

Physiology and Endocrinology: Poultry Physiology

358 Blue-and-gold Macaw (*Ara ararauna*) postmortem semen collection. J. M. Silva¹, S. K. Cunha¹, C. D. Corcini¹, A. S. Varela Junior², A. P. N. Albano¹, A. L. S. Valente¹, and D. C. Bongalharo^{*1}, ¹Universidade Federal de Pelotas, Pelotas, RS, Brazil, ²Universidade Federal de Rio Grande, Rio Grande, RS, Brazil.

The blue-and-gold Macaw (*Ara ararauna*) is a Neotropical parrot from South America. It is rated as Least Concern by Bird Life International; however, there is a very heavy wild-caught trade of this species. In December 2008, one adult male was brought to the Wildlife Rehabilitation Center from the Federal University of Pelotas shortly after death. The main objective of this work was to perform a postmortem semen collection in this bird, aiming to recover sperm for cryopreservation. As a complement, testicular histology was observed to confirm that the macaw was sexually active. Necropsy was made approximately 3 h after death; at this time the testes, epididymis and deferent ducts were removed and placed in a Petri dish. Epididymis and deferent ducts were washed with Lakes diluent and the suspension was brought to the laboratory, where recovered sperm was evaluated by motility. Dry slides were made to observe sperm morphology, using 5 different stains: orcein, eosin, eosin-nigrosin, Giemsa, and Coomassie Blue. The testes were fixed in 10% buffered formalin; after 24 h they were dehydrated in crescent concentrations of alcohol, immersed in xylol at 100%, impregnated in Paraplast Xtra, and sliced (5 μ m). The cuts were stained with Harris hematoxylin and eosin and observed in optical microscope. Few sperm were recovered from the epididymis and deferent ducts and motility was lower than 1%, therefore it was not possible to proceed with cryopreservation. The low motility could be attributed to the time elapsed between the death and the necropsy. Eosin was the only stain that allowed clear visualization of the sperm cell, which presented normal morphology. Histology of the testes showed intense seminiferous tubules activity; a multi stratified epithelium containing sperm in different stages of development could be observed, as well as polygonal Sertoli cells. The results show that the testis were fully functional and producing sperm with normal morphology. It was also demonstrated that it is possible to collect motile sperm from dead birds, however, to attempt cryopreservation, it is still necessary to acquire higher motility.

Key Words: *Ara ararauna*, postmortem semen collection, testis histology

359 To move or not to move? Gait analysis of the modern broiler and its implications. H. Paxton*, M. A. Daley, S. A. Corr, and J. R. Hutchinson, *Royal Veterinary College, Hatfield, Hertfordshire, UK.*

Ever since Darwin, it has been well recognized that artificial selection could bring about changes in animal behavior, physiology and morphology by the simple selection of human-desired traits. But what happens when these selection pressures begin to alter the locomotion of an animal and potentially result in an increase in musculoskeletal pathologies? The modern broiler has changed dramatically reaching its market weight faster and having an increased meat yield. Research indicates that these changes may predispose some of the broilers to leg pathologies which are a welfare concern. We therefore investigate whether the modern broiler and its 2 great-grandparent lines have adopted different locomotor strategies as a result of their altered morphology. We also investigate whether any changes might prevent these birds from walking economically or stably and perhaps predispose them to locomotor pathology. We collected force plate and kinematic data from 27 chickens (350 steps), including a commercial broiler strain and 2 great-grandparent lines (A

and B) over a range of walking speeds, to quantify the 3D dynamics of the center of mass and determine how these birds modulate the force and mechanical work of locomotion. Walking speeds were slow (0.10–0.99 ms^{-1}) and ranges differed between groups with the fastest walking speeds recorded in the broiler population. There was greater variability in step width than step length, likely linked to increased mediolateral forces and control strategies for stability. We only found right-left limb asymmetries in the great-grandparent lines, with line B showing a decrease in step length with speed ($P = 0.01$) that is not commonly seen in ground birds. Based on current empirical models for cost, the differences in locomotor mechanics among lines suggest that Line B is likely to have a 10% higher cost of locomotion due to significantly higher stride frequencies ($P < 0.001$) and shorter step lengths. Thus selection for desirable traits in the broiler chicken lineage has carried along different gait characteristics that we have quantified for the first time.

