception rates of lactating dairy cows. J. M. DeJarnette*[1], R. L. Nebel[1], D. Laansma[2], and C. E. Marshall[1], [1]*Select Sires, Inc.*, *Plain City, OH*, [2]*Northstar Cooperative*, *East Lansing, MI*.

This study evaluated the effects of heterospermic insemination on conception rate of Holstein cows. Three Holstein sires with above average conception potential as determined by one or more sire fertility ranking systems were selected for use in this trial. On each of two days, 2 ejaculates were collected by artificial vagina from each sire and evaluated for volume, concentration, and motility before and after pooling within sire. Aliquots were strategically removed from the within sire pools to create an across sire pool containing equal numbers of total sperm from each of the 3 sires (heterospermic). All 4 samples (3 homospermic, 1 heterospermic) were extended to 40 x 10^6 sperm/mL in an egg-yolk-Citrate-glycerol extender. Samples were loaded into color-coded 0.5 mL French straws (n = 500/sample/collection day; 4000 total), and frozen in liquid nitrogen vapor. Straws were randomly used in 5 herds of lactating Holstein cows during the months of Apr. through Oct., 2007. At the time of AI, straw color and collection date were recorded in the on-farm records management system. Data (n = 3,384) were extracted from electronic downloads and analyzed in least-square means models including the effects of herd, sire, batch within sire, parity, postpartum interval, season and all two-way interactions. Conception rates were influenced ($P < 0.05$) by interactions of herd with parity, season, and postpartum interval. All interactions and main effects of sire and semen batch were not significant ($P > 0.05$). Conception rates for sires A, B, C and the heterospermic mix were 26.8%, 25.3%, 29.3% and 25.4%, respectively (n = 846 ± 15/sire). In conclusion, heterospermic insemination may provide a means to guard against poor conception rates should a subfertile sire be selected. However these data provide no evidence that the conception rate of a heterospermic mixture will be greater than that obtained from homospermic use of the highest conception bull(s) in the mix.

Key Words: Heterospermic Insemination, Mixed Semen, AI

TH177 Effects of preincubation of sperm at 38.5 or 40°C before insemination on developmental competence of bovine embryos derived from in vitro fertilization. K. E. M. Hendricks* and P. J. Hansen, *University of Florida*, *Gainesville*.

The ability of the preimplantation embryo to develop depends in part on genetic and non-genetic inheritance from gametes. Here, the hypothesis was tested that aging of ejaculated sperm would affect embryo competence for development. Oocytes were inseminated with frozen/thawed sperm maintained for 4 h at 38.5°C or 40°C. Control oocytes were inseminated with sperm not subjected to 4 h incubation. The percent of cleaved embryos that became blastocysts at d 8 after insemination (Bl/Cl) was used to assess embryo competence. In Exp. 1 (n = 9 bulls), oocytes were fertilized for 22 h and the resultant embryos cultured in 5% CO_2 in air. The percent of oocytes that became blastocysts (Bl/Ooc) and Bl/Cl was lower for oocytes inseminated with preincubated sperm than for oocytes inseminated with control sperm ($P<0.01$). There were no differences between oocytes inseminated with sperm at 38.5°C when compared to 40°C. For Exp. 2 (n = 13 bulls), embryos were cultured in 5% O_2. The percent of oocytes that cleaved (66.1 ± 3.2, 60.2 ± 3.2%, and 74.5 ± 3.2% for 38.5°C, 40°C and control; $P<0.05$), Bl/Ooc (23.6 ± 2.3, 14.9 ± 2.3%, and 27.9 ± 2.3%, $P<0.01$), and Bl/Cl (32.9 ± 2.9, 22.2 ± 2.9%, and 37.4 ± 2.9%; $P<0.05$) was lower for oocytes insemi-

nated with preincubated sperm. Similarly, Bl/Ooc ($P<0.05$) and Bl/Cl ($P<0.05$) was lower for oocytes inseminated with sperm at 40°C than for oocytes inseminated with sperm at 38.5°C. A high cleavage rate in a parthenogenesis group (38.9 ± 3.2%) made interpretation of data difficult because of the possibility that fertilization rate was overestimated. Accordingly, Exp. 3 (n = 8 bulls) was carried out with fertilization reduced to 8 h. There were no differences between oocytes inseminated with sperm preincubated at 40°C compared to oocytes inseminated with sperm preincubated at 38.5°C. Results indicate that embryos produced with aged sperm can have reduced competence for development although aging effects were independent of temperature within the range of 38.5-40°C. Support: USDA NRICGP 2007-35203-18070 and BARD US 3986-07.

