

conditions throughout the test. The hens were provided nutrients to meet the needs of all the hens based upon compilation of the breeder recommendations. The eggs from these strains were collected every 28 days from the previous 24 hrs production. Once collected the quality and compositional properties of the eggs such as pH, solids, whipping height, Haugh units and angel food cakes were evaluated. Angel food cake volumes had greater variation in the first laying cycle (first 12 months) than in the second laying cycle (second 12 month period). None of the other quality measurements analyzed were a good predictor of cake volume. Egg weights were also influenced by strain. The strains with the highest ($P<0.05$) egg weights were the W-98, and ISA White at 61.2 and 61.0 g, respectively. The lowest egg weights were associated with the W-36 hen's eggs weighing 57.1 g during the first cycle.

Key Words: egg quality, albumen functionality

250 Functionality and quality of whole eggs and yolk from five different layer strains over two production cycles. L. K. Kerth*¹, P. A. Curtis¹, and K. E. Anderson², ¹*Auburn University, Auburn, Alabama*, ²*North Carolina State University, Raleigh*.

Breeders in the modern commercial environment have been developing and selecting layer strains for the production of large egg sizes to enhance yield. However, the impact of such breeding programs on egg composition has not been evaluated. To date the research that has

been conducted only looks at a few characteristics and does not look at those characteristics over a complete laying cycle or as the hen ages. Therefore, in this study five commercial layer strains were selected based upon their egg weight, availability, and market share and were monitored for a complete two year laying cycle (17-114 weeks of age) including a molt. The five strains that were utilized in this study are Hy-Line W-36, Hy-Line W-98, Hy-Line CV-20, Bovans White and ISA White. Eggs were collected every 28 days from the 35th North Carolina Layer Performance and Management Test in which the strains were maintained under identical husbandry and environmental conditions throughout the study. After collection, the quality, functional, and compositional properties of the eggs were determined. During the first production cycle (17-66 weeks of age) the functionality of the whole egg as tested by sponge cake volume was very erratic ($P<0.05$) between the strains. However, the volume of the sponge cakes became much more consistent among the strains ($P>0.05$) during the second cycle (70-114 weeks of age). Egg weights and whole egg solids were also significantly ($P<0.05$) influenced during both cycles by strain. Emulsion strength of mayonnaise was impacted by hen age and strain during both production cycles. Yolk solids had no difference ($P>0.05$) among the five strains during the first cycle, but there was a significant difference ($P<0.05$) during the second. Vitelline membrane strength was also impacted over the complete laying cycle by hen age and strain ($P<0.05$). Both hen age and strain appeared to influence the functional properties of the eggs produced.

Key Words: egg quality, yolk functionality, whole egg functionality

Physiology, Endocrinology, and Reproduction: Reproduction

251 Are thermal manipulations to improve thermotolerance during chick's embryogenesis only a question of fine tuning? S. Yahav*, *Institute of Animal Science ARO the Volcani Center, Bet Dagan, Israel*.

Thermal manipulations during chick's embryogenesis are based on the following hypothesis: a. during embryogenesis, inducing long lasting physiological memory, based on epigenetic adaptation, is achievable; b. the meaning of long lasting memory can be defined, most probably, as alteration in the thermoregulatory hypothalamic threshold response to changes in the environment; c. thermal manipulations during the sensitive periods of embryogenesis, using specific level and duration of heat exposure will achieve the improvement of thermotolerance acquisition during life span. It was previously well documented that thermal manipulations during the 1st week post-hatch reach the targeted aim of improved thermotolerance. However, technical problems to adopt these manipulations in chicks, coupled with the hypothesis that during embryogenesis thermal manipulations maybe more efficient for the improvement of thermotolerance, targeted the chick embryogenesis as a promising period for manipulations. Thermal manipulations during 8 to 10, and 16 to 18 d of embryogenesis were conducted using 6 h exposure to 39.5C on each day. These treatments didn't affect hatchability or development upon hatch or later on. Challenging the treated chicks with hot conditions for 3 d demonstrated a better thermotolerance (significantly lower body temperature coupled with lower thyroid hormones concentration) and stress resistance of the manipulated embryos at 16 to 18 d of embryogenesis. However, challenging the chickens at marketing day (42 d of age) did not show any thermotolerance efficiency/advantage of the manipulated chickens.