Key Words: broiler chicken, locomotion, lameness

360 Effects of commercial in ovo injection of carbohydrates on broiler embryogenesis. W. Zhai*, R. Pulikanti, S. Womack, D. E. Rowe, and E. D. Peebles, *Mississippi State University, Mississippi State.*

The effects of in ovo injection of different carbohydrate solutions on hatch rate, hatch time, BW, body moisture, yolk sac weight, and yolk sac moisture of Ross \times 708 broiler chicks, hatched from eggs laid by a 34-wk-old breeder flock, were investigated. Eggs containing live embryos were injected in the amnion on d 18.5 of incubation using an automated multiple-egg injector with 0.1, 0.4, 0.7, or 1.0 mL of commercial diluent or a carbohydrate dissolved in diluent. Commercial diluent containing 25% of the following carbohydrates were injected into eggs: glucose, fructose, sucrose, maltose, and dextrin. The results showed that no carbohydrate type or volume affected hatch time. Absolute and relative BW on hatch day were positively related to injection volume ($P < 0.0001$). However, hatch rate was negatively related to injection volume ($P < 0.0001$). To realize a 90% hatchability of fertilized eggs, injection volume could not exceed 0.7, 0.4, 0.4, 0.7, and 0.7 mL for glucose, fructose, sucrose, maltose, and dextrin, respectively. Yolk-free BW was negatively related to fructose and sucrose injection volume ($P < 0.004$), but was not related to diluent, glucose, maltose, and dextrin injection volume. Similarly, absolute and proportional yolk sac weights were positively related to fructose, sucrose, and dextrin injection volume ($P < 0.01$), but were not related to diluent, glucose, and maltose injection volume. Yolk sac moisture was positively related to injection volume for all injectables including diluent ($P < 0.03$). However, body moisture and yolk-free body moisture were not related to injection type or volume. In conclusion, the use of carbohydrates added to commercial diluent for the in ovo injection of broiler hatching eggs requires the use of appropriate volumes to promote growth and nutrient utilization without adversely affecting rate of hatch.

Key Words: BW, hatch rate, in ovo injection

361 The effect of egg weight loss on embryonic development in Chinese painted quail (*Coturnix chinensis*) exhibiting parthenogenesis. J. B. Wells, H. M. Parker*, A. S. Kiess, and C. D. McDaniel, *Mississippi State University, Mississippi State.*

Parthenogenesis, the development of an unfertilized egg, has been studied extensively in turkeys. Recently it has been revealed that

parthenogenesis occurs in the eggs of Chinese painted quail and the percentage of eggs exhibiting parthenogenesis is negatively correlated with egg production as well as clutch size. In broiler breeders it has been reported that the first egg of a sequence loses less moisture during incubation. Because the incidence of parthenogenesis is greater and egg weight loss is less in the first egg of a sequence, it is possible that parthenogenesis is also affected by egg moisture loss. Therefore, the objective of this study was to determine if a relationship exists between egg weight loss and parthenogenesis. In this experiment individual eggs were collected from 157 hens daily and labeled with hen number and date. Eggs were stored for 0 to 3 d at 20°C before incubation at 37°C. To determine egg weight loss, eggs were weighed on the day of collection and again after 10 d of incubation. Pearson's correlation coefficients were used to determine if relationships exist between egg weight loss and the percentage of eggs exhibiting parthenogenesis, parthenogen size, egg storage, and clutch position. The percentage of egg weight loss was negatively correlated with the incidence of parthenogenesis in eggs ($r = -0.56$; $P < 0.001$), parthenogen size ($r = -0.49$; $P < 0.001$), and egg storage ($r = -0.24$; $P < 0.003$). However, the percentage of egg weight loss was positively correlated with average clutch position ($r = 0.32$; $P < 0.001$). In conclusion, it appears that eggshell quality possibly influences the incidence of parthenogenesis in Chinese Painted quail eggs because as the percentage of egg weight loss decreases, the incidence of parthenogenesis increases.

