

Physiology: Physiology II

103 Effect of microgravity on mammillary cones of Japanese Quail eggs. S. Westmoreland^{*1}, T. Halupnik¹, J. Walker¹, and P. Hester², ¹*The University of Texas, Arlington*, ²*Purdue University, West Lafayette, Indiana*.

A study was conducted in cooperation with NASA, National Aeronautics and Space Administration, to determine if normal incubation of fertile eggs of Japanese quail could occur in microgravity. Our role was to examine the eggshells using scanning electron microscopy (SEM) to establish whether normal calcium loss had occurred. Based on a previous baseline study with Japanese quail, we predicted that the earliest calcium loss from mammillary cones of shells would be at 12 d of incubation. There were 5 experimental groups in the NASA study. The eggs of all groups were incubated at 37 to 37.5°C and turned hourly. Eggs in groups 1 and 2 were incubated in space in an Avian Development Facility (ADF) unit aboard the Orbiter Endeavour flight STS-108 for 12 days. Group 1 was in 0 g; group 2 was spun in a centrifuge at 1 g. Groups 3 and 4 were incubated in the ground control laboratory in an ADF unit. Group 3 was stationary at 1 g, while group 4 was spun vertically in a centrifuge, resulting in variable force from 0 to 2 g. Group 5 was incubated on Earth in a standard laboratory incubator. The incubation was interrupted at days 4, 7, and 12 in each of the test groups. The day 4 and 7 shells were injected with 4% paraformaldehyde in 50 mM Cacodylate buffer, pH 7.4 to terminate incubation. Incubation of day 12 eggs was terminated by opening the shells; no preservative was used. Egg-shell samples from each group were treated with 6% sodium hypochlorite for removal of shell membranes. The SEM examination revealed that calcium loss had occurred in some mammillary cones from each group, regardless of treatment or length of incubation. Fixative most likely played a role in the dissolution of Ca from the mammillary cones of shells from embryos incubated for 4 and 7 d. An ash analysis of the Ca content of the day 12 shells obtained from live embryos showed no significant difference among treatment groups. These results indicate that embryos incubated in microgravity for 12 d utilized Ca from the shell during development.

Key Words: Microgravity, Calcium, Embryogenesis

104 Use of a vascular access port for the measurement of pulsatile luteinizing hormone secretion in old broiler breeders. C. Senthilkumaran^{*1}, S. Peterson¹, M. Taylor², and G. Bédécarrats¹, ¹*University of Guelph, Guelph, ON, Canada*, ²*Ontario Veterinary College, Guelph, ON, Canada*.

Techniques used to measure pulsatile hormone release over extended periods of time routinely involve cannulation or physical restraint, resulting in sepsis and stress. We thus adapted a method for serial blood sampling in chickens using a vascular access port (VAP) surgically implanted under the skin of the neck, and connected to a catheter inserted in the right jugular vein. The system was used to measure luteinizing hormone (LH) profiles in six 21-month-old broiler breeders at the end of their laying period (laying rate ranging from 0 to 60%). VAPs were implanted under general anaesthesia and, after a period of recovery, serial blood samples (every 10 min for 6 h) were collected using an extension line connected to a push pull system. Birds were unrestrained and had free access to food and water. Every hour, blood cells were recovered by centrifugation, reconstituted in saline solution, and returned to each donor of origin through the VAP. LH levels were subsequently measured in plasma by radioimmunoassay. With the exception of one hen that developed valvular endocarditis, no sign of disease or infection was observed throughout the course of the study, and VAPs remained functional in all birds for at least 3 months. Radioimmunoassay results revealed that LH was released in a pulsatile manner with pulse amplitudes ranging from 0.5 to 2.5 ng/ml at a frequency of 1 pulse every 30 min. Interestingly, in out-of-lay hens, LH pulses were less defined, with reduced amplitude compared to laying hens. Our results suggest that VAPs are a safe, reliable, and non-stressful technique for serial blood sampling and long-term studies. Furthermore, in broiler breeders at the end of their laying period, LH secretion is pulsatile.

Key Words: LH, Blood sampling, Broiler breeder

105 Soy phytoestrogen effects on progesterone receptor and ovalbumin synthesis in chick oviduct. L. Stevenson^{*}, A. Doernste, S. Oates, A. Peterson, and W. Berry, *Auburn University, Auburn, Alabama*.