Moreover, these chickens lost their relatively lower body temperature with age, meaning, they lost their efficiency to better cope with heat challenge. These results raise the following questions: a. is it possible that there is no way to induce long lasting thermoregulatory memory by thermal manipulations during embryogenesis? or b. is it only a question of thermal manipulation fine tuning? Further research is needed to shed light on this topic, although there are evidences that fine tuning may achieve the targeted goal.

Key Words: embryogenesis, thermotolerance, thermal manipulations

252 Incubator temperature and oxygen concentration affect the physiology of selected muscles of broiler embryos at the plateau stage in oxygen consumption. V. L. Christensen*, D. T. Ort, M. M. Mann, M. J. Wineland, P. E. Mozdziaik, S. L. Funderburk, J. L. Grimes, and E. R. Oviedo, *North Carolina State University, Raleigh*.

An apparent paradox in energy budgets of avian eggs and metabolism occurs at the plateau stage in incubation as heat output increases, but oxygen utilization does not. When confronted with life-threatening situations, embryonic organ growth and function may be antagonistic. Temperature manipulations have been shown to affect the posthatch phenotype through muscle alteration. Little is known of the effect of oxygen on embryonic muscle physiology. The objective of the current study was to test the effect of incubation temperature and fractional oxygen concentration at the plateau stage in development on muscle physiology. Eggs from two strains of broilers (high and low eggshell conductance (G) strains) were incubated normally for 18 d. At that

time they were transferred to four incubation cabinets to operate at 36, 37, 38 and 39° C in trial 1 or with 17, 19, 21 or 23% oxygen in trial 2. At external pipping and at hatching randomly selected embryos or chicks were weighed and sampled for pipping, breast and thigh muscle glycogen and lactate. Blood samples were collected to test for the enzyme activities of creatine kinase (CK) and lactate dehydrogenase (LDH). Increasing temperature decreased ($P < 0.0001$) breast, thigh and pipping muscle glycogen at the plateau stage, but it did not affect CK or LDH. At hatching temperature and strain interacted ($P < 0.01$) such that the high G strain was less sensitive to increases in temperature than the low G, but it did not affect CK or LDH. Oxygen had divergent effects on muscle lactate and glycogen. Increasing oxygen concentrations decreased ($P < 0.01$) glycogen concentrations in some muscles but increased it in others with no consequent effects on CK or LDH. It is concluded that temperature greater than 37 C and oxygen concentrations less than 21% in the incubator atmosphere at the plateau stage in embryo development affect muscle physiology.

Key Words: embryo, muscle physiology, temperature

253 Incubator temperature and oxygen concentration affect the growth of selected muscles of broiler embryos at the plateau stage in oxygen consumption. V. L. Christensen*, M. J. Wineland, E. R. Oviedo, S. L. Funderburk, D. T. Ort, J. L. Grimes, P. E. Mozdziak, and M. M. Mann, *North Carolina State University, Raleigh.*

