Physiology and Endocrinology II

840 The physiology of heat stress: A shift in metabolic priorities at the systemic and cellular levels. R. P. Rhoads*[1] and L. H. Baumgard[2], [1]*Virginia Polytechnic Institute and State University, Blacksburg,* [2]*Iowa State University, Ames.*

Environmental heat stress (HS) undermines efficient animal production resulting in a significant financial burden to agricultural producers. The reduction in performance parameters, either lactation or lean tissue accretion, during HS has traditionally thought to result from the typical animal response of reduced nutrient intake during environmentally driven hyperthermia. Recently, this notion has been challenged with observations indicating heat-stressed animals may exploit novel homeorhetic strategies to direct metabolic and fuel selection priorities independently of nutrient intake or energy balance. Alterations in systemic physiology support a shift in metabolism, stemming from coordinated interactions at whole-body and tissue specific levels. Such changes are characterized by increased basal and stimulated circulating insulin concentration in addition to the ostensible lack of basal adipose tissue mobilization coupled with a reduced responsiveness to lipolytic stimuli. Hepatic and skeletal muscle cellular bioenergetics also exhibits clear differences in carbohydrate production and use, respectively due to HS. The apparent dichotomy in intermediary metabolism between the 2 tissue types may stem from factors such as TCA cycle substrate flux and mitochondrial respiration. Thus, the HS response markedly alters post-absorptive carbohydrate, lipid and protein metabolism through coordinated changes in fuel supply and utilization across tissues in a manner that is distinct from commonly recognizable changes that occur in animals on a similar reduced plane of nutrition. Perhaps most intriguing is that the coordinated systemic, cellular and molecular changes appear conserved across physiological states and among different ruminant and monogastric species. Ultimately, these changes result in the reprioritization of fuel selection during HS, which may be important for whole-body metabolism and overall physiological adaptation to hyperthermia.

841 Single and double, fixed-time insemination of postpartum sows given intravaginal triptorelin gel. N. R. Augspurger*[1], M. E. Johnston[1], M. E. Swanson[2], and S. K. Webel[1], [1]*JBS United Inc., Sheridan, IN,* [2]*Pennatek LLC, Radnor, PA.*

The objective was to compare farrowing rates and litter sizes from 1 or 2 fixed-time inseminations of sows treated with 200 μg triptorelin gel (TG: OvuGel) at 96 h post-weaning with those of untreated sows inseminated daily while in estrus. Four hundred 3 weaned sows were blocked by genetic line (Ausgene, PIC), parity (parities 1–7; average parity 3.1), and previous lactation length (range 13 to 25 d; average length 19.4 d), and allocated to one of 4 treatments: 1) Controls (n = 102) inseminated the day they were observed in estrus and at 24 h intervals for the duration of estrus; 2) TG24 sows (n = 100) treated with TG 96 h post-weaning and inseminated once 24 ± 2 h post-TG; 3) TG30 sows (n = 101) treated with TG 96 h post-weaning and inseminated once 30 ± 2 h post-TG; and 4) TG24+30 sows (n = 100) treated with TG 96 h post-weaning and inseminated 24 and 30 h post-TG. The number of inseminations per sow allotted was 1.7, 1.0, 1.0, and 2.0 for treatments 1–4, respectively. Estrus was detected in 89%, 94%, 90%, and 88% of the sows in treatments 1–4, respectively. There was no effect of treatment on farrowing rate ($P = 0.743$). Farrowing rates were 83.3%, 81.0%, 77.2%, and 81.0% for treatments 1–4, respectively. Average number of pigs born alive was 11.1, 10.8, 10.7, and 11.0 for treatments 1–4, respectively ($P = 0.822$). Control sows were inseminated if detected in estrus, whereas sows assigned to TG treatment were inseminated at a fixed-time, regardless of estrus. The number of sows farrowed/number not in estrus was 0/11, 2/6, 5/10, and 4/12 for treatments 1–4, respectively. These data indicate that sows treated with TG 96 h after weaning and inseminated at a fixed-time, independent of estrus status, have farrowing rates and litter sizes comparable to sows inseminated multiple times during behavioral estrus. Furthermore, 2 fixed-time inseminations (24 and 30 h post-TG) did not improve sow performance over a single insemination given at either 24 or 30 h post-TG.

