



Modelling broodiness in reproductive turkeys (*Meleagris gallopavo*)

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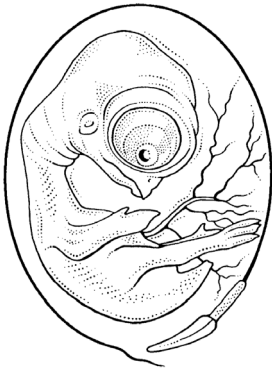
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INCUBATION AND FERTILITY RESEARCH GROUP

{WPSA Working Group 6 (Reproduction)} 2010

Meeting – Tours, France,

23 August 2010



The IFRG meeting 2010 was held in the beautiful city of Tours in France. The topics now reported included the storage and early embryo development; new developments in incubation temperature; and the influence of glucose application to poultry embryos. Two aspects of cryopreservation were considered: primordial germ cells, and *in vivo* cryopreserved cock semen. The latter was tested to assess its feasibility for use in commercial production or preservation of genetic stocks. With regard to commercial poultry production, the data recorded in one study supported the hypothesis that fertility would be higher and would persist for longer in pens where the birds were able to find a physical refuge from other birds. Other factors affecting the production of turkeys, ducks, pheasants, ostriches, chickens and ducks were discussed, thus fulfilling the meeting's objective of reporting research into the incubation and fertility of all avian species.

Keywords: *avian fertility, egg incubation, avian embryonic development, commercial poultry breeder management*

The hen's vagina and its role in sustained fertility

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The hen's vagina serves several functions: (1) conduit during oviposition; (2) site of semen deposition; (3) site of sperm transport, selection, and storage; and (4) mucosal immunity. Over the past decade new information has been presented that addresses the last three functions. While aware of its coiled nature, it was only recently that the anatomy of the vagina and utero-vaginal junction [UVJ: location of the sperm-storage tubules (SSTs)] was exposed. Of significance is that the UVJ-SST, which is clearly located at the anterior end of the vagina in the absence of a uterine egg mass, becomes contiguous with the uterine mucosa when an egg mass is in the uterus. The influence of the uterine environment relative to sperm entry into and egress from the SSTs will be discussed. Generally, <1% of the sperm inseminated reach the SSTs and

this is due to an intense selection process orchestrated by the vagina. While the majority of sperm are dispelled from the vagina within minutes of semen transfer, other factors such as sperm mobility, micro-anatomy of the vaginal mucosal folds, sperm surface characteristics, immunological events, and the female's cryptic-sperm selection function in the sperm selection process. Biologically, sustained fertility in the hen is a complex series of temporal and spacial events that ultimately result in some number of viable sperm in the infundibulum, the site of fertilization, at the time of oviposition. As sperm numbers in the infundibulum begin to wane, either due to low sperm numbers in the SSTs, impaired sperm transport and selection by the vagina, or the inability of the SSTs to store sperm, fertilization rates will fall.

New developments in incubation temperature

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Several scientific papers show that the so-called thermal conditioning of the embryo during the specific phases of embryonic development induces long-term adaptations so that the post-hatch performance is positively influenced. One method of thermal conditioning in a commercial hatchery is the practice of 'Circadian Incubation'. Circadian incubation means that embryos are thermally conditioned by daily short periods of heat or cold shocks. For the first time the results of a thermal conditioning experiment in a large commercial incubator are presented. In a large commercial incubator (capacity 115.200 eggs) Ross-308 eggs from three different flocks and four different flock ages were thermal conditioned for 3 hours per day from day 16.5 until day 18.5 of incubation.

At the end of the thermal conditioning period average egg shell temperatures 39.8–40.1°C (103.6–104.2°F) were measured. After the application of thermal conditioning (circadian incubation) a small, not significant, higher number of first class chicks were collected compared to the control. Feed conversion was positively influenced but the response varied between the flocks. The reasons for the different response of the three flocks are not known but is subject of further research.

This was the first application of circadian incubation, thermal conditioning, in a large commercial incubator: results are promising. In future research, robustness and the capacity of the thermoregulation will be tested. For this chickens will be challenged with heat and cold at critical ages in the farm.

Long-term influence of glucose application during the critical period in chicken embryos on body weight regulation

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During 'critical periods' of neuronal development, perinatal hyperglycaemia leading to elevated leptin concentration may cause a mal-programming of the regulatory centre of body weight and metabolism, especially in the hypothalamus. The basic mechanisms of these mal-programming are still unknown. Central regulation of metabolism and body weight in birds and mammals is similar to a large extent, as with the *Nucleus accumbens* in mammals, the *Nucleus infundibularis* (NI) is the primary regulatory centre in birds. However, in birds no information on sensitivity of NI-neurons to changes in glucose concentration is available.

The aim of this project is to investigate the influence of different glucose concentrations during the 'critical period' of prenatal development on the postnatal glucose sensitivity of NI neurons in birds. In chicken embryos between

days 14 and 17, solutions with high glucose concentrations (above the physiological mean level of 10 mmol L⁻¹) were injected via catheters into the blood vessels of the chorioallantoic membrane. Such high glucose concentrations may induce mal-programming of the feed intake and metabolism regulating system. It might lead to changes in glucose sensitivity of NI-neurons. Because in birds no information on glucose sensitivity of NI-neurons is available, the first part of the project is focused on investigations of sensitivity of NI-neurons on altered glucose concentrations in chickens, which were incubated without prenatal glucose applications (control group). The second part of the project is concentrated on chickens, which were exposed to prenatal hyperglycaemia or which were NaCl injected (injection control). Based on these results, it will be possible to draw conclusions on the impact of prenatal hyperglycaemia

on mal-programming of metabolism, feed intake and body weight.

The experiments on investigation of neuronal glucose sensitivity were carried out in brain slices (400 μm) of 20- to 30-days-old chickens of both sexes. Using extracellular recording neuronal activity of NI-neurons was measured under superfusion with artificial cerebrospinal fluid (ACSF), which contained glucose concentration of 1, 5 or 30 mmol L^{-1} , with 11 mmol L^{-1} as control (identical with the usually used ACSF).

Results of the first part of the project are presented as follows. In the control group, a total of 115 NI-neurons were determined. Under glucose concentration of 1 mmol L^{-1} , 18 were glucose sensitive ($n = 33$), under 5 mmol L^{-1} , 28 ($n = 53$) and 30 mmol L^{-1} , 21 ($n = 29$), respectively. Similar to mammals (Fioramonti *et al.*, 2007), 'high

glucose sensitive neurons', which increased or decreased the neuronal activity if glucose concentration was increasing from the control level and 'low glucose sensitive neurons', which increased or decreased the neuronal activity if glucose concentration was decreasing from the control level up to 1 mmol L^{-1} were found. Further experiments in chickens incubated under the influence of prenatal hyperglycaemia and NaCl applications are scheduled.

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Embryonic development and weight changes in ostrich egg components during incubation

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An important tool for identifying incubation problems that cause low hatchability is knowledge of the age and degree of development of an embryo at the time of death (Ar and Gefen, 1998). In this study, we describe the development of the ostrich embryo and changes in weight of the egg components from day 8 to day 42 of incubation. Eighty-four ostrich eggs were weighed and incubated at 36°C and 24% RH in an electronic incubator. Two eggs were weighed daily to determine weight changes associated with water loss, opened, the embryo and albumen weighed and the stage of embryonic development noted. In addition, between 12 and 14 randomly chosen eggs were candled daily with a 100 watt candling light, and photographed. A total of 486 images from the photographs were digitalized and assessed using the software package AnalySIS[®]. The percentage of the egg volume occupied by the air cell was determined from the images.

Only 65 of the eggs opened (77.4%) showed evidence of embryonic development and were used further. The eyes became visible on day eight (Table 1). The beak was

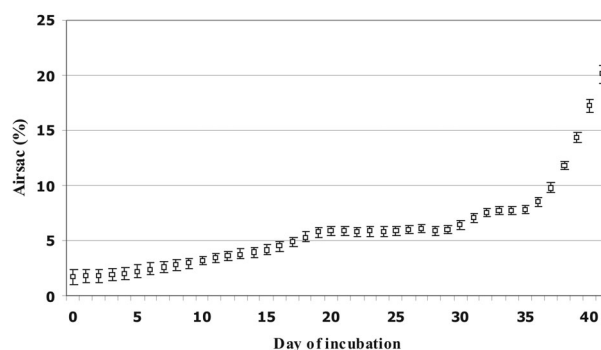


Figure 1 The percentage of the egg volume occupied by the air sac in ostrich eggs during the 42-day incubation period, as modelled by a cubic spline.

Table 1 Development stages of ostrich embryos

Day 8	Limbs were clearly discernable, the eyes became visible
Day 10	Growth in length of the limbs could be observed
Day 12	The beak was clearly discernable
Day 14	The blood vessel network and the amniotic sack enclosing the embryo covered the upper surface of the yolk. All the body organs were present at this stage; mean weight (\pm SD)=2.59 g or 0.174% of initial egg weight ($n = 1$)
Day 18	Feather buds could be seen
Day 20	Rudimentary feathers could be discerned
Day 21	Embryo weight = 17.9 ± 2.2 g or $1.29 \pm 0.24\%$ of initial egg weight ($n = 3$)
Day 24	Plumules were present. The beak began to harden
Day 28	The embryo turned with its spine parallel to the long axis of the egg; embryo weight = 142 g or 10.8% of initial egg weight ($n = 1$)
Day 35	The embryo bent its neck to the right over the right foot, with its beak pointing towards the air cell, to attain the correct pipping position; embryo weight = 382 ± 19 g or $27.3 \pm 2.4\%$ of initial egg weight ($n = 3$)
Day 38	All the albumen was completely incorporated and the remaining yolk began to be absorbed into the body cavity through the navel
Day 40	All yolk was absorbed into the body cavity through the navel; embryo weight = 923 ± 41 g or $64.5 \pm 3.5\%$ of initial egg weight ($n = 2$)
Day 42	Hatching

discernable after day 12 and feather buds could be seen by day 18 of incubation. Nails appeared on the toes of the embryo on day 28 and from day 35 of incubation the embryo turns to reach the correct pipping position for day 42. Evaporative water loss resulted in a reduction in egg weight of 0.31% per day and totalled 13% of initial egg mass over the incubation period. Shell weight remained relatively constant at approximately 18% of initial egg weight as did the weight of the eggshell membranes at approximately 1% of initial egg weight. Embryo weight increased exponentially from 29% to 79% of initial egg weight during incubation, with most growth, taking place in the last week of incubation. Albumen weight decreased linearly from approximately 50% of initial egg weight at a rate 1.4% per day and was completely incorporated by day 35 of incubation. The percentage of the egg occupied by the air sac changed from 1.76% for fresh eggs to 20.1% for eggs incubated for 40 days. These data allows the estimation of the time of death of embryos and may help identifying particularly sensitive periods.

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Modelling broodiness in reproductive turkeys (*Meleagris gallopavo*)

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Turkey (*Meleagris gallopavo*) is the second most popular poultry species in Europe with about 15% of this market. This position is, in part, the consequence of moderate production costs originating from rapid growth rates and effective feed efficiency. Unfortunately, selection on growth traits may impair reproductive performances including fertility and laying rates. In fact, one of the major problems is the decrease in egg production which is mainly due to broodiness, an incubation behaviour that is still present in modern turkey breeder strains (up to 20% to 30% of breeder hens). Broodiness is generally expressed during the first weeks of the reproductive season. It is generally characterized by a marked reduction up to a total arrest of feed consumption during the days preceding broodiness.

One of the objectives of our study was to collect field information from a relatively high population (>7,000) of turkey breeder hens in order to possibly anticipate or, at least limit broodiness and therefore facilitate flock management. Birds used were from two commercial strains, one defined as “heavy” (Big6, Aviagen-BUT, Cheshire, UK, $n = 3,360$ females) while the other is considered as a “medium” type (BUT9, Aviagen-BUT, 4,200 females). Each strain was housed within two barns divided into 42 laying pens. Each broody female was individually identified and referenced by a coloured wing tag. The incidence and pattern of incubation behaviour along with the delay to return in lay were recorded throughout the period of reproduction.

The results of this study indicate the possibility to develop a model of broodiness. A higher percentage of incubation behaviour was observed in the Big6 hens compared to the BUT9 (16% *versus* 12%). In both strains, the average duration of stay in broody pens increased with hen's age. Hens expressing incubation behaviour after 18–20 weeks of lay tended not to

return to egg production. Among females having experienced broodiness, those having experienced it twice stayed longer in broody pens than those having experienced it once. Among hens getting broody once, 17–19% (BUT9) or 23% (Big6) went back to broodiness a second time and among the latter ones, 15–22% (BUT9) or 20–26% (Big6) went back to broodiness a third time.

Preliminary investigation on fertility and hatchability by the use of cryopreserved cock semen

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In poultry, semen cryopreservation technique is rarely used in breeding practice due to low fertility rates, which are far from those obtained in domestic mammalian species. Besides, the overall fertility rates with frozen/thawed poultry semen are highly variable because of the large variety of bird species and the lack of suitable protocols for each of them. Therefore, at present time the use of frozen/thawed semen is not reliable enough for use in commercial production or preservation of genetic stocks. As a contribution to overcome these differences in the present study we tested *in vivo* cryopreserved cock semen.

Meat-type male breeders (Cobb 700) at 49 weeks of age were bred in single cages and fed on a commercial breeder diet. Semen collected by dorso-abdominal massage technique, was pooled and processed using a freezing procedure in pellets, as previously used in pheasants (Marzoni *et al.*, 2009) with the following changes: dilution 1:2 (sperm:diluent, v/v) in pre-freezing Lake's diluent (Lake *et al.*, 1981) added with 50 mM Glycine, drop volume 100 μ L. At thawing, one pellet at a time was melted into a glass tube heated in a water bath at 60°C. Sperm concentration by optic microscopy using Burker

chambers was assessed on fresh diluted semen. Sperm viability and morphology by nigrosin/eosin staining procedure and mobility by Accuden[®] procedure were evaluated on fresh and thawed semen. The fertilizing ability of cryopreserved spermatozoa was tested *in vivo* by six consecutive artificial inseminations at 4-day intervals, performed on five 58 weeks old Isa-Warren hens (one pellets/hen). Egg fertility was determined by candling eggs on day 7 of incubation. Eggs considered unfertile were broken up for macroscopic examination of the germinal area.

Traits of fresh semen and thawed pellets are reported in Table 1. Cryopreservation produced a considerable worsening of semen quality, nevertheless, spermatozoa viability values are comparable to results observed by other authors. Morphologically normal cells are affected by the frozen/thawed procedure, even though less evident than the viability. These data show that each inseminating dose (one pellet) provides approximately 155 million spermatozoa/female containing 42 million of live and normal spermatozoa.

The fertility was observed in the eggs of hens when they were at 83% egg production. The semen cryopreservation procedure resulted in 33% fertility, a decrease of approximately 70%, which even though high, can be considered acceptable in accordance to the species. In fact, other authors report a fertility of 30.4% using the same cryoprotectant and 600 million spermatozoa doses. Additionally, it is important to mention that the hens used in this study, were not the same genetic strain as the males. As demonstrated by other authors this fact

Table 1 Semen traits of the meat type male breeders used for semen cryopreservation

		Fresh semen mean \pm SD	Thawed pellet mean \pm SD
Semen pH		7.11	–
Sperm concentration	10 ⁹ /mL	4.65	–
Viable spermatozoa	(%)	82.3 \pm 5.9	36.2 \pm 5.8
Morphologically normal spermatozoa	(%)	92.9 \pm 4.0	75.2 \pm 8.4
Mobility	(A _{550nm})	0.250 \pm 0.001	0.075 \pm 0.004

can affect performance, actually matings between homologous or heterologous strains, can improve or worsen fertility rates (Blesbois *et al.*, 2007).

The most important parameter to be considered at the end of the freezing procedure is the hatchability, which indicates the real success of the whole process. In this study, more than a half of fertile eggs hatched. Few studies have investigated this target; nevertheless, we believe this value to be a good result, which encourages us to continue further studies.

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Increased spatial complexity of breeding pens affects fertility of commercial pheasants

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Commercially reared pheasants (*Phasianus colchicus*) are typically kept in breeding pens that are open and lack any cover for the birds to find refuge from other birds (Deeming, 2009). As a result in larger group sizes there is scope for aggressive behaviour between males trying to establish territories or trying to court females. This environment is likely to be highly stressful for the birds and will impact upon their welfare compromising their survival and potentially their reproductive productivity. Despite such conditions there has been very little research into the welfare of breeding pheasants in a commercial environment.

This study examined the effects of sight barriers within pens of breeding pheasants on a game farm in the UK over the 10-week laying season in the spring of 2010. Other studies by us (Deeming *et al.*, 2010) running concurrently with the study described here showed that although bird mortality was unaffected by the barriers the plumage scores (a sign of welfare) of males and females were improved. In addition, there were changes in the behaviour time-budgets of the birds and in the levels of observed aggression. The barriers appeared to be having some benefits to pheasant welfare and it was also suggested that they would provide refuges for courting pheasants leading to more efficient copulation. It was, therefore, predicted that fertility would be higher and would persist for longer in those pens with barriers compared with pens where barriers were absent.

There were 11 pens (measuring 13.2 × 13.2 m) with barriers in an X-shape made from metal sheets and

straw bales (Figure 1), and 11 control pens that were left open as per the typical layout on the farm. At the start of the 10-week breeding season each pen had 56 hens and eight cocks (7:1 sex ratio), which were provided with pelleted feed and water *ad libitum*. Reproductive performance was recorded on a weekly basis as egg production, numbers of eggs rejected for hatching together with measures of fertility, embryonic mortality and hatchability expressed as a percentage of eggs set to incubate. Repeated measures analysis of variance (ANOVA) was used to assess the effect of the barriers on the various measures of reproductive performance.



Figure 1 Photograph of a pheasant pen with barriers. The straw bales and metal sheet were absent from the open pens. The metal half oil drums served as nest sites.

Egg production per pen and the numbers of rejected eggs exhibited typical patterns for pheasants but were not significantly ($P > 0.05$) affected by the presence of the barriers. By contrast, fertility expressed as a percentage of eggs set was significantly higher and persisted for longer in the barrier pens ($P < 0.05$). At week 3 the barrier pens had 1% better fertility but even though fertility in both pen types declined with time by week 10, fertility was 4% higher in the barrier pens. Patterns of embryonic mortality were unaffected by the presence of the barriers ($P > 0.05$) but percentage hatchability was significantly ($P < 0.05$) 3% lower in the open pens.

The data recorded in this study supported the original hypothesis that fertility would be higher and would persist for longer in pens where the birds were able to find a physical refuge from other birds. The results are comparable to a study where panels placed in broiler breeder houses increased egg production, fertility and hatchability (Leone and Estévez, 2008). Whether these effects were

due to increased rates of mating or improvements in the efficacy of copulation, remains unclear and is worthy of further research.

Establishing sight barriers in breeder pens for commercial pheasants would appear not only to offer improvements in bird welfare but also significant commercial advantages. Increases in hatchability associated with higher fertility were not only statistically significant but were commercially very important.

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Influence of short-term warm stimulation during the last 4 days of incubation time on body temperature and oxygen consumption in broiler and layer embryos

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In our previous study, we showed that short-term warm stimulation during the last 4 days of incubation might improve the post-hatching adaptability and performance in broiler chicks (Tzschentke and Halle, 2009). Hatchability, for instance, was increased by more than 1.5% and a significantly higher percentage of hatched male chicks were observed. Further, as a long-lasting consequence of the prenatal temperature training, final body weight at slaughter was increased, especially in the male broilers, and feed conversion was significantly lower in male and female broilers in comparison to the normal incubated control group. In relation to these investigations we were interested in the influence of prenatal short-term warm stimulation on the development of embryonic physiological mechanisms. The aim of this study was to investigate the effect of short-term warm stimulation during the final incubation period on the development of body temperature and metabolism in broiler and layer embryos.

The investigations were carried out using 20 embryos of a layer line (Lohmann White Leghorn) and 22 embryos of a high yield broiler line (Cobb). All eggs were incu-

bated under normal temperature conditions (37.5°C) prior to treatment. In one group of each line short-term warm stimulation (38.5°C, 2 h per day) was applied during the last 4 days of incubation (day 18 (layer) and day 17 (broiler) until hatching). The second group was incubated continuously at 37.5°C (control group). Single chicken eggs were placed in metabolic chambers and embryonic temperature (temperature of the chorioallantoic fluid, Taf), oxygen consumption (for some embryos) and ambient (incubation) temperature were continuously measured.

In the control groups, Taf and oxygen consumption increased with increasing age. Taf of the Cobb embryos reached significantly higher levels than the Taf of the layer line, which is in agreement with our previous investigations. During short-term warm stimulation Taf of embryos of both lines increased significantly in comparison with Taf before and after the treatment. Interestingly, in both lines after short-term warm stimulation (when Taf again reached a constant level under normal incubation temperature), Taf was significantly lower than in the control group, especially during the last day of incubation.

Oxygen consumption was decreased by short-term warm stimulation. In a previous study on embryos of a layer line and the Muscovy duck, a short-term increase in incubation temperature during the last days of incubation was also observed to decrease in oxygen consumption (Janke *et al.*, 2002). The depression in metabolism might be an effective heat defense mechanism in poultry embryos, which could be trained by short-term warm stimulation and may result in long-lasting effects on regulation of body temperature and metabolism.

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Changes in vitelline membrane strength during the storage of eggs from two broiler lines

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The long storage of hatching eggs results in a decline in hatchability and the extent that storage affects hatch can vary between genetic lines. This paper will describe a study using two broiler lines that differ in their response to long egg storage; Line A shows a much faster decline in hatch with storage than Line B. During the storage process, various changes have been observed to take place within the egg and within the embryo, including thinning of the albumen, increase in albumen pH, movement of water from the albumen to yolk, weakening of the vitelline membrane and the death of embryo cells. The intention of this study was to look at one of these factors, the change in vitelline membrane strength (VMS) during storage, to see if it could account for the different response to long storage between the two broiler breeds.

Two trials were undertaken, both using Line A and Line B eggs. In trial 1, eggs were obtained from breeder flocks at 34 weeks of age and the eggs were stored for 3, 9 and 23 days before VMS was measured. In trial 2, eggs were obtained from breeder flocks 33–34 and 53 weeks of age and the eggs stored for 3, 7 and 14 days before VMS was measured. VMS was measured using a TA-XT2i texture analyzer (Texture Technologies Corp., Scarsdale, NY) with 1 mm rounded end stainless steel probe and 5 kg load cell (calibrated using a 2 kg weight), 0.1 gram trigger force, and 3.2 mm s⁻¹ test speed. All eggs were stored at 15.5–18.3°C without humidity control. In trial 2, the hatchability was also measured on eggs stored for 3 and 14 days from both lines and flock ages.

In both trials, VMS declined significantly with egg storage. In trial 1, there was a significant interaction between broiler breed and length of storage. Line A eggs showed a significant decline in VMS between 3 and 9 days of storage but not between 9 and 23 days whereas Line B eggs only showed a significant decline in VMS between 9 and 23 days of storage. In the second trial there were no significant interactions between broiler breed, flock age or length of storage. VMS was significantly lower in Line B than Line A eggs and in eggs from younger breeder flocks than older breeder flocks.

The hatch loss between 3 and 14 days of storage did not differ between the two broiler lines from the young breeder flocks but was significantly greater in Line A than Line B (24% versus 11%) when eggs were obtained from the older breeder flock. However, the hatch changes observed between Lines A and B did not correspond to the changes observed in VMS.

While the eggs were being opened to measure VMS, some yolks would rupture immediately suggesting these membranes were very weak. In trial 1, the incidence of ruptured yolks increased with storage and was higher in Line A eggs. However, similar trends were not observed in trial 2.

The two trials did show that VMS declined with egg storage and this may be associated with the loss in hatch with prolonged storage. However, the evidence from the two trials did not give conclusive support to the hypothesis that Line A eggs were less able than Line B eggs to withstand long storage due to weaker VMS.

Hatchability of turkey poults and the impact of early feeding on post-hatch survival and performance are affected by egg size and stage of lay

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Eggs from mid-lay turkey flocks have been shown to have lower embryonic mortality, higher yield and better liveability results compared to early and late-lay flocks. Many factors influence this overall effect, including egg size and the early provision of food for the newly hatched poults. In Phase 1 of the present study, 3,456 eggs from a Hybrid flock at three weeks of lay (WOL) were randomly selected and divided into two groups based on weight—small (<80 g) and large (>80 g). Eggs were set in a Robbins H-10 incubator for the incubation period and weighed at the time of traying, day 7, day 14, transfer and take-off. Hatched poults from small and large egg groups were randomly divided into two subgroups, fed and unfed and each of these subgroups further divided into three replicate groups placed in respective brooding rings at placement. Mortality and weight gain at 7 days post-hatch were recorded. Fed groups received Novus Oasis hatching supplement

(100 g/100 poults) after take-off while unfed groups received no supplement. The overall process was then repeated using mid-lay eggs from the same flock at WOL 19. In Phase 2, the entire protocol was repeated with early (WOL 3) and mid-lay (WOL 19) eggs from a second flock.

Results indicated that egg size was a more important factor in performance in early-lay than mid-lay poults. Hatchability results were better for eggs from the mid-lay period but applying a pro-active incubation profile can improve the performance of poults from early-lay eggs, including results for their early post-hatch liveability. Early access to a feed supplement had beneficial effects in terms of both liveability and 7 day post-hatch weight gain, though this was significantly influenced by hatchling size. Poults from small, early-lay eggs showed the greatest 7 day weight gain when expressed as a percentage of hatching weight.

The rate of egg fertilization in the Pekin duck is higher in between- than within-strain matings

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We investigated the relationship between the numbers of sperm holes made in the inner perivitelline layer and sperm trapped in the outer perivitelline layer of the vitelline membrane (Wishart, 1987; Bramwell *et al.*, 1995) in the Pekin duck. The group of commercial Pekin ducks ("Luv-a-duck" Victoria, Australia) consisted of the high and low body weight strains. In the High Body Weight (HBW) strain, the males ($n = 3$) weighed 5.1 ± 0.5 kg (Mean \pm SD) while the females ($n = 3$) weighed 5.0 ± 0.3 kg. In the low body weight (LBW) strain, the males ($n = 3$) weighed 3.3 ± 0.6 kg and the females ($n = 3$) 3.2 ± 0.2 kg. Every male was mated with every

female in the following male \times female combinations: LBW \times LBW, LBW \times HBW, HBW \times LBW, HBW \times HBW (three pairings per combination) over a period of 7 and 14 days. Pairs were maintained individually in the outdoor enclosures (The University of Western Australia, Native Animal Facility, Shenton Park).

The sperm and sperm holes were counted in the perivitelline membrane collected from above the germinal disc of non-incubated eggs ($n = 159$) stored for up to 3 days. After collecting the membrane on a filter ring and cleaning with PBS, the membrane was placed on a glass slide, stained with DAPI (Wishart, 1987) and then with



Figure 1 A newly hatched Pekin duck. Photo courtesy of Jumpingmaniac.

Schiff's reagent (Bramwell *et al.*, 1995) and allowed to dry overnight on the lab bench at 18–20°C (room temperature) and 40–50% relative humidity as per processing protocol of the emu membrane (Malecki and Martin, 2005). The holes were viewed using standard light microscopy whereas the sperm using fluorescence, and counted in four successive fields of view under 20X objective starting in the centre of the GD. The hole to sperm ratio data were subjected to a single factor (mating combination) analysis of variance (ANOVA) with Fisher's LSD for the means comparison test (SuperAnova, Abacus Concepts, Berkeley CA, USA).

The hole to sperm ratio depended on mating combination ($P < 0.01$). The mean (\pm SEM) hole to sperm ratio in the LBW \times LBW combination was similar ($P < 0.05$) to HBW \times HBW combination (1.22 ± 0.09 versus 1.15 ± 0.07 respectively). This relationship was also similar in the LBW \times HBW and HBW \times LBW combinations (1.72 ± 0.16 versus 1.53 ± 0.10 respectively). However, the within-line matings had lower ($P < 0.05$) hole to sperm ratio than the between-line matings. We conclude that because more sperm were gaining access to the egg cytoplasm in between rather than within-line matings, the inter line matings had higher fertilization rate than the intra line matings.

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Glucose oxidation in chicken embryos incubated at a normal or high eggshell temperature—preliminary results of a tracer study

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In practice, eggshell temperature (EST) often increases at the end of incubation due to heat production of the developing embryos and a poor cooling capacity or air velocity in the incubator. High ($\geq 38.9^\circ\text{C}$) compared to normal (37.8°C) EST negatively affects survival and hatchling development (Lourens *et al.*, 2005), and this may be related to changes in nutrient utilization or more specific changes in glucose metabolism. This study was performed to measure *in ovo* glucose metabolism using [$\text{U-}^{13}\text{C}$]glucose at a normal or high EST.

Two studies were conducted to develop the technique. Broiler eggs incubated for 13 days were used (Ross flock 59

weeks; $n = 300$). Eggs were divided between two identical open-circuit climate respiration chambers (CRC) and incubated at a normal (37.8°C) or high (38.9°C) EST. In study I, a solution containing [$\text{U-}^{13}\text{C}$]glucose (1.05 mg in 250 μL sterile water) was injected as a single bolus in the allantoic fluid at day 14.5 of incubation. In study II, a solution containing [$\text{U-}^{13}\text{C}$]glucose (0.73 mg in 250 μL sterile water) was repeatedly injected as a daily bolus in the chorio-allantoic fluid for 4 consecutive days from day 14.5 of incubation onward. From day 14 until 19 of incubation, the $^{12}\text{CO}_2$ and $^{13}\text{CO}_2$ production were measured every six minutes. ^{13}C enrichment in CO_2 was

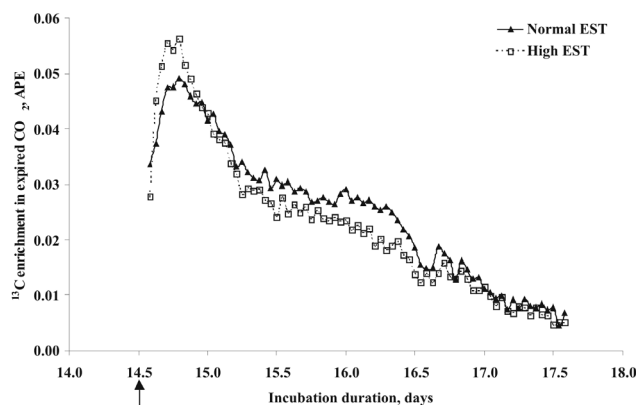


Figure 1 Study I: ^{13}C enrichment in expired CO_2 after injecting a solution of $[\text{U}-^{13}\text{C}]$ glucose in the chorio-allantoic fluid at day 14.5 of incubation of embryos incubated at a normal (37.8°C) or high (38.9°C) eggshell temperature (EST) from day 13 of incubation onward ($n = 1$). The arrow on the X-axis indicates the time of injection.

corrected for the natural background of ^{13}C enrichment during the two days prior to the $[\text{U}-^{13}\text{C}]$ glucose injection for each treatment. ^{13}C enrichment was expressed as atom percentage excess (APE). ^{13}C enrichment in expired CO_2 in both studies was calculated per hour and not analyzed statistically, because the studies were not repeated.

In study I, ^{13}C enrichment in expired CO_2 showed a peak in both EST treatments within 8 h after $[\text{U}-^{13}\text{C}]$ glucose injection (Figure 1). This indicates that the injected glucose was partially oxidized and that this technique can be used to measure glucose oxidation in chicken embryos. In study II, the same pattern of ^{13}C enrichment was observed as in study I (Figure 2). However, with each subsequent injection ^{13}C enrichment started higher and declined faster (i.e. narrower oxidation peak). This indicates that glucose oxidation increased during embryonic development and supports findings that glycolytic activity in muscle tissue and liver increase during incubation (Pearce, 1977). Such increased oxidation may suggest higher glucose requirements in the

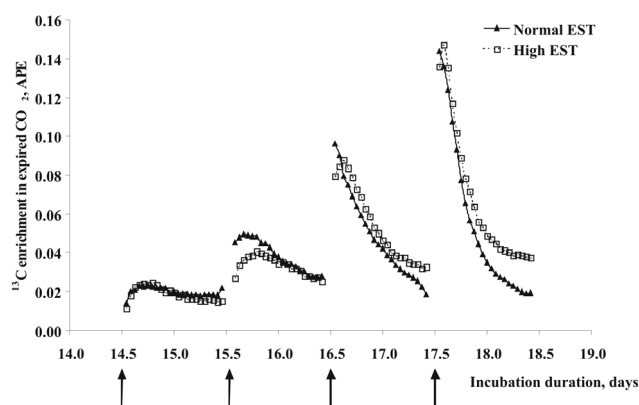


Figure 2 Study II: ^{13}C enrichment in expired CO_2 , after injecting for 4 consecutive days, from day 14.5 of incubation onward, a solution of $[\text{U}-^{13}\text{C}]$ glucose in the chorio-allantoic fluid of embryos incubated at a normal (37.8°C) or high (38.9°C) eggshell temperature (EST) from day 13 of incubation onward ($n = 1$). The arrows on the X-axis indicate the times of injection.

developing chicken embryo towards the end of incubation. Because differences in ^{13}C enrichment between EST treatments tended to increase during incubation, we started a larger study to evaluate the effect of EST on glucose oxidation. During that experiment, ^{13}C enrichment in expired CO_2 , plasma glucose, and hepatic glycogen was measured. Furthermore, yolk free body mass, residual yolk weight, plasma glucose and plasma lactate concentrations, and hepatic glycogen concentrations were evaluated. Results of this larger study are currently processed.

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Incubation temperature and leg health in broilers

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Leg problems are a primary welfare and production concern for poultry production world-wide. Until recently, all interventions to reduce prevalence of leg abnormalities and lameness have been conducted at farm level during

the grow-out. Very few have been pursued at the hatchery or breeder level where problems may actually start. Poultry bones initiate their ossification processes during embryonic development. The incubation temperature

conditions during the plateau stage of oxygen uptake (last 4 days) are known to affect yolk utilization, intestinal maturation, thyroid-IGF1-GH hormonal axis, heart, and muscle development. Several factors that control endochondral ossification of long bones can be also affected by incubation conditions, mainly during the last days of incubation when bones have their fastest elongation rate. If yolk utilization is reduced, the embryo will not metabolize the lipids, trace minerals, and vitamins involved in bone modeling and remodeling when they are needed the most. It has been shown that small differences in incubation temperature applied throughout incubation influence growth and development of long bones and prevalence of disorders such as tibial dyschondroplasia in broilers.

Several experiments at NC State University have lead us to the conclusion that incubation conditions such as elevated temperatures and hypoxia during the plateau stage of incubation affect avian long bone development. Suboptimal incubation conditions have a greater impact on embryo development of broiler strains with low eggshell conductance. Under commercial incubation conditions, hot spots, low ventilation, and reduced airflow are linked.

We have demonstrated that high eggshell temperatures (40°C) and hypoxia (17% O₂) during the last phase of incubation cause down-regulation of the gene expression of matrix metalloproteinases Mmp13 and Mmp9 in the proximal tibia growth plate at hatch. The gene expression of collagen type X and vascular endothelial growth factor (VEGF) receptor 1 and 2 is also down-regulated in these conditions, but only in strains of chickens exhibiting low eggshell conductance. Additionally, the immunofluorescence of the proteins collagen type X as well as the

transforming growth factor β is reduced. All these proteins are essential for the formation of the organic matrix of bone and the vascularization needed prior to ossification. This may explain why high temperatures and hypoxia alter chondrocyte density in the growth plate, reduce tibia, femur and shank weights, and length; and consequently decrease bone mass (Oviedo-Rondón *et al.*, 2008). In all our experiments, we have observed that pre-hatch stress impairs embryonic development and increases relative asymmetry. Higher asymmetry of bone traits has also been linked to locomotion problems, tonic immobility, tibial dyschondroplasia, and worsening of gait scores.

Bone weight, length, thickness, and strength of broilers at processing age can also be affected by incubation conditions. Recently, our group also observed differences in tendon development caused mainly by incubation conditions independent of broiler strain or breeder nutrition. The effects of incubation on leg health have also been observed in six and eight week-old broiler flocks during several other experiments under both University and commercial conditions (Oviedo-Rondón *et al.*, 2009).

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Effects of mono(2-ethylhexyl) phthalate (MEHP) on chicken seminiferous tubule cultured *in vitro*

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For several decades, chemical compounds have been used and released into the wild without concern for the effect on wildlife. Recently, it has been estimated that 10% of species of wild birds will be endangered in the next hundred years. The causes of this decline are multiple: destruction or alteration of habitat, overhunting, climate change and contact with toxic substances. The effects of these substances may not be lethal but induce a decrease in the reproductive functions, thus reducing the

number of animals in future generations. The toxicity of a chemical substance is assessed by *in vivo* tests on multiple species models, but new European laws suggest the establishment of *in vitro* alternative methods and a reduction of animal testing.

Toxicological tests using *in vitro* strategy is well established with rodents and human tissue, but not clearly with wild species such as birds. Hence, we have established the *in vitro* culture of germ cells and

somatic cells from chicken testis and we have evaluated the sensitivity to phthalates (mono-(2-ethylhexyl) phthalate, MEHP) in comparison to previous studies using rodent and human system. Consequences of MEHP stimulation (1 μ M to 100 μ M) was assessed on Germ and Sertoli cells apoptosis, Sertoli cell proliferation, and interaction.

After 96 hours of MEHP incubation, Sertoli cells present a disruption of the cell layer at the 10 μ M concentration as observed after phalloidine staining and the percentage of germ cells was decreased as assayed by FACS analysis with 1 μ M of MEHP. However, it is only at 100 μ M MEHP concentration that elevated caspase-3 activity in cell culture was detected suggesting activation of apoptosis mechanism. Moreover, at 10 μ M MEHP,

proliferation of Sertoli cells and number of AMH-positive cells were reduced. AMH is a marker of Sertoli cells. We can note that *in vivo*, Sertoli cells provide support and nutrition of germ cells and play a key role for spermatogenesis.

In conclusion, these results suggest that the level of sensitivity of chicken testicular cells cultures would be close to those of rat model (10 μ M, Chauvigne *et al.*, 2009), with more parameters affected on germ cells and Sertoli cells than in rodent cells culture and better sensitive than human model (100 μ M, Lambrot *et al.*, 2009). Finally, the validation of the avian model with MEHP suggest its complementary use to the mammals' model to evaluate toxic substances in the respect of the new European laws.

The effect of hypercapnic conditions during early incubation of long-stored eggs

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Egg storage beyond 7 days has a negative effect on hatchability and chick quality. These negative effects may be caused by a sub-optimal level of albumen pH during early incubation. Gillespie and McHanwell (1987) measured an intra-embryo pH of 7.9–8.4 between 26 and 53 hours of incubation. The albumen pH is around 9.0 within the first day of incubation and declines slowly after approximately 2 days of incubation. During early incubation, the embryo, therefore, has to maintain a barrier between the inside of the embryo (pH 7.9–8.4) and its exterior (yolk pH around 6.3 and albumen pH around 9.0). A long-stored embryo may be unable to maintain this barrier due to the negative effects of prolonged egg storage on embryo viability. A high CO₂ concentration in the incubator can reduce albumen pH and this may have a positive effect on embryonic development and hatchability. In the current study, it was investigated whether hypercapnic conditions during the first 5 days of incubation affected albumen pH, embryonic development, and embryonic mortality when eggs were stored for 15 days.

Eggs ($n = 2,560$) were collected from a Ross broiler breeder flock of 36 weeks of age and stored for 15 days. Eggs were incubated in two separate setters (HatchTech-

4,800) for the first 5 days of incubation. In one setter, CO₂ was injected to maintain CO₂ concentrations between 0.70% and 0.80%. In the other setter, CO₂ increased from 0.05% to 0.20%, due to CO₂ production of the embryos. In both setters, relative humidity was maintained between 65% and 75%. Average eggshell temperature was maintained at 37.8°C. On day 6 of incubation, all eggs were transferred to one setter (Hatch-Tech-57,600). At 18, 42, 66, and 90 hours of incubation, 20 eggs per treatment were used to determine albumen pH and the stage of embryonic development. On day 6 of incubation, clear eggs were removed. After 520 hours of incubation, all unhatched eggs were collected. Clear eggs and unhatched eggs were opened to determine infertility or the stage of embryonic mortality.

Results showed that between 18 and 90 hours of incubation, albumen pH decreased in both treatments and the CO₂ treatment had a lower albumen pH than the control treatment. The stage of embryonic development was not affected by the CO₂ treatment. The CO₂ treatment had a lower hatchability of fertile eggs than the control treatment (87.9% versus 91.0%; $P = 0.02$). This lower hatchability was caused by a higher embryonic

mortality on day 3 of incubation (3.5% *versus* 2.2%; $P = 0.08$), between days 4 and 9 of incubation (2.7% *versus* 1.6%; $P = 0.13$), and on day 20 of incubation (0.9% *versus* 0.3%; $P = 0.01$). In addition, more unhatched embryos were malpositioned in the CO₂ treatment than in the control treatment (1.2% *versus* 0.4%; $P = 0.05$).

The hypercapnic conditions used in the current study had no affect on embryonic development and increased

embryonic mortality. It seems that an albumen pH of 9.0 is not sub-optimal for embryonic development in long-stored eggs.

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Hatching synchronization in birds

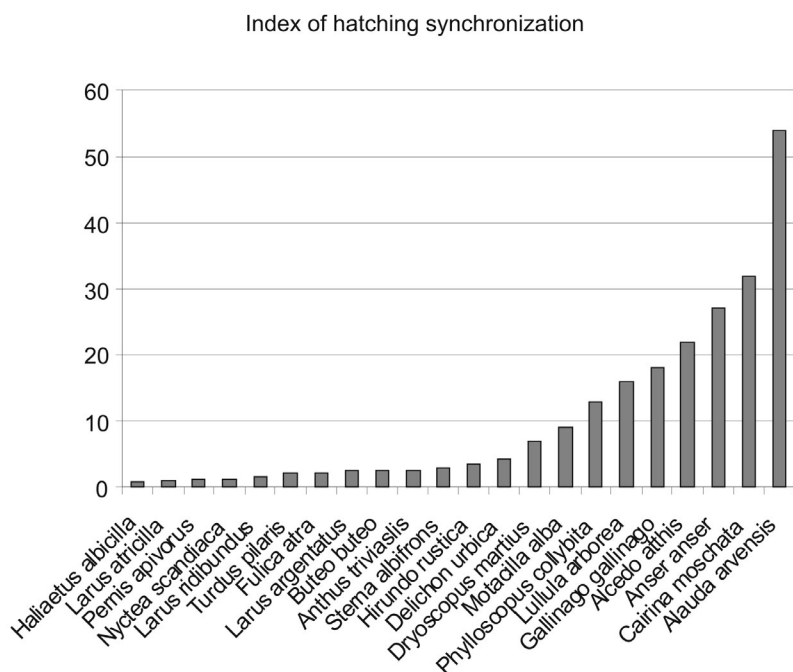
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The index of synchronization (egg laying interval/hatching interval) can be used to describe the ability of a bird species to synchronize hatching, *i.e.*, to narrow the time span of all young hatching (Rumpf, 1992). The index was calculated for 86 bird species. Examples are presented in Figure 1. Comparing bird species we found out that the classification into altricial, semialtricial and precocial species has nothing to do with their ability to synchronize hatching.

Low indices indicate bird species with low ability to synchronize hatching and higher indices indicate species, which are able to synchronize hatching very well. The mechanisms of hatching synchronization are discussed. The ability to synchronize hatching depends on the breeding strategy during the laying period and on the possibility of using clicking sound communication (Rumpf and Tzschentke, 2010).



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Figure 1 Index of hatching synchronization (laying interval/hatching interval), modified from Rumpf (1992).

Antioxidants and hatchability

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A great body of evidence indicates that avian maternal nutrition is the major determinant of the health and development of the progeny. The egg composition is designed in such a way that all nutrients necessary for the development of the future embryo are accumulated within the egg yolk and white. However, an optimal egg composition for the maximum hatchability is not known yet. Among different nutrients in the maternal diet which could significantly affect chick embryo development and their viability in the early posthatch life, antioxidants have been suggested to play a central role. Indeed, increased accumulation of polyunsaturated fatty acids in the embryonic tissues makes them sensitive to lipid peroxidation and requires an effective antioxidant defence. Some antioxidant components are coming to the egg and further to the developing embryo from the diet (vitamin E, carotenoids and selenium), others (ascorbic acid, glutathione, antioxidant enzymes) are synthesized during embryonic development.

It is necessary to stress that effective antioxidant interactions and formation of the integrated antioxidant system are the major steps providing antioxidant defence during embryonic development. In particular, vitamin E is effectively transferred from the diet to the egg yolk and further to the developing embryo. In fact, vitamin E accumulation in the chicken liver during embryonic development is considered as an adaptive mechanism to deal with stress conditions of the hatching process. Carotenoids are also transferred from the diet to the egg yolk but their involve-

ment in the antioxidant defence of the embryo needs further investigation. It has been shown that inclusion of organic selenium into the breeder's diet is an effective means of increasing its concentration in the egg and embryonic tissues and increasing protection from oxidative stress.

In postnatal development, a strategy of antioxidant defence is changed from antioxidant accumulation to the synthesis of antioxidant enzymes with more sophisticated and effective regulation. Antioxidant systems as well as immune and digestive systems of the chicken are not mature at hatch and are actively developing during first 2 weeks posthatch. It has been proven that maternal diet determines chicken development during egg incubation and for the first few days posthatch. Recently it has been shown that changes in vitamin and mineral composition for the broiler breeders affected gene expression in the gut of the newly developing chickens at 3 and 14 days posthatch. Those 11 genes affected by maternal nutrition are responsible for intestinal turnover, cell proliferation and development, metabolism and feed absorption. Therefore, these data are a clear example of maternal programming in poultry, a process which requires further investigations.

In conclusion, it seems likely that antioxidant composition of the breeder diet and formation of the integrated antioxidant defence system in the developing embryo are important processes providing high egg hatchability and posthatch chicken viability.

Photoperiodic response of male broiler breeders

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The expression of photorefractoriness, a feature of seasonal breeding in birds, is important in production species, where egg production and fertility are affected by the photoperiod. Years of selection in meat-type birds have reduced the potential reproductive function, and the environmental manipulation of such genotypes is essential to maximise productivity. While it is known that egg-type

pullets no longer exhibit photorefractoriness, and that female broiler breeders do, there is not much information about the response of male broiler breeders to photoperiod. Such information is important when designing lighting programmes for breeding stock, and this study aimed to gain more insight into the male response to photoperiod.

A series of experiments was designed in order to achieve this. The response of male broiler breeders to rearing on constant photoperiods was measured as well as the effects of age at photostimulation and photostimulation photoperiod on age at sexual maturity. The response to an increase in photoperiod during the production cycle was also investigated. Evidence of photorefractoriness in males was observed, but this was not the case in all birds, possibly due to high variation in some of the fertility traits

measured. It seems possible that males do not respond to photoperiod to the same extent as females. Unexpected observations of a response in some birds to early photostimulation, and the high variation observed, prompted another experiment in which age at sexual maturity of offspring from parents that either responded or not to early photostimulation was measured as a potentially revolutionary method of selection, whilst checking that this response is not negatively linked to broiler growth rates.

Impact of prenatal temperature stimulation on development of body functions: activation of hypothalamic NO-synthase in Muscovy duck embryos

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In the course of prenatal and early postnatal ontogeny, environmental factors might influence the development of the respective physiological control systems for the entire life period, especially by changes in neuroorganization and expression of related effector genes (Tzschentke and Plagemann, 2006). In precocial birds, the final incubation period is characterized by well-developed body functions (e.g. temperature regulation, Tzschentke, 2007) as well as dramatic changes in the quality of regulatory processes (Tzschentke and Plagemann, 2006). Environmental stimulation could improve the maturation and adaptability of body functions ('training effect').

In the brain, nitric oxide (NO) is prominently involved in neurotransmitter release and synaptic plasticity throughout life. We assume a crucial role of NO in environmental induced neuronal plasticity and also in the birds' thermoregulatory network during different developmental periods.

The aim of the study was to investigate the influence of prenatal temperature stimulation on neuronal NO-synthase (nNOS) expression in the preoptical area of the anterior hypothalamus of Muscovy duck embryos.

Experiments were performed on embryonic day (E) E20, E23, E28 and E33 using histochemistry for identification of the nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-d) as marker of NOS-containing neurons. Until the experiments all duck embryos were incubated under standard temperature conditions (37.5°C). During 3 hours before the start of the experiments one group was incubated at 37.5°C (control group), the second was warm experienced at 39°C and the third was cold experienced at 34°C.

In normal and warm incubated duck embryos, nNOS activity could be first detected on E23, but after cold stimulation nNOS activity was detected earlier at E20. Cold incubated embryos had a higher level of nNOS activity than the controls at all ages. The warm-stimulated embryos showed an initial increase in nNOS activity over controls at E23 but by E28 there was no difference from controls and by E33 had a lower level of nNOS activity indicating a proximate non-adaptive reaction.

These findings are similar with results we obtained in another study with respect to the blood flow in the vessels of chorioallantoic membrane (Tzschentke, 2007). In Muscovy duck embryos, the blood flow increased or decreased while warming or cooling on E26 until E30 (proximate non-adaptive). After this period until hatching, the reaction became proximate adaptive. But it was exclusively found under cooling (decrease in blood flow). During warming the proximate non-adaptive reactions of blood flow continued.

It can be concluded, that the 'training effect' of temperature stimulation in Muscovy duck embryos depends on the applied temperature (warming or cooling) and that probably in the late-term Muscovy duck embryo NO acts as a mediator of the neuronal cold pathway in the anterior hypothalamus, which can be improved by prenatal cold stimulation.

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How does the embryo digest and absorb the egg nutrients resources during incubation?

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The growth and development of the broiler embryo and the hatched chick are dependent on the nutrients deposit in the fertile egg. While the fertile egg has a defined nutrient composition, the rate and mechanism of absorption of these nutrients by the embryo is largely unknown. Furthermore, the embryonic systems that digest and absorb these nutrients are relatively unknown in terms of their developmental profile and capacities.

Nutrients which support the embryo during incubation period are recovered from yolk and albumen. At the last period of incubation the albumen enters the amniotic sac which is consumed by the embryo and by that enters the intestine. Yolk is absorbed via three ways; one way is absorption by endocytosis through the yolk sac membrane (YSM) directly into the blood. Second way is digestion and absorption by YSM cells and the third way is the entrance of yolk contents into the intestine thorough the yolk stalk.

While the uptake of lipids by the YSM has been well studied, little is known about the uptake of protein, amino acids, minerals and carbohydrates. Research in our laboratory is focused currently on the mechanism by which the developing embryo, from E12 to day of hatch (DOH) derives its nutrition via the nutrients uptake systems of the YSM and embryonic intestine. We investigate the change, from E12 to DOH, in the nutrients and minerals provided to the embryo by the yolk and amnion and examine the expression of genes involved in the digestion and absorption of nutrients and minerals in the YSM and embryonic intestine.

Results showed that from E0 (fresh fertile egg) to E17 almost 50% of the protein was absorbed by the embryo

from the yolk. However, increase in protein content was observed between E17 and E20 probably due to infiltration of protein and water from the non-yolk egg compartments (albumen and/or amnion) into the YS,

The majority of fat (65% of the yolk fat content) was absorbed in a linear manner from the yolk between E11 to E17. Then, at E17–E20, only a small amount of fat was absorbed while at DOH 15% of yolk fat content was absorbed during just 24 h. The amount of carbohydrates in the yolk increased during incubation (E15–E20) reaching a peak at 19E. These results points toward differential uptake of yolk nutrients during the incubation period.

Results, utilizing real time RT-PCR method, showed that YSM expresses many of the digestive enzymes and transporter genes normally associated with the small intestine. Among them are the sucrase-isomaltase (SI) and aminopeptidase (AP) brush border digestive enzymes, the CAT-1, EAAT3, PepT1 amino acids and di-peptide transporters, the sodium-glucose transporter (SGLT1) and also the zinc (ZNT-1) and phosphorus (SLC34A2) transporters. This demonstrates that the YSM serves as an important site for nutrient digestion and assimilation from E12 until E18.

Towards the end of incubation (E17–DOH), the small intestine expressed these genes in an elevated pattern and contributes to the abilities of digestion and absorption of the intestine. Results point toward a shifting dominants between the YSM and small intestine tissues from E12 to DOH and contribute to the comprehension of embryo nutrition during incubation.

Synthesis and storage of carbohydrates in the chick embryo during incubation

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Development and growth of poultry embryos and hatchlings are dependent upon the nutrient deposits in the fertile egg. The fresh egg (E0) contains very low levels of carbohydrates (COH), a necessary source of energy for early chick embryonic development. Towards

the end of incubation, the high demand for energy to support the dramatic physiological changes of the hatching process, and the reduced oxygen availability of the egg, drives the embryo towards catabolism of glucose, which is dependent upon the amount of

glucose held in reserves of glycogen in the liver and upon the degree of glucose generated by gluconeogenesis from protein and glycerol.

Although processes of glucose synthesis and glycogen storage have been studied in the liver and muscle of the chick embryo, only few studies have examined these processes in the extra-embryonic tissues of the embryo.

Therefore, the current study examined the role of the yolk sac (YS) in the supply of COH to the chick embryo during incubation. For that matter, the levels of glucose and glycogen were measured in YS and liver of broiler embryos from E11 until day of hatch (DOH). Eight fertile eggs were sampled on 0E (fresh eggs), 11E, 13E, 15E, 17E, 19E, 20E and on DOH. Egg, embryo, YS and liver were weighed. Glycogen and glucose concentrations (mg/g tissue) in the YS and liver were determined, and the absolute amounts (mg) of glycogen or glucose in the YS and liver were calculated.

Results show that levels of glucose in the YS increased from 25 mg on 11E to 60 mg on 19E, and then decreased by nearly 30 mg at DOH. Only trace amounts of glycogen were found in the yolk of the fresh egg, whereas on 11E

glycogen amount in the YS was 25 mg, and then increased by 10-fold reaching 250 mg on 19E. Between 19E to DOH, glycogen levels decreased by 100 mg. Results also show that COH amounts in the liver had a similar dynamic pattern compared to the YS, yet the liver tissue had significantly lower levels of glycogen (20–50 folds lower along the examined incubation period).

These results may be indicative of glucose synthesis in the YS, which is then stored in the form of glycogen. To elucidate the mechanisms involved in YS COH synthesis and storage, gene expression of enzymes involved in glycogen synthesis, glycogen degradation, and gluconeogenesis, were characterized in the yolk sac membrane (YSM). Results clearly indicate that YSM tissue expresses gluconeogenic and glycogenic enzymes that are normally associated with the liver.

It can be concluded that during the last week of embryonic development, the YS is also a gluconeogenic and glycogenic extraembryonic organ, which stores COH for provision to the embryo, to be used as an additional or perhaps a major source of available energy in the days prior to hatch and during the hatching process.

Effect of strain on eggshell temperature during incubation and chick measurements

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Incubation temperature which affects embryonic growth should be between 37 and 37.5°C. However, not only the incubation temperature but also the temperature that the embryo experience is very important. Increased shell temperature caused by metabolic heat produced by embryos could influence hatchability and chick quality. Metabolic heat production of embryos from different broiler breeder strains may be differing due to growth potential. The aim of the present experiment was to investigate eggshell temperature deviations from incubation temperature in eggs from two broiler breeder strains. Duration of hatching process and chick measurements was also determined.

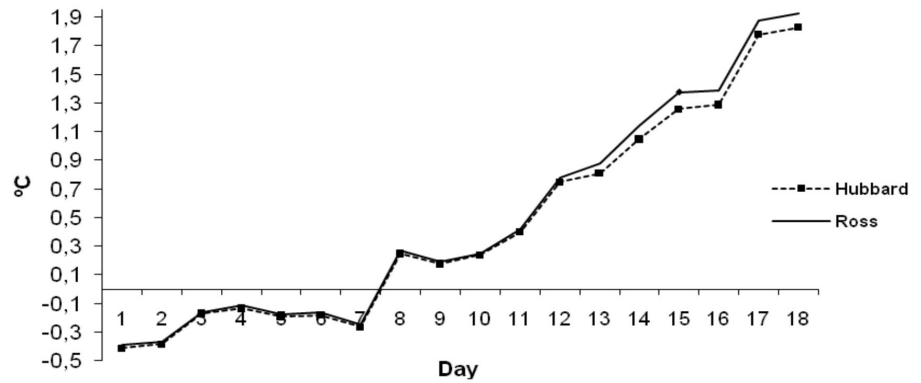
A total of 1200 eggs were obtained from Ross and Hubbard broiler breeders at 36 and 58 wk (300 eggs/strain/age). Eggs were weighed individually and incubated at the same incubator. Egg weights for 36 and 58 wk breeders were 61.6 g and 71.9 g for Hubbard and 61.4 and 71.9 g for Ross breeders, respectively. Twelve

eggs per strain/age were used to measure albumen and yolk weights. Egg shell temperature was measured on 60 eggs/strain/age/day from day 1 to day 18 of incubation. At 18 days of incubation, all eggs were reweighed to determine egg weight loss. Eggs were individually checked every 2 h between 464 and 500 h of incubation and number of embryos pipped externally and chicks hatched were recorded. Embryonic mortalities and hatchability were determined. On day of hatch, lengths of chick and shank and weights of chick, yolk sac, heart and liver were obtained.

Although mean egg weight was similar (65.8 and 66.6 g for Hubbard and Ross, respectively) between strains, higher relative albumen (58.1 *versus* 56.6% for Hubbard and Ross) but lower relative yolk weights (30.2 *versus* 31.9% for Hubbard and Ross) were obtained for Hubbard. Higher egg weight loss was obtained for Hubbard eggs compared to Ross (13.51 *versus* 11.86% for Hubbard and Ross).

Table 1 Egg shell temperature for eggs from Hubbard and Ross and incubation temperature during 1 to 18d of incubation

Genotype	Temperature (°C) from 1 to 18 days of incubation																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Hubbard	37.61	37.66	37.89	37.83	37.88	37.84	37.40	37.62	37.65	37.72	37.82	38.15	38.17	38.36	38.52	38.53	39.01	39.07
Ross	37.63	37.67	37.91	37.85	37.90	37.88	37.41	37.66	37.66	37.73	37.84	38.20	38.23	38.43	38.64	38.63	39.10	39.17
SEM	0.00	0.00	0.00	0.00*	0.00*	0.00*	0.00*	0.00*	0.00	0.00	0.01	0.01*	0.02*	0.03*	0.03*	0.03*	0.05	0.04*
Incubation temperature	38.03	38.04	38.06	37.96	38.07	38.02	37.66	37.37	37.46	37.48	37.43	37.40	37.34	37.29	37.25	37.24	37.22	37.24

*Means within a day differ significantly ($P < 0.05$).**Figure 1** Egg shell temperature deviations from incubation temperature by strains.

Eggshell temperature increased gradually during the incubation (Figure 1). Significant differences between shell temperatures of strains were obtained from 4 to 8 and from 12 to 18d of incubation, being higher for Ross eggs. Egg weight did not influence eggshell temperature because incubation temperature was adjusted during the incubation.

The time required for attaining the external pipping and hatching was shorter for Ross chicks by 2 and 1 h, respectively. There was no difference between strains for embryonic mortalities and hatchability. Although chick weight and shank length was similar between strains, chick length was longer for Ross chicks. Similar relative weights of yolk, heart, and liver were obtained for strains. In conclusion, higher eggshell temperature with shorter time required to attain the external pipping and hatching obtained for Ross strain may be related to the higher metabolic rate of the embryos. The results showed that embryos produced by different stocks produce different amount of heat. Therefore, it may be beneficial to incubate eggs from different stocks under different incubation conditions.

Hatching results of genotypes with different live weights used for developing slow growing meat type chickens

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Meat type ROSS breeders were crossed with egg type breeders Barred Plymouth Rock (BAR) and Rhode Island Red (RIR) genotypes to develop slow growing meat type chickens. The male and females were reared in different houses until the onset of lay. RIR and BAR genotypes selected for live weight and heavier pullets were chosen for breeding. At the second week of laying period, males were transferred to the house of hens for natural mating and eggs were collected after one week. The storage period for eggs was seven days. The eggs were placed to incubator and fertile eggs were determined by candling at the day of 18. The fertility ratio was the ratio of fertile egg to total eggs placed to incubator. Hatching rate is the

ratio of hatched chicks to the fertile eggs and incubation yield is the ratio of hatched chicks to the total eggs. The results were given in Table 1.

Significant differences between cross breeding groups for all hatching traits were found ($P < 0.01$). Lowest fertility ratios were found in Ross \times BAR and Ross \times RIR groups (45.43% and 66.61% respectively). It is thought that the lower fertility in these groups can be related to the weight differences between male and females. Heavier cockerels had difficulty mating with lighter hens.

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Table 1 Fertility and hatchability of different breed male \times female crosses. Breed crosses within a row with different letters are significantly different

Hatching trait	Ross \times Ross	BAR \times Ross	RIR \times Ross	BAR \times BAR	Ross \times BAR	Ross \times RIR	RIR \times RIR	SEM	P
Fertility ratio (%)	95.88 a	85.05 cd	95.06 b	77.88 d	45.43 f	66.61 e	89.26 bc	0.91	**
Hatching rate (%)	87.85 bc	90.89 ab	94.14 a	84.50 c	91.19 ab	89.98 abc	85.11 c	0.70	**
Incubation yield (%)	84.26 ab	77.97 b	89.84 a	65.94 c	41.81 d	60.50 c	76.30 b	1.01	**

The potential to use turkey (*Meleagris gallopavo*) primordial germ cells to produce a genomic archive and germ line chimeras

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Diseases such as avian influenza can destroy turkey flocks, resulting in the loss of valuable genetic material from the gene pool. Primordial germ cells (PGCs), the stem cell precursors to gametes, take a complex migratory route through the developing embryo, from the epiblast,

to the germinal crescent, blood vascular system and eventually settling in the embryonic gonads, where they will differentiate into sperm and eggs. Germline chimeras produced by intravascular transfer of both fresh and frozen PGCs have been reported in other avian species

(chicken and quail), but with low efficiency. The aims of this study were: to test the feasibility of using turkey PGCs to produce a frozen archive of turkey strains; to see if these cells retained the potential to produce germline chimeras once reinjected into host embryos, and to see if host PGCs could be ablated in order to improve the efficiency of the process. The following experiments were performed: (1) Identification of PGCs within turkey embryos; (2) development of an efficient method for isolation of turkey PGCs; (3) demonstration that PGCs could be frozen, recovered and retain viability; (4) reinjection into host embryos and detection of injected PGCs; and (5) establishment of an efficient method for host PGC ablation.

Isolated PGCs were identified using Periodic acid Schiff reagent, and the immunological marker OLP-1, which recognised PGCs from the blood and cryosections of stage 30 (HandH) gonad tissue. Ficoll density gradient centrifugation was used to isolate bloodstream PGCs from stage 12–18 embryos. PGC numbers peaked within the circulation at stages 13, 14 and 15 with 32 ± 4.9 , 33 ± 6.4 and 26 ± 5.4 (mean \pm SEM) PGCs recovered respectively. The highest concentration of PGCs was present at stage 13 where 32 ± 4.9 PGCs were isolated per microlitre of blood. PGCs were frozen in a freezing medium containing DMEM, 20% foetal calf serum and 10% dimethylsulfoxide and demonstrated $90\% \pm 1.7$ viability after 3 months frozen in liquid nitrogen. Freshly isolated and frozen/thawed PGCs repro-

populated host stage 30 gonads after reinjection into the heart at stage 15. Dil and Q-tracker fluorescently labelled PGCs were detected in whole mount gonads dissected from *ex-ovo* cultured embryos by confocal microscopy. Dil labelled cells repopulated gonads less frequently, with $36\% \pm 13.2$ of surviving gonads containing the Dil labelled PGCs, and $7\% \pm 3.8\%$ of reinjected PGCs reaching the gonadal tissue of the positive embryos. Q-tracker labelled cells were detected more frequently in surviving embryos with $67\% \pm 21.1$ of embryos having positive signals. $44\% \pm 4.9$ of reinjected Q-tracker labelled PGCs colonised the embryonic gonads in positive embryos. Immunosurgical removal of PGCs was achieved using a combination of anti-OLP-1 antibody and baby rabbit complement. This resulted in a reduction of 79% of circulating PGCs when compared to controls. The knockdown in the bloodstream was also detectable in stage 30 gonads where 53 ± 25.5 PGCs were detected in comparison to 243 ± 24.7 from PBS injected controls.

This study demonstrates the feasibility of using turkey PGCs as a means of archiving genetic material from different strains of turkey since frozen PGCs once reintroduced into host embryos can colonise the gonads of the host, suggesting that it would be possible to produce turkey germline chimeras. The development of a novel Immunosurgical method to remove endogenous PGCs from host embryos could also improve the efficiency of germline chimera production.