

to phytate with a higher K_m (74 vs. 34 μM , $P < 0.01$) than PhyA, this bacterial enzyme displayed a greater ($P < 0.01$) V_{max} (1,070 vs. 120 $\mu\text{mol min}^{-1} \text{mg}^{-1}$), k_{cat} (840 vs. 170 sec^{-1}) and k_{cat}/K_m (1.1×10^7 vs. $0.5 \times 10^7 \text{ M}^{-1} \text{sec}^{-1}$) than that of PhyA. PhyA and AppA2 had optimal temperatures of 65°C (pH 5.0) and 58°C (pH 3.5), respectively. Both enzymes had two pH optima at 37°C: PhyA with a peak at 2.0 and a greater peak at 5.5, while AppA2 had a peak at 3.4 and a greater peak at 5.0. PhyA was nearly twenty times more resistant to competitive inhibition by myo-inositol hexasulfate than AppA2 ($K_i = 3.9$ vs. 0.2 μM). Likewise, 4 times more (1.4 vs. 0.3 M) guanidine hydrochloride was required to produce the same 50% inhibition of enzyme activity for PhyA than AppA2. Overall, AppA2 possesses superior kinetic properties compared to PhyA at pH 3.5, which helps in catalyzing phytate hydrolysis at commonly observed gastric conditions of simple-stomached animals.

Key Words: Phytase, Kinetics, Enzymology

883 Effects of dietary electrolyte balance and molasses in diets with corn-based distillers dried grains with solubles on growth performance in nursery and finishing pigs. C. Feoli*¹, J. D. Hancock¹, S. M. Williams¹, T. L. Gule¹, S. D. Carter², and N. A. Cole³, ¹Kansas State University, Manhattan, ²Oklahoma State University, Stillwater, ³USDA/ARS, Bushland, TX.

Two assays were conducted to determine the effects of dietary electrolyte balance (dEB) and molasses in diets with corn-based

distillers dried grains with solubles (DDGS, Sioux River Ethanol, Hudson, SD) on growth performance of nursery and finishing pigs. For the first experiment, 126 nursery pigs (35 d old and avg BW of 10.2 kg) were assigned with six pigs/pen and seven pens/treatment. Treatments were a corn-soybean meal-based control and diets with DDGS as 30% of the formula without and with 0.93% sodium bicarbonate to bring dEB to 64 meq/kg [(Na + K) × (Cl + S)] as in the control. Diets were formulated to 1.4% Lys, 0.75% Ca, and 0.35% available P. Pigs fed the control diet had greater ADG ($P < 0.03$) and ADFI ($P < 0.08$) but did not differ in G:F ($P > 0.6$) compared to those fed diets with DDGS. Addition of sodium bicarbonate did not improve growth performance ($P > 0.3$). For the second experiment, a total of 70 gilts (avg BW of 60.5 kg) were assigned with two pigs/pen and five pens/treatment. The pigs were fed the experimental diets for 26 d, fed a common diet for 6 d, and then reassigned to a different treatment for an additional 26-d assay. The end result was 10 pens per treatment. Treatments were a corn-soybean meal-based control and diets with DDGS as 40% of the formula without and with 5% molasses and sodium bicarbonate (none, 1, and 2%) arranged as a 2 x 3 factorial plus control. Diets were formulated to 0.9% Lys, 0.6% Ca, and 0.22% available P. Pigs fed the control diet had greater ADG and ADFI ($P < 0.001$) but did not differ in G:F ($P > 0.4$) compared to those fed diets with DDGS. Adding molasses and(or) sodium bicarbonate did not affect ADG ($P > 0.5$) or ADFI ($P > 0.14$) and adding molasses actually decreased ($P < 0.03$) G:F. In conclusion, adding sodium bicarbonate and(or) molasses to diets with DDGS did not improve growth performance in nursery or finishing pigs.

Key Words: Distillers Dried Grains, dEB, Pig

Physiology & Endocrinology - Livestock and Poultry: Reproductive Physiology

884 Emerging concepts regarding the integration of neuroendocrine signals that regulate gonadotropin secretion in domestic livestock. C. A. Lents*¹ and C. R. Barb², ¹The University of Georgia, Athens, ²USDA-ARS, Russell Research Center, Athens, GA.