Temperature is the major regulatory factor in egg incubation and embryo development because of its effect on general growth and the coincidental impact on tissue formation. Prior research indicated that temperature manipulations affected the posthatch phenotype through the alteration of muscle (Maltby and Strickland, 2004). Little is known of the effect of oxygen on muscle cell expression, but it is thought that oxygen may be a primary determinant of the growth of the embryo. The impact of temperature and oxygen on embryo muscle development is highly important because of the proliferating pool of precursor muscle cells available late in incubation. The objective of this study was to test the effects of incubation temperature and oxygen concentration on the development of muscles during incubation. Eggs from two strains of broilers (high and low eggshell conductance (G) strains) were incubated normally for 18 d. At that time they were set in four incubation cabinets calibrated to operate at 36, 37, 38 and 39°C in trial 1 or with 17, 19, 21 or 23% oxygen in trial 2. At external pipping and at hatching randomly selected embryos or chicks were weighed and sampled for pipping, breast and thigh muscle weights. Increasing temperature depressed ($P < 0.0001$) BW, pipping and thigh muscle weights but not breast muscle weight. Increased fractional concentrations of oxygen depressed ($P < 0.01$) pipping muscle weights but increased ($P < 0.0001$) thigh muscle weights with no effect on breast muscle weight. Oxygen interacted with strain as 19% increased ($P < 0.01$) thigh muscle weight in the low G line but it required 23% oxygen to increase the weight in the high G line. It is concluded that temperature and oxygen have divergent effects the growth of muscles.

Key Words: embryo, muscle growth, temperature

254 Determining true fertility of clear eggs identified at 7 days incubation histologically. K. L. Knight and T. A. Scott*, *University of Sydney, Camden, NSW, Australia.*

In order to best differentiate infertility from very early embryo mortality it is important that we can clearly classify true fertility, particularly for

those eggs identified as clear with candling. This study determined the effectiveness of using Propidium Iodide (PI) (Sigma P4170, St. Louis MI, 63103), a fluorescent DNA-specific red stain, in identification of true fertility of eggs identified as clear at 7 d candling. True fertility is determined by the presence of cell nuclei that uptake the stain and illuminates the nuclei. Propidium iodide acts by intercalating between base pairs of DNA. The cells of fertile eggs are diploid and it is estimated that at the time of oviposition, embryos may contain up to 70,000 cells. Briefly, the procedure requires a portion of the shell at the large end of refrigerated 7-d candled clear eggs to be removed and the outer perivitelline membrane moved to expose the germinal disk. Germinal disks were visually screened for signs of early embryonic development; those germinal disks showing no development were transferred to a microscope slide. Propidium iodide was used to stain the germinal disk at a concentration of 5 µg PI/mL 0.9% NaCl solution. For each slide, 5 µL of the dilute PI was used and a coverslip placed on top. Based on 4,800 eggs (single age flock) from each of two storage times (3 and 7 d at 18 C) 8.3 % were classified macroscopically as infertile, 4.25 % as early dead. Approximately 50% (347) of the 798 eggs identified as "clear" were fixed, stained and histologically examined; of these 41 were reclassified as fertile with embryo mortality occurring before oviposition or during storage; this was equal to almost 12% of eggs classified as "clear" and estimated to be 1% of total eggs candled. Incidence of eggs re-classified as fertile with PI staining were numerically higher with 7 d as compared to 3 d of storage before incubation. This study has provided a definitive tool to assess true fertility from eggs incubated to 7 d of age.

Key Words: true fertility, stains, embryo

255 Effect of cooling and developmental age on quail embryo heart rate. B. C. Wentworth*, *University of Wisconsin, Madison.*

Managers of avian incubation have known for many years that it is very important to keep the temperature at a constant appropriate level. We have known that avian embryo development varies with respect to incubation temperature. The objective of this study was to report the effect of cooling and developmental age on quail embryo heart rate. The heart rates of 302 Wisconsin white egg line Japanese quail (*Coturnix coturnix japonica*) embryos were recorded from day four of incubation to day 15 of incubation. The heart rate was determined with an infra red heart rate sensor in an instrument called "Buddy". The instrument would not detect heart rate before day four. Respiration after day 15 compromised the heart rate recording. The heart rate of 210 ± 23 beats/min on day four increased with a significant ($P < 0.001$) linear trend to 371 ± 28 beats/min on day ten with a NS decrease (367 ± 17 beats/min) on day 15. Cooling the embryos at room temperature (22°C) for five min. resulted in an average heart rate decrease of $16 \pm .69$ beats/min. The decrease in heart rate with cooling of four day-old embryos was $11 \pm .73$ beats/min with a significant ($P < 0.01$) numerical decrease in heart rate ($13 \pm .64$ beats/min.) at day eight to ($18 \pm .72$ beats/min). On day 13 of development there was a rate plateau until day 15. Among the 302 embryos recorded there was NS dam and sire affect on heart rate from day four to 15, or on the heart rate decrease with cooling for five min. These results demonstrate that a short period of cooling has a dramatic effect on slowing the heart rate of quail embryos.

