Physiology and Endocrinology: Sperm Fertility, Embryos and Development

989 Comparison study of alternative cryoprotectants for cryopreserving bull spermatozoa. M. M. Awad*, *Animal Production Dept. Faculty of Agriculture, Suez Canal University, Ismailia, Egypt*.

Mammalian spermatozoa experience osmotic stress when the glycerol is added to the cells before freezing and removal from the cells after thawing. To minimize osmotic damage, alternative cryoprotectants, having lower molecular weights and greater membrane permeability than glycerol, were evaluated to determine their effectiveness for cryopreserving bull spermatozoa. The primary goal of this study was to compare glycerol as the most common cryoprotectant of bull spermatozoa to ethylene glycol and methyl as alternative cryoprotectants. Bull semen was diluted with tris-egg yolk extender containing 3% glycerol, 3, 2 and 1% ethylene glycol and 3, 2 and 1% methyl. Bull semen was frozen in pellets form using the cold surface of cattle fat. Computer assisted sperm analysis (CASA) assay was used to study the post-motility properties of bull spermatozoa. Bull spermatozoa exhibited higher percentages ($P < 0.01$) of all motile spermatozoa properties when frozen in extender containing 3% glycerol compared with 3, 2 and 1% ethylene glycol or 3, 2 and 1% methyl cryoprotectants. It is concluded that glycerol concentrations of 3% in the extender is the most suitable cryoprotectant for cryopreservation of bull spermatozoa.

Key Words: cryopreservation, bull spermatozoa

990 Effects of anti-lipid peroxidation supplements on frozen-thawed boar spermatozoa. B. D. Whitaker*, R. Taupier, and S. J. Casey, *Ferrum College, Ferrum, VA*.

This study evaluated the effects of 2 anti-lipid peroxidation supplements when added to the thawing and incubation medium of frozen-thawed boar spermatozoa. Semen pellets were thawed and incubated for 1 h in media with either 1.0 m*M* α-tocopherol or diethylenetriamine (DETA, nitric oxide analog). After incubation, the acrosome reaction was inducted using calcium ionophore A23187 and acrosomes were evaluated using Wells-Awa staining. The amount of spermatozoa with fragmented DNA was evaluated using silver staining after single-cell gel electrophoresis. The 1.0 m*M* DETA supplementation had a significantly higher ($P < 0.05$) percentage of acrosome reacted spermatozoa (84.4 ± 4.1%) compared with the control (78.3 ± 4.2%) and the 1.0 m*M* α-tocopherol supplementation (78.0 ± 3.9%). There was no difference between the control and the 1.0 m*M* α-tocopherol supplementation. The control had a significantly higher ($P < 0.05$) percentage of spermatozoa with fragmented DNA tails (59.3 ± 4.3%) compared with the 1.0 m*M* DETA (28.7 ± 4.1%) and the 1.0 m*M* α-tocopherol supplementation (28.0 ± 3.8%). There was no difference between the 1.0 m*M* DETA and the 1.0 m*M* α-tocopherol supplementation. These results indicate that supplementing with either 1.0 m*M* DETA or α-tocopherol during semen thawing protects against DNA fragmentation and increases the percent of spermatozoa capable of completing the acrosome reaction.

Key Words: swine, antioxidant, sperm

991 Reproductive performance of sows inseminated with various doses of frozen-thawed semen. K. S. Fisher*[1], T. S. Stewart[1], P. H. Purdy[2], H. D. Blackburn[2], W. L. Singleton[1], B. L. Sparks[1], P. J. Gunn[1], and G. A. Bridges[1], [1]*Purdue University, West Lafayette, IN*, [2]*National Animal Germplasm Program, NCGRP, ARS, USDA, Fort Collins, CO*.