Key Words: parthenogenesis, moisture loss, clutch sequence position

362 Relationships of Ross × 708 broiler post-hatch development to embryonic temperature, incubation length, and eggshell water vapor conductance. R. Pulikanti, E. D. Peebles*, W. Zhai, A. Bello, C. N. Obi, and A. O. Sokale, *Mississippi State University, Mississippi State.*

To establish relationships between post-hatch broiler development, egg incubation length (IL), and eggshell water vapor conductance (G), Ross × 708 broiler hatching eggs were randomly set on 8 replicate tray levels of an incubator (20 eggs per replicate). On 10.5 d of incubation, transponders were implanted in the air cells of 4 embryonated eggs per replicate for determination of internal egg temperatures (embryo temperatures; IT) through d 18.5. External egg temperatures were determined using 2 water filled vials and 2 infertile eggs containing transponders per each replicate level. Hatch was monitored every 12 h between 18.5 and 21.5 d of incubation. The chicks were placed in corresponding replicate floor pens and grown through 49 d of age. On 28 and 48 d post-hatch, at least 2 chicks per pen were necropsied for determination of BW, and the relative weights and moisture contents of the liver, breast muscle, and left gastrocnemius muscle. Also, IL, G, relative G (RG), and the conductance constant (C) of each embryonated egg were determined. Mean IL, G, RG, and C were 20.7 d, 14.2 mg water/d × Torr, 24.6 mg water/d × Torr × 100 g egg weight, and 5.05. The IT was negatively correlated with relative chick BW and relative gastrocnemius muscle weight on d 28 post-hatch. However, IL was positively correlated with relative gastrocnemius muscle weight on d 28 but negatively correlated with gastrocnemius muscle moisture on d 48 post-hatch. Nevertheless, RG and C were negatively correlated with liver and breast muscle moisture contents on d 28 post-hatch. Correlations were considered significant at $P \leq 0.06$. Within physiological limits, RG was negatively associated with chick hydration status on d 28 post-hatch. The observed relationships were more pronounced initially during early post-hatch life and subsided as the chick reached market age.

Key Words: broiler, eggshell conductance, embryo temperature

363 NADH oxidase generated superoxide reduces nitric oxide availability in lungs of hypoxic broilers chickens. J. Bautista-Ortega*, E. A. Ellis, and C. A. Ruiz-Feria, *Texas A&M University, College Station.*

Xanthine (XO) and NADH oxidase (NOX) are important sources of superoxide in cardiovascular diseases including pulmonary hypertension syndrome (PHS). Previously we localized XO and NOX in the pulmonary artery endothelium of hypoxic broilers. Nitrotyrosine is a biomarker for peroxynitrite which is negatively correlated with the availability of nitric oxide (NO). Cytochemical localization of XO and NOX (reflectance units), and colloidal gold based immunocytochemical localization of nitrotyrosine (N of colloidal gold particles) were used to determine oxidative and nitrosative (reduced availability of NO) stress in lungs of broilers exposed to hypoxia (d 7 to d 36, simulated 3,000 m above sea level) and fed a control diet (CTL), or control plus arginine (ARG, 0.8% wt/wt), or CTL plus ARG and vitamins C (500 mg / L water) and E (200 IU / kg of feed) (AEC). Also, a group of normoxic broilers were fed the CTL diet (NOR). Nitrosative stress was also determined in hypoxic broilers with PHS and in clinically healthy ones. The XO activity was higher in NOR birds (586 ± 43) than in both AEC (456 ± 39) and ARG birds (394 ± 31), whereas CTL birds had the lowest XO activity (313 ± 27). The NOX activity or NO availability was not affected by dietary or hypoxia conditions in clinically healthy birds. Nevertheless, hypoxic birds that developed PHS had higher nitrotyrosine (145 ± 19) than hypoxic but clinically healthy birds (56 ± 11). Increased levels of superoxide generated by NOX may have resulted in decreased availability of NO as measured by nitrotyrosine. To our knowledge, this is the first time that XO and NOX activity has been semi-quantitatively determined in situ in lung samples of hypoxic broiler chickens. Supplementation with ARG and antioxidant vitamins C and E tended to produce NOX-derived superoxide levels similar to those in the normoxic chickens probably preserving NO in the system but significance was not reached probably due to limited number of birds. The dual role of XO, which produces superoxide and uric acid (antioxidant), may have buffered the effects of superoxides in clinically healthy birds.