Chickens are continually exposed to the estrogenic soy isoflavone genistein through the diet. Inappropriate exposure to environmental estrogens, such as genistein, is known to alter morphology and function of the mammalian reproductive tract. Previous experiments in this laboratory have demonstrated that genistein can induce growth of the chick oviduct. However, information is lacking as to specific reproductive tract morphological and functional effects of genistein exposure in chicks. To begin to address this lack of information, experiments were done to characterize the effects of genistein exposure on oviduct morphology and function. Day old female chicks were assigned to treatments: soy based chick diet with daily subcutaneous injection of sesame oil vehicle (SV); soy-free diet/ vehicle injection (V); daily oral gavage of 0.5 mg diethylstilbestrol (DES); 1.0 mg genistein (G1); or 10.0 mg genistein (G10). At 10 days of age, a subset of chicks from each treatment received a single injection of progesterone to induce ovalbumin synthesis in the oviduct. At 12 days of age, DES treatment increased oviduct and liver weight compared to vehicle-injected control. Oviduct weights of G10 chicks were significantly increased compared to SV. Immunohistochemistry of formalin fixed oviduct samples revealed that the DES and G10 treatments significantly increased specific staining for progesterone receptor and ovalbumin in the chick oviduct as compared to SV, V, and G1 treatments. (Supported by the Alabama Agricultural Experiment Station and USDA AD-421 project S-289).

Key Words: Oviduct, Phytoestrogen, Development

106 Interconversion of corticosterone and dehydrocorticosterone in liver, kidney and intestine of the chicken. A. Katz^{*}, R. Meidan, and B. Robinson, *The Hebrew University of Jerusalem, Jerusalem, Israel*.

Activation and deactivation of glucocorticoids (GC) by their interconversion between 11-hydroxy-GC and 11-keto-GC is executed by 11 β -hydroxysteroid dehydrogenases (11 β HSDs). Hitherto, in mammals, three 11 β HSDs were identified: (a) 11 β HSD1 that in presence of NADPH, reduces 11-keto-GC to their active 11-hydroxy-GC form. In some tissues 11 β HSD1 lacks NADPH supply and is presented with NADP⁺. Under these circumstances 11 β HSD1 oxidizes the 11-hydroxy-GC. (b) 11 β HSD2 that serves as unidirectional oxidase that in the presence of NAD⁺ oxidizes C11. (c) 11 β HSD3 that is NADP⁺-dependent unidirectional oxidase.

Our current research is aimed to a) characterize the reduction/oxidation of C11 in GC by various chicken tissues and their subcellular fractions and to find the role of 11 β HSDs in this conversion. b) document their involvement in stress responses. c) examine the mRNA expression of 11 β HSD isoforms in these tissues using RT-PCR.

Our data show that: (1) Reduction of dehydrocorticosterone (DHC) to corticosterone (CS) occurs in duodenum and liver. (2) This reduction occurs in the cytosol and was NADH-dependent in duodenum but in liver occurs in the presence of either NADH or NADPH. (3) Oxidation of CS in kidney membranous organelles in the presence of either NAD⁺ or NADP⁺.

Chickens 11 β HSD1, 2 and 3 sequences were deduced from the genebank and PCR products of all the three were found in liver and kidney.

The NADH-dependent activity profile found in the cytosol of liver and duodenum does not coincide with any of the 11 β HSDs reported for mammals, which are all membrane-bound, losing their activity upon truncation of trans-membrane domain and reduce GC only in NADPH-dependent manner. Therefore, chickens may use different iso-enzyme for activation of GC

Key Words: Chicken, Glucocorticoids, 11 β HSDs

107 The efficacy of intracerebroventricular injections of arginine vasotocin on plasma corticosterone levels in undisturbed male and female broilers (*Gallus gallus*). F. Madison*, A. Jurkevich, and W. Kuenzel, *University of Arkansas, Fayetteville*.