Key Words: embryo, heart rate, temperature

256 Torpor in quail embryos and young quail chicks. B. C. Wentworth*, J. L. Cigan, and T. J. Schaaf, *University of Wisconsin, Madison*.

The purpose of this study was to explore the state of torpor in Japanese quail (*Coturnix coturnix japonica*). In this experiment, quail embryos and young quail chicks demonstrate phenomena that simulate torpor that has been documented in families represented by Chimney Swift, Hummingbirds and Whip-poor-wills. The heart rate was determined with an infra red heart rate sensor in an instrument called "Buddy". Six day-old embryos were placed in a cold-room at 13°C for 24 h. After 12 h the embryo body temperature was 13°C and heart rate at 13°C averaged 14 ± 9 beats/min and the average control embryo heart rate at 37.5°C was 311 ± 15 beats/min. The torpor quail embryos had a 24 h delay in hatching at 58%. The controls hatched on time at 81%. Twelve six day-old quail were selected that appeared uniform in size and vigor and were placed in a low temperature environment (13°C) for a period of 6 h. Quail from which the test group was selected were retained in a brooder to serve as a control comparison to those the low temperature (13°C) environment. During the time when the 12 quail were in the state of torpor the heart rate (EKG) for both torpid and control quail was determined with a Tektronix TDS-200 Digital Oscilloscope. The quail in the state of torpor had a heart rate of 14 ± 6 beats/min. The control quail had an average heart rate of 525 ± 24 beats/min. After emerging from the reduced-temperature environment, the torpid quail were placed under an infrared (IR) light producing a brooder-like temperature of 33°C. During observation under the IR, one quail showed signs of life with beak movement in three min. After ten min all quail were breathing and after 25 min some quail were able to stand and peep loudly. After 40 min all quail could walk around and some eat and drank. Ten of the 12 original quail recovered from their torpid state and appeared normal, but two appeared to have neurological problems and were euthanized. These data demonstrate that quail and quail embryos can tolerate 13°C cold environments.

Key Words: torpor, embryo, chick

257 Effect of breeder hen age and incubation temperature on embryonic temperature and development of White Pekin ducklings. K. A. Kroesen* and M. S. Lilburn, *The Ohio State University, Wooster*.

In each of two experiments, internal egg temperatures were measured in commercial duck hatching eggs during incubation. In Experiment 1, eggs from very young and prime age flocks were individually weighed at setting and incubated at 99.5°F. At multiple ages during incubation, internal egg temperatures were measured on a sample of 20 eggs per hen age. Internal egg temperature increased in a linear fashion from 99.5-100° on Day 5 to 101.5°F on Day 21 in eggs from both hen ages. On Day 14, the mean temperature difference between flock ages was small (0.8°F) but > 80 % of the prime eggs had internal temperatures > 100°F compared with < 50% of the young eggs. At 21 d, > 80% of the eggs from both hen ages had internal temperatures between 101.4 and 102.2°F. Differences in yolk-free embryo weight (prime vs. young) were 0.7 g at 23 d (34.8 vs 34.1 g) and 1.6 g (46.1 vs. 44.5 g) at 26 d of incubation but there were no differences in embryo length at either embryonic age (Day 23, 15.5 cm; Day 26, 16.2 cm). In Experiment 2, eggs from young and prime flocks were incubated at 99.5° or 101.0°F. Measurements on Day 13 of incubation confirmed that we were successful in creating different internal egg temperatures of approximately 98.5° and 100°F. In embryos from both age hens, the

