582 The effects of coenzyme Q10 supplementation on in vitro fertilization in porcine oocytes. Caitlin A. Streacker* and Brian D. Whitaker, *The University of Findlay, Findlay, OH.*

Polyspermic penetration as a result of in vitro fertilization (IVF) has shown to be a major obstacle in the production of porcine embryos. The objective of this study was to reduce the incidence of polyspermic penetration by supplementing coenzyme Q10 during oocyte maturation. Oocytes (n = 50/well) were maturated in tissue culture media 199 supplemented with 0, 10, 50 or 100 m*M* of coenzyme Q10 during the last 24 h of maturation. Frozen thawed semen from a single boar was used for fertilizing groups of approximately 30 oocytes in a concentration of 200 sperm/oocyte. Approximately 12 h after IVF, oocytes were evaluated for fertilization kinetics and subsequent embryonic development. A total of 600 oocytes over 3 replications were used in this study with one well/treatment/replicate. Data were analyzed using ANOVA with the main effects including treatment well and replicate. Chi-squared analysis was used to determine percentages of embryos reaching the different developmental states for each treatment. Oocytes supplemented with 50 m*M* coenzyme Q10 had significantly higher ($P < 0.05$) penetration rates (100 ± 14.97%) and pronuclear formation (MPN; 58.33 ± 19.54%) compared with 100 m*M* coenzyme Q10 supplementation. There was no difference between not supplementing coenzyme Q10 and 10 m*M* supplementation with respect to penetration and MPN formation rates. There was no difference between the treatments groups when considering the incidence of on polyspermic penetration. Cleavage at 48 h after IVF and blastocyst formation at 144 h after IVF were evaluated. Supplementing 100 m*M* coenzyme Q10 during oocyte maturation significantly decreased ($P < 0.05$) the percentage of embryos cleaved by 48h after IVF (3.33 ± 19.06%) and the percent of embryos at the blastocyst stage of development by 144 h after IVF (0.00 ± 15.76%) compared with all the other treatment groups. There was no difference between the other treatment groups with respect to cleavage and blastocyst formation rates. These results indicate that 50 m*M* coenzyme Q10 supplementation during oocyte maturation will not be detrimental to IVF and embryo culture in pig oocytes.

Key Words: coenzyme Q10, IVF, swine

583 Melatonin supplementation during oocyte maturation improves embryonic development in pigs. Rachel L. Lane* and Brian D. Whitaker, *University of Findlay, Findlay, OH.*

High levels of reactive oxygen species (ROS) in and around maturing oocytes lead to oxidative stress, which decrease fertilization success rates. Melatonin reduces levels of ROS during in vitro fertilization in swine. The objective was to determine the effects of melatonin during oocyte maturation on: IVF kinetics and embryonic development. Oocytes (n = 50/well) were supplemented during the last 24 h of maturation with 0, 75, 100, or 150 n*M* melatonin and then subjected to IVF and embryo culture. Frozen-thawed semen from a single boar was used (approximately 30 oocytes/well, 200 sperm/oocyte). A total of 600 oocytes over 3 replicates were used in this study with one well/ treatment/ replicate. Post IVF, a percentage of the embryos were evaluated for penetration, polyspermy, and male pronuclear formation rates. The remaining embryos were evaluated 48 h after IVF for cleavage and 144 h for blastocyst formation. Data were analyzed using ANOVA with the main effects including treatment, well and replicate. Chi-squared analysis was used to determine percentages of embryos reaching the different developmental stages for each treatment. There were no differences between treatment groups with respect to penetration and polyspermy. Supplementation of 150 n*M* melatonin produced a significant ($P < 0.05$) percent of embryos with MPN (25. 93 ± 21.17%) compared with those supplemented with 75 n*M* (66.67 ± 21.17%) or 100 n*M* (79.17 ± 21.17%). There was no difference between the 75 n*M* and 100 n*M* treatment groups. Supplementation of 150 n*M* (22.00 ± 21.37%) produced significantly ($P < 0.05$) less of cleavage between all of the other treatment groups 48 h after IVF. Embryo supplementation of 75 n*M* melatonin during maturation had significantly higher ($P < 0.05$) percentage of blastocyst formation by 144 h after IVF compared with those supplemented with 150 n*M* (10.00 ± 17.32%) of melatonin. There was no difference in the percent of embryos reaching the blastocyst stage of development by 144 h after IVF between no supplementation, 100 n*M*, and 75 n*M* of melatonin. These results indicate that the supplementation of 75 n*M* melatonin during the later stages of maturation improves embryo development by increasing cleavage and blastocyst rates 48 and 144 h after IVF, respectively.