Key Words: Bovine, Sperm Preincubation, Embryo Developmental Competence

TH178 Paternal influence on the in vitro embryonic development of vitrified oocytes based on estimated relative conception rate. V. M. Anchamparuthy*, A. Dhali, R. E. Pearson, W. M. Lott, and F. C. Gwazdauskas, *Virginia Polytechnic Institute & State University*, *Blacksburg*.

Variation with in vitro fertilization (IVF) characteristics among bulls was reported, but has not been reported with vitrified oocytes. This study was designed to test the influence of sire based on ERCR after AI with the frozen thawed semen on the in vitro embryonic developmental competence of 2 classes of vitrified oocytes (n = 1,968) derived from follicles of different diameters, small (less than 4 mm) and medium (4 to 10 mm). Follicle size had a significant ($P \leq 0.05$) impact on the developmental rates of embryos from cleavage to the blastocyst stage. The oocytes from medium sized follicles had a 9.9% increase in the cleavage rate compared to oocytes from small follicles. The increase in the blastocyst rate was only 5.9% between the oocytes from small and medium-sized follicles. The sires showed significant ($P \leq 0.05$) effects on embryonic developmental rates. Similarly, sire by follicle size interaction was significant for blastocyst rates and sire by treatment interaction was significant on cleavage and blastocyst rates. There were differences among bulls. Fertilized oocytes (n = 1,685) using semen from Bulls I, II, III, and IV showed cleavage rates of 49.3, 55.0, 50.0, and 40.9%, respectively ($P \leq 0.05$). Both morula and blastocyst stages of development were significantly different ($P \leq 0.05$) among bulls. While the developmental rates to morula were 26.4, 29.9, 23.4 and 16.7% for Bull I, II, III, and IV, respectively, and the blastocyst developmental rates were 18.1, 17.9, 10.0, and 10.0%, respectively. Bull I had the highest blastocyst development from vitrified oocytes (10.9%; $P \leq 0.05$) and the corresponding rate for its control oocytes was 25.3%. The blastocyst rates from the vitrified oocytes were 6.0, 2.5, and 1.0%, respectively, for Bull II, III and IV, with the corresponding rates in the control oocytes of 29.9, 17.4, and 18.9%, respectively. Our results show that differences in development exist in source of oocytes and sires used for IVF after vitrification. No correlation exists between post-IVF outcome and field fertility based on ERCR.

Key Words: Bull, Oocyte, Vitrification

TH179 Effects of dietary fats differing in n-3/n-6 ratio on oocyte quality in dairy cows. M. Zachut*[1,2], I. Dekel[1], H. Lehrer[1], A. Arieli[2], A. Arav[1], and U. Moallem[1], [1]*Agriculture Research Organization*, *Bet Dagan, Israel*, [2]*Faculty of Agriculture, Hebrew University*, *Rehovot, Israel*.