Key Words: hypoxia, nitrotyrosine, pulmonary hypertension

364 Genistein effects on fatty liver syndrome induced by estrogen. L. M. Stevenson*, S. S. Oates, J. B. Hess, and W. D. Berry, *Auburn University, Auburn, AL.*

Soy phytoestrogens, such as genistein, have been demonstrated to have a protective effect against fatty liver syndrome in rats. A study was performed with old laying hens to determine if genistein had a protective effect against avian fatty liver syndrome induced by exogenous estrogen. Twenty-four 5 year-old laying hens were randomly selected and divided into 6 treatment groups. Each treatment group consisted of 4 hens. Genistein doses were given by a daily gavage for 14 d. The treatments were: Sham Control (gavage and injections of sesame oil), Estrogen Control (7.5 mg estrogen/kg body weight per dose), Genistein Control (20 mg genistein/kg body weight daily), Low Genistein (7.5 mg estrogen/kg body weight per dose and 10 mg genistein/kg body weight daily), Medium Genistein (7.5 mg estrogen/kg body weight per dose and 15 mg genistein/kg body weight daily), and High Genistein (7.5 mg estrogen/kg body weight per dose and 20 mg genistein/kg body weight daily). Estrogen doses were estrogen dipropionate and were given by injection in the subcutaneous tissue in the back of the neck 3 times during the experiment. All treatments were dissolved in sesame oil. Birds were weighed at placement and at the end of the study. There were no significant differences in body weights for any treatments at

placement ($P > 1.0$) or at the end of the study ($P > 0.6$). Blood samples were collected at placement and at the end of the study. Plasma genistein was analyzed by high performance liquid chromatography (HPLC). Levels of genistein in the plasma were significantly increased in birds on the genistein treatments ($P < 0.05$). Livers were removed, weighed, and samples were collected after the study. There were no significant differences in liver weights ($P > 0.5$) or livers as a percent of their final body ($P > 0.4$). Pictures were taken of all of the livers and visually scored to determine the degree of fatty liver. Birds in the Estrogen Control treatment had an increase in yellow coloring of the liver as compared with the other treatments.

Key Words: genistein, estrogen, liver

365 Gene expression of thyroid hormone regulating elements in reproductively photosensitive and photorefractory turkey hens. S. W. Kang*, S. Kosonsiriluk, and M. E. El Halawani, *University of Minnesota, St. Paul.*

Thyroid hormones are important in the reproductive neuroendocrine response to changing photoperiod in birds. Regulation of thyroid hormone homeostasis in the brain is mainly determined by thyroid hormone transporter (transthyretin (TTR)), type II iodothyronine deiodinase (Dio2), type III iodothyronine deiodinase (Dio3) and thyrotropin-releasing hormone (TRH). TTR plays a key role in transporting thyroid hormones into the brain via the choroid plexus (CP). Tuberal hypothalamus (TuH) is a well known site in the brain where Dio2 is involved in converting thyroxin (T4) into triiodothyronine (T3). In this study, to gain insights into seasonal photoperiodic mechanism(s) that govern reproductive photoresponsiveness and photorefractoriness of turkey hens, we investigated gene expression of thyroid hormone regulating elements (i.e., TTR, Dio2, Dio3, and TRH) in the brain of photosensitive and photorefractory hens using real-time PCR. The expression of each gene was determined in microdissected brain areas from reproductively inactive short day photosensitive hens (SD), long day photostimulated photosensitive hens (LD), and long day photorefractory hens (RF) at 2 circadian time points (CT14, CT19). In TuH of LD, Dio2 was significantly greater (2.8 fold, $P < 0.01$) and Dio3 was repressed (42%, $P < 0.05$) when compared with that of SD. In LD, TTR was 2.5 fold greater relative to that in SD ($P < 0.01$). And, TTR expression was downregulated (3.5 fold, $P < 0.01$) in long day RF relative to LD. Also, TTR was greater (45%, $P < 0.05$) during the photosensitive phase (CT14) than during the dark phase (CT19). CP Dio2 was significantly higher (2.1 fold, $P < 0.01$) in LD compared with that of SD and RF. TRH in the paraventricular nucleus was 2.8 fold greater in LD compared with that of RF ($P < 0.01$), and the levels in RF were 22% ($P < 0.05$) lower when compared with SD. In summary, Dio2 in CP as well as TuH appears to be an important gene involved in the expression of photorefractoriness. TTR in CP might be a significant thyroid hormone transporter involved in photoperiodic response of turkey hens.

