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Differentiation of embryonic cells during incubation

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By definition gastrulation is the formation of primitive differentiated tissues by overall movements of embryonic cells during the first 24 hours of incubation. This is a process that is required for optimal embryo formation in the hen.

If we have a closer look at the chicken embryo after 24 hours of incubation we can see a difference from an unincubated embryo.

Within 24 hours the flat disc located on top of the yolk of an unincubated egg has developed to an elongated bilaterally symmetrical structure: the embryo.

In this one-day old chick embryo we can easily recognise the primitive streak as the origin of the future backbone of the chick.

The transition from a flat disc, the blastoderm, to an elongated one-day chicken embryo is the result of the gastrulation process initiated by the increasing incubation temperature.

The higher embryo temperature initiates an enormous movement of the pluripotent blastomeres to form three differentiated cellular layers: the germ layers.

The different germ layers receive instructions while they migrate towards their final position and are no longer pluripotent. The outer germ layer, ectoderm, will develop to surface layers (skin) and brain and nervous systems.

The endoderm receives instructions to develop the digestive tract, associated organs and lungs. The layer of cells between the ectodermal and endodermal layer, the mesoderm, generates connective tissues, blood cells, heart, kidneys, bones and muscles.

Also the non-reproductive tissues of the gonads is generated from the mesoderm, and the future reproductive cells, oocyte or spermatozoa, are set aside before the gastrulation starts.

By definition: the totipotent fertilised oocyte can develop into all cells in the body, including reproductive cells (primordial germ cells) and extra-embryonic cells.

The pluripotent cells can no longer develop to extra-embryonic tissues or primordial germ cells. The multipotent cells in the three germ layers are restricted to form specific cell types like lung, brain and blood cells.

In summary, gastrulation comprises the process of differentiation of the pluripotent blastomeres to the multipotent ectodermal, endodermal and mesodermal cells.

Each of these germ layers can be recognised, not only by their position in the embryo but also by differences in the proteins produced.

The movement of the pluripotent blastomeres in the unincubated blastoderm initiate differential gene expression.

The differential gene expression is the result of the gastrulation related cellular movements. The movement of the cells creates, for each cell, a different time-dependentm'micro-environment', which results in the formation of three basic germ layers.

Each of these layers is recognised by their specific form and function of the cells as a result of the production of cell-specific proteins. Gastrulation-related movement of the blastomeres starts with the increased temperature of the blastoderm.

However, the initiation of cellular movements is not random but starts at a defined 'organiser' region in the blastoderm differentiated during embryo development in the hen.

Recently it has been shown that the in utero development of the pluripotent cells in the blastoderm depends on integrated networks of differential gene expression and protein formation.

These integrated networks are already initiated before fertilisation, while the yolk is deposited in the follicles of the ovary. ■

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Assessment of food safety risk and microbiome links

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Intestinal microbial colonisation is an important development process that takes place in early life.

Colonising bacteria are believed to come mostly from the mother or the environment. Since current poultry production relies on artificial incubation, the contribution of the breeder hen microbiota to offspring intestinal microbiome has been questioned.

This study was designed to investigate the link and contribution of breeder hen microbiota to broiler chicken flocks under commercial conditions. Twenty excreta samples were collected directly from the cloaca using sterile swabs from six Ross 308 breeder flocks ranging between 35 and 52 weeks of life. These flocks were fed the same feed and kept under similar management conditions. Meconium samples were collected from 20 chicks from the eggs produced by these same breeder flocks on day of hatch at the hatchery.

Finally, cloaca swab samples were collected from 20 broilers from these same birds at 21 and 32 days of age. DNA was extracted from each of these samples and submitted to one step PCR amplification of 16S bacteria DNA while incorporating a fluorescent dye.

The individual labelled DNA samples were hybridised on a microarray chip containing a predefined list of 100 bacteria selected to be related to broiler performance and food safety.

The image of fluorescence signals were captured and submitted to quality control and data analysis. The patterns were identified by Principal Component Analysis (PCA) and the flocks were compared by ANOVA with flock (1-6) and category (breeder, hatchery, offspring 21 days and offspring 32 days) and their interaction as fixed effects. Means were compared by FDR 0.05%.

The results showed that in five out of the six flocks there was a strong link between microbiota of the breeder and that of the offspring.