The pulsatile discharge of GnRH from hypothalamic neurons is obligatory for the synthesis and release of the pituitary gonadotropins. Many conditions have been characterized that reduce gonadotropin secretion and result in anovulatory states which contribute to inefficiencies in livestock production. Nutrient intake, body energy reserves, suckling, and season are all major regulators of gonadotropin secretion in mammals. Lack of adequate gonadotropin secretion during the pre-pubertal and postpartum periods adversely impact the reproductive efficiency of domestic herds and flocks. These various anovulatory states typically have one fundamental similarity; a lack of hypothalamic release of GnRH. For the most part, pituitary function remains intact, as indicated by the release of gonadotropin from the anterior pituitary gland in response to exogenous GnRH. Identifying the hormonal and metabolic factors that mediate the central effects of these factors on the GnRH neuronal network in the brain has been an intensely studied area. Additionally, season and metabolic status seems to enhance the central mechanism of estradiol negative feedback on LH secretion. Despite much progress, identifying the mechanisms that directly mediate these actions on GnRH neurons in a consistent and well regulated fashion has proven difficult. For example, GnRH neurons appear to lack estrogen receptors, suggesting that feedback effects of estrogen are mediated through interneuronal systems. A role

for the peptide hormone kisspeptin as a major regulator of gonadotropin secretion has recently emerged. Recent reports have demonstrated a potential role of kisspeptin in regulating the onset of puberty as well as mediating metabolic and photoperiodic control of reproduction in rodents. The purpose of this review is to summarize what is currently known regarding kisspeptin in domestic livestock and postulate the role it may have in regulating reproductive function of these larger mammalian species.

Key Words: Kisspeptin, Gonadotropin, Reproduction

885 Effects of human chorionic gonadotropin (hCG) and gonadotropin releasing hormone (GnRH) on follicle and corpus luteum dynamics and concentrations of progesterone in pre-pubertal Angus heifers. C. R. Dahlen*², J. E. Larson¹, G. Marquezini¹, and G. C. Lamb¹, ¹North Central Research and Outreach Center, University of Minnesota, Grand Rapids, ²Northwest Research and Outreach Center, University of Minnesota, Crookston.

We determined the effects of administering human chorionic gonadotropin (hCG) on subsequent follicle and corpus luteum dynamics and concentrations of progesterone in pre-pubertal heifers. Forty-seven purebred, pre-pubertal Angus heifers were stratified by age and weight and assigned randomly to one of three treatments: 1) heifers received a 100 μg injection of GnRH (GnRH; $n = 16$); 2) heifers received a 1,000 IU injection of hCG (H1000; $n = 16$); and 3) heifers received a 500 IU

injection of hCG (H500; n = 15). From d -1 to 9 relative to treatment daily blood samples were collected to determine concentrations of progesterone and ovaries of each heifer were examined daily by transrectal ultrasonography using a 7.5Mhz transducer. Diameter of all follicles larger than 5 mm and all corpora lutea (CL) were measured and recorded. A greater percentage ($P < 0.05$) of heifers in the H1000 treatment (87.5%) ovulated compared with GnRH heifers (43.8%), whereas H500 heifers (73.7%) were intermediate. A greater percentage ($P < 0.05$) of H1000 (87.5%) and H500 (73.3%) heifers developed a CL compared with GnRH heifers (18.8%). The largest follicle present on ovaries of H1000 and H500 heifers was smaller ($P < 0.05$) from d 2 to 5 than that of GnRH heifers. Concentrations of progesterone peaked on d 6 for all treatments. Heifers treated with H1000 (1.72 ng/ml) had peak concentrations of progesterone that were greater ($P < 0.05$) than H500 heifers (1.34 ng/ml), which were greater ($P < 0.05$) than heifers treated with GnRH (0.31 ng/ml). Mean volume of luteal tissue was greater ($P < 0.05$) in H1000 heifers (1.54 cm³) than in H500 heifers (1.15 cm³), which was greater ($P < 0.05$) than in heifers treated with GnRH (0.23 cm³). We concluded that hCG was more effective than GnRH in its ability to ovulate follicles, increase volume of luteal tissue in the subsequent developing CL, and concentrations of progesterone in pre-pubertal heifers. In addition, hCG appears to be more effective when administered at 1,000 IU than 500 IU.