The objective of the current study was to examine the effects of feeding encapsulated fats containing different n-3/n-6 ratio on oocyte quality in dairy cows. Twenty-four multiparous Israeli-Holstein cows, averaging 114 DIM, were assigned to one of three treatment groups: 1) **Control -** (n = 7) were fed a lactating cow diet; 2) **E-LNA -** (n = 8) were supplemented with 1 kg/d of encapsulated fat containing 40% linseed oil which is high in linolenic acid; or 3) **E-LA -** (n = 9) were supplemented with 1 kg/d of encapsulated fat containing 40% sunflower oil which is high in linoleic acid. Ovum pick-up (**OPU**) and *in vitro* fertilization (**IVF**) sessions (n = 13) began 94 d after the commencement of the dietary treatments, and were conducted on 5 cows per treatment twice a week. Plasma FA profile was significantly affected by dietary treatments; the percentages of C18:2, C20:3 and C20:4 in plasma were lower in E-LNA cows than in E-LA or control ($P < 0.004$), whereas the percentage of C18:3 in plasma was more than 5 fold higher in E-LNA cows as compared to E-LA or control cows ($P < 0.0001$). The average number of follicles aspirated during the OPU procedure from each cow at each session was higher in E-LNA cows than in control ($P < 0.01$; 11.4, 13.8 and 11.8 for control, E-LNA and E-LA, respectively), and the number of recovered oocytes per cow was higher in control than in E-LA ($P < 0.05$) but did not differ from the E-LNA. The average number of grade 1 oocytes per cow was lower in E-LA than in both other groups ($P < 0.05$). A total of 388 oocytes were selected for IVF across treatments. The percentage of maturated oocytes did not differ among treatments; however the oocytes cleavage rate was higher in E-LNA oocytes than in control ($P < 0.05$; 43.0, 52.3 and 49.5% for control, E-LNA and E-LA, respectively). In conclusion, dietary fat with a high n-3/n-6 ratio (linseed oil) seems to positively affect ovarian follicle numbers and IVF cleavage rate.

Key Words: Linolenic Acid, Oocytes Quality

TH180 Influence of cysteine in conjunction with growth factors during in vitro production of bovine embryos. W. M. Lott*, V. M. Anchamparuthy, M. L. McGilliard, I. K. Mullarky, and F. C. Gwazdauskas, *Virginia Polytechnic Institute & State University*, *Blacksburg*.

Cysteine (Cys) supplementation to in vitro maturation (IVM) media increases cellular glutathione production, which reduces reactive oxygen species. Similarly, the beneficial effects of growth factors for improving the rate of blastocyst development have been reported, but not in conjunction with Cys. The aim of this study was to determine whether supplementation of Cys during oocyte maturation and growth factors, epidermal growth factor (EGF) and insulin-like growth factor-I (IGF-I), during embryo culture, would improve cleavage and blastocyst rates of in vitro produced bovine embryos. Bovine oocytes from slaughterhouse ovaries were matured in TCM-199, with or without the addition of Cys (0.6 mM) at 0 h (0 h Cys) or 12 h (12 h Cys) of maturation. Matured oocytes were fertilized in BSA. After in vitro fertilization, embryos were allocated to culture treatments using synthetic oviductal fluid medium. Culture treatments included fetal calf serum (FCS, 4%); IGF (100 ng/mL); EGF (10 ng/mL); IGF+EGF (100 ng/mL+10 ng/mL); 0 h Cys with

IGF; EGF; or IGF+EGF; and 12 h Cys with IGF; EGF; or IGF+EGF. Developmental scores were assigned to each embryo on d 2, 4, and 8 of culture. Even though rates for cleavage and blastocyst stage were not different ($P \geq 0.05$) among treatments, significant improvements in the proportion of oocytes undergoing cleavage (12 h Cys IGF+EGF, 75.3%) and blastocyst (0 h Cys IGF, 40.3%) formation were achieved when Cys was added to the maturation media, as compared to control (FCS: cleavage, 61.7% and blastocyst, 34.5%) without supplementation. Similarly a combined effect of IGF+EGF for the cleavage (72.6%) and blastocyst (37.8%) development was obtained. In conclusion, supplementation of Cys during IVM of oocytes, in conjunction with growth factors, excluding IGF-I, during in vitro culture, resulted in a similar embryonic development to that of FCS, and could effectively be used as a replacement for FCS.

Key Words: Cysteine, Embryo, Growth Factor

TH181 Simulated microgravity conditions affect preimplantation bovine embryo development *in vitro*. S. Jung*, S. D. Bowers, and S. T. Willard, *Mississippi State University*, *Mississippi State*.