Key Words: sow, reproduction, OvuGel

842 Effects of glucuronic acid and *N*-acetylglucosamine supplementation on the in vitro maturation and fertilization of pig oocytes. A. Mello,* K. Dalton, and B. D. Whitaker, *The University of Findlay, Findlay, OH.*

Increasing the perivitelline space (PVS) surrounding pig oocytes could improve the cortical reaction and decrease the incidence of polyspermic penetration during in vitro fertilization (IVF). Oocytes were supplemented during the last 24 h of maturation with 0.01 mM of PVS components, glucuronic acid or *N*-acetylglucosamine (GlcNAc) and then subject to IVF and subsequent embryonic development. Oocytes (n = 300) were evaluated for zona pellucida and PVS thickness after maturation in addition to the presence of cortical granules using the intensity of fluorescence. Fertilization characteristics were evaluated 12 h after IVF and rates of embryonic cleavage and blastocyst development were observed at 48 h and 144 h after IVF, respectively. There were no significant differences in zona pellucida thicknesses or fluorescent intensities of cortical granules; however, unsupplemented oocytes had a significantly thinner ($P < 0.05$) PVS (10.28 ± 0.86 μm) compared with the glucuronic acid (16.44 ± 0.87 μm) and GlcNAc (15.14 ± 1.20 μm) supplemented oocytes. Oocytes supplemented with glucuronic acid had a significantly less ($P < 0.05$) incidence of polyspermic penetration (15.00 ± 6.88%) compared with unsupplemented (30.00 ± 8.07%) and GlcNAc supplemented oocytes (40.00 ± 7.93). Oocytes supplemented with GlcNAc had significantly less ($P < 0.05$) male pronuclear development (60.00 ± 4.03%) compared with unsupplemented (70.00 ± 4.77%) and glucuronic acid supplemented oocytes (75.00 ± 5.07%). No significant differences in embryo cleavage rates at 48 h after IVF were seen but oocytes supplemented with glucuronic acid had a significantly higher ($P < 0.05$) percentage of blastocysts at 144 h after IVF (40.82 ± 6.29%) compared with the unsupplemented oocytes (21.74 ± 6.49%) and the GlcNAc supplemented oocytes (19.15 ± 6.42%). 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: cortical granule, polyspermy, IVF

843 Litter characteristics and thermoregulatory behavior of first parity sows exposed to a controlled heat stress (HS) during gestation. M. C. Lucy*[1], T. J. Safranski[1], J. N. Rhoades[1], J. W. Ross[2], N. K. Gabler[2], R. P. Rhoads[3], and L. H. Baumgard[2], [1]*University of Missouri, Columbia,* [2]*Iowa State University, Ames,* [3]*Virginia Tech, Blacksburg.*

First parity sows suffer from seasonal infertility that is associated with HS. The objective was to test the effects of HS on thermal regulation during gestation and assess carryover effects after farrowing in first

parity sows. Nulliparous gilts (n = 23) were brought into environmental chambers, inseminated and assigned to one of 2 ambient temperature treatments [HS, n = 12 (27 to 37°C; relative humidity, RH 85 to 55%) or TN, n = 11 (15 to 20°C; RH 60 to 50%)] that were applied during gestation. Rectal, ear and shoulder temperatures (RT, ET, and ST) and respiration rate [RR; breaths per minute (BPM)] were collected (0900 and 1500h). Piglets and placentas were weighed after farrowing. There was an effect of treatment ($P < 0.001$) on RT (38.4 ± 0.1 vs 38.1 ± 0.1°C), ET (35.9 ± 0.1 vs 29.6 ± 0.1°C), ST (35.8 ± 0.1 vs 29.1 ± 0.1°C), and RR (58 ± 2 vs 30 ± 2 BPM) in gestation (HS vs TN). Sows were moved to a thermoneutral farrowing room (23°C) during the last wk of gestation. Gestation was shorter ($P < 0.05$) for HS (115.7 ± 0.5 d) compared with TN (117.4 ± 0.5 d). The number of live born (11.5 ± 0.8), still born (0.3 ± 0.1), and mummies (0.4 ± 0.2) per litter were similar for HS and TN. There was an effect of treatment on birth weight ($P < 0.01$) and placental weight ($P < 0.10$) because HS sows had lighter piglets (1180 ± 50 vs 1409 ± 53 g) and lighter placenta (224 ± 12 vs 255 ± 13 g) compared with TN. The piglet weight difference (HS vs TN) was maintained for wk 1 (2171 ± 109 vs 2761 ± 113 g; $P < 0.001$) and wk 2 (3809 ± 205 vs 4488 ± 205 g; $P < 0.05$) but not at weaning (4824 ± 205 vs 5259 ± 214 g; $P > 0.10$). The RT, ET, and ST (respectively) increased ($P < 0.001$; 38.2 ± 0.1 to 39.5 ± 0.1°C, 31.8 ± 0.3 to 35.0 ± 0.3°C, 30.2 ± 0.3 to 33.8 ± 0.3°C; d −5 to d 10) after farrowing (during lactation). There was a carry-over effect of gestational HS on RT in farrowing (non-HS conditions) because HS sows had a greater increase in RT after farrowing (39.8 ± 0.1 vs 39.2 ± 0.1°C on d 2; $P < 0.001$). In summary, gestational HS shortened gestation and reduced piglet birth and placenta weight. There were carry-over effects of gestational HS on sow thermoregulation during lactation. This project was supported by USDA NIFA 2011–67003–30007.