Key Words: Human Chorionic Gonadotropin, Estrous Synchronization, Beef Heifers

886 Increasing ovulation rate reduced follicle size and increased blood progesterone concentrations but had no effect on fertility in cattle selected for twins. S. E. Echternkamp*, R. A. Cushman, and M. F. Allan, *USDA, ARS, U.S. Meat Animal Research Center, Clay Center, NE.*

Smaller ovulatory follicles (F) and lower progesterone concentrations during the luteal phase after breeding reportedly decrease fertility and embryonic survival in cattle. Diameter of individual F and corpora lutea (CL), blood progesterone concentrations, and conception to AI were compared among cows with ovulation rate (OR) records of one (n = 74), two (n = 253), three (n = 88), or four (n = 6) CL in 2004 to 2006; herd mean for OR was 2.09. Number and diameter of F and subsequent CL were determined by ultrasonography at 12 h after onset of estrus and 8 to 15 d after AI, respectively, and number of fetuses at 60 d after AI. Progesterone was quantified by RIA in a single blood sample collected at CL diagnosis. Data were analyzed by SAS PROC MIXED procedures; main effects in the models were OR, day, fetal status, and year. Follicle diameter was smaller ($P < 0.01$) in 2006. Follicle and CL diameter were correlated ($r = 0.53$; $P < 0.01$) and decreased ($P < 0.01$) with increasing OR (1 CL = 17.1 ± 0.3 and 23.3 ± 0.4 mm, 2 CL = 14.0 ± 0.1 and 20.2 ± 0.4 mm, 3 CL = 12.6 ± 0.2 and 18.1 ± 0.3 mm, 4 CL = 11.7 ± 0.5 and 16.7 ± 0.7 mm, respectively), but diameter were similar for fertile (fetus) and infertile ovulations (13.9 ± 0.1 and 19.8 ± 0.4 mm vs. 13.8 ± 0.1 and 19.4 ± 0.4 mm, respectively). Fetal number per female increased ($P < 0.01$) with OR (0.56 \pm 0.11, 1.04 \pm 0.06, 1.47 \pm 0.10, and 1.80 \pm 0.26 for 1, 2, 3, and 4 CL, respectively), but the fetus:ovulation ratio (0.52 \pm 0.3) was unaffected. OR did not affect pregnancy rate (65.6 \pm 0.3%) at 60 d after AI. Progesterone concentrations increased ($P < 0.01$) with OR (1 CL = 7.1 ± 0.3 , 2 CL = 8.9 ± 0.2 , 3 CL = 9.7 ± 0.3 , 4 CL = 8.5 ± 0.7 ng/ml) and from d 8 (6.3 \pm 0.5 ng/ml) to 14 (10.6 \pm 0.5 ng/ml). Progesterone concentrations did not differ between pregnant and nonpregnant

cows (8.8 \pm 0.3 vs. 8.2 \pm 0.3 ng/ml). Decreased follicle size and increased progesterone in cows with natural multiple ovulations did not affect conception.

Key Words: Cattle, Fertility, Ovulation Rate

887 Altered liver gene expression and reproductive function in postpartum suckled beef cows on different planes of nutrition. M. Bionaz*¹, F. Samadi², M. J. D'Occhio^{2,3}, and J. J. Loo¹, ¹University of Illinois, Urbana, ²The University of Queensland, Gatton Campus, Australia, ³CRC for Beef Genetic Technologies, Gatton Campus, Australia.