higher incubation temperature resulted in increased yolk-free embryo weight at Day 20 (21.8 vs 19.3 g; $P < 0.001$) and embryo length (13.4 vs 12.7 cm; $P < 0.001$) and the correlation between embryo length and yolk-free embryo weight was 0.84 ($P < .001$). It was also observed that 18.3% of all embryos were upside-down in the egg in the 101.0°F treatment, similar to the 29.3% incidence reported in turkey embryos incubated at 101.3°F.

Key Words: Pekin ducks, embryo, temperature

258 Defining physiological parameter relationships of the pre- and post-hatch broiler chick including the impacts of sex and time of hatch. R. W. Keirs*, E. D. Peebles, L. W. Bennett, S. K. Whitmarsh, K. A. Viscione, and P. D. Gerard, *Mississippi State University, Mississippi State*.

A deeper assessment is needed of physiological parameters significantly impacting the embryo and chick including the influences of sex (CS) and time of hatch (TOH). This study included 10 replicates of 5 chicks that were identified with their respective individually isolated egg. Percentage egg weight loss (PEWL) was measured through 18 d of incubation, and BW, rectal temperature (RT), hematocrit (HCT), serum refractive index (RI), and plasma glucose (GLU) were measured through 72 h post-hatch. Chicks received nutrition and heated brooding at 12 h post-hatch. The significant correlations found are as follows. Overall (across TOH and CS): RT at 48 h with HCT at 0, 12, 24, and 72 h; 24 h GLU with 0-6, 6-12, 12-18, and 0-18 d PEWL; hatch RI with hatch RT; hatch RI with HCT at 0, 24, 48, and 72 h; and 72 h RT with HCT at 0, 12, 24 and 72 h. Within TOH: 20 d chick 48 h RT with 12 h HCT; and 72 h RT with 24, 48, and 72 h HCT. 20 d 6 h chick 24 h GLU with PEWL between 0 and 18 d; 24 h RI with both 24 h GLU and 24 h RT; RI at hatch with 0 and 24 h HCT; and 24 h RI with 0, 48, and 72 h HCT. 20 d 12 h chick 24 h GLU with hatch RI; 24 h GLU with 72 h RT; hatch RI with 24 h HCT; and 48 h RT with HCT at hatch. Within CS: Male 24 h GLU with 6-12 and 0-18 d PEWL; 24 h RI with 48 h RT; and hatch RI with 48 h HCT. Female 24 h RT with 0, 12, 24, and 72 h HCT; hatch RI with 0, 12, 24, and 48 h HCT; 24 h RI with 0-6, 6-12, 12-18, and 0-18 PEWL; and 24 h HCT with 0-6, 6-12, 12-18, and 0-18 PEWL. Male mean hatch RT (37.04°C) was significantly lower than that of females (37.54°C), and RI at 24 and 72 h was significantly lower in males than in females. RT in chicks that hatched at 20 d 12 h was significantly lower than those that hatched at 20 d 6 h or earlier. The relationships of physiological markers indicative of performance through the incubational and post-hatch periods were influenced by bird sex and time of hatch. The significance and consistency of relationships among physiological markers is imperative to ascertain their impact on performance.

Key Words: blood, broiler chick, embryo

259 Determination of eggshell microstructural characteristics and associated physiological profiles in MG-vaccinated egg-laying chickens. S. L. Westmoreland*¹, S. B. May¹, M. I. Gracey¹, D. L. Hawkins¹, E. D. Peebles², S. W. Park², and S. L. Branton³, ¹*The University of Texas, Arlington*, ²*Mississippi State University, Mississippi State*, ³*South Central Poultry Research Laboratory USDA, Mississippi State, Mississippi*.

This experiment determined the effect of vaccination of commercial layers with F-strain *Mycoplasma gallisepticum* on eggshell thickness.