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Key Words: transthyretin, turkey reproduction, deiodinase 2

366 Identification of a nonclassical glucocorticoid responsive region of the growth hormone gene during chick embryonic development. K. A. Heuck-Knubel* and T. E. Porter, *Department of Animal & Avian Sciences, University of Maryland, College Park.*

Growth hormone (GH) affects growth and contributes to a lean phenotype in broiler chickens; however, exogenous GH has little effect on post-hatch growth. Elucidation of the regulation of the GH gene could

result in alternative approaches for maximizing growth in broilers. GH secretion occurs naturally between embryonic day (e) 14 and e16, concomitantly with a rise in adrenal corticosterone (CORT) secretion. Treatment of chicken embryonic pituitary (CEP) cells with CORT induces GH secretion prematurely. Inspection of the regulatory region of the GH gene reveals no glucocorticoid (GC) response element (GRE). Pretreatment of e11 cells with a protein synthesis inhibitor, CHX, resulted in blockage of the CORT-induced increase in GH mRNA. This leads to the hypothesis that a GC-responsive intermediary protein is necessary for the CORT induced increase in GH. Characterization of the upstream region of the GH gene may identify such a protein. -1727/+48 bp of the GH gene was cloned into a luciferase reporter plasmid and transfected into e11 CEP cells. Treatment with 100 nM CORT increased luciferase activity 10-fold ($n = 3$; $P < 0.05$). Truncation of this construct to -954 abolished activity. Thirteen additional constructs were tested, revealing an inhibitory region from -1467 to -1430 and a GC-responsive region (GCRR) from -1045 to -954 ($P < 0.05$; $n = 3$). Other constructs showed that the GCRR is position-, orientation-, and context-dependent. Potential transcription factor motifs in the GCRR include ETS1, ELK4, and a GC-binding region (GBR). Mutation of the ETS1 site or the GBR in the -1045/+48 reporter resulted in a loss of Luciferase activity. Nuclear proteins bind to a GCRR probe in a CORT-regulated manner in gel shift assays, and unlabeled competitor can compete off binding. In a single experiment, an ELK4 antibody (ab) did not result in a supershift, but an ETS1 ab abolished protein binding to the probe. The chicken GH gene is GC responsive and contains a putative ETS1 and GBR site at -1017 to -985. Investigation of protein binding to the GCRR is underway using gel shift assays and chromatin immunoprecipitation.

Key Words: luciferase, corticosterone, pituitary

367 Detection and expression of the glucocorticoid receptor in the laying hen's oviduct. L. A. Bola*, D. V. Arbona, and J. B. Hoffman, *North Carolina State University, Raleigh.*

The hen's oviduct is critical in the process of egg formation and ovipositioning. Specifically, the components of the oviduct including the infundibulum, magnum, isthmus, uterus/shell-gland, and vagina are responsible for both interior egg quality as well as exterior shell quality. Due to the sensitivity of the oviduct during hormonal and cellular changes that occur during the formation of the egg, stress susceptibility may have a significant impact on egg quality and formation. Specifically regulation of hormonal action on the oviduct is subject to paracrine modulation by steroids such as glucocorticoids. The presence of glucocorticoid receptors (GR) in the oviduct that bind corticosterone, the avian glucocorticoid, may be an indicator of oviductal tissue sensitivity to stress. The expression of GR was characterized in the oviducts of 6 Leghorns at 27 weeks of age. Tissue samples were removed from the infundibulum, isthmus, magnum, uterus/shell-gland, and vagina from each hen by manual dissection. Subsequently, RNA extraction and real-time RT-PCR analyses were performed. Our results showed expression of the GR in each of the tested regions of the oviduct tissue. These results suggest that corticosteroids may directly act on the oviduct to influence the process of egg formation and overall egg quality.

Key Words: glucocorticoid receptor, oviduct, egg quality

368 Detection and expression of glucocorticoid receptors in the germinal disc (GD) and non-germinal disc (NGD) regions of the laying hen's hierarchical ovarian follicles. J. B. Hoffman*, D. V. Arbona, and L. A. Bola, *North Carolina State University, Raleigh.*

The influence of corticosterones on fertilization and developmental events post-fertilization has yet to be fully characterized in the laying hen. Because the left adrenal gland is embedded in the ovary, it is possible that corticosterones may act through a paracrine mechanism to influence fertilization, sex-ratio distributions, and embryological development. However, paracrine mediation by corticosterones is dependent upon the presence of glucocorticoid receptors (GR) in the germinal disc (GD) region of the F1 hierarchical follicle where fertilization occurs. Additionally, differences in expression of GR in the GD versus the non-germinal disc region (NGD) which is not involved in the process of fertilization may further elucidate the degree to which corticosterones are involved in regulating these processes. In this study, the expression of GR was characterized in the granulosa tissue of the GD and NGD from the F1-F4 hierarchical follicles of 6 Leghorns at 32 weeks of age. Tissue samples (3 cm x 3 cm) were removed from the GD and NGD by manual dissection. Subsequently, RNA extraction and real-time RT-PCR analyses were performed. Our results showed expression of the GR in the GD and NGD regions of the F1-F4 hierarchical follicles suggesting that corticosterone may influence the process of fertilization and post-fertilization events.