Alternative *in vitro* embryo culture environments require further investigation to determine whether they may improve, or be deleterious to, embryo development. The objective of this study was to investigate bovine preimplantation embryo development in a simulated (and varied) microgravity environment *in vitro*. A Rotating Cell Culture System (RCCS) bioreactor with a High Aspect Ratio Vessel (HARV) was used to generate a low shear simulated microgravity condition. *In vitro* matured and fertilized bovine oocytes were cultured in mSOF (modified synthetic oviductal fluid supplemented with 8 mg/ml BSA and amino acids) at 39°C and 5% CO_2 in humidified air. On d 2 pi (post insemination), cleaved embryos (2–, 4–, and 8–cell stage embryos n=1524) were equally distributed to one of following culture conditions: Control, 100 µl microdrops of mSOF medium covered by mineral oil in a Petri dish; High Speed, 100 µl microdrops of mSOF medium with mineral oil overlay in HARV rotated at 34 rpm on a horizontal axis; Low Speed, 100 µl drops of mSOF medium with mineral oil overlay in HARV rotated at 3.7 rpm on a horizontal axis. The final developmental rates were assessed on d 9 pi. The experiment was replicated four times and arc–sine transformed data were analyzed by ANOVA and Fisher′s PLSD test. The proportions of 2– to 8–cell stage embryos in the High Speed and Low Speed groups (100.0 and 99.6%, respectively) were significantly higher ($P < 0.05$) than 2– to 8–cell stage embryos in the Control group (67.7%) on d 9 pi; with only 1 embryo reaching the morulae stage for the Low Speed group. Thus in the Control group, a greater ($P < 0.05$) proportion of morulae and blastocyst stage (15.8% and 16.5%, respectively) embryos were achieved than in the High (0%) and Low (0% blastocyst and 0.4% morulae) groups. There were no significant differences ($P > 0.05$) in embryo development rates between the High and Low Speed groups with respect to any of the embryo stages noted on d 9 pi. These results indicate that simulated microgravity culture conditions have a negative impact on preimplantation bovine embryo development *in vitro*.

Key Words: Bovine Embryo, *In vitro* Culture, Microgravity

TH182 Insulin-like growth factor-1 reduces the anti-development effects of menadione on development of bovine preimplantation embryos. J. I. Moss* and P. J. Hansen, *University of Florida*, *Gainesville*.

Insulin-like growth factor-1 (IGF-1) can reduce effects of heat shock on development of preimplantation bovine embryos. The present objective was to determine whether IGF-1 can block other adverse environmental conditions affecting embryonic development. The approach was to evaluate effects of IGF-1 on embryos treated with menadione. This polycyclic aromatic ketone serves as a precursor for vitamin K2 and can inhibit development of mouse embryos (Toxicology 191:77). Bovine embryos were produced in vitro and cultured in KSOM-BE2 medium. At d 5 after fertilization, embryos ≥ 16 cells were placed in fresh drops ± 100 ng/ml of recombinant human [Arg3]-IGF-1 (analog with reduced affinity for IGF binding proteins). Menadione was added 1 h after addition of IGF-1 at a final concentration of 0, 1, 2.5 and 5 µM. Embryos were cultured for 24 h, washed, and then cultured in fresh KSOM-BE2 medium until d 8. The experiment was replicated 14 times with 120-204 embryos per treatment. The percent of embryos that became blastocysts at d 8 was affected by the menadione x IGF interaction ($P<0.001$). Menadione reduced development in a concentration-dependent manner and IGF-1 reduced effects of 1 and 2.5 µM. Percent of embryos becoming blastocysts was 50.1 ± 2.4%, 36.2 ± 2.9%, 5.8 ± 2.9%, and 0 ± 2.6% for 0, 1, 2.5 and 5 µM in the absence of IGF-1 and 37.0 ± 2.5%, 46.3 ± 2.5%, 13.0 ± 2.5% and 0 ± 2.6% in the presence of IGF-1. In conclusion, the anti-developmental action of menadione on development of bovine embryos can be blocked by IGF-1. Research supported by BARD US-3986-07 and USDA NRICGP 2007-35203-18070.

Key Words: Insulin-Like Growth Factor-1, Preimplantation Embryos, Menadione

TH183 Effect of the addition of hyaluronan to bovine embryo culture on in vitro survival after cryopreservation and in vivo survival following transfer to recipients. L. Bonilla*[1], J. Block[1,2], and P. J. Hansen[1], [1]*University of Florida*, *Gainesville*, [2]*EmboGen LLC*, *Gainesville, FL*.