Key Words: melatonin, IVF, swine

584 Cumulus-oocyte complex gene expression in bovine preovulatory follicles after administration of porcine luteinizing hormone. Amir Behrouzi*[1], Marcos G. Colazo[1], Ana Ruiz-Sanchez[2], and Divakar J. Ambrose[1,2], *[1]Alberta Agriculture and Rural Development, Livestock Research Branch, Edmonton, AB, Canada, [2]Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada.*

In previous work, the use of porcine luteinizing hormone (pLH) in lieu of gonadotropin releasing hormone (GnRH) for synchronizing ovulation improved pregnancy rates in lactating dairy cows. Later, we found that pLH treatment not only altered the LH profile, but also influenced expression of the BMP-15, GDF-9 and TGF-β1 proteins known to promote cumulus expansion and oocyte competence in nonlactating cows. We currently hypothesized that pLH would alter the expression of target genes in the cumulus-oocyte complexes (COC), which regulate oocyte competence. Cyclic nonlactating Holstein cows (n = 8) were subjected to ovarian stimulation with 200 mg FSH. Follicles = 10 mm were aspirated at 21 ± 1 h after giving either 100 μg GnRH or 25 mg pLH im, and COC recovered and frozen until qRT-PCR analysis. Serial blood samples were collected from before giving GnRH/pLH until 20 h after, for plasma LH analysis using an anti-bovine LH monoclonal antibody that cross-reacts equally with both bovine and porcine LH. Plasma LH concentrations and qRT-PCR data were analyzed using MIXED and GENMOD procedures of SAS, respectively. When LH concentrations (ng/mL) were compared in GnRH- and pLH-treated cows, effects of time and time-by-treatment interactions were detected ($P < 0.01$) with mean LH being higher in GnRH than pLH cows (2.8 ± 0.2 vs. 2.0 ± 0.2) from 30 min until 4 h post-treatment. However, mean plasma LH from 5 to 20 h post-treatment was greater ($P < 0.01$) in pLH- than in GnRH-treated cows (1.1 ± 0.2 vs. 0.4 ± 0.1). Treatment with pLH altered *HAS-2* and *GREM-1* mRNA abundance ($P < 0.01$) by about 26- and 7-fold, in COC of pLH- and GnRH-treated cows, respectively. Whereas the relative expression of *AREG* abundance tended ($P = 0.06$) to increase in GnRH-treated cows, *EREG*, *BTC*, *PGr*, *COX-2* and *PTX-3* mRNA expression were not affected by treatment. This study provides evidence that a prolonged, higher-than-basal LH profile in pLH-treated cows increased the expression of *HAS-2* and *GREM-1*, genes known to

improve oocyte competence, which may explain the higher pregnancy rates previously reported when pLH was used to induce ovulation.