The effect of caloric restriction on long-term liver gene expression was evaluated using Droughtmaster cows (n = 5/diet) assigned to improved (IP) or moderate pasture (MP) from 6 mo of gestation through 14 wk of lactation. Ovarian follicular status and blood IGF-1, insulin, and glucose postpartum were determined weekly. Liver was biopsied at 1, 6, and 14 wk postpartum and RNA used to measure expression of genes (qPCR) with key functions in fatty acid metabolism (*PPARA*, *CPT1A*, *ACOX1*, *ADIPOR2*, *HNF4A*, *SREBF1*), cholesterol metabolism (*APOB*, *HMGCR*, *PPARD*, *SREBF2*), insulin signaling (*IRS1*, *FRAP1*), gluconeogenesis (*PCK1*, *FBP1*, *PC*), reproductive function (*IGF1*, *GHR*, *SHBG*, *SIRT1*), signal transduction (*IGFBP1*), regulation of cell growth (*IGFBP2*), and oxidative stress (*SOD1*). Cows on IP had greater ($P < 0.05$) BW (+57 kg) and BCS (3.7 vs. 2.3) at parturition, and greater plasma concentrations of insulin (5.9 vs. 3.8 μ U), glucose (3.9 vs 3.4 mmol/L), and IGF-1 (191 vs. 145 ng/mL) postpartum. All IP cows resumed ovulation between 12-15 wk postpartum compared with only one MP cow. Effects of caloric restriction on liver gene expression were most evident at wk 6 when IP cows had greater ($P < 0.05$) expression (1.5 to 2.9-fold) of *ACOX1*, *ADIPOR2*, *CPT1A*, *FRAP1*, *GHR*, *HNF4A*, *IRS1*, *PPARA*, *PPARD*, *SCD*, *SHBG*, *SREBF1*, and *SREBF2*. Temporal effects of diets on gluconeogenesis, cholesterol synthesis/metabolism, and oxidative stress were most apparent between wk 1 and 6, with MP resulting in lower (time \times diet $P < 0.06$) mRNA (-1.4 to -2.3-fold) of *HMGCR*, *PC*, *APOB*, *PPARD*, and *SOD1* at wk 6. *IGFBP1* increased between wk 1 and 14 with both IP and MP. However, on wk 6, *IGFBP1* was 3.7-fold greater with MP vs. IP (time \times diet $P < 0.05$). *IGFBP2* was greater with MP vs. IP at wk 1 (2-fold), but decreased linearly through wk 14 compared with IP. *SCD* and *SREBF1* expression was lower with MP throughout lactation, suggesting reduced desaturation and fatty acid synthesis in liver. Delayed resumption of ovulation with caloric restriction might be linked to altered metabolic homeostasis driven at least in part by liver genomic adaptations.

Key Words: Liver, Reproduction, Caloric Restriction

888 Luteal function at day 30 of pregnancy in relation to serum progesterone in dairy cows at risk for late embryonic or early fetal mortality. J. D. Rhinehart*¹, J. A. Flores¹, R. A. Milvae², and E. K. Inskeep¹, ¹West Virginia University, Morgantown, ²University of Connecticut, Storrs.

Pregnancy failures during placentation have been associated with low concentrations of peripheral serum progesterone (P₄) in lactating dairy cows. Experiments were done to determine if luteal function and/or metabolic clearance rate of injected P₄ differed for cows with high or

low concentrations of serum P₄ on approximately d 30 of gestation. Luteal tissue was removed from pregnant cows with ≥ 4.0 ng/mL (H) or ≤ 2.5 ng/mL (L) serum P₄ during d 28 to 34 post-insemination. Luteal tissue was analyzed for P₄ by radioimmunoassay and mRNA expression of preproendothelins 1 and 3, endothelin converting enzyme, endothelin receptors A and B, cyclooxygenase-2, aldoketoreductase 1B5, 15-hydroxyprostaglandin dehydrogenase, and prostaglandin E synthase by real-time RT-PCR. Dispersed luteal cells were incubated for 2 h with bovine luteinizing hormone (bLH) or arachidonic acid (AA), increasing (10^{-10} to 10^{-7}) concentrations of endothelin-1 (ET-1), and combinations of ET-1 and bLH or AA. Neither luteal content of P₄ (mean 106.0 ± 3.3 μ g) nor mRNA for genes investigated were correlated with serum P₄ at lutectomy. Both basal and LH-stimulated P₄ secretion from dispersed luteal cells were inhibited ($P < 0.05$) by ET-1 in a dose dependent manner. This inhibition was greater ($P < 0.05$) for luteal cells from L vs. H cows cultured with ET-1 and AA. To evaluate P₄ catabolism, cows were injected s.c. with 150 mg P₄ every 12 h beginning at lutectomy. In jugular blood collected every 4 h until h 48, serum P₄ was maintained at lower ($P < 0.05$) concentrations for L vs. H cows. Area under the curve was less ($P < 0.05$) for L (49.6 ± 6.2) vs. H (83.6 ± 12.5) cows. Metabolic clearance was more important in regulating peripheral concentrations of P than luteal production. However, luteal sensitivity to co-culture with ET-1 and AA was enhanced for cows with low serum P₄. Management practices that reduce metabolic clearance rate of P₄ might decrease late embryonic or early fetal mortality as efficiently as supplementation with exogenous progestogens. Supported by USDA-CSREES-NRI 2002-35203-12230.

Key Words: Embryonic and Fetal Mortality, Progesterone, Lactating Dairy Cow

889 Effect of seminal plasma and transforming growth factor (TGF)- β 1 treatment on pregnancy outcome in beef cattle. J. F. Odhiambo*¹, I. Holásková¹, J. D. Rhinehart¹, D. H. Poole², J. M. DeJarnette³, E. K. Inskeep¹, and R. A. Dailey¹, ¹West Virginia University, Morgantown, ²Ohio State University, Columbus, ³Select Sires Inc, Plains City, OH.

The role of post-mating inflammatory response on pregnancy outcome has been described in rodents, pigs and humans. Transforming growth factor (TGF)- β 1 was identified as the active inflammatory-inducing moiety derived from seminal vesicles (Tremellen et al., 1998). The present studies tried to examine in beef cattle the significance of pre-sensitization of the uterus before or at breeding with seminal antigens on pregnancy outcome. Spring calving beef cows ($n = 1083$) were synchronized for estrus and assigned randomly to treatments during 2003 to 2006. In Trial 1, treatments included 0.5 ml seminal plasma (SP), 80 ng/ml rhTGF- β 1 suspended in bovine serum albumin (BSA) and 0.5 ml BSA (control). In Trial 2, two treatments SP and BSA were examined. Trial 3 compared treatment with SP or no treatment (control). Treatments were infused at onset of estrus (12 h before insemination) in Trial 1 or at inseminations (Trial 2 and 3). Pregnancy data were examined by contingency analysis and least squares analysis of variance using the GLM procedures of SAS for effects of treatment, time of treatment, age of cow, year, and their second and third order interactions. Differences were considered significant at $\alpha = 0.05$. In Trial 1, pregnancy rates did not differ (53.1, 54.7 and 54.8 % for BSA, SP and TGF- β 1 respectively). In Trial 2, TGF- β 1 tended to improve pregnancy outcome compared to SP or BSA in 2005 (49.2 vs. 33.1

and 38.4 %, respectively). In a separate 2005 study, pregnancy rates were 59.4 and 67.0 % for BSA and SP, respectively and tended to be affected by time of insemination. In Trial 3, pregnancy outcome was not different between SP or control, 61.4 and 52.4 % respectively. However, interactions of farm by treatment existed. The data obtained from these studies did not provide any conclusive evidence for the effect TGF- β 1 or seminal plasma on pregnancy outcome in beef cattle, although TGF- β 1 might improve pregnancy rates when fertility is compromised.

Key Words: Seminal Plasma, TGF- β 1, Pregnancy

890 Prolactin and luteinizing hormone profiles during the reproductive cycle in the native Thai chicken. S. Kosonsiriluk¹, N. Sartsoongnoen¹, N. Prakobsaeng¹, I. Rozenboim², M. E. El Halawani³, and Y. Chaiseha*¹, ¹Suranaree University of Technology, Nakhon Ratchasima, Thailand, ²The Hebrew University of Jerusalem, Rehovot, Israel, ³University of Minnesota, Saint Paul.

Unlike Gallinacous-temperate zone birds, the reproductive cycle of the native Thai chicken, an equatorial non-photoperiodic continuous breeder consists of three reproductive stages including laying (LAY), incubating (INC) and rearing of young (R). In temperate zone birds, luteinizing hormone (LH) and prolactin (PRL) levels vary during the four reproductive stages with the high PRL levels observed during the incubation phase are responsible for the suppression of gonadotropic hormones and ovarian steroids, follicular atresia, termination of egg laying activity and induction of incubation behavior. PRL action on the reproductive neuroendocrine system has been shown to be mediated by its feedback effects on the hypothalamus, pituitary and ovary. The objective of this study was to establish baseline information on the neuroendocrine changes (LH and PRL levels) associated with reproductive stages of the native Thai hens. Chickens were classified into three stages: LAY, INC and R ($n=10$). Blood samples were collected for determining plasma PRL and LH levels by Enzyme-Linked Immunosorbent Assay. Daily records were kept of egg production and nesting activity during the reproductive cycle. The results revealed that PRL levels (ng/ml) were low in R (24.1 ± 1.9), intermediate in LAY (40.4 ± 12.6) and highest in INC (351.9 ± 37.1 , $P < 0.05$). There were no changes ($P > 0.05$) in LH levels across the reproductive stages. Levels were 3.4 ± 0.3 , 3.7 ± 0.4 and 3.2 ± 0.1 ng/ml, whereas ovarian weights were ($P < 0.05$), 35.9 ± 0.9 , 3.1 ± 1.2 and 1.9 ± 0.9 gm for LAY, INC and R, respectively. The finding that ovarian regression occurred in INC and R hens in the absence of a decline in LH levels is interpreted as an adaptive mechanism(s) allowing for reinitiating egg laying activity in case of nest destruction at any time and irrespective of the season. The finding further suggest the antigonadotropic effect of PRL is limited to its effect on the ovary.

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Key Words: Bird, Luteinizing Hormone, Prolactin

891 The effect of active immunization against vasoactive intestinal peptide and inhibin on semen production of young and aged roosters. I. Rozenboim* and N. Avital, Hebrew University of Jerusalem, Faculty of Agriculture, Department of Animal Science, Rehovot, Israel.

Low levels of fertility are causing major economic losses in poultry breeder farms. The objective of this study was to investigate the effect of active immunization against VIP and inhibin on semen production and quality of roosters at different ages. Exp. 1. Sixty WL roosters at 19 wk of age were divided to 4 groups (n=15): 1) control, 2) VIP immunized, 3) Inhibin immunized and 4) immunized against VIP and inhibin. Semen quality was measured every 2 wks, blood samples were collected every other week for plasma steroid levels. At 41 wks of age roosters were killed, hypothalamai pituitaries and testicular tissue were removed and stored for mRNA analysis of GnRH, VIP and LH, FSH, prolactin and LH and prolactin receptors respectively. Exp. 2. Sixty WL roosters at 67 wk of age were treated similarly as in exp 1. All measurements conducted in exp. 1 were similarly conducted. Exp. 3. Twenty eight WL roosters at 97 wk of age were divided to 4 groups (n=7): 1) control, 2) VIP immunized, 3) control + prolactin (1mg/day/bird for 7 days) and 4) VIP immunized + prolactin (1mg/day/bird for 7 days). Results: combined immunization (VIP+inhibin) in young roosters significantly (P<0.05) increased semen quality compare to control birds (volume: 0.78 ± 0.01 ml vs. 0.6 ± 0.01 ml, concentration: 3.7×10^9 vs. 3.5×10^9 (cells/ml) and mobility 0.6 vs. 0.2 O.D.). In elderly birds VIP immunization significantly (P<0.05) reduced semen concentration, volume, motility and mobility compare to all other treatment groups. In addition active immunization against inhibin significantly increased mobility value compare to control group (1.4 vs. 0.9 O.D.) and semen motility (8 vs. 7.2) Prolactin administration (Exp. 3) to old roosters previously actively immunized against VIP significantly improved semen quality manifested by increase in concentration from $2.1 \times 10^9 \pm 0.1$ to 3.1×10^9 (cells/ml), mobility from 0.9 to 1.15 and motility from 6 to 7.5 . As in mammalian species, prolactin was found to be involved in semen production of old roosters. The mechanism of this phenomenon involves testicular prolactin and LH receptors, since prolactin significantly elevated testicular LH receptors mRNA in this study.

Key Words: Rooster, Semen, Prolactin

892 Chicken epiregulin (ER) gene: cDNA cloning, genomic organization, and regulation of its mRNA expression in ovarian granulosa cells. Y. Wang*, J. Li, and F. C. Leung, *The University of Hong Kong, Hong Kong, HK-SAR, China.*

Growing evidence suggests that epiregulin (ER), a ligand of epidermal growth factor receptor (EGFR), is involved in controlling follicular development in mammals. However, there is no information on its expression in the ovary of non-mammalian species including chicken. In this study, we first cloned the full-length cDNA of chicken epiregulin gene from ovary. The cloned cDNA is 948 bp in length and encodes a membrane-anchored precursor of 153 amino acids, which shares high sequence identity (~59%) with its mammalian counterpart. Genomic structural analysis shows that chicken epiregulin gene spans approximately 8 kb on chromosome 4 and consists of 5 exons. Using RT-PCR assay, ER mRNA was detected to be expressed in embryonic (embryonic day 20), sexually immature (3 week), and mature ovaries (6 chickens used at each stage), suggesting a role of ER in ovarian development. Compared with the other six EGFR ligands, epiregulin appeared to be expressed at a low level. However, using semi-quantitative RT-PCR assay, epidermal growth factor (EGF), transforming growth factor- α (TGF- α) and heparin-binding epidermal growth factor-like growth factor (HB-EGF) have been demonstrated to be capable of inducing ER mRNA expression significantly in cultured

ovarian granulosa cells from preovulatory follicle (F1) in a time-dependent manner. The expression of epiregulin reached the maximum at 4 h and was sustained at 24 h post-treatment. Moreover, phorbol 12-myristate 13-acetate (PMA), a potent protein kinase C (PKC) activator, could also significantly induce epiregulin mRNA expression in a dose-dependent manner, suggesting that PKC (or PKC-activated) signaling pathway may be involved in controlling ER expression in the chicken ovary. These findings suggest that ER is a potential paracrine/autocrine factor involved in controlling ovarian development and its expression is regulated by local ovarian factors including EGFR ligands from chicken ovary.

Key Words: Epiregulin, Chicken, Ovary

893 Effects of different cryopreservation methods on the glyocalyx of chicken spermatozoa. J. Pelaez and J. A. Long*, *Beltsville Agricultural Research Center, Beltsville.*

The carbohydrate-rich zone on the sperm surface is essential for immunoprotection in the female tract and early gamete interactions. We recently have shown the glyocalyx of chicken sperm to be extensively sialylated and contain residues of mannose, glucose, galactose, fucose, N-acetyl-galactosamine, N-acetyl-glucosamine and N-acetyl-lactosamine. Our objective here was to evaluate the effects of cryopreservation on the sperm glyocalyx. Semen was pooled from 6 roosters, diluted 1:1 (Lakes pre-freeze diluent), cooled to 5°C and aliquoted for cryopreservation using 6% DMA, 11% DMSO or 11% glycerol. For the DMA method, semen was equilibrated for 1 min with DMA and rapidly frozen by dropping 25 μl aliquots into liquid nitrogen. For the DMSO and glycerol methods, semen was equilibrated for either 1 min (DMSO) or 20 min (glycerol), loaded into 0.25 ml straws and frozen (5 to -35°C , $7^{\circ}\text{C}/\text{min}$; -35 to -140°C , $20^{\circ}\text{C}/\text{min}$; nitrogen plunge). Thawed (rapid, DMA; moderate, DMSO, glycerol) semen was stained with 1 of 12 FITC-conjugated lectins ($100 \mu\text{g}/\text{mL}$; 30 min; 25°C ; 100×10^6 cells/mL). Samples counterstained with PI were assessed by flow cytometry. On the day of cryopreservation, aliquots of fresh semen were stained with the panel of lectins and PI. For each lectin, the Mean Fluorescence Intensity (MnFI) of live sperm was compared among fresh and frozen/thawed (fr/th) treatments (n=5 replicates). For the majority of lectins (10/12), the MnFI was higher (P<0.05) for fr/th than fresh sperm. Exceptions included lectins specific for sialic acid and α -fucose, where DMSO and glycerol treatments, respectively, had MnFI similar (P>0.05) to fresh sperm. Among the fr/th treatments, the MnFI of sperm cryopreserved with DMSO was higher (P<0.05) for 4/10 lectins, including those specific for N-acetyl-lactosamine and N-acetyl-glucosamine. These data indicate that surface carbohydrates are altered during cryopreservation, and that cryoprotectant type and fr/th rates affect the degree of modification. While the specific functions of these glycoconjugates are not known, it is likely that the observed differences in fr/th sperm contribute to the reduced fertility of cryopreserved chicken semen.

894 Testicular development in meishan and commercial crossbred prepubertal boars. J. J. Ford*, *U.S. Meat Animal Research Center, Clay Center, NE.*

Total daily sperm production and testicular size of adult boars increase in proportion to the number of Sertoli cells within the testis. Meishan

(MS) boars experience puberty at a younger age than commercial crossbred (CB) boars in association with earlier cessation of Sertoli cell proliferation and smaller postpubertal testicular size. The objectives of the current study were 1) to define changes in the production of anti-Mullerian hormone (AMH) and p27kip1, markers of Sertoli cell differentiation, in prepubertal MS and CB boars ($n > 3/\text{breed/d}$), and 2) to relate these changes with the pubertal expansion of the seminiferous tubules. Presence of AMH and p27kip1 were assessed by immunohistochemistry and densitometric analyses using a Bioquant Nova color imaging system. Testicular weights were similar ($P > 0.10$) in MS and CB boars at 7, 28 and 49 d of age although CB boars had greater ($P < 0.01$) body weight at these ages. Testes weights of MS increased more rapidly than in CB after 49 d of age and were greater ($p < 0.02$) at 70, 91 and 112 d of age. Diameter of seminiferous tubules increased ($P < 0.01$) at a younger age and to a greater extent in MS than in CB boars. Relative amount of AMH, a product of less mature Sertoli cells, increased in both breeds from 7 to 28 d. AMH then declined thereafter at a more rapid rate ($P < 0.001$) in MS than in CB boars and was nearly absent at 70 d in MS and at 112 d in CB boars. Seminiferous tubules containing p27kip1 were first detected at 28 d in some MS and at 70 d in some CB boars ($P < 0.01$) and increased in both breeds through 112 d. The earlier onset of pubertal development in MS boars was characterized by expansion of seminiferous tubules at a younger age in association with a more rapid decline in AMH production and an earlier increase in p27kip1, indicative of an earlier onset of Sertoli cell differentiation in MS relative to CB boars.

Key Words: Boar, Testis, Puberty

895 Transcript profiling of testes from boars divergently selected for testosterone production. M. S. Ashwell*, S. Druyan, C. M. Ashwell, and J. P. Cassady, *North Carolina State University, Raleigh.*

The objective of this research is to identify changes at the gene level associated with 10 generations of divergent selection for testosterone production in response to a GnRH challenge. After ten generations of selection, average testosterone levels were 75 and 200 ng/ml for the low and high lines, respectively. Lines were subsequently maintained using random selection. Testicles from five boars from each line in generation 21 were collected at 1, 30, and 120 days of age. Tissues were snap frozen in liquid nitrogen and stored at -80°C for transcript profiling using a 13,297-oligonucleotide swine microarray. RNA was extracted from all boars from both lines, pooled by age and line, fluorescently labeled with either Cy3 or Cy5, and hybridized to the arrays. Hybridization intensity data were LOWESS-normalized within and across the arrays and analyzed using ANOVA. In total, 25 genes showed differential expression with a false discovery rate of 8%. Ten of these 25 genes have no known function or characterization. Most of these 25 genes were found to be differentially expressed across ages and by the interaction of age and line. The ability to detect differences in gene expression between the lines that are related to the observed differences in testosterone level is in part a result of the degree of replication in the array. Two genes associated with steroid biosynthesis, steroidogenic acute regulatory protein and aromatase type II, were shown to be differentially expressed. Validation of these findings is underway using real-time PCR methods.

Key Words: Swine, Gene Expression, Steroid Biosynthesis

Production, Management & the Environment - Livestock and Poultry: The Evolving National Animal Identification System

896 The Canadian Livestock Traceability System. J. M. Stitt*, *Canadian Cattle Identification Agency, Calgary, Alberta, Canada.*

The Canadian Cattle Identification Agency (CCIA) is a not-for-profit National Agency, incorporated in 1998, and led by a Board of Directors, representing all sectors of the livestock industry. The mandate of CCIA is to establish and maintain an efficient Animal Health and Food Safety Identification and Traceability system. With the program fully implemented on July 1, 2002, CCIA has been successfully established as a leader in Animal Identification and Traceability. Guided by National Standards and operating Under the ID Regulations within the Federal Health of Animals Act, the CCIA, in partnership with the Canadian Food Inspection Agency (CFIA), has achieved 98-100% compliance nationally. The CCIA system provides multi-species services and currently houses the beef, dairy, bison and sheep trace back data and is offering services to pork and poultry. In 2003, the Canadian cattle industry committed to the transition from barcode dangle tags to Radio Frequency Identification Devices (RFID). The program is industry supported, sustainable and has proven invaluable through the recent BSE investigations. The Canadian System incorporates the 3 Key Pillars for Traceability; Animal Identification, Premises Identification and Animal Movement. Additionally, it offers Value-Added services, as required by industry. Age Verification is one example of a Value-Added service assisting in assuring market access. The program implementation was not easy and as we expand on the national infrastructure we continue to face challenges. The successful

implementation and commitment to ongoing development of the National Livestock Traceability System can be attributed to:

- support from all sectors of the cattle industry across Canada
- national communications strategy
- shared industry/government partnership
- commitment for industry to lead the program
- commitment to keep the program user-friendly, cost-effective and scaleable
- the unfortunate but timely global Animal Health and Food Safety issues.

CCIA is committed to ensuring that all program components continue to meet and exceed evolving Domestic and International requirements.

Key Words: Traceability, Identification, CCIA

897 Issues surrounding existing and potentially disruptive RFID technologies for the identification of food producing animals. D. A. Blasi*, *Kansas State University, Manhattan.*

The primary objective of a voluntary US National Animal Identification System (NAIS) when fully operational is the integration of the