Stress is a common factor faced by the poultry industry and it has been demonstrated that male birds seem more susceptible to stress than females. Little, however, is known about sex-related differences in the neuroendocrine control of the hypothalamo-pituitary-adrenal axis (HPA) during the stress response in broilers. There is evidence showing that the hypothalamic neuropeptide, arginine vasotocin (AVT) stimulates the HPA. The purpose of this study was to determine the efficacy of AVT administered intracerebrally for releasing corticosterone (CORT) *in vivo* in undisturbed male and female birds. Broiler chickens were fitted with a chronic cannula placed in the lateral ventricle. Birds were housed individually in cages behind a one-way glass partition and noise was avoided during the sampling. Each bird in the study received a single 5.0 μ l intracerebroventricular (ICV) injection of either saline (SAL) or 10 ng AVT. Blood was sampled remotely every fifteen minutes for two hours beginning from time of injection from a catheter implanted in the jugular vein and plasma CORT was determined by radioimmunoassay. There was no difference between male and female birds injected with saline (control). AVT injected into females did not induce any changes in CORT levels as compared with saline injected controls. In males, AVT induced a significant increase in plasma CORT at 15 min post injection. CORT levels remained elevated during 2 hrs of sampling. Plasma CORT levels in males injected with AVT were significantly higher than females injected with the same dose beginning 30 min post injection until 2 hr. Of interest is that CORT levels in AVT injected birds peaked initially, decreased, and peaked again before the end of the 2 hr sampling period, resulting in a rhythmic fluctuation of CORT. In this study it appears that the pituitary-adrenal axis of males is more responsive to low doses of AVT than females when injected ICV. Supported in part by NSF grant #IBN 01111006 and Arkansas Agricultural Experiment Station.

Key Words: Stress, Hypothalamo-pituitary-adrenal axis, Sex differences

108 Regulation of transforming growth factor beta on decorin expression during myogenesis in poultry. X. Li* and S. Velleman, *The Ohio State University, Ohio Agricultural Research and Development Center, Wooster*.

Transforming growth factor β (TGF- β) is an inhibitor of both skeletal muscle myoblast proliferation and differentiation. In addition, myoblast proliferation and differentiation are influenced by cell interactions with the extracellular matrix. Decorin, a member of small leucine-rich proteoglycans in the extracellular matrix, interacts with TGF- β , regulating myoblast responsiveness to TGF- β . The chicken genetic muscle weakness, Low Score Normal (LSN), has lower proliferation and differentiation rates, and altered expression of TGF- β and decorin during myogenesis, compared to the normal birds. The LSN has been used as a model to investigate the roles of TGF- β and the extracellular matrix in myogenic satellite cell proliferation and differentiation. Elevated mRNA expression of both TGF- β and decorin were detected in LSN satellite cells during myoblast proliferation and differentiation using a real-time quantitative polymerase chain reaction, compared to that of normal satellite cells. To investigate the relationship of TGF- β and decorin during myogenesis, the concentration of TGF- β in cell culture was altered by the addition of exogenous TGF- β 1 or anti-TGF- β 2 antibody during satellite cell differentiation. The expression of decorin was reduced with TGF- β 1 treatment. In contrast, the inhibition of TGF- β by anti-TGF- β 2 antibody induced decorin expression, as well as that of LSN satellite cells. Since decorin binds to TGF- β and regulates cellular responsiveness to TGF- β , the effect of TGF- β on decorin expression may play an important role in TGF- β -dependent regulation of skeletal muscle differentiation.

Key Words: Decorin, Transforming growth factor beta, Muscle

109 Effect of Melengestrol Acetate (MGA) on the production of yolk proteins by the liver. J. M. Koch*¹, J. S. Moritz¹, D. C. Lay Jr.2, and M. E. Wilson¹, ¹West Virginia University, Morgantown, ²USDA-ARS-LBRU, West Lafayette, Indiana.

Inducing hens to molt increases egg quality, egg production and extends the productive life of hens. Recently, we have demonstrated that MGA, an orally

active progestin, decreased gonadotropic support for the ovary, which decreased the steroidogenic support for the oviduct and resulted in the cessation of lay. Estrogen produced by the large yellow follicles stimulates the production of the yolk proteins vitellogenin II and apolipoprotein II. The objective of this experiment was to determine the expression of yolk proteins in response to a MGA induced molt. Hy-Line W-36 laying hens (n=48) at 40 weeks of age were fed either 0 or 8 mg MGA per day for 28 days in a balanced diet and then returned to a normal diet until day 44. Four birds per treatment on days 1, 8, 16, 28, 36 and 44 were euthanized and the liver was removed and snap frozen in liquid nitrogen until RNA was extracted. Expression of vitellogenin II and apolipoprotein II, relative to actin, was determined using real-time RT-PCR. Data for relative expression of the two genes were analyzed by defining regression models with day as a continuous variable and treatment as a categorical variable. Following the regression analysis, T-tests were used to make pair-wise comparisons of the resultant slope coefficients. Vitellogenin II expression was reduced ($P < 0.05$) in hens fed 8 mg of MGA compared to those fed 0 mg of MGA. Expression of vitellogenin increased after removal of MGA from the diet. There was no difference ($P > 0.10$) in the expression of apolipoprotein II between the two groups throughout the experiment. One potential reason for the lack of similarity in the pattern of expression of these two yolk proteins may be that vitellogenin II is used only as a yolk protein and therefore depends on the follicular estrogen; however, apolipoprotein II is a component of the very-low-density lipoprotein particle and its synthesis may not be as dependent on follicular estrogen. Therefore, utilizing MGA as an alternative method to induce molt results in the similar changes in liver function that result from a feed withdrawal induced molt.