Genebanks have a limited supply of frozen-thawed boar semen (FTS), thus it is critical to determine the optimal dose of FTS required for reproductive proficiency. Crossbred sows (n = 106) were used in 3 replicates to determine the efficacy of 4 doses of FTS with a single AI. After weaning, estrous detection was conducted using a boar and sows received a single transcervical insemination 24 h following the onset of estrus. At AI, sows were randomly assigned to receive FTS from 1 of 2 boars (boar 1, n = 36; boar 2, n = 61) at a post-thawing dose of 0.25 (n = 24), 0.50 (n = 25), 0.75 (n = 24), or 1.0 (n = 24) × 10^9 motile cells. Thirty d after AI, sows were slaughtered and viable fetuses and corpora lutea were counted. The effects of replicate (REP), boar, dose, and all interactions on pregnancy rate and number viable fetuses were evaluated using the Glimmix and Mixed procedures of SAS. Pregnancy rate did not differ among semen doses (0.25- 25%, 0.5- 44%, 0.75- 54%, 1.0- 42%), but was decreased ($P < 0.05$) for boar 1 (22.2%) compared with boar 2 (52.5%). The number of viable fetuses recovered was decreased ($P < 0.05$) for boar 1 (4.6 ± 1.4) compared with boar 2 (8.6 ± 0.7). There was a REP x dose interaction ($P < 0.05$) for number of fetuses. The 1.0 and 0.75 doses resulted in the greatest number of fetuses in REP 1 and REP 3, respectively, with no difference among doses in REP 2. Across replications, a linear effect of dose on viable fetuses was observed ($P < 0.05$) and a tendency for a quadratic effect ($P < 0.1$). To ascertain the dose most appropriate to use if semen was limited, the total viable fetuses per sow inseminated was multiplied by the number of potential doses available if only 1 × 10^9 motile sperm cells existed. Although only non-significant differences among doses were found (0.25- 0.91 ± 2.1, 0.50- 3.4 ± 1.7, 0.75- 4.8 ± 1.7, 1.0- 3.3 ± 1.7), results suggested, from the perspective of maximizing both the number of sows that can be inseminated with a limited supply of semen and reproductive proficiency, that the optimal dose of semen to utilize per insemination was between 0.50 and 0.75 × 10^9 motile sperm cells.

Key Words: germplasm preservation, semen, swine

992 Analysis of proteomic changes during sperm capacitation associated with sperm fertility. Y. J. Park*, S. A. Oh, W. S. Kwon, S. J. Yoon, Y. H. Kim, E. A. Mohamed, Y. A. You, and M. G. Pang, *Department of Animal Science & Technology and BET Research Institute, Chung-Ang University, Ansung, Gyeonggi-Do, Korea*.

Spermatozoa are required to undergo the processes of capacitation before fertilization. Since spermatozoa are transcriptionally silent, the functional metamorphosis of these cells during capacitation is accomplished entirely by post-translational modifications. Despite the importance of this process, very few studies have attempted to define the precise nature on the molecular level. The objective of this study was to characterize the supposed differences in fertility with respect to capacitation-related proteins. We undertook differential proteome profiling before and after capacitation of spermatozoa from fertile bulls with extreme non-return rates (NRR): a low fertility group (45.10±4.95) and a high fertility group (82.45±6.26). For identification of capacitation related proteins associated with fertility, capacitation was induced by 10 μg/mL heparin for 20 min and was confirmed by chlorotetracycline assay where significantly increasing in capacitated sperm was observed. Two-demensional gel electrophoresis (2-DE) was carried out with triplicate samples of pooled spermatozoa before and after capacitation from 3 low and 3 high fertility bulls. Protein expression levels were compared using PD-Quest software. The marked difference in spot intensity was arbitrarily set as a >3-fold difference following analysis data from the software manufactures. Twelve protein spots showed differences

between high and low fertility groups and seven of these proteins were identified by LC/MS-MS. Glyceraldehyde 3-phosphate dehydrogenase, phosphatidylethanolamine-binding protein 1, bovine mitochondrial F1-ATPase complexed with Aurovertin B and actin-related protein T2 were more expressed in high fertility group. On the other hand phospholipid hydroperoxide glutathione peroxidase, ropporin and enolase 1 were more expressed in high fertility group. These proteins are in correlation with sperm physiology, and these proteins may be used as sperm fertility markers.