As broilers aged their whole microbial profile became more similar to that of breeders, with the biggest differences around 21 days.

Looking at individual bacteria differences, there were, on average, only three bacteria significantly different between breeders and hatched chicks, four with broilers at 21 days and six bacteria probes differences with broilers at 32 days. This general pattern included more streptococcus and clostridium species in breeders compared to offspring, while more bacteroides and lactobacillus were found in broilers compared to breeders.

Although these results demonstrate a link between breeder and offspring broiler microbiome, it is still expected that this connection is mostly done via the environment since they all belong to the same integrated poultry operation. At least under these conditions it was demonstrated that the microbiome connection between breeders and their offspring flocks is not lost.

In one flock, higher salmonella in breeders was followed by significantly higher salmonella at hatch, which could indicate vertical transmission. The flock that did not follow the usual pattern of microbial maturation was the youngest breeders (35 weeks). That was also the only breeder flock with high levels of lactobacillus and enterococcus, though these differences did not affect the offspring performance of this flock.

Therefore, we conclude that in an integrated poultry operation there was a strong link between breeder and offspring microbiome. This link can be positive or negative depending on the microbial profile of the breeders.

Since the offspring flock that deviated from breeder microbiome had equivalent good performance, it appears to be possible to change broiler microbiome when breeder profile is not desired, while maintaining broiler performance. ■

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Goose embryo development at oviposition

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Embryo development and hatched chick quality are influenced by parental genotype, age, nutrition, environmental conditions, and flock management. It was the aim of this study to determine if breeder genotype, age of breeder, or eggs laid near the onset of egg production versus eggs laid near the end of egg production influence the stage of embryo development at oviposition.

To compare genotypes (Exp. 1) 50 eggs of comparable sizes were collected from 3-year-old commercial line White Koluda (WK) breeders and from two breeds involved in a genetic resources conservation program, Zatorska (Za) and Bilgoraj (Bi).

Age comparison (Exp. 2) was conducted with 50 eggs of comparable sizes collected from 1, 2, 3, and 4-year-old WK breeders. To compare laying periods (Exp. 3), 150 WK eggs were collected the first week of March and 100 WK eggs collected the last two weeks of June.

All eggs were stored for 72 hours at 16°C and then staged using Eyal-Giladi and Kochav (EGK, Roman numerals) and Hamburger and Hamilton (HH, Arabic numerals) procedures.

● **Exp. 1 Genotype:** Individual breed differences were evident with Stage X embryos comprising 42.4%, 33.3%, and 38.7% in the eggs examined from the WK, Bi, and Za breeds, respectively. For all breeds combined, 38.8% of the embryos were in Stage X but in the next order in WK there was stage XI (18.2%), while in geese from the genetic reserve it was stage XIII (Bi – 14.3; Za – 26.4%).

● **Exp. 2. Age:** In eggs from 1, 2, and 3-year-old WK breeders, the majority of embryos (38.7, 32.4 and 42.2%, respectively) were at Stage X. In contrast, the majority of embryos observed in the 4-year-old WK eggs were in Stage XI (36.1%).

● **Exp. 3. Laying period:** With WK eggs staged in March and in June, the highest percentage of embryos were in Stage X (33.7% and 43.6%, respectively). In addition, more developmentally advanced stages (XI-XIII) was similar in both periods. However, embryos developmentally at the onset of primitive streak formation (Stage 2 HH) were only observed in the eggs from the end of the laying season.

Interestingly, earlier stages of development (Stages VI-IX) were observed exclusively in the eggs collected in March (early egg production).

Results obtained encourage further experiments on factors affecting the stage of goose embryo development at oviposition and its impact on gosling hatchability. ■

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Rooster sperm quality after six hours of storage

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There are many extenders of different compositions, sometimes with special additives, both commercial and developed by individual investigators, that can be used for short (liquid) or long (in a frozen stage) term storage of chicken semen.

Nevertheless, problems with natural mating of broiler breeder flocks, as well as the growing interest of consumers in new, original poultry products means that interest in bird reproduction by artificial insemination and developing more effective extenders, is not diminishing.

We aimed to investigate the effect of enrichment of EK extender (Lukaszewicz, 2002) with some natural additives on chicken sperm characteristics after six hours storage at 4°C. Pooled ejaculates were collected from 10 meat type roosters (Hubbard FLex), twice a week by dorso-abdominal massage. Males were kept in individual cages and controlled environmental conditions; water was provided ad libitum, food - 130g/day/male.

Freshly collected semen sample was divided into nine parts: 1) net semen; 2) diluted in 1:2 ratio with EK extender; 3) EK + 200mg/ml of lyophilised quail egg white (LQEW); 4) EK + 100mg/ml LQEW; 5) EK + 50mg/ml LQEW; 6) EK + 100mg/ml of lyophilised quail egg yolk (LQEY); 7) EK + 5 mg/ml LQEY;) EK + 25mg/ml LQEY; 9) EK + 100mg/ml of lyophilised whey of cow colostrum. In the diluted semen samples (after 15 minutes and six hours storage) sperm morphology (on the basis of nigrosine-eosin histological smears; at 1250x magnification, Nikon Eclipse E100 light microscope), and motility (with Sperm Class Analyzer, version 5.1, Microptic, Barcelona, Spain) was determined. 12 replications were made.

Although, the addition of tested organic substances had positive effect on sperm quality comparing to net semen or sample diluted exclusively with EK extender, none of them prevented an adverse changes after 6 hours of storage. In all extenders the decrease in percentage of total live and live normal sperm and in their motility was observed, but the significance of these decreases was dependent on the particular extender.

The most beneficial effect on sperm morphology after 6 hours storage was found in EK supplemented with 100mg/ml of LQEY (90.1% of total live and 68.0% of live normal sperm) and next, in EK with 200mg/ml of LQEW (87.5% and 68.2%, respectively), while sperm motility was the highest (77.1%) in EK with 50mg/ml of LQEW. ■

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The effect of rapid cooling rate on broiler hatching eggs

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This study investigated the effect of the egg cooling profile of broiler hatching eggs after oviposition on embryonic development and hatchability of fertile eggs. Hatching eggs were obtained from Ross 308 broiler breeders at 28 weeks (young) and 64 weeks (old) of age.

A total of 3,150 eggs that had been laid within a 15 minute period were collected and then randomly assigned to two temperature controlled chambers with either control (360'-480') or rapid (45'-120') cooling to 24 and 18°C EST, respectively. Eggs remained in the chambers until the EST of both cooling groups were similar, then eggs were transported to the hatchery and were stored for six days at 16°C and 75% RH.

Each tray of 150 eggs was considered to be a replicate and there were five replicate trays per cooling profile treatment in each flock age. Some (25 embryos in each batch) of the eggs were opened before and after cooling profile treatment to determine the stage of the blastoderm. The eggs were randomly set in a single commercial incubator. Data from the completely randomised design were subjected to ANOVA using the GLM procedure of SAS. The stage of embryonic development was advanced by control cooling and by the older flock. In younger flock eggs, fertile hatchability was significantly decreased by rapid cooling due to higher early and late embryonic mortality (P≤0.05).

However, early embryonic mortality and percentage of second grade chicks was reduced (P≤0.05) and fertile hatchability was numerically higher by rapid cooling compared to the control in older flock eggs. In conclusion, the data from this study demonstrated that rapid cooling after lay retarded the stage of blastoderm development in eggs from both young and old broiler breeder flocks.

This was apparently detrimental, as indicated by higher early and late embryonic mortality, in the case of the young flock but beneficial in the case of the old flock. The hatchability differences between young and old flock eggs induced by a rapid cooling rate might depend on the differences of embryonic development at oviposition. ■

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Eggshell temperature and leg bone characteristics

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Leg problems leading to a poor walking ability or lameness can negatively affect broiler chicken welfare and performance.

Controlling this issue may be possible by improving leg bone characteristics, using a multi-factorial approach throughout the complete poultry production chain.

One of the predisposing factors may be incubation temperature (Oviedo-Rondón et al., 2009). Recent findings suggest that chicken development can be improved with a high eggshell temperature (EST) of 38.9°C in the second week of incubation or a low EST of 36.7°C in the third week of incubation (Maatjens et al., 2016; Nangsuay et al., 2015).

However, effects of these EST or combinations of these EST on post-hatch performance and, in particular, leg health are unknown. The current study evaluated effects of different EST patterns during incubation on leg bone characteristics of male broiler chickens at slaughter age. Eggs of a Ross 308 broiler breeder flock (44 weeks) were incubated in a 2 × 2 factorial design with a control (37.8°C) or high (38.9°C) EST in the second week of incubation and a control (37.8°C) or low EST (36.7°C) in the third week of incubation.

Results showed that body weight and proximal length of the tibia at slaughter age were not affected by EST treatment (all P>0.10; n=128). Lateral cortex thickness (+3.1%) and proximal bone head thickness on the metatarsal side (+1.3%) was slightly higher in the high compared to the control EST in the second week of incubation (both P=0.04).

The tibial breaking strength was 5.8% higher for the high EST compared to the control EST in the 2nd week of incubation (P<0.001) and 3.7% lower for the low compared to the control EST in the third week of incubation (P=0.02). An interaction was found for proximal bone head thickness on the femoral side; the combination of a high EST in the second week of incubation with a control EST in the third week of incubation gave the highest value (P=0.05). It can be concluded that EST during incubation can affect leg bone characteristics in broiler chickens at slaughter age.

The slightly thicker bone combined with a higher bone strength as a result of a high EST in the second week of incubation might be related to changes in bone ossification during incubation or higher post-hatch activity (Molenaar et al., 2018). The lower bone strength after a low EST in the third week of incubation might be related to a longer incubation duration (+5 hours), resulting in a longer period that the chicken may experience a relatively low body mineral status (Yair et al., 2012). ■

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Incubation temperatures and poult quality

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It has been reported that the temperature is a crucial factor affecting poultry embryo development, hatchability, quality and farm performance. The majority of the studies are made in Gallus gallus domesticus and are extrapolated to Meleagris gallopavo without previous verification. In incubation trials often environmental air temperature is used as treatment applied to the eggs.

Therefore, the purpose of this study was to compare the effect of four different levels of embryo temperature 99.2-99.4°F; 99.4-99.6°F; 99.8-100.2°F; and 100.5-101.0°F (shell temperature) frequently observed in commercial hatcheries from day one to 25, upon hatchability, poult weight and seven days mortality.

Turkey eggs (2,400) from one breeder flock (41 weeks of age) were collected, stored for three days at 60°F, prewarmed for six hours to 70°F, selected for a uniform egg weight (large and small eggs were culled at setting), and set in trays at 75 eggs/tray.

Eggs were divided into four incubators and incubation air temperature was adjusted daily to correspond to four different eggshell temperatures (EST) from 1-25 days of age: 99.2-99.4°F; 99.4-99.6°F; 99.8-100.2°F; and 100.5-101.0°F (all treatments incubated at 53% RH and turned hourly).

Each day, shell temperatures were taken from the same 10 eggs from the centre of the treatment set. If adjustments were made, EST were checked an hour later for accuracy.

After 25 days of incubation, eggs were candled and the developing embryos were transferred into one hatcher with hatch trays stacked in a randomised manner; non-developing eggs were broken and stage of embryonic development determined.

At hatch (28 days and four hours), a sample of poults (25/treatment; five poults randomly chosen/hatch tray) were euthanised and residual yolk sacs were weighed and yolk-free poult weight calculated. Hatch residue was evaluated to determine stage of development.

The total hatch of eggs showed that those incubated at an egg shell temperature (EST) of 99.2-99.4°F and 99.4-99.6°F had a higher hatchability than the eggs incubated at 100.5-101°F at P≤ 0.10 level.

The hatch for fertile eggs incubated at the 99.2-99.4°F and 99.4-99.6°F EST were significantly higher than the 99.8-100.2°F and 100.5-101°F EST treatments at P≤ 0.05. It has been reported in broilers that a high embryo temperature (102.2°F), but not low embryonic temperature (98.06°F) produces a higher embryonic mortality.

The treatment 99.2-99.4°F and 99.4-99.6°F generated poults heavier at pulling time than EST treatment 99.8-100.2°F and 100.5-101°F. The yolk free body mass of the poults under the temperature treatments of 99.2-99.4°F and 99.4-99.6°F was higher than 99.8-100.2°F, but only 99.2-99.4°F was higher than 100.5-101°F poults.

The lower body weights and yolk free body mass found for the high temperature treatment are in agreement with studies made in broilers. It is suggested that the higher temperature accelerates the embryo growth and development so reducing the time for nutrient use plus it causes a lower efficiency of protein utilisation for growth. ■

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