Key Words: cumulus-oocyte complex, porcine LH, GnRH

585 α-Lipoic acid improves the post-thaw quality and survival of Nili-Ravi buffalo bull sperm. Muhammad Hammad Fayyaz[1], Sajid Iqbal[1,2], Muhammad Binyameen[3], Nasim Ahmad*[1], and Mushtaq Ahmad[1], *[1]Department of Theriogenology, University of Veterinary and Animal Sciences, Lahore, Pakistan, [2]Semen Production Unit, Qadirabad, Sahiwal, Pakistan, [3]Buffalo Research Institute, Kasur, Pakistan.*

Cryopreseravation exerts damages to sperm including crystal formation, oxidative stress, osmotic shock and lipid peroxidation. α-Lipoic acid (ALA); an antioxidant that plays a role in ATP production and breakdown of free radicals thus reduces oxidative stress. The aim of this study was to optimize the concentration and effect of ALA on post-thaw quality and survival at 37°C of buffalo (*Bubalus bubalis*) bull sperm. Semen of 3 mature Nili Ravi breeding bulls was collected twice a week using artificial vagina. The ejaculates of 3 bulls were pooled bull wise (replicates = 6), divided into 6 aliquots, diluted with the Tris-citrate-egg yolk extender supplemented with different concentrations of ALA (0, 0.5, 1, 1.5, 2 and 2.5mM) and cryopreserved in 0.5-mL French straws using standard procedure. Two straws per replicate were thawed and pooled and incubated in modified synthetic fluid (mSOF) at 37°C in a humidified CO_2 incubator. Semen was evaluated for motility, plasma membrane integrity (PMI; hypo-ospmotic swelling test), viability and acrosomal integrity simultaneously (FITC-PNA/PI assay) and DNA integrity using acridine orange assay; at 0, 1.5, 3 and 4.5h of incubation. The data were presented as Mean ± SEM and analyzed 6 × 4 factorial repeated measure ANOVA for 6 concentrations and 4 times using MIXED procedure in SAS Enterprise Guide (version 4.2) taking time as repeated measure. The results revealed that extender supplemented 0.5 m*M* and 1 m*M* ALA resulted in higher ($P < 0.05$) sperm motility (55 ± 1.3% and 55.5 ± 1.3% respectively than control; 48.5 ± 1.37%, 1.5 m*M*; 49 ± 1.37% and 2 m*M*; 43.5 ± 1.37%), PMI (59.8 ± 1.8% and 60 ± 1.8% respectively), viability (67.3 ± 1.5% and 68.5 ± 1.5% respectively) and DNA integrity (99.29 ± 0.48% and 99.39 ± 0.48%) than control and other groups after thawing. The survival of sperm was also recorded higher ($P < 0.05$) due to 0.5 and 1 m*M* ALA resulting in motility (23.5 ± 1.3% and 25.5 ± 1.3%), PMI (34.3 ± 1.8% and 37.8 ± 1.8%), viability (38.6 ± 1.5% and 42.6 ± 1.5%) and DNA integrity (97.34 ± 0.48%and 98.0 ± 0.48%) than control and other groups at 4.5 h of incubation. In conclusion, α-lipoic acid enhances the post-thaw quality and survival of buffalo bull sperm.

Key Words: α-lipoic acid, buffalo sperm, in vitro incubation

586 Comparison of fertility of liquid and frozen semen when varying the interval from CIDR removal to insemination. Brittany N. Richardson*[1], Erin L. Larimore[1], Julie A. Walker[1], Matthew D. Utt[2], J. Mel DeJarnette[2], and George A. Perry[1], *[1]Department of Animal Sciences, South Dakota State University, Brookings, SD, [2]Select Sires Inc., Plains City, OH.*

