Physiology and Endocrinology II

647 Can prenatal social stress impact sex characteristics in piglets? L. A. Mack*[1], S. D. Eicher[2], A. K. Johnson[3], D. C. Lay Jr.[2], B. T. Richert[2], and E. A. Pajor[4], *[1]Purdue University, W. Lafayette, IN, [2]LBRU, USDA-ARS, W. Lafayette, IN, [3]Iowa State University, Ames, [4]University of Calgary, Calgary, AB, Canada.*

Prenatal stress (PNS) alters sex traits in rodents by androgenizing offspring resulting in reduced reproduction. In production, gestating sows are often exposed to social stress of mixing. This study examined if mixing gestating sows alters sexual development in piglets. At 34 ± 10 d of gestation, 6 groups of 18 sows (n = 108) were put in 1 of 4 treatments: stable (S), hydrocortisone acetate (HCA), unstable (U) or unstable companion (UC). In an incomplete block design, 18 sows were housed in 6 pens of 3 sows for 3 wk. Each pen contained 3 S, 3 HCA, or 1 U and 2 UC sows. Stable, HCA, and UC sows did not move; unstable sows moved weekly into a new pen with unknown UC sows. To simulate stress, 70 mg HCA was orally given twice daily to HCA sows for 3 wk. Data were analyzed in SAS using Mixed Model Procedure. Cortisol concentration was greatest in HCA sows ($P < 0.0001$) and after initial mixing ($P < 0.05$). Sows' progesterone level did not differ by treatment or time. Lesion scores increased after mixing in all treatments ($P < 0.05$). On wk 3, U sows had more head and upper leg lesions than the other treatments ($P < 0.05$). Sow's treatment had little effect on piglets: litter size, sex ratio, BW, and mortality did not differ. Pigs were weighed on d 1, d 3, weaning (d 19 ± 8), and 5 mo post-weaning (6 mo). There was no treatment effect on weight from birth to weaning but at 6 mo HCA and UC pigs tended to weigh more ($P < 0.10$) and from weaning - 6 mo had greater ADG than S pigs ($P < 0.05$). Testes weight, teat number, and teat asymmetry did not differ by treatment or gender. Males born from dominant sows, defined by feeding behavior, tended to have more teats than those of low ranked sows ($P < 0.10$). Anogenital distance (ANO) in male pigs was greater in UC than U pigs ($P < 0.05$) with the other treatments intermediate. Female ANO showed no differences. Social stress induced by weekly mixing had little impact on sexual, morphological measures of the offspring in this study.

Key words: prenatal stress, swine, reproduction

648 Heat stress increases small intestinal permeability and circulating endotoxin in growing pigs. S. C. Pearce*, V. Mani, L. H. Baumgard, and N. K. Gabler, *Iowa State University, Ames.*

Heat-stress causes a decreased intestinal integrity and induces "leaky" gut. This may lead to reduced growth performance and bacterial sepsis, but whether this occurs in pigs and the mechanisms responsible for it, are ill-defined. Crossbred gilts (n = 48; 35 ± 4 kg BW) were housed in constant climate controlled rooms in individual pens and exposed to 1) thermal neutral (TN) conditions (20°C; 35–50% humidity) with ad libitum intake (n = 18), 2) HS conditions (35°C; 20–35% humidity) with ad libitum intake (n = 24) or 3) pair-fed (PF in TN conditions [PFTN], n = 6: to eliminate confounding effects of dissimilar feed intake [FI]). Pigs were sacrificed at 1, 3, or 7d of environmental exposure and freshly isolated jejunum samples were mounted into modified Ussing chambers. Segments were then analyzed for transepithelial electrical resistance (TEER) and intestinal fluorescein isothiocyanate (FITC)-labeled lipopolysaccharide (LPS) transport expressed as endotoxin apparent permeability coefficient (APP). Additionally, circulating concentrations of endotoxins were measured in plasma blood samples. Irrespective of day, plasma endotoxin concentrations increased 46% ($P < 0.05$) in HS pigs compared with TN pigs, while TEER decreased 24% ($P < 0.05$) and endotoxin APP increased 81% ($P < 0.01$), respectively. Furthermore, d 7 HS pigs tended to have increased APP ($P = 0.06$) compared with PFTN controls. These data indicate that HS decreases intestinal integrity and increases endotoxin permeability. Together, this translated into an increase in circulating endotoxin. We hypothesize that these events lead to increased acute inflammation which is responsible for reduced pig performance during warm summer months.