Key Words: fertility, capacitation, proteome

993 Prognosis of bull fertility using sperm penetration assay. Y. J. Park*, S. A. Oh, S. J. Yoon, W. S. Kwon, Y. H. Kim, E. A. Mohamed, Y. A. You, and M. G. Pang, *Department of Animal Science & Technology and BET Research Institute, Chung-Ang University*, *Ansung, Gyeonggi-Do, Korea*.

The prediction of sperm fertility is of paramount importance for breeding animal herds when artificial insemination is applied. While the fertility assays provide valuable quantitative data, they yield limited information concerning the functional competence of the spermatozoa. The objective of this study was to standardize a method for predicting in vivo fertility in bulls using the in vitro penetration assay. To increase the assay sensitivity, each step in the procedure was standardized. We found that maximum penetration of hamster zona-free oocytes (SPA) obtained from heparin-treated (10 µg/mL) sperm cells for 20 min ($P < 0.05$). The SPA result showed significant correlation with historic average non-return rate (NRR) ($P < 0.05$). To determine the normal range for the SPA, lower limits of the sperm fertility index from SPA were established as 2.55 to cut-off more than 70% NRR using the receiver operating characteristic curve. The overall accuracy was 93.33% for the low and high NRR, sensitivity and specificity were 92.86 and 93.75 respectively. The protocol increases the potential to discriminate between bulls with good and poor fertility. Although the conditions for the capacitation and penetration of the spermatozoa are greatly different from the in vivo situation, nevertheless the test provides meaningful information on the bull fertility.

Key Words: sperm, fertility, sperm penetration assay

994 Semen quality index of broiler breeder cockerels subjected to different collection techniques. A. Ijaz*, M. U. Sohail, H. Rehman, M. Aleem, A. Riaz, and M. S. Yousaf, *University of Veterinary and Animal Sciences*, *Lahore, Pakistan*.

Present study aims at evaluating 3 different semen collection techniques; abdominal-massage, live-mount and dummy-mount technique, in broiler breeder cockerels. Six cockerels (50 week old), randomly selected from a natural breeder farm, were kept in individual floor pens. After one week of acclimatization, cockerels were trained for semen collection using the abdominal-massage technique and dummy-mount technique. Thereafter, the cockerels were subjected to abdominal-massage, live-mount and dummy-mount technique in phases for 14 d each, with 2 weeks period of rest among techniques. Semen was daily collected from 6 cockerels pooled. Semen was evaluated for number of semen ejaculates, semen volume per ejaculate, visual appearance of semen, sperm motility and sperm concentration. Visual appearance of semen was indexed as watery, milky or opaque. Diluted semen was placed on pre-warmed slide and examined under microscope for motility. Sperm concentration was estimated using Neubauer hemocytometer. Data regarding semen volume, visual appearance, sperm motility and concentration were analyzed using ANOVA, while data regarding number of ejaculates were subjected to chi-squared test. Live-mount technique gave higher ($P < 0.01$) number of ejaculates and volume per ejaculate (44, 0.26 ± 0.24) compared with abdominal-massage technique (26, 0.04 ± 0.01) and dummy-mount technique (37, 0.15 ± 0.03). Visual appearance index of semen, sperm motility and sperm concentration remained unaffected ($P > 0.05$) independent of the techniques employed. In conclusion, live-mount technique is better for cockerel semen collection compared with abdominal-massage and dummy-mount technique.

Key Words: cockerel, semen, collection method

995 Effect of supplemental sialic acid on the fertility of in vitro stored turkey semen. J. A. Long* and T. Conn, *Beltsville Agricultural Research Center*, *Beltsville, MD*.