Cryopreservation allows for long-term storage of semen. However, cryopreservation and thawing imposes stress on spermatozoa, and prematurely initiates the process of capacitation; possibly decreasing sperm lifespan. Liquid semen is not exposed to these stressors, leading to a potential longer lifespan in the female reproductive tract and thus increasing the window for successful insemination. The objective of this study was to compare fertility of liquid and frozen semen when varying the interval from CIDR removal to insemination using the 7-d CO-Synch + CIDR protocol. Within age group cows (n = 389) were randomly assigned to insemination at 36 or 60 h after CIDR removal with either liquid (36L and 60L) or frozen semen (36F and 60F). Cows were monitored for estrus activity from CIDR removal to 60 h after CIDR removal. Cows that failed to exhibit estrus received an injection of GnRH (100 μg) and a blood sample was collected for analysis of estradiol concentration. Data were analyzed using the GLIMMIX procedure of SAS with sire and herd as random variables. There was no difference in pregnancy rates between liquid or frozen semen (53% and 52%), but cows inseminated at 60 h had greater ($P < 0.01$) pregnancy rates than cows inseminated at 36 h (72% and 31%). There was no interval by semen type interaction ($P = 0.57$). Estrus was detected in 63%, 61%, 56%, and 62% of 36F, 36L, 60F, and 60L, respectively (only 5% and 1% of 36F and 36L were detected in estrus before insemination). Overall cows that exhibited estrus had greater pregnancy rates compared with cows that did not ($P < 0.01$; 79% and 24%). Among cows that did not exhibit estrus, those inseminated with liquid semen tended to have greater pregnancy rates than those inseminated with frozen semen ($P = 0.06$), and ones that became pregnant had elevated ($P < 0.01$) concentrations of estradiol at 60 h (10.7 ± 1.9 vs 7.9 ± 2.9 pg/mL). In summary, there was no difference in pregnancy success between liquid and frozen semen. However, cows that exhibited estrus and inseminated at 60 h after CIDR removal had greater pregnancy success compared with cows that did not exhibit estrus.

Key Words: pregnancy, liquid semen, estrus

587 The effects of seasonal heat stress on sperm nuclear shape in boars. Teyanna M. Loether, Ricky L. Monson, Cathy Miller-Gaudette, and John J. Parrish*, *University of Wisconsin-Madison, Madison, WI.*

Negative effects on germ cell development have been correlated with elevated scrotal temperatures in domestic livestock species, including the boar. To fully comprehend the effect of environmental heat stress on male fertility, a total of 1,181 boar ejaculates, with an average of 49.2 ± 1.7 per week, were collected during June–November 2012 from a commercial boar stud. Boars were housed in facilities equipped with cool cell technology and facility temperature was measured every 30 min in 2012. Environmental temperature data were obtained from the National Oceanic and Atmospheric Administration for 2012 and 2014, and expressed as a weekly average of the maximum daily temperature. Sperm were assessed using Fourier Harmonic Amplitude (FHA) analysis to determine sperm nuclear shape and harmonic values were generated (HA0–5). Using the first collection week as the control, a GLM procedure with Dunnett's mean separation test was performed to measure changes occurring in HA0-HA5. Changes were found in HA0 and HA2 for wk 8–21, or the weeks of July 25-October 24 ($P < 0.05$). The HA0 at week one was 2.80 ± 0.01 μm and peaked during wk 14 at 2.87 ± 0.01 μm. Effects on HA0 appeared 3 weeks after external temperatures rose above 90°F (facility temperature = 82°F); this corresponds to when affected round spermatids first appear in the ejaculate as mature sperm. Fluctuations in HA0 followed the oscillations in temperature until the average maximum external and internal temperature fell below 68°F. To confirm these results, samples from the same facility were collected in 2014 3 weeks after external temperatures rose above 90°F. A period of heat stress was effectively captured as changes in HA0 and HA4 were again found 3 weeks post-heat event ($P < 0.05$). Increases seen in HA0, which is a reflection of the overall size of the sperm head, indicate that boar sperm nuclei enlarge during seasonal heat stress. Fourier Harmonic

Amplitudes, particularly HA0, 2, and 4, can be utilized as an effective tool in predicting specific periods where nuclear shape may be affected by heat stress. Furthermore, FHA analysis could be utilized in the future to correlate changes in nuclear shape as a result of heat stress to fertility.