Key words: heat stress, pig, intestinal permeability

649 The effect of naloxone on reproductive behavior and plasma prolactin levels in third lactation sows. V. O. Fuentes Hernadez*, R. Orozco Hernandez, and A. Bernal Canseco, *Centro Universitario de los Altos, Universidad de Guadalajara, tepatitlan Jalisco, Mexico.*

The present study was undertaken to study the effect of small doses of naloxone on behavior, prolactin plasma levels, interval of weaning to first estrus, and duration of estrus in third lactation sows. Thirty York × Landrace sows weaned at 25 to 27 d pospartum, were selected and separated at random in 2 groups of 15. One group served as control and the other received every 12 h 2 mg of naloxone im. Treatment with small doses of naloxone started 3 d before and continued for 3 d after weaning similarly the control group was injected with 2 mL of a saline solution. Naloxone treated sows showed estrus 88.8 ± 6.2 h after weaning ($P < 0.01$), control sows estrus was evident 102.37 ± 7.2 h after weaning. Duration of estrus in treated and nontreated was 85.6 ± 3.8 and 42.6 ± 3.7 h respectively. Prolactin levels decreased rapidly after weaning in both groups, but plasma PR in naloxone treated sows were below control levels (15 ± 2 and 7 ± 0.3 ng respectively $P < 0.1$). Behavior scores showed that naloxone treated sows accepted mounting with a significant reduction in aggressive behavior as compared with controls. It was concluded that endogenous opioids are important modulators of sow sexual behavior.

Key words: sow, naloxone, behavior

650 Differential expressed proteins in porcine follicular fluid during folliculogenesis. J. M. Feugang*[1], K. Pendarvis[2], S. T. Willard[3], and P. L. Ryan[1,4], *[1]Department of Animal and Dairy Sciences, Mississippi State University, Mississippi State, [2]Life Science Biotechnology Institute, Mississippi State University, Mississippi State, [3]Department of Biochemistry and Molecular Biology, Mississippi State University, Mississippi State, [4]Department of Pathobiology and Population Medicine, Mississippi State University, Mississippi State.*

Ovarian follicular fluid (FF) is a dynamic and suitable microenvironments for growth and acquisition of oocyte developmental competence. Disparities in oocyte quality of diverse follicle sizes are partly attributed to FF composition, whose changing contents during folliculogenesis are still not well-characterized. Follicular fluid is an optimal source for identifying determinants of oocyte and follicle growth. Therefore, we compared proteome profiles of porcine FF of various follicular developmental stages. Follicles were dissected from healthy sow ovaries and classified as small (SFF; ≤3mm), medium (MFF; 4-6 mm), and large (LFF;7-12mm). Follicular fluid was aspirated from individual follicles, centrifuged and collected for protein quantification and estradiol assay. All procedures were performed at 4°C and

repeated 4X. Samples with highest estradiol content were selected for a non-gel based proteome analyse. Peptides significantly detected ($P \leq 0.05$) were subjected to protein identification, and highly differentially detected (DD) proteins ($P \leq 0.05$) were selected for functional annotation. Approximately two thousand proteins were detected in each FF group. Significant protein numbers (1,493-1,656) were specific to each FF group, and 23-26% of proteins (558-570) were common amongst groups. Of these shared proteins, 28 to 33% were significantly differentially detected ($P \leq 0.05$). Gene ontology (GO) analyses of highly significantly DD indicated associations with various cellular components, molecular functions and biological processes. Pairwise comparisons indicated that MFF vs SFF had the highest total GO annotation number (184) compared to LFF vs SFF (61), and LFF vs MFF (48). Based on their abundance in FF, few proteins such as fibronectin 1, inhibitor of carbonic anhydrase precursor, and histidine rich glycoprotein were highly DD in LFF, MFF, and SFF, respectively. In conclusion, the study indicates important quantitative and qualitative changes in FF composition during folliculogenesis, and whose specific components may serve as candidate markers of follicle and oocyte growth. Work supported by USDA-ARS Biophotonics Initiative #58-6402-3-0120.