Key Words: Molting, MGA, Vitellogenin

110 Expression profiles of prolactin receptors during chicken embryonic development. C. Y. Wang*, Y-J Wang, and F. C. Leung, *The University of Hong Kong, Hong Kong, HK-SAR, China*.

Prolactin (PRL) interacts with prolactin receptor (PRLR) in a broad variety of target tissues to exert diverse physiological functions in vertebrates. In order to elucidate the physiological roles of prolactin in chicken embryonic development, we have examined the expression profiles of prolactin receptor by semi-quantitative reverse transcription-polymerase chain reaction (semi-quantitative RT-PCR). On day 8 of chicken embryos, the prolactin receptor mRNA could be easily detected in allantoic membrane, intestine, kidney, liver, lung, heart, brain, and anterior pituitary. Interestingly, during embryonic development (from day 8 to day 20 of incubation), the expression levels of prolactin receptor mRNA in all tissues examined were maintained at a high level, which is comparable to those in adult chicken tissues. By contrast, the levels of prolactin mRNA expression on day 8 in the anterior pituitary were very low and the maximal expression was detected on day 20. The differential expression patterns of prolactin and prolactin receptor mRNA during chicken embryonic development demonstrate that pituitary prolactin plays roles in the late development of chicken embryos and further suggest that prolactin receptor may be involved in early embryonic development.

Key Words: PRL, PRLR, Embryo

111 Regulation of chicken Heparin-binding EGF-like Growth Factor (HB-EGF) Expression by EGF and TGF α in the cultured ovarian granulosa cells. Y-J. Wang*, J. Li, and F. C. Leung, *The University of Hong Kong, Hong Kong, HK-SAR, China*.

Our previous study demonstrated that both epidermal growth factor (EGF) and its receptor (EGFR) are expressed in the chicken ovary, suggesting that EGF might play a paracrine/autocrine role in controlling ovarian functions. To further examine whether other EGF family members were involved in vertebrate ovarian development, we investigated the expression of heparin-binding EGF-like Growth factor (HB-EGF), a member of EGF family, in the chicken ovary. Interestingly, like EGF, HB-EGF was also expressed in all of the developing follicles from the adult chicken ovary and its highest expression was noticed in the smallest follicles (<1mm). The co-expression of EGF and HB-EGF promotes us to elucidate their possible interactions in the chicken ovary. Using semi-quantitative RT-PCR, we noticed that mouse epidermal growth factor

(mEGF) strongly increased the expression of HB-EGF in the cultured ovarian granulosa cells in clear time- and dose-dependant manners. The effect of EGF (150 nM) reached the maximal level at 2 h of treatment. In consistent with this finding, recombinant human transforming growth factor $\hat{I}\pm$ (rhTGF α) (10 nM) also strongly induced HB-EGF expression and its action was much more potent than EGF. Interestingly, phorbol-12-myristate-13-acetate (PMA, 100 nM), a protein kinase C (PKC) activator, could mimic the stimulatory effects of EGF and TGF α on HB-EGF expression. However, the stimulatory effect of EGF on HB-EGF expression was substantially reduced by an MEK inhibitor (PD98059, 50 μ M), but not by a PKC inhibitor, suggesting that the regulation of HB-EGF expression by EGF is primarily mediated by MEK-MAPK signaling pathway. In summary, the expression of HB-EGF in the chicken ovary, together with the up-regulation of HB-EGF expression by EGF and TGF $\hat{I}\pm$, suggests that ovarian HB-EGF, like EGF, might play an important role in ovarian development and its expression level is tightly controlled by intra-ovarian EGF and other EGF family members, probably, including HB-EGF itself.