Key Words: sow, heat stress

844 Comparison between conventional sex-sorted semen and a higher dose\ lower concentration sex-sorted semen on conception rates and calf gender ratio. J. A. Lucena*[1], A. G. Kenyon[1], J. P. Reynolds[2], J. D. Champagne[1], T. L. Lehenbauer[1], and S. S. Aly[1], *[1]Veterinary Medicine Teaching & Research Center, School of Veterinary Medicine, University of California, Davis, [2]Western University of Health Sciences, Pomona, CA.*

The objective of this study was to compare conception rates and gender ratios among Jersey heifers and lactating cows inseminated with conventional (Low Dose High Sort [LDHS]) or High Dose Low Sort (HDLS) sex-sorted semen. Study subjects consisted of nulliparous heifers and lactating cows that received their first service after a 50-d voluntary waiting period (VWP). Females were allocated systematically to each treatment group. Subjects that failed to conceive were rebred to the same sire and type of sex-sorted semen for up to 2 additional services. Females that were not pregnant after 3 breeding attempts were censored. A total of 1,879 services were performed on 1,029 eligible females (LDHS; n = 499, HDLS; n = 530). Experiment groups were comparable at enrollment with respect to age at first AI among heifers ($P = 0.45$) and DIM at first AI for multiparous cows ($P = 0.53$). Nulliparous heifers bred to HDLS had a 17% higher risk of conceiving compared with LDHS (HR = 1.17; $P = 0.12$); cows had a 22% higher risk of conception to HDLS (HR = 1.22; $P = 0.05$). Insemination to HDLS resulted in fewer female calves compared with LDHS (77.4% vs. 88.4%, $P < 0.01$). The odds ratio for a female calf born to a dam inseminated to HDLS compared with LDHS was 0.44 ($P < 0.01$). The use of HDLS sex-sorted semen resulted in a minimal improvement in the instantaneous risk for pregnancy. Furthermore, the use of HDLS resulted in 11% less females compared with LDHS which may be explained by the lower gender bias in HDLS compared with LDHS. Nevertheless, the reduction in proportion of pregnancies with female calves may be cost-effective given the simpler processing technique required to sort semen into HDLS compared with LDHS.

Key Words: sex-sorted semen, sperm dose, conception rate

845 Effect of a post-weaning high-energy diet on age at puberty, testicular characteristics, and semen production in Holstein bulls. B. R. Harstine*[1], M. Maquivar[1], L. A. Helser[2], M. D. Utt[1], C. Premanandan[3], J. M. DeJarnette[2], and M. L. Day[1], *[1]Department of Animal Sciences, The Ohio State University, Columbus, [2]Select Sires Inc., Plain City, OH, [3]Department of Veterinary Biosciences, The Ohio State University, Columbus.*