The fertility of turkey semen stored for longer than 6 h in vitro is dramatically lower than that of fresh semen. Previously we have shown that physiological changes occur on the sperm membrane when turkey semen is held for 24h at 4°C. In particular, the carbohydrate content of the sperm glycocalyx undergoes significant alterations, including loss of terminal sialic acid residues. The objective of the present study was to determine if the glycocalyx can incorporate exogenous sialic acid and whether incubation with sialic acid in vitro will improve the fertility of stored turkey semen. Semen was collected from toms and extended with Beltsville Poultry Semen Extender supplemented with 0, 60, 70, 80, 90 or 100 µg/ml of sialic acid. At 30 min intervals for up to 3h, aliquots were removed, stained with LFA (a sialic acid-specific lectin), counterstained with propidium iodide (PI) and assessed by cell flow cytometry. The mean fluorescence intensity (MnFI) of viable (PI negative) sperm was compared with control sperm samples at each interval. Significantly higher MnFI occurred at 30 and 60 min ($P < 0.05$) in the presence of sialic acid; however, further increases in MnFI were not noted at later time points. A dose-response effect ($P < 0.05$) occurred for the 60, 70 and 80 µ/ml treatments only. Twelve-week fertility trials were conducted using semen held at 4°C for 24h with and without sialic acid. Of the 5 doses evaluated, 80 µg/ml supported the highest fertility rates (mean 81.2 ± 9.1%) for the first 6 weeks of insemination. Taken together, these data suggest a possible strategy for improving the fertility of in vitro stored turkey semen.

Key Words: glycocalyx, NANA, semen storage

996 Vitrification of bovine blastocysts: Effects of cooling with an aluminum block submerged in liquid nitrogen versus liquid nitrogen cooled air and lowering sodium and calcium concentrations in vitrification media. S. G. Kruse* and G. E. Seidel, *Colorado State University*, *Fort Collins*.

Our objective was to improve procedures for vitrifying bovine blastocysts produced using standard in vitro procedures. In Experiment 1, we studied a new base medium with lowered sodium and calcium concentrations based on the hypothesis that this would result in a lower chance of sodium and calcium toxicity. Base media contained either 1) normal concentrations of sodium (120 m*M*) and calcium (2 m*M*) (CONT; n = 151) or 2) 60 m*M* sodium choloride + 60 m*M* choline chloride and 0.5 m*M* calcium (LOW; n = 139). Blastocysts were exposed to 5 M ethylene glycol made in CONT or LOW base medium (V1) for 3 min at 22°C and moved to 6.5 M ethylene glycol + 0.5 M galactose + 18% Ficoll made in CONT or LOW base medium (V2) at 22°C and immediately loaded into 0.25 mL straws. After 35 s, embryos were vitrified by either 1) standard cooling in liquid nitrogen cooled air (AIR) for 1 min or 2) cooling via contact of straw walls with columns drilled into an aluminum block immersed in liquid nitrogen (BLK) for

2 min, and then directly plunged into liquid nitrogen. Embryos were warmed by holding straws in air at 22°C for 10 s, placing them in a water bath at 37°C for 20 s, mixing embryos with 1.0 M galactose diluent in the straw for 2 min and expelling into CONT or LOW base medium. Embryos were recovered, rinsed through holding medium, and cultured in chemically defined medium for 24 h before evaluation. Post warming survival did not differ ($P > 0.1$) between base medium (CONT = 22.5%; LOW = 25.9%). Experiment 2 was conducted as Experiment 1 (some overlap of embryos used) except the AIR (n = 134) versus BLK (n = 138) comparison was made. There was no difference ($P > 0.1$) in survival due to vitrification method (AIR = 32.1%; BLK = 31.9%). We recommend use of the BLK vitrification method as it is both easier to use and more consistent.

