## Session 1: Thermostimulation

(in order of presentation, speaker underlined)

### Thermal treatments before incubation improve performance of Cobb broilers to 35 days of age.

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Embryo development during pre-incubation storage largely depends upon temperature. The potential beneficial impact of manipulating temperature in a programmed manner during the pre-incubation periods of broiler chickens has been realized during the past several decades. Furthermore, convincing evidence that temperature could influence the sex ratio of avian offspring has recently become available. This study was designed to elucidate the effect of temperature that was elevated above standard conditions prior to incubation on hatchability, sex ratio, growth and development post-hatching, and secondary sexual phenotypic characteristics. Two experiments were conducted using Cobb 500 broiler hatching eggs that had been stored for 4 and 9 days, respectively. Two treatments were applied: Control maintained at standard conditions of no pre-heating and 37.5 C throughout incubation; pre-heating (**Pre)** for 12 h prior to incubation to 30.2 C. **Pre** treatment increased early embryonic deaths, but significantly improved hatchability. The point of 50% hatchability was achieved earlier (by 8 hours) in this treatment. The BW of males and females at 35 d of age in both experiments was greater from the thermal treatment. This was coincident with increased relative breast muscle weight. The secondary sexual phenotypic characteristics of comb and wattles were also affected by the thermal treatments, being heavier in both cases. The testes were larger in thermal treated males, which may be associated with increased plasma testosterone concentration. However, testosterone concentration was significantly higher in females too, in both experiments. It was concluded that thermal treatment pre-incubation period had, in general, positive effects on hatchability, growth, carcass yield, and secondary sexual characteristics of broilers.

**Keywords:** broilers, embryo, pre-heating, sex determination, broiler live performance.

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## Pre-natal thermal manipulation of turkey embryos- effects on embryo development and post-hatch performance

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Previous studies conducted in our laboratory on meat type chickens showed that embryonic thermal manipulations of 39.5°C for 12h/d, during the time window of hypothalamus–hypophysis-thyroid axis development and maturation significantly lowered metabolic rate of the embryo and the chicken, improving feed conversion rate (FCR) post-hatch (Piestun *et al*. 2009; 2013). The aim of this study was to investigate the effect of intermittent thermal manipulation during turkey embryogenesis on embryo development and post hatch performance.

B.U.T. turkey eggs (±10 g) were divided into three treatments (n=250): Control; 6H-intermittent elevation of temperature by 1.7°C and RH by 9% above the control conditions (TM) for 6 h/d (6H), from E10 to E22 inclusive (240 till 552 h of incubation); and 12H-intermittent TM for 12 h/d (12H) at the same time period. From E23 onwards all eggs were incubated under control conditions. After hatch chicks were randomly placed in cages (40x28x45 cm) in 4 computer-controlled environmental rooms that maintained temperature with an accuracy of ± 1.0°C and raised under standard conditions until 35 days of age. Weekly the chicks and feed were weighed and FCR was calculated, while blood samples were taken from the brachial vein.

Embryo growth was not affected by TM. However, during TM eggshell temperature, embryonic heart rate and oxygen consumption were elevated according to the manipulation as the embryos were in their ectothermic phase. However, by the end of the TM period and until hatch (the endothermic phase) these parameters were significantly lower in both treatments than in the control, indicating lower metabolic rate and heat production. 12H embryos hatched 8 hours earlier than control and 6H group without any negative effects on chick body weight or hatchability. Nevertheless, 12H treatment resulted in higher proportion of chicks with unhealed navels and spread legs. Body temperature at hatch was significantly lower in the TMs chicks compared to the control suggesting lower heat production and metabolic rate. During the growth period, there were no differences in body weight. However, feed intake of TMs turkeys was significantly lower, resulting in lower FCR. This coincided with lower plasma thyroid hormones concentration and lower body temperature suggesting lower metabolic rate that may explain the improved FCR.

It was concluded that TM during turkey's embryogenesis may alter the thermoregulatory set point, and thus lowered the embryo metabolic rate with long lasting post-hatch effect resulting in lower FCR. However, fine tuning of the manipulation is required for treatment optimization.