Experiments were conducted to determine whether hyaluronan could: 1) improve survival after cryopreservation, and 2) increase pregnancy rates following transfer to recipients. In experiment 1, embryos (n = 3,564 putative zygotes) were cultured in modified synthetic oviductal fluid (SOF) with 1.0 mM alanyl-glutamine in 5% (v/v) oxygen with either no surfactant, 1 mg/mL polyvinyl alcohol (PVA) or 4 mg/mL bovine serum albumin (BSA) and ± 1 mg/mL hyaluronan. Blastocyst and expanded blastocyst-stage embryos on day 7 were vitrified using open pulled straws (Mol. Reprod. Dev. 1998, 51:53-58). Vitrified embryos were thawed and then cultured for 72 h in modified SOF containing 10% (v/v) fetal bovine serum and 50 ±M dithiothreitol. There was no effect of hyaluronan on re-expansion at 24 and 48 h or hatching rate at 72 h. Re-expansion rate at 24 and 48 h and hatching rate at 72 h was reduced ($P<0.0001$) by BSA (24 h, 72.9 ± 2.6 vs. 22.6 ± 4.8%, 48 h, 77.3 ± 2.7 vs. 20.7 ± 4.9%, 72 h, 72.5 ± 2.7 vs. 21.5 ± 5.0%). For experiment 2, heat-stressed lactating Holsteins were synchronized for timed-embryo transfer using the OvSynch protocol. Embryos produced in vitro were

cultured in modified SOF with 4 mg/mL BSA, 100 ng/mL insulin-like growth factor-1, and ± 1 mg/mL hyaluronan. At day 7 after expected ovulation, a single embryo was transferred to each recipient with a palpable corpus luteum. Pregnancy was diagnosed by rectal palpation at d 35-90 of gestation. There was a treatment x embryo stage interaction ($P< 0.03$). Hyaluronan increased pregnancy rates for recipients that received morula and blastocyst-stage embryos (15/92=16.3% vs. 22/78=28.2%) but had no effect for recipients receiving expanded blastocysts (31/82=37.8% vs. 25/83=30.1%). There was no effect of hyaluronan on in-vitro survival after vitrification, but BSA reduced cryosurvival. Hyaluronan increased pregnancy rate among recipients that received morula and blastocyst-stage embryos, but not for recipients that received expanded blastocysts (USDA 2006-55203-17390).

Key Words: Hyaluronan, Vitrification, Embryo Transfer

TH184 Effect of progesterone concentration during follicular development on fertilization and embryo quality in dairy cows. R. L. A. Cerri*[1,2], R. C. Chebel[2], F. Rivera[2], C. D. Narciso[2], R. A. Oliveira[2], and J. E. P. Santos[1], [1]*University of Florida*, *Gainesville*, [2]*University of California Davis*, *Tulare*.