The experiment involved multiple variables in addition to the vaccination, including three different diets (basal diet; 2% PF added; and 2% PF plus phytase and D3) and two ages of lay (24 and 50 wk). Cross-section micrographs were taken on each eggshell and thickness measurements were made. These data were statistically analyzed using SAS statistical software to determine the effects of the variables on shell thickness. For treatment effects, both sham-inoculated and vaccinated data were examined for age-treatment interactions. It was found that for sham-inoculated shells, layers fed diet 1 had thinner shells in young versus old birds, however, in layers fed diets 2 and 3, young birds had thicker shells. Only in diet 3 was this difference significant ($P = 0.006$). For vaccinated shells, it was found that young birds produced thinner shells in layers fed all three diets. Again, only in diet 3 was this difference significant ($P = 0.0011$). For diet effects, shells from birds fed each of the three diets were examined for age-treatment interactions. For diet 1, sham-inoculated birds had thinner shells than vaccinated birds at both ages (24 and 50 wk). For diets 2 and 3, sham-inoculated had thicker shells at age 24 wk, but thinner shells at age 50 wk than vaccinated birds. None of these results were significant. For age effects, shells taken from layers at both ages were examined for diet-treatment interactions. At age 24 wk, sham-inoculated birds fed diet 1 had thinner shells than vaccinated birds, but the reverse was true for diets 2 and 3. At age 50 wk, sham-inoculated birds fed all 3 diets had thinner shells. None of these results were significant.

Key Words: F-strain *Mycoplasma gallisepticum*, commercial layer, eggshell quality

260 Chicken amphiregulin (AR) gene: cDNA cloning, promoter analysis, and regulation of its mRNA expression in the cultured ovarian granulosa cells. Y. Wang*, J. Li, and F. C. Leung, *The University of Hong Kong, Hong Kong, HK-SAR, China.*

Recent evidence demonstrates that amphiregulin (AR), a member of epidermal growth factor (EGF) superfamily, plays an important role in the regulation of ovarian functions in mammals. However, in non-mammalian species, studies on its role and hormonal regulation in the ovary are rather limited. In the present study, a full-length cDNA coding for the precursor of amphiregulin (210aa) has been cloned from the chicken ovary. To elucidate its potential role in the ovary of chicken, using semi-quantitative RT-PCR, we first examined the expression of amphiregulin during ovarian development. The mRNA level of amphiregulin was lowest at the embryonic stages; however, at the post-hatching stages, its expression dramatically increased, suggesting that amphiregulin may play an active role in the initiation or progression of the ovarian follicle growth. Using an in vitro granulosa cell culture system, the regulation of amphiregulin expression by gonadotropin and local growth factors was also investigated. Interestingly, unlike those findings in mammals, follicle-stimulating hormone (FSH) only weakly stimulated amphiregulin expression in the cultured ovarian granulosa cells from F1 follicles. By contrast, EGF, TGF α and HB-EGF strongly induced the expression of amphiregulin in a clear time- and dose-dependant manner. In agreement with the above finding, the promoter activity of amphiregulin gene could be enhanced significantly by EGF treatment in the cultured ovarian granulosa cells, indicating that the regulation of amphiregulin expression by EGF may occur primarily at the transcriptional level. Collectively, results from our study provide evidence that ovarian amphiregulin may be actively involved in the follicle growth and its expression is regulated by local

ovarian growth factors, probably including amphiregulin itself, in the ovaries of non-mammalian species.