We previously reported that feeding Holstein bulls a high energy (HE) diet beginning at 58 d of age increased LH secretion at 125 d, increased testosterone concentration from 181 to 210 d, and increased scrotal circumference (SC) from 147 to 230 d. In the present experiment, the impact of this diet on age at puberty and on testicular measurements, seminiferous tubule diameter, and semen production in mature bulls is reported. From 58 to 230 ± 0.3 d of age, calves received either a high energy diet (HE, n = 8; 2.2 Mcal/kg NEm and 1.37 Mcal/kg NEg, targeted ADG 1.5 kg/d) or a control diet (n = 7; 1.70 Mcal/kg NEm and 1.09 Mcal/kg NEg, targeted ADG 0.75 kg/d). Thereafter, bulls were fed a similar diet. Beginning at 241 ± 5 d of age, semen collection was attempted and continued every 14 d using a teaser animal and artificial vagina until each bull attained puberty. Puberty was defined as the day an ejaculate containing 50×10^6 spermatozoa with 10% motility was obtained. Ages of bulls during these collections ranged from 233 to 396 d. SC was measured on d 262, 305, 332, and 367. To assess mature semen production, semen was collected thrice weekly during the 4 weeks before slaughter at 580 ± 18 d of age. Epididymal and testicular measurements were collected after slaughter, and seminiferous tubule diameter was measured using fixed and stained sections of testes. Age at puberty did not differ between treatments (310 ± 35 d), but SC was greater ($P < 0.05$) in the HE treatment during the puberty assessment period. As mature bulls, sperm production did not differ between treatments in the collections preceding slaughter. However, testis weight (318.0 ± 13.5 vs. 267.5 ± 14.4 g), epididymal weight (31.6 ± 1.1 vs. 28.0 ± 1.2 g), and testis volume (305.0 ± 11.9 vs. 244.9 ± 12.9 cm^3) were all greater ($P < 0.05$) in the HE treatment. Seminiferous tubule diameter did not differ between treatments (252.23 ± 2.44 μm). These data suggest that a HE diet initiated at 2 mo of age increased testis size, but did not accelerate puberty, increase sperm production, or increase seminiferous tubule diameter.

Key Words: bull, diet, testes

846 Oviductal protein and ovarian hormone concentrations during the first five days of the estrous cycle in first and third estrous ewe lambs and mature ewes. J. G. Berardinelli,* *Montana State University, Bozeman.*

The objectives of this study were to determine if ampullary (AMP) and isthmic (IST) protein concentrations patterns, and progesterone (P4) and estradiol (E2) concentrations patterns differ over the first 5 d of the estrous cycle among first (pubertal; FE) and third estrous (TE) ewe lambs and mature ewes (ME). Crossbred, spring-born ewe lambs (n = 40) and mature (4- to 6-yr-old; n = 20) were assigned randomly at estrus to treatments arranged in a 3 (cycle type; CT) x 5 (day of cycle) factorial

(n = 4 ewes per treatment). Observation of estrus occurred twice daily with the aid of mature, epididymectomized rams beginning in October. Each ewe was bi-laterally salpingectomized on either Day 0 (estrus), 1, 2, 3 or 4 after estrus. A jugular blood sample was collected from each ewe immediately before surgery on these days. Right and left AMP and IST segments were flushed with 4 and 2 mL of Delbecco's PBS (pH = 7.2), respectively. PMSF was added to a final concentration of 10 mM to each flushing and flushings were flash frozen in liquid N2. Protein in flushings was assayed using the BCA method (Pierce, Rockford, IL). Serum samples were assayed for P4 and E2 concentrations by RIA. There was an interaction ($P < 0.01$) between CT and day of cycle for AMP and IST protein concentrations. These interactions were caused by greater ($P < 0.05$) protein concentrations in the AMP and IST of ME than in FE ewes on Day 2 and lower ($P < 0.05$) concentrations in ME than in FE ewes on Day 4. There was an interaction ($P < 0.01$) between CT and day of cycle for P4 and E2 concentrations and P4:E2 ratios. P4 increased ($P < 0.05$) more rapidly from D 2 to 4 in ME than in FE and TE ewes. E2 increased ($P < 0.05$) from D 2 to 3 more rapidly in ME than in FE and TE ewes; whereas, E2 deceased ($P < 0.05$) from D 3 to 4 in ME and TE ewes, while E2 increased ($P < 0.05$) from D 2 to 4 in FE ewes. P4:E2 ratios increased ($P < 0.05$) from D 2 to 4 in ME and TE ewes than in FE ewes. In conclusion, concentration patterns of AMP and IST protein and ovarian steroid hormones during the first 5 d of the estrous cycle differ between FE and ME ewes. These results indicate the possibility that reduced fertility in ewe lambs at their first estrus may be caused by an inappropriate protein milieu in the AMP and IST that may be detrimental to normal early embryonic development.