Key Words: blastocyst, vitrification, bovine

997 Efficacy of embryo transfer in lactating dairy cows during summer using fresh or vitrified embryos produced in vitro with sex-sorted semen. B. M. Stewart[1], J. Block[2,4], P. Morelli[1], A. E. Navarrette[1,3], M. Amstalden[3], L. Bonilla[4], P. J. Hansen[4], and T. R. Bilby*[1,3], [1]*Texas AgriLife Research and Extension, Texas A&M System*, *Stephenville*, [2]*OvaTech, LLC*, *Gainesville, FL*, [3]*Texas A&M University*, *College Station*, [4]*University of Florida*, *Gainesville*.

Objective of the study was to determine whether transfer of fresh or vitrified embryos produced in vitro with sex-sorted semen could improve pregnancy rates during summer in lactating dairy cows versus artificial insemination (AI). Lactating dairy cows (n = 722) were enrolled during summer at 2 commercial dairies in Texas. Cows were randomly assigned to one of 3 treatments: AI (n = 227), embryo transfer-vitrified (ET-V; n = 279) or embryo transfer-fresh (ET-F; n = 216). Embryos were produced in vitro using sex-sorted semen and cultured in BBH7 culture medium until d 7 after insemination. For vitrification, grade 1 expanded blastocysts were vitrified using the open-pulled straw method. Fresh embryos were grade 1 blastocysts and expanded blastocysts. Cows were submitted to an estrous synchronization protocol and either time-AI or AI following detected estrus (day of estrus = d 0). On d 7, cows were ultrasounded for presence or absence of a corpus luteum (CL). A vitrified or fresh embryo was transferred to cows with CL in ET-V and ET-F groups. Cows were synchronized if progesterone was <1 ng/mL on d 0 and presence of CL on d 7. There was no treatment by farm interactions. At initial pregnancy diagnosis (40 ± 7 d), proportion of cows pregnant was greater ($P < 0.01$) for ET-F versus both ET-V and AI for all cows (42.1 vs. 29.3 and 18.3%) and synchronized cows (45.5 vs. 30.9 and 22.9%). Also, proportion of cows pregnant was greater for ET-V than AI among all cows ($P < 0.01$) and tended ($P = 0.10$) to be greater among synchronized cows. At second pregnancy diagnosis (97 ± 7 d), proportion of cows pregnant among all cows was greater ($P < 0.05$) for ET-F and ET-V versus AI (36.4 and 25.7 vs. 17.0%) and ET-F was greater ($P < 0.05$) than ET-V. Among synchronized cows, proportion of cows pregnant was greater for ET-F than ET-V and AI (39.4 and 27.0 vs. 21.2%); there was no difference between ET-V and AI. There was no effect of treatment on embryo loss. Transfer of vitrified and fresh embryos produced in vitro using sex-sorted semen can improve fertility in lactating dairy cows during summer.

Key Words: embryo, heat stress, dairy

998 The importance of fibroblast growth factors on bovine embryo development in vitro. S. D. Fields*, P. J. Hansen, and A. D. Ealy, *University of Florida*, *Gainesville*.