Objectives were to evaluate the effects of differing progesterone (P4) concentrations on follicular dynamics and embryo quality. Lactating Holstein cows (n = 154), were randomly assigned to 1 of 2 treatments. Cows had their estrous cycle presynchronized and were then submitted to a modified Ovsynch (GnRH + CIDR, 7 d prostaglandin F2a [PG] + CIDR removal, 2 d GnRH, 12 h timed AI) starting on d 7 of the estrous cycle. Cows enrolled in the high P4 (HP) treatment received no further treatments and cows in the low P4 (LP) treatment received additional PG injections on d 2, 2.5 and 3 of the estrous cycle preceding the Ovsynch and on d 0, 2, 2.5 and 3 after the first GnRH of the Ovsynch. Ovaries were evaluated by ultrasonography and blood was sampled for P4 and estradiol (E2) concentrations throughout the study. Uteri were flushed 6 d after AI and recovered embryos/oocytes were evaluated. Concentrations of P4 were greater ($P<0.01$) for HP cows from d 2 to 7 after first GnRH of Ovsynch (4.3 vs. 1.5 ng/mL); concentrations of E2 at PG (3.0 vs. 3.8 pg/mL) and at the last GnRH (4.6 vs. 5.3 pg/mL) were less ($P<0.01$) for HP than LP. Proportion of cows in estrus at timed AI was less ($P = 0.01$) for HP than LP (5.3 vs. 38%). The ovulatory follicles of HP cows were smaller ($P<0.01$) at PG (14.6 vs. 17.2 mm) and GnRH of the Ovsynch (16.9 vs. 19.7 mm), which resulted in smaller ($P=0.03$) CL diameter 6 d after AI (22.8 vs. 24.3 mm). Double ovulation after the last GnRH of the Ovsynch was less ($P=0.01$) for HP than LP (4.5 vs. 18.6%). Fertilization rate was similar ($P=0.67$) and averaged 82.7%. Excellent and good quality embryos as a proportion of embryos ($P=0.48$) and of embryo-oocytes ($P=0.66$) were not different. Number of blastomeres was similar ($P=0.79$) for HP and LP, but proportion of live blastomeres tended to be greater ($P = 0.08$) for HP than LP (99.5 vs. 94.8%). Reducing P4 concentrations before and during the Ovsynch program altered E2 concentrations and follicular dynamics, but resulted in minor changes in embryo quality.

Key Words: Dairy Cow, Embryo, Progesterone

TH185 Milk production and rectal temperature during pregnancy in lactating dairy cow recipients. D. T. G. Jardina*[1], F. L. Aragon[2], M. B. Veras[2], S. Soriano[3], N. Sobreira[3], A. B. Scarpa[1], P. L. T. Justolin[1], and J. L. M. Vasconcelos[1], [1]*FMVZ, Unesp*, *Botucatu, SP, Brazil*, [2]*Policlinica Pioneiros*, *PR, Brazil*, [3]*Farm Colorado*, *SP, Brazil*.

The objective of this study was to evaluate factors that can influence pregnancy per embryo transfer (P/ET) in lactating Holstein cows. The trial was conducted between February and November 2007. Cows were milked 3 times a day and fed with total mixed ration. Recipient Holstein cows (n = 468) were synchronized with HeatSynch + CIDR protocol [CIDR in + GnRH (1 mL Fertagyl) - 7d - CIDR out + PGF2α (5 mL Lutalyse) - 24h - Estradiol Cypionate (0.5 mL ECP)]. Cows were evaluated by ultrasonography for detection of CL and embryo transfers were done, non-surgically, 9 d after injection of ECP. P/ET was determined by presence of an embryo 21 d after transfer by ultrasonography and was analyzed by logistic regression. The regression model included the effects of month, body condition score, parity, days in milk (DIM), milk production, milk components (fat, protein, somatic cell count, urea), serum progesterone, rectal temperature, CL diameter, embryo (fresh vs. frozen/thawed) and interactions. Embryo influenced ($P<0.01$) P/ET; 48.8% (150/307) with fresh and 28.1% (45/160) with frozen/thawed embryos. There was an interaction ($P<0.05$) between milk production and rectal temperature, that were grouped by median. Number of cows, milk production, rectal temperature, DIM, serum progesterone, CL diameter and P/ET were: 1) low milk production/normal rectal temperature: n=128, 28.6kg of milk/d, 38.5°C, 264 DIM, 3.03ng/mL, CL 4.27cm2 and 42.2%; 2) low milk production/high rectal temperature: n=105, 27.0kg of milk/d, 39.3°C, 292 DIM, 3.4ng/mL, CL 3.9cm2 and 36.1%; 3) high milk/normal temperature with n=144, 42.2kg of milk/d, 38.4°C, 138 DIM, 3.1ng/mL, CL 4.6cm2 and 47.7%; and 4) high milk production/high rectal temperature with n=91, 41.8kg of milk/d, 39.2°C, 141 DIM, 3.3ng/mL, CL 3.8cm2 and 38.4%, respectively. These results indicated that high milk production did not affect pregnancy after ET in recipient lactating cows with normal rectal temperature.

Key Words: Embryo Transfer, Rectal Temperature, Milk Production