Key Words: oviductal protein, ovarian steroids, ewe lambs

847 Effect of phytoestrogens on basal and GnRH-induced gonadotropin secretion from ovine pituitary cells in culture. S. A. Arispe,* B. M. Adams, and T. E. Adams, *University of California, Davis.*

The objective of our study was to assess the effect of dietary phytoestrogens (PEs) on basal and stimulated gonadotropin secretion. We used ovine pituitary cells in culture to assess the effect of PEs on basal and GnRH agonist-induced LH and FSH secretion. Pituitary tissue was collected from wether lambs, enzymatically dispersed and aliquots of the resulting cell suspension were used to seed 24 well culture plates. After attachment, media containing vehicle, 50 or 5000 pM estradiol (E2), or increasing concentrations (1, 10, 100, or 1000 nM) of PE (coumestrol [CM] or zearalenone [ZR]) was added to 4 replicate wells. Each experiment was duplicated. Media was collected after treatment with E2 or PE for 48 h and basal FSH secretion was determined by RIA. The effect of estrogen on gonadotrope responsiveness was also assessed by incubating cells with E2 or PE for 12 h followed by addition of a GnRH agonist (Des-Gly^{10}, D-Ala^{6} GnRH ethylamide [GnRH-A] at 0.1, 1, 10, 100, 1000 pM) and incubation for 6 h. Data were analyzed using linear mixed model procedures. For basal secretion, fixed effects were treatment (TRT) and experiment (EXP). Random effect was plate (PL). For GnRH-A induced LH secretion, fixed effects were TRT, GnRH-A, and TRT:GnRH-A. Random effects were EXP, EXP:GnRH-A, PL, and PL:GnRH-A. Our data indicate that 100 nM CM and ZR decreased ($P < 0.001$) basal FSH secretion by 40%. Treatments with 50 pM E2 or 100 nM CM or ZR increased ($P < 0.01$) the sensitivity of the response to GnRH-A by 27-fold. The effects of E2 and PE on basal and agonist-induced gonadotropin secretion were blocked ($P < 0.01$) by the estrogen receptor (ER) antagonist ICI 182,780. To examine the role of specific ER isoforms in the PE-induced effects, we incubated cells with ERα or β agonists (propyl pyrazole triol [PPT] and diarylpropionitrile [DPN], respectively) and assessed basal and GnRH-A induced responses. PPT was 10–100 times more effective than DPN in decreasing basal FSH secretion and increasing GnRH-A induced LH secretion ($P < 0.01$). Overall, these data demonstrate that PEs mimic E2 and affect gonadotrope function in a manner that is likely mediated by ERα.

Key Words: phytoestrogen, gonadotropin, estrogen receptor

848 Effect of acidic pH on uterine response to interferon-τ. A. Ahmadzadeh,* T. Davis, K. Carnahan, and C. Autran, *University of Idaho, Moscow.*

Low uterine pH associated with high blood urea and dietary protein, can reduce fertility in dairy cows. The objective was to determine the effects of acidic pH on protein expression of the endometrial cells of the bovine uteri in response to interferon-τ (IFN-τ).Using bovine endometrial (BEND) cells as a model, the experiment was designed to determine the effects of acidic pH on the production of 2 IFN tau stimulated proteins, ISG15 and Mx1. Dimethadione (DMD), a weak non-metabolizable acid, was used to decrease culture medium pH. Bovine endometrial cells were cultured in media containing 0 (pH = 7.3 ± 0.05), 10 (pH = 7.2 ± 0.05), 15 (pH = 7.0 ± 0.05), or 20 (pH = 6.8 ± 0.05) mM DMD and subsequently challenged with 0 or 10,000 antiviral units of recombinant IFN-τ and were incubated for 24 h. Once harvested, BEND cells were lysed and the lysate was analyzed for protein content. Protein was separated by SDS-PAGE and subjected to Western blot analysis and immunoblotting to assess the production of Mx1 and ISG15. DMD decreased ($P < 0.01$) the pH of the culture media. Based on optical density, IFN-τ increased ($P < 0.01$) Mx1 and ISG15 production regardless of DMD treatment after 24 h. There was effect of DMD ($P < 0.01$) and DMD by IFN-τ interaction ($P < 0.01$) on both Mx1 and ISG15. The 15 and 20 mM DMD reduced ($P < 0.01$) IFN-τ-induced Mx1 expression, whereas 20 mM DMD reduced ($P < 0.01$) ISG15 expression in response to IFN-τ indicating that acid pH abrogated BEND cell Mx1 and ISG15 production in response to IFN-τ. These results show that acidic pH disrupted IFN-τ-stimulated Mx1 or ISG15 production, and it may indicate that high dietary protein may compromise fertility by inducing lower pH in uterine secretions altering proteins essential for pregnancy.

Key Words: pH, interferon-τ, bovine endometrial cells