The objective was to test the hypothesis that fibroblast growth factors (FGF) regulate early embryonic development. The first 2 experiments were performed to determine if embryo-derived FGFs are required during development. In Exp 1, bovine embryos were produced in vitro and cultured in modified synthetic oviductal fluid (mSOF) containing either 20 μ*M* SU5402, an FGF receptor inhibitor, or carrier (0.2% DMSO) at D 0 or 4. There was no effect of SU5402 on cleavage rate at Day 3. SU5402 reduced ($P = 0.04$) the percent of oocytes that were blastocysts on D 7 compared with controls when added at D 0 (5.9 ± 2.1 vs. 16.9 ± 2.4) but not when added at Day 4. Exp 2 tested if blocking FGF action at the blastocyst stage would affect subsequent cell number. D 8 blastocysts were placed into individual culture drops of mSOF containing 0.2% DMSO or 20 μ*M* SU5402. SU5402 decreased ($P = 0.04$) the number of cells at D 11 (211.1 ± 27.5 vs. 297.8 ± 25). Exp 3 was performed to test if supplemental FGF2 would enhance development to the blastocyst stage. Embryos were cultured in mSOF containing 0, 5, or 100 ng/mL FGF2. There was no effect of FGF2 on cleavage or percent of oocytes that were blastocysts at D 7 or 8. For Exp 4, 8–16 cell embryos were placed in fresh mSOF containing 0, 5, or 100 ng/mL FGF2 at D 5 after insemination. There was no effect of concentration of FGF2 on percent of oocytes that were blastocysts at D 7 or 8, number of trophectoderm cells or inner cell mass cells, or the ratio. Effects of higher concentrations of FGF2 were examined in Exp 5. Embryos received 0 ng/mL of FGF2 or 500 ng FGF2 at D 0, D 4, or D 0 and 4. There was no effect of FGF2 on cleavage. Addition of FGF2 at both D 0 and 4 (27.4 ± 1.3) increased ($P \leq 0.03$) the percent of oocytes that became blastocysts on D 7 compared with control (19.7 ± 1.3) or FGF2 on D 4 (20.4 ± 1.3), but did not differ from FGF2 treatment on D 0 (23.2 ± 1.3). In summary, FGFs are important for normal blastocyst development. One of these, FGF2, increased the competence of embryos to become blastocysts at high concentrations.

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Key Words: fibroblast growth factor-2, embryo

999 Changes in cotyledonary vascular architecture with advancement of placentomal (PLAC) type during gestation in the ewe. S. Hein*[1], A. Uthlaut[1], P. W. Nathanielsz[1,2], and S. P. Ford[1], [1]*Center for the Study of Fetal Programming, Dept. of Anim. Sci., University of Wyoming*, *Laramie,* [2]*Center for Pregnancy and Newborn Research, Dept. of OB/GYN, University of Texas Health Sciences Center*, *San Antonio*.

PLAC advance morphologically from Type A to Types B, C, or D during the second half of gestation in sheep in association with exponential fetal growth. Several research groups have attempted to understand the changes in the cotyledonary vascular bed with advancing PLAC type using routine histological evaluation of individual PLAC sections which fail to provide a 3 dimensional view of the vascular architecture, leading to conflicting results. We utilized vascular casting to visualize cotyledonary capillary bed 3-dimensional structure. Six multiparous ewes of similar age and body condition were necropsied at d135 gestation and a Type A, B, C, and D PLAC collected from each. The cotyledonary arterial vasculature of each PLAC was perfused with Biodur (Heidelberg, Germany), forming a flexible vascular cast. The surrounding tissue was removed by storage in a 5% KOH solution for 4–6 weeks. The vascular cast images were then visualized on a tabletop Scanning Electron Microscope (TM-1000), and analyzed for capillary area density (CAD, cotyledonary capillary area/cotyledonary area) and capillary diameter (CD) using ImageJ (NIH). Data are M Â ± SEM; differences determined by ANOVA. Cotyledonary CAD and CD (Table 1) and arteriole branching increased as PLAC progressed from type A

to type D. The cotyledonary villous capillary trees of later stage PLAC (C and D) extend for greater distances along villi, became increasingly organized, and consolidated into large nodes. These changes suggest an increased capacity for maternal to fetal nutrient transfer with PLAC advancement in late gestation.

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Table 1. CAD and CD of Differing Placentome Types

	A	B	C	D	P-value
CAD (%)	58.25±1.54[a]	59.88±1.78[a*]	64.16±1.54[b*]	67.12±1.26[b]	a,b<0.05, *<0.10
CD (μm)	8.18±0.36[a]	7.59±0.42[a]	9.31±0.36[b]	10.70±0.30[c]	a,b,c<0.05

Key Words: placentomal type, cotyledonary vasculary, sheep