Key Words: boar reproduction, seasonal heat stress, sperm nuclear shape

588 Expressional regulation of chemerin and its receptors in the liver and adipose tissues of young cattle by weaning and nutrition. Yutaka Suzuki*[1], Daichi Kato[1], Mitsuhiko Kondo[1], Hizuru Hatanaka[1], Satoshi Haga[1,2], Takafumi Gotoh[3], and Sanggun Roh[1], *[1]Graduate School of Agricultural Science, Tohoku University, Sendai, Miyagi, Japan, [2]Grassland Management Research Division, NARO Institute of Livestock and Grassland Science, Nasushiobara, Tochigi, Japan, [3]Kuju Agricultural Research Center, Kyushu University, Takeda, Oita, Japan.*

Chemerin is highly expressed and secreted from liver and adipose tissues, and acts through its receptors in an endocrine, paracrine, autocrine manner in ruminant. We previously reported the administration of chemerin analog peptide induced insulin secretion in vivo. However, there are currently no data on the expressional regulation of chemerin and its receptors depending on weaning and nutrition. In the present study we investigated the expressional levels of chemerin and its receptors (CMKLR1 and CCRL2) gene in the liver and adipose tissue of weaning calves and young cattle fed with different diets. Japanese Black calves were divided into (1) pre-weaning and post-weaning group (euthanized before and after weaning; 1.5 or 3.5 mo of age, respectively), or (2) concentrate-fed group received concentrate (CP 12%, TDN 73%; 2.5% of BW daily) plus Italian ryegrass (CP 9.4%, TDN 55.3%; ad libitum) and hay-fed group received Italian ryegrass ad libitum from 3 to 10 mo of age. The liver and/or adipose tissue (subcutaneous, mesenteric, perirenal and epididymal) samples were collected from cattle to quantitate mRNA expressional levels of chemerin, CMKLR1 and CCRL2 by qRT-PCR. Statistical analysis was performed by Mann-Whitney U test (significant difference; $P < 0.05$). Chemerin mRNA level was higher in mesenteric adipose tissue of post-weaning group and CCRL2 mRNA level was higher in the liver of post-weaning group, compared with pre-weaning group. Chemerin mRNA level in liver was higher in the concentrate-fed group than in hay-fed group at 10 months of age. This study revealed the effects of weaning and dietary energy source on gene expression of chemerin and CCRL2, suggesting the role of chemerin in the regulation of insulin secretion in cattle around weaning and in different nutritional states.

Key Words: chemerin, weaning, nutrition

589 Modulation of the metabolic response to vaccination in naïve beef steers using an acute versus chronic stress model. Nicole C. Burdick Sanchez*[1], Jeffery A. Carroll[1], Nathan D. May[2], Shelby L. Roberts[2], Heather D. Hughes[2], Paul R. Broadway[1], Kate P. Sharon[1,3], Michael A. Ballou[3], and John T. Richeson[2], *[1]USDA-ARS, Livestock Issues Research Unit, Lubbock, TX, [2]West Texas A&M University, Department of Agricultural Sciences, Canyon, TX, [3]Texas Tech University, Department of Animal and Food Sciences, Lubbock, TX.*

Available energy plays a critical role in the initiation and maintenance of an immune response to a pathogen a process that is further altered by activation of stress system. This study was designed to determine the effect of an acute versus chronic stress model on the metabolic response to vaccination in naïve beef steers. Steers (n = 32; 209 ± 8 kg) were blocked by BW and assigned to 1 of 3 treatments: (1) Chronic stress (CHR), 0.5 mg/kg BW dexamethasone (DEX) administered i.v. at 1000h on d 3 to d 6; (2) Acute stress (ACU), 0.5 mg/kg BW DEX administered i.v. at 1000h on d 6; or (3) Control (CON), no DEX. On d 2, steers were fitted with jugular vein catheters and moved into individual stanchions in an environmentally controlled facility. Blood samples were collected at −74, −50, and −26 h, at 0.5-h intervals from −4 h to 6 h, and at 12, 24, 36, 48, and 72 h relative to vaccination with Pyramid 5 + Presponse SQ at 1200h on d 6. Data were analyzed by the MIXED procedure of SAS specific for repeated measures. Feed intake was not different ($P = 0.44$) between CON (4.9 ± 0.2 kg), ACU (4.9 ± 0.2 kg) and CHR steers (5.1 ± 0.2 kg). There was a treatment × time interaction ($P < 0.001$) for serum glucose concentrations. Specifically, glucose concentrations increased at −50 h in CHR steers and at 12 h in ACU steers, and remained elevated through 72-h post-vaccination period compared with CON steers. The change in NEFA concentrations was affected by treatment ($P < 0.001$) and time ($P < 0.001$) such that the change in NEFA was greater in CHR (0.06 ± 0.01 mmol/L), followed by CON (−0.01 ± 0.01 mmol/L) and ACU steers (−0.04 ± 0.01 mmol/L). Blood urea nitrogen (BUN) was affected by treatment ($P < 0.001$) and time ($P < 0.001$) such that BUN concentrations were greatest in CHR (12.0 ± 0.1 mg/dL) followed by ACU (10.4 ± 0.1 mg/dL) and CON steers (9.6 ± 0.1 mg/dL). These data demonstrate that activation of the stress and immune axes using an acute or chronic stress model can increase energy mobilization before and following vaccination in naïve steers, potentially affecting energy availability needed to mount an adequate antibody response to vaccination.