Key Words: amphiregulin (AR), promoter, granulosa cells

261 Cloning of chicken epigen and regulation of its mRNA expression in the cultured ovarian granulosa cells. Y. Wang*, J. Li, and F. C. Leung, *The University of Hong Kong, Hong Kong, HK-SAR, China.*

Epigen, also named epithelial mitogen, is the latest identified ligand for epidermal growth factor receptor (EGFR). Although much attention has been paid to the mitogenic and antiapoptotic effects of ovarian EGF and TGF- α , little is known about the expression of epigen in the vertebrate ovary. In this study, the cloned full-length cDNA coding for chicken epigen is 2,278bp in length and encodes a putative membrane-anchored precursor (151 amino acids), which shares 61%~65% sequence identity with its mammalian counterpart. Unlike other 6 chicken EGFR ligands, epigen has a long 5' untranslated region (5'-UTR, 594 bp). Most strikingly, promoter analyses revealed that this long 5'-UTR displayed promoter activity and removal of partial or whole 5'-UTR greatly impaired the basal promoter activities, providing a possibility that this 5'-UTR represents only one of multiple transcriptional initiation sites or promoters used by epigen gene. Using semi-quantitative RT-PCR assay, we also noticed that epigen was one of most abundantly expressed EGFR ligands among all of the EGFR ligands examined in the chicken ovary. And its abundant expression could be detected not only in the adult ovary, but also in the developing ovaries including those from embryonic stages (from day 8 to day 20), strongly suggested that ovarian epigen may have a tonic role during whole process of ovarian development. Moreover, epigen was also highly expressed in the cultured granulosa cells from 6mm, F4+5, and F1 follicles. Like other chicken EGF family members, administration of exogenous EGF or TGF- α could also significantly stimulate epigen expression in the cultured granulosa cells despite the high basal level of epigen in the cultured conditions. Present results provide evidence, for the first time, that ovarian epigen, like other EGF family members, is also a potent paracrine/autocrine factor involved in the ovarian development of vertebrates.

Key Words: epigen, ovary, EGF

262 Characterization of fourteen chicken PAC1 receptor spliced variants: Evidence for the conserved 13bp intronic sequence as a critical factor to determine signaling properties and splicing patterns of PAC1 receptor. Y. Wang*, J. Li, and F.C. Leung, *The University of Hong Kong, Hong Kong, HK-SAR, China.*

It is well documented that the actions of PACAP are mediated by three types of receptors, named PAC1-R, VPAC1-R, and VPAC2-R, respectively. Among of them, PAC1-R has attracted much attention due to multiple spliced variants with different signaling properties in mammals. In contrast, little is known about spliced variants of PAC1-R and their functionalities in birds. In this study, using RT-PCR, we have isolated 14 new spliced variants from brain and ovary of adult chickens. In the 3rd intracellular loop, the insertion of 5 types of cassettes (14, 27, 46, 59 and 60aa), which are coded either by exon 14, 15, and 16 alone or in combination, results in 5 spliced variants identified in chicken brain. To test their functionalities, these variants

were cloned into pcDNA3.1 vector and co-transfected with pGL3-CRE luciferase construct into CHO cells. In the presence of these variants (except for 14aa-variant), ovine PACAP38 could strongly increase luciferase activities in a dose-dependant manner with EC50 values lower than 1 nM, suggesting that receptor variants could activate cAMP-PKA signaling pathway. Strikingly, an insertion of 13bp (partial retention of intron 18) into PAC1-R short form and spliced variants results in the cloning of additional 6 spliced variants (with altered C-terminal tails), which could not increase luciferase activities after

PACAP treatment. Moreover, 3 variants (457, 356 and 418aa) (due to deletion of exon 18, or exon 17 and 18) were also cloned from chicken ovary. Again, the insertion of 13bp generates a variant with a C-terminal tail (418aa), while absence of 13bp causes a premature stop codon (356aa). Interestingly, the identical 13bp could also be found in PAC1-R gene of zebrafish and frog, suggesting that it plays a critical role in switching the signaling pathway and determining the splicing pattern of PAC1-R in lower vertebrates.

Key Words: PAC1-R, spliced variant, ovary

Embryo Symposium: Managing the Embryo for Performance

263 Managing incubation: Where are we and why? R. M. Hulet*, *Pennsylvania State University, University Park.*

Improvements in broiler rate of gain for the past ten years have made the incubation period a larger percentage of the overall growth period for commercial poultry and has increased its importance for improving growth efficiency. Historically, hatchery managers have observed decreases in hatchability and chick performance although temperature profiles in the setters and hatchers have not appreciably changed. Decreases in hatchability, first week livability, hatch time, and overall chick quality have precipitated the need for a change in the way hatcheries are managed. Historically, the poultry industry within the US and UK had successfully utilized multi-stage incubation. Currently, use of single-stage incubation in Europe has increased because research has shown they more precisely meet the demands of the developing embryos. Therefore, research has changed the focus for multi- and single-stage hatcheries in order to determine the proper hatchery conditions to optimize embryo development, chick quality, and their affects on post-hatch performance. Studies have investigated how increases in shell temperature, independent of machine temperature, can result in increases in embryonic mortality, lower heart yield (heart / body weight), lower yolk-free body weight, and increased yolk weight. Factors that have contributed to the increase in heat stress to the developing embryos include egg mass, age of the embryo, air flow, breeder flock fertility, etc. Other studies have shown that the variation in chick performance can be explained by heat stress in the hatchery. Therefore, the symposium will show how improvements in our knowledge of the requirements of the developing embryo can help improve not only hatchability and first week livability, but post-hatch performance of commercial poultry.

Key Words: shell temperature, post-hatch performance, hatchery management

264 Incubation parameters and chick quality. R. Meijerhof*, *Nutreco, Boxmeer, The Netherlands.*

Hatch results give an underestimation of the influence of the incubation process on the total production chain, as the influence of the quality of the day old chicken and with that of the incubation process on the performance parameters of broilers is significant. Therefore, the quality of the incubation process should be much more expressed in terms of development and quality of embryos then in chicks of (fertile) eggs set. The major contributing factor to the quality of the day old chick is the embryo temperature. This temperature is the result of the

balance between heat production of the embryo on one side and air temperature, heat capacity of air, air velocity and water evaporation on the other side. A lack of control of the embryo temperature results in a dramatic decrease in chick quality and broiler performance. Several attempts have been made to quantify the quality of the day-old chick in an easy and repeatable way. The length of the chicken has shown to be a useful and practical applicable tool, as it is related with the performance potential of the bird. More qualitative scores as the Pasgar or Tona score are useful as well, but focus more on the survival opportunity in the first week of the broilers life then on the performance potential.

Key Words: incubation parameters, chick quality

265 Attainment of thermoregulation. B. Tzschentke*, *University of Berlin, Humboldt, Germany.*

In poultry the early development of adaptive body functions, like the thermoregulatory system, is characterized by the following peculiarities.

I The development of peripheral as well as central nervous thermoregulatory mechanisms starts in the course of the prenatal ontogeny. However, their maturity is attained during early postnatal development. In the perinatal period environmental factors have a high impact on development of temperature regulation.

II Acute changes in the environmental conditions induce as a rule, first uncoordinated and immediately non-adaptive reactions. Later the uncoordinated (immediately) non-adaptive reactions change into coordinated (adaptive) reactions. Prenatal environmental influences may have a 'training effect' on the postnatal efficiency of the thermoregulatory system. These 'training effects' are necessary for the complete development of body functions like thermoregulation.

III Functional systems of the organism develop from open loop systems without feedback control into closed systems controlled by feedback mechanism. During this 'critical period', the actual environment modulates the development of the respective physiological control systems for the entire life period, especially by changes in neuroorganization and expression of related effector genes. Knowledge on these mechanisms might be specifically used to generate long-term adaptation of the organism to the postnatal climatic conditions (epigenetic temperature adaptation: ETA). In poultry ETA was developed by changes in the incubation temperature. Compared to birds, which were incubated at 37.5°C, a low incubation temperature induced postnatal cold-adaptation and warm incubation temperature induced postnatal heat-adaptation. ETA was shown in changes in the neuronal