Key Words: EGF, HB-EGF, Ovarian granulosa cells

112 Effects of injected gluconeogenic supplements on the physiology of broilers from young breeders. E. Peebles¹, W. Berry², R. Keirs^{*1}, L. Bennett¹, S. Park¹, and P. Gerard¹, ¹Mississippi State University, Mississippi State, ²Auburn University, Auburn, Alabama.

The potential for the use of various practical nutrient sources as gluconeogenic supplements in broilers from young breeder flocks was explored. Injections (0.2 mL) containing physiological saline or a gluconeogenic energy source (casein hydrolysate, ovalbumin hydrolysate, or crude ovalbumin) were given subcutaneously (in the back of the neck) to 320 broiler chicks at hatch. Concentrations of gluconeogenic substances in solution were approximately 200 g / L. Biotin was added to injected crude ovalbumin solutions. Chicks were hatched from eggs that were obtained from a single young breeder flock at 27 wk of age. At hatch, chicks were divided into 16 floor pens with 20 chicks in each of four replicate pens per treatment group, and were brooded under commercial conditions. Tissue condition at the site of injection, BW, relative liver weight, rectal temperature, hematocrit, plasma refractive index, and liver glycogen content were determined at 16 d post hatch. Treatment injection caused no local histological reaction. In addition to the absence of any treatment effects on performance through 16 d, as previously reported, none of the injected supplements affected any of the parameters examined on d 16. In this study, the use of injected casein hydrolysate, ovalbumin hydrolysate, or crude ovalbumin for

gluconeogenic supplementation did not affect the BW, liver weight, body temperature, hydration status, or liver glycogen content of chicks from young parents that were provided adequate brooding conditions.

Key Words: Broiler, Gluconeogenesis, Supplement

113 Serotonin receptor subtypes influence prolactin secretion in the turkey. M. El Halawani^{*1}, O. Youngren¹, J. Proudman², and Y. Chaiseha³, ¹University of Minnesota, St. Paul, ²United States Department of Agriculture, Beltsville, Maryland, ³Suranaree University of Technology, Thailand.

Serotonin (5-HT) stimulation of prolactin (PRL) secretion is mediated through the dopaminergic (DAergic) system, with 5-HT ligands having no direct effect on pituitary PRL release. Infusion of 5-HT into the third ventricle (ICV) or electrical stimulation (ES) of the medial preoptic area (MPOA) or the ventromedial nucleus (VMN) induces an increase in circulating PRL in female turkeys. These increases in PRL do not occur when a selective antagonist blocks the D₁ dopamine (DA) receptors in the infundibular area (INF). In the current study utilizing Nicholas large white laying female turkeys, the ICV infusion of DOI, a selective 5-HT₂ agonist, caused PRL to rise. Pretreatment with ketanserin, a selective 5-HT₂ receptor antagonist, blocked DOI-induced PRL secretion, attesting to the specificity of the response. DOI-induced PRL secretion was prevented when the D₁ DA receptors in the INF were blocked, suggesting that the DAergic activation of the VIP/PRL system is mediated by a stimulatory 5-HT₂ receptor subtype. The DOI-induced PRL rise did not occur when DPAT was concurrently infused. DPAT is a 5-HT₁ agonist with a high affinity for the 5-HT_{1A} receptor subtype. This subtype appears to mediate the inhibitory influence of 5-HT on PRL secretion. When DPAT was microinjected directly into the VMN, it blocked the PRL release effected by ES in the POA. These data suggest that when 5-HT₂ receptors are activated, they influence the release of DA to the INF. When 5-HT_{1A} receptors are stimulated, they somehow inhibit the PRL-releasing actions of 5-HT₂ receptors. This inhibition could take place centrally, or it could occur postsynaptically at the pituitary level. It is known that D₂ DA receptors in the pituitary antagonize PRL release. A release of DA to the pituitary, initiated by 5-HT_{1A} receptors, could effectively inhibit PRL secretion. In conclusion, ES may stimulate 5-HT fibers and/or 5-HT₂ receptors in POA or VMN, leading to activation of the DAergic system and subsequent PRL secretion. Stimulation of the 5-HT_{1A} receptors definitely prevents ES- or DOI-induced PRL secretion, but the site of action is as yet unknown. USDA grant #04-35203-14771

Key Words: Serotonin, Prolactin, Turkey