Key Words: cattle, metabolism, vaccination

590 Mimicking acute and chronic stress exposure in naïve beef steers alters the acute phase response (APR) associated with vaccination. Jeffery A. Carroll*[1], Nicole C. Burdick Sanchez[1], Nathan D. May[2], Shelby L. Roberts[2], Heather D. Hughes[2], Paul R. Broadway[1], Kate P. Sharon[1,3], Michael A. Ballou[3], and John T. Richeson[2], *[1]USDA-ARS, Livestock Issues Research Unit, Lubbock, TX, [2]West Texas A&M University, Department of Agricultural Sciences, Canyon, TX, [3]Texas Tech University, Department of Animal and Food Sciences, Lubbock, TX.*

This study was designed to determine the effect of an acute versus chronic stress model on the APR associated with vaccination in naïve beef steers. Steers (n = 32; 209 ± 8 kg) were blocked by BW and assigned to 1 of 3 treatments: (1) Chronic stress (CHR), 0.5 mg/kg BW dexamethasone (DEX) administered i.v. at 1000h on d 3 to d 6; (2) Acute stress (ACU), 0.5 mg/kg BW DEX administered i.v. at 1000h on d 6 only; or (3) Control (CON), no DEX. On d 2, steers were fitted with indwelling rectal temperature (RT) devices and jugular catheters, and then moved into individual stanchions in an environmentally controlled barn. Blood samples were collected and serum isolated at −74, −50, and −26 h, at 0.5-h intervals from −4 h to 6 h, and at 12, 24, 36, 48, and 72 h relative to vaccination with Pyramid 5 + Presponse SQ at 1200 h on d 6. A second blood sample was used to measure complete blood cell counts. Data were analyzed using the Mixed procedure of SAS specific for repeated measures. There was a treatment × time interaction ($P < 0.01$) for RT such that DEX treatment in CHR and ACU steers decreased RT on d3 and d6, respectively, compared with CON steers. Vaccination on d 6 increased RT in CON but not in CHR or ACU steers with RT remaining elevated in CON for the remainder of the study. There was a treatment × time interaction ($P < 0.01$) for total white blood cells (WBC), neutrophils, lymphocytes, and monocytes. Specifically, DEX

increased WBC and neutrophils in CHR and ACU steers, yet decreased lymphocytes in CHR steers. Monocytes initially increased in response to DEX treatment in CHR and ACU steers. Also, eosinophils were greater ($P < 0.01$) in CON than ACU and CHR steers. There was a treatment × time interaction ($P < 0.01$) for interferon-γ (IFN-γ) such that IFN-γ was greater in CON and CHR than ACU steers. Concentrations of tumor necrosis factor-α were elevated ($P < 0.01$) in CHR compared with ACU and CON steers. These data suggest that ACU stress may prime, while CHR stress may cause hyper-activation, of the APR associated with vaccination in naïve beef calves, thus potentially negatively affecting the overall immunological response to vaccination.

Key Words: cattle, immunity, vaccination