Key Words: germinal disc, glucocorticoid receptor, fertilization

369 Molecular cloning and characterization of chicken and zebrafish prostaglandin receptors. A. H. Y. Kwok*, Y. Wang, and F. C. Leung, *The University of Hong Kong.*

Prostaglandin E2 (PGE2) and F2 α (PGF2 α) are autocrine/paracrine mediators responsible for regulation of vital physiological processes, ranging from systemic effects such as fever generation and ACTH-related stress response, to tissue-localized effects such as inflammation, vascular homeostasis and reproduction. Four receptor subtypes have been identified for PGE2, namely prostaglandin E receptor subtypes 1 (EP1), EP2, EP3 and EP4, and prostaglandin F receptor (FP) for PGF2 α . Though the receptors were extensively studied in mammals, little is known about their functionality and expression in non-mammalian vertebrate species. Complementing our previous study on chicken EP2 (cEP2), cEP3, cEP4 and cFP (including cFPa and a novel middle-truncated isoform, cFPb) in which full-length cDNAs were first cloned and preliminarily characterized, in the present study, we furthered our investigation, first, by quantitative real-time PCR, the relative expression of cFPa and cFPb were detected in 12 adult chicken tissues and hen oviduct, wherein both isoforms were found to be widely expressed. Interestingly, the putative functional ortholog cFPa was expressed in abundance in testis, in contrast, only minimal signal of cFPb was detected; an inverse expression ratio was observed in ovary. As EP1 appears to be absent in chicken (neither found in genome database nor detected by degenerate primers

designed), we proceeded to examine whether EP1 was present in other lower vertebrates, such as fish. Three full-length EP1 cDNAs were identified from zebrafish (*Danio rerio*), they were named zEP1a, zEP1b and zEP1-like (zEP1-L) respectively, based on their corresponding sequence identities to mammalian EP1 orthologs (from 38% to 42%). By semiquantitative reverse transcription (RT-) PCR, zEP1a and zEP1b were found in all 9 adult tissues examined, while zEP1-L was detected only in brain and kidney. Subsequent functional assays for the cloned receptors are under way, which, in combination with the above results, would help to elucidate the physiological roles of PGE2 and PGF2 α and their receptors in target tissues of non-mammalian vertebrate.

Key Words: chicken, prostaglandins, prostaglandin receptors

370 Effect of seminal plasma progesterone on sperm hole penetration in White Leghorns. E. M. Anderson* and K. J. Navara, *University of Georgia, Athens.*

While there has been significant interest in identifying the hormonal contributions passed on with gametes by female birds, the hormone content of fluids contributed during reproduction by the male remains relatively unstudied. We aimed to characterize the hormone content, and the potential function of those hormones, in avian seminal plasma. First, we measured the concentrations of 5 hormones within seminal plasma collected from White Leghorn roosters, including progesterone (P4), testosterone (T), dihydrotestosterone (DHT), estrogen (E) and corticosterone (B). P4 levels were higher in seminal plasma compared with other hormones analyzed, ranging from 0 to 14.8 ng/ml (all other levels were <1.1 ng/ml). Given this relatively high concentration of seminal plasma progesterone, we then attempted to determine how progesterone in seminal plasma may function in fertility. White Leghorn hens were assigned to 2 treatment groups: progesterone-treated or control (n = 24 hens per treatment). Semen was collected via abdominal massage from 24 White Leghorn roosters. Each semen sample was divided into 2 equal volumes, one treated with progesterone (0.4 ng/50 μ L) and the other with a control of diluent and artificially inseminated into each treatment group of hens. Eggs were collected 2 d after insemination. A perivitelline layer sperm hole assay, a method that can be used as a predictive measure of fertility, was performed for each egg and the number of sperm holes was counted under a microscope. The effects of treatment were then analyzed and the number of sperm holes were significantly less in the progesterone treated group compared with the control on the second day after insemination ($P = 0.0307$). These results suggest that progesterone has a detrimental effect on the ability of sperm to penetrate the IPVL, and that males that deposit more progesterone into seminal plasma may have a decreased capability to fertilize an egg.

Key Words: seminal plasma, progesterone, white leghorn