Key words: follicular fluid, proteome, oocyte growth

651 Effects of glucuronic acid supplementation on the in vitro maturation and fertilization of pig oocytes. A. R. Clark* and B. D. Whitaker, *The University of Findlay, Findlay, OH.*

The objective of this study was to assess the in vitro maturation and fertilization (IVF) of pig oocytes supplemented with glucuronic acid during the last 24 h of maturation. Oocytes were transferred after 20 h from the beginning of maturation into hormone-free maturation media containing glucuronic acid (0, 0.01, 0.1, 1 mM) for an additional 24 h of maturation. Oocytes (n = 300) were evaluated for thickness of the zona pellucida, size of the perivitelline space, fertilization characteristics at 12 h after IVF, and embryo development at 48 h and 144 h after IVF. There were no significant differences between treatment groups when comparing zona pellucida thickness, however supplementation of 0.1 and 0.01 mM glucuronic acid significantly increased ($P < 0.05$) the size of the perivitelline space compared with no supplementation. Supplementation of 0.01 mM glucuronic acid significantly increased ($P < 0.05$) the size of the perivitelline space compared with 1.0 mM glucuronic acid supplementation. There were no significant differences between the treatment groups when evaluating sperm penetration or male pronucleus development but 0.01 mM glucuronic acid supplementation significantly decreased ($P < 0.05$) polyspermic penetration compared with the other treatment groups. Although there were no significant differences in cleaved embryos at 48 h after IVF, there were significantly higher ($P < 0.05$) numbers of embryos derived from oocytes supplemented with 0.01 mM glucuronic acid (38.78 ± 5.50%) at the blastocyst stage by 144 h after IVF compared with 0 mM (22.00 ± 5.68%), 0.1 mM (25.00 ± 5.81%), and 1.0 mM (18.60 ± 5.87%). The results of this study suggest that there are positive effects of 0.01 mM glucuronic acid supplementation during the oocyte maturation on successful IVF and subsequent embryo development in pigs.

Key words: glucuronic acid, swine, embryo development

652 Vitrification versus freezing for cryopreserving bovine embryos. S. G. Kruse* and G. E. Seidel Jr., *Colorado State University, Fort Collins.*

Our objective was to compare vitrification and freezing for cryopreserving bovine embryos. Crossbred, nonlactating beef cows were superovulated and embryos recovered 7d post estrus. Embryos of quality #1 or #2 per IETS standards were cryopreserved via vitrification (VIT; n = 40) or slow freezing (SLF; n = 42). For VIT, embryos were exposed to 5 M ethylene glycol in SynGro for 3 min at 22°C and moved to 6.5 M ethylene glycol + 0.5 M galactose + 18% Ficoll in SynGro at 22°C, and in 20 µl, immediately loaded in 0.25 mL straws between 2 columns of 1 M galactose in SynGro. After 35 s, embryos were vitrified by cooling for 2 min via contact of straw walls with columns drilled into an aluminum block immersed in liquid nitrogen; straws were then plunged into liquid nitrogen. Embryos frozen via SLF were exposed to 1.36 M glycerol in modified Dulbecco′s PBS + 0.4% BSA (PBS) for 10 min at 22°C, loaded in 0.25 mL straws, and placed into a freezing machine. Straws were cooled to −6°C at 4°C per min, held at −6°C for 5 min, seeded, held at −6°C for 10 min, and cooled to −30°C at 0.5°C per min and plunged into liquid nitrogen. Embryos were warmed/thawed by holding straws in air at 22°C for 8 s and placing them in 37°C water for 20 s. VIT embryos were mixed with 1 M galactose in SynGro in the straw for 2 min and directly transferred. SLF embryos were expelled, and glycerol was removed in steps: 0.8 M glycerol + 0.3 M sucrose for 6 min; 0.4 M glycerol + 0.3 M sucrose for 6 min; 0.3 M sucrose for 6 min; and PBS for 2 min, then loaded in 0.25 mL straws. Embryos were nonsurgically transferred into cows culled for unknown reasons, but with normal-appearing reproductive tracts. Recipients were d 7 ± 0.5, and each received 2 embryos into the uterine horn ipsilateral to the CL. Pregnancy diagnosis was at d 37 ± 2 via ultrasonography. Survival rate per embryo (normal fetus with heartbeat) did not differ (Fisher′s Exact; $P \geq 0.1$) between methods (VIT = 47.5%; SLF = 38.1%; 16 of the 19 pregnant cows carried twins). Therefore, VIT was similarly efficacious to SLF for cryopreservation of bovine embryos, and simpler, requiring less equipment, time, and expense.

Key words: embryo transfer, vitrification, bovine

653 Effects of cyanocobalamin supplementation on frozen-thawed boar spermatozoa. A. M. Hyde, L. E. Elsea*, and B. D. Whitaker, *The University of Findlay, Findlay, OH.*

The objective of this study was to assess the in vitro fertilization (IVF) of pig oocytes using frozen-thawed boar sperm supplemented with cyanocobalamin to the incubation media. Frozen semen pellets were thawed and incubated for 1 h in fertilization media containing cyanocobalamin (0, 0.5, 1.0, 2.0 µM) then evaluated for forward progressive motility and viability. Forward progressive motility of the 0.5 and 1.0 µM cyanocobalamin supplements were significantly higher ($P < 0.05$) than the 0 and 2.0 µM cyanocobalamin supplements. Viability of sperm supplemented with 0.5 µM cyanocobalamin was significantly higher ($P < 0.05$) than all other groups. Oocytes were matured and fertilized with frozen-thawed boar semen that was previously incubated for 1 h in fertilization media containing cyanocobalamin (0 or 0.5 µM; 100 oocytes/treatment). Fertilization characteristics were evaluated 12 h after IVF of oocytes and embryo development was analyzed at 48 h and 144 h post-IVF. There were no significant differences between treatment groups when evaluating sperm penetration, polyspermic penetration, or male pronucleus development. Embryos derived from the oocytes fertilized with 0.5 µM cyanocobalamin supplemented sperm had a significantly higher percentage ($P < 0.05$) of cleaved embryos compared with those without cyanocobalamin supplementation at 48 h after IVF. There were no significant differences in the percent of embryos reaching the blastocyst stage by 144 h after IVF

between treatment groups. The results of this study suggest that there are positive effects of 0.5 μM cyanocobalamin supplementation during incubation of frozen-thawed boar semen on early development of IVF derived pig embryos.

Key words: cyanocobalamin, in vitro fertilization, swine

654 GnRH therapeutics to advance the timing of pregnancy in the seasonally anovulatory mare. J. F. Thorson*[1,2], L. D. Prezotto[1,2], R. D. Cardoso[1,2], B. R. C. Alves[1], M. Amstalden[1], and G. L. Williams[1,2], *[1]Texas AgriLife Research, Beeville, [2]Texas A&M University, College Station.*

Onset of the winter anovulatory period in mares is associated with a marked diminution in adenohypophyseal synthesis and release of LH. Native GnRH, unlike its synthetic agonists, stimulates the synthesis and secretion of LH in mares without pituitary refractoriness. Herein we tested the hypotheses that 1) the average Julian day of conception can be accelerated by up to 50 d in winter anovulatory mares treated continuously with native GnRH beginning on February 1 and 2) mares will sustain luteal function and pregnancy following treatment withdrawal. Forty-two winter anovulatory mares were stratified by age and BCS across 2 locations in a randomized block design and assigned to 1 of 3 groups (n = 14/group): 1) Control: untreated; 2) GnRH-14: GnRH delivered subcutaneously in saline at a rate of 100 μg/h for 8 wk (Feb. 1–Mar. 29) using 4 consecutive 14-d pumps (Alzet 2ML2), or 3) GnRH-28: GnRH delivered as in 2, but using 2, 28-d pumps (Alzet 2ML4). Upon development of a 35 mm follicle and expression of estrus, mares were bred the following day and treated with hCG. Pregnancies were confirmed by ultrasonography on d 14, 24, 33, and 45, with blood samples collected to assess luteal function. Mares treated with GnRH (GnRH-14 and GnRH-28) exhibited marked increases ($P \leq 0.04$) in the frequency of development of a 35-mm follicle, submission rate for live cover/AI, ovulation, and pregnancy compared with Control mares on treatment d 28 (March 1, 2010) and 56 (March 29, 2010). Interval to first 35 mm follicle was 51.8, 15.1, and 16.0 d for Control, GnRH-14 and GnRH-28, respectively, excluding 2 GnRH-28 mares that failed to develop 35-mm follicles during the treatment period. By the end of the treatment period (Mar. 29), only 14% of Control mares were confirmed pregnant compared with 75% of GnRH treated mares. Further, serum concentrations of progesterone were similar to ($P \geq 0.07$) or greater than ($P \leq 0.05$) that of Control mares from d 14 to 46 post-breeding for GnRH-28 and GnRH-14, respectively. These data illustrate that continuous administration of native GnRH is a practical and highly efficient option for managing seasonal anovulation in mares.

Key words: GnRH, mare, seasonality