**Keywords**: turkey, embryo, pre-natal, thermal manipulation, turkey live performance

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## Temperature training before hatching: successful method to optimize hatching results and performance in broiler chickens

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Temperature manipulation during the last days of embryonic development has long-lasting impact on development and post-hatching performance. The last days before hatching is a critical period in the formation of regulatory systems, such as the development of feedback mechanisms like thermoregulation and it is shaped via "imprinting" by the actual incubation environment. Short-term manipulation of incubation temperature improves post-hatching performance, which has been demonstrated in broiler chickens. Short-term warm stimulation during the last days of embryonic development effects a higher hatching rate, mostly in favour of more hatched male chickens, and a better feed conversion which implies a higher body weight at slaughtering. Short-term temperature training promotes adaptation to environmental fluctuations and finally improves "robustness", because of being closer to a natural correspondence with the physiological needs of the embryo. For the use of the approach of "temperature training" before hatching for incubation of high yielding poultry species and to investigate underlying physiological mechanisms a collaborative research project on "Circadian Incubation" is running together with Pas Reform Hatchery Technologies and the Friedrich-Loeffler Federal Research Institute for Animal Health.

The aim of the present investigation is to analyse the long-lasting influence of temperature training on hatching results, post-hatching performance and related neurophysiological processes in the *nucleus infundibuli hypothalami*, which is located in the hypothalamus and responsible for regulation of metabolism, body weight and feed intake. The immunohistochemistry research was focused on Neuropeptide Y, which is one of the most potent orexigenic peptides found in the brain, and which stimulates food intake.

The investigations were carried out in ROSS 308 broiler chicks. In four commercial trials within this project one group (n=900) was prenatally short-term temperature exposed with + 1°C over standard for 2 hrs daily during the last three days before hatching. The control group (n=900) was incubated under conventional conditions. Additional, commercial trials with 30000 chickens of each group were done separately by Pas Reform. Data about hatching rate, feed conversion and body weight gain were collected during different commercial trials. In addition to the production data brain tissue, for Neuropeptide Y analysis, was also collected during slaughter at the end of the commercial trials and also in experimental lab trials. For the analysis of Neuropeptide Y expression and the percentage of immune positivity the slices were stained by using the indirect method (antigen-antibody reaction).

In commercial trials the hatching rate was improved by an increase in hatchability of about 3.4%. Furthermore, a better feed conversion and an increase in body weight at slaughter age, especially in males was observed. For females the effects on performance were less marked, but the changes were essentially the same way.

The measurement of hypothalamic Neuropeptide Y expression found a numerical difference consisting of a lower Neuropeptide Y expression in temperature stimulated chickens compared to the conventional incubated ones. The highest difference was between the

perinatal temperature stimulated males and the conventional incubated males. This finding corresponded with the better feed conversion in prenatally temperature stimulated chickens, which was clearly related to the prenatal imprinting of metabolism to a lower level.

However, experience indicates that the success of the method in relation to hatchability and performance is likely affected by basic conditions like parent stock management and age of parent stock, which affects in the quality of set eggs.

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## Long-term influence of prenatal temperature stimulation on reproductive performance in juvenile female broilers: A novel immunohistochemical amplification technique enabled the detection of hypothalamic type-II iodothyronine deiodinase (Dio2) expression

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During critical periods the prenatal environment may have long-lasting influence on body functions. For instance, before hatching short-term temperature experienced broiler chicks are characterized by an improved hatching rate, mostly producing more hatched male chickens, as well as post-hatching growth rate and feed conversion (Tzschentke and Halle, 2009). Furthermore, we hypothesis a beneficial influence of the prenatal "temperature training" during the last days until hatching on other body functions, like reproduction. It is well established that the up-regulation of hypothalamic type 2 iodothyronine deiodinase (Dio2) is one of the earliest steps of the photosexual stimulation cascade in birds, but due to the lack of a commercial bird-specific antiserum and the difficulty of detecting sparsely expressed Dio2 proteins, very little is known about the cellular distribution of Dio2 within the pre-sexual maturation period. Therefore, the current study used a modified immunohistochemistry technique to investigate the influence of short-term elevation in incubation temperature, during the last days of embryonic development, on the localization and the density of mediobasal hypothalamic Dio2, as a maturation stimulant for later reproductive performance in female broilers.

The experiments were carried out in 35 days old female Cobb broilers, which were exposed to short-term warm stimulation (2 hrs at 38.2-38.4°C) daily from day 18 of incubation until hatching. Normal incubated (37.3-37.4°C) Cobb broilers were used as control. After hatching all birds were kept under similar conditions according to a standard temperature regime used in commercial broiler production. On day 35 the broilers were slaughtered and the brains removed for the immunohistochemical procedure that utilizes an additional amplification step involving an anti-multispecies biotinylated antibody. The distribution of Dio2 was examined at x40 magnification in an average of ten (N=7-13) caudal hypothalamic slices per brain. For each slice, Dio2 optical density was estimated in each of the microscopic fields examined all over the ependymal layer of the 3rd ventricle, at the

region of subcommisural organ (SCO) and median eminence (ME), using KS-400 V3.0 image analysis software (Carl Zeiss Vision, Oberkochen, Germany).

Accordingly, the caudal but not rostral hypothalamic slices revealed that exclusively elevating incubation temperature by 1°C for 2 h daily, during the last days of embryonic development, induced a statistically significant increase in expression of Dio2 (p-value ≤ 0.0001) within the SCO and the ME. However, such abundant expression was only detected using the proposed amplified immunohistochemical technique.

In conclusion, short-term temperature manipulation during the last 3 days of embryonic development might play a vital role in advancing the photoinduction of Dio2. The increased expression of Dio2 protein in only caudal hypothalamic slices in short-term temperature stimulated birds leads us to propose a novel physiological prospective involving the suppression of thyroid hormone and the elevation of follicle-stimulating hormone secretion to considerably advance the age of photoinduced egg production via embryonic thermal manipulation, which could be also of practical relevance for broiler breeder females.

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## Session: Influence of incubation conditions on performance

(in order of presentation, speaker underlined)

### Manipulating incubation conditions to alter sex ratio of Taiwan Country Chickens.

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Red feathered Taiwan country chicken (RTCC) and black feathered Taiwan country chicken (BTCC) are both Taiwan locally developed slow-growth breeds of chickens (Lee, 2006). Compared with the commercial broiler type chickens, they take a longer time (13-16 weeks) to grow to the market weight (2.6-3.0 kg). RTCC is characterized with heavier body weight and fleshy legs and thighs, while BTCC is lighter and smaller. Most chickens purchased from the markets in Taiwan for domestic use are cooked in the Chinese traditional cooking. Taiwanese prefer to use legs and thighs of RTCC to prepare certain kinds of chicken cuisines where the whole leg is cooked, so there is a demand in the market for male RTCC since the whole legs of male chickens weigh 15% more than the leg of female chickens (Lee and Chen, 1984). For these reasons, price of male RTCC chicks is always higher than female RTCC chicks at hatcheries. On the other hand, female BTCC chicks are more popular due to their smaller carcass size for whole chicken slow cooking. The purpose of this study was to alter the sex ratio at hatching by manipulating the incubation environment. Total of 420 eggs from RTCC (210) and BTCC (210) were incubated at 96℉, 98℉ and 100℉ during the 0-19 days of incubation, at the last two days of incubation, temperature was reduced by 1 degree and the humidity was increased by 2%. The genotypic sex and the phenotypic sex were determined upon hatching by DNA-based sexing (Quitana *et al.*, 2008) and gonadal observation, respectively. A sex reversal chick was any chick whose phenotypic sex was different from its genotypic sex. Sex reversal rate was calculated as percentage of sex reversal chicks in total number of its original genotypic sex; and the male ratio was determined by the percentage of male chicks in total chicks; only the hatched live chicks were used for calculation. Our results showed that the hatchability of all environmentally manipulated groups were not as good as the eggs incubated at normal condition (98℉). However, the female to male sex reversal rate was 33% at higher incubation temperature group for RTCC. When the thermal manipulation time was adjusted to only the first week of incubation (n=120), the hatchability of the RTCC fertilized eggs at 100℉ were 80% and the male ratio for the thermally-manipulated group was 62% (Table 1). The embryos were also analyzed for microRNAs expression. The microRNAs expression were found to be affected by the temperature. A higher incubation temperature up-regulated the male differentiation related miR-31 and miR-202* but down-regulated the female differentiation related miR-101. There was no clear pattern of sex reversal rate in BTCC for all treatment groups. An attempt to enhance male ratio of RTCC by increase incubation temperature to a higher temperature was successful but at the cost of poorer hatchability (Table 1). Further studies are needed to optimize the temperature, the manipulation timing and the relative humidity for the best RTCC male ratio.

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**Table 1. Sex ratio of Red feathered Taiwan Country Chickens (RTCC) in different incubation conditions.**

| Thermal manipulation timing | temperature (℉) | Hatchability (%) | Sex reversal rate (%) | | Male ratio |
|---|---|---|---|---|---|
| | | | ♀→♂ | ♂→♀ | |
| 0-19 day of incubation | 96‡ | 71.9 | 21.1 | 19.2 | 55.6 |
| | 98‡ | 86.2 | 12.0 | 20.0 | 50.0 |
| | 100‡ | 50.0 | 33.0 | 0.0 | 60.9 |
| 0-7 day of incubation | 100‡‡ | 79.2 | 11.1 | 0.0 | 61.9 |
| | 102‡‡ | 39.7 | 73.3 | 0.0 | 82.6 |

‡ 70 eggs were used at day 0 of incubation for each temperature, after removal of unfertilized egg, the number of eggs for 96, 98 and 100 ℉ were 64, 68 and 66, respectively.
‡‡ 60 eggs were used at day 0 of incubation for each temperature, after removal of unfertilized egg, the number of eggs for 100 and 102 ℉ were 53 and 58, respectively.

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## Effect of Hatching Time on Yolk Sac Percentage and Broiler Performance

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This study investigated the effects of broiler chick hatching time on percentage yolk sac and subsequent live performance.

Broiler hatching eggs were obtained from commercial flocks of Ross 344 males and Ross 308 females at 59 and 55 wk of age in Experiments 1 and 2, respectively. Eggs were stored for 1-3 d at 18 C and 75% RH prior to setting in incubators under standard conditions. Early hatch time was 472-480 h or 471-477 h, Middle hatch time was 488-492 h or 480-486 h, and Late hatch time was 496-510 h or 494-510 h, respectively, in Experiments 1 or 2. Chicks were deemed to be hatched when they exhibited healed navels and dryness about the head and neck. At 510 h of incubation, the chicks that had completed the hatching process were removed from the trays, feather sexed, counted, permanently identified with neck tags, weighed, and placed in floor pens on new wood litter shavings under the same feeding and management program. Chicks were necropsied and yolk sac weight determined at placement in pens and at 1 d in Experiment 1 and immediately at hatch (477h, 486h and 510h) at each hatch time and also at placement in Experiment 2. For each hatch group, chicks were assigned to 8 pens of 10 male and 10 female chicks each or 9 pens of 9 male and 9 female chicks each for a total of 480 and 486 chicks in Experiments 1 and 2, respectively. BW and feed consumption were determined at 1, 7, 28, and 35 d of age or 7, 14, 21, and 35 d of age in Experiments 1 and 2, respectively. Mortality was counted and weighed twice daily. Data from the 3 (hatch time) x 2 (sex) completely randomized design were subjected to analysis of variance using the GLM procedure of SAS (1996). Statements of difference were based upon $P \leq 0.05$.

Percentage yolk was greater in Late compared to Early and Middle hatch chicks at placement and at 1 d in Experiment 1. Percentage yolk was similar in all groups in Experiment 2 at hatch but Early hatch chicks had less percentage yolk at placement. Broiler chick BW was greater at placement in Late hatch chicks compared to Early hatch chicks in both experiments. However, this advantage disappeared by 7d. BW was greater in the Middle hatch compared to Late hatch chicks with Early hatch chicks intermediate at 7 and 35 d in Experiment 1. Although Early and Middle hatch chicks exhibited greater BW loss between hatch and placement and lower BW at 0 d compared to Late hatch chicks, Early hatch chicks had significantly larger BW than Late hatch chicks with Middle hatch chicks intermediate at 35 d in Experiment 2. Late hatch chicks consumed less feed and exhibited lower relative growth rate to 7 d and exhibited greater cumulative mortality in both experiments.

These data showed that Late hatch chicks had greater percentage yolk sac and BW at 510 h of incubation, which was followed by less feed consumption to 7 d. Live performance of Late chicks, judged by mortality and BW, was reduced compared to Early and Middle hatch chicks.

**Keywords**: broilers, hatch time, yolk sac, feed consumption, mortality

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## Effect of incubation temperature on leg bone development in broiler hatchlings

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At slaughter age, leg problems (pathologies that result in impaired walking ability) are highly prevalent in broilers. Poor skeletal leg health in later life may be related to suboptimal bone development during incubation (Oviedo-Rondón *et al.,* 2009). The present experiment aimed to investigate the effect of eggshell temperature (EST) throughout incubation on leg bone development in broiler hatchlings.

298 Ross 308 eggs of a 44 week old breeder flock were incubated from day 0 of incubation until hatch at 1 of 5 EST: Very low (36.1ºC); Low (36.9ºC); Normal (37.8ºC); High (38.6ºC); Very high (39.4ºC). Hatchability differed between treatments: 15% for Very low, 57% for Low, 83% for Normal, 72% for High and 36% for Very high. The hatched chicks were killed within 12 hours after hatch and used for determination of yolk free body mass (YFBM) and chick length as measures of chick quality, and femur, tibia, and metatarsus length, diameter, and weight.

Low, Normal, and High resulted in higher YFBM (+5.5, +4.1, and +0.5 g, respectively; $P < 0.001$) and chick length (+15, +14, and +13 mm, respectively; $P < 0.001$) compared to Very high. EST showed a consistent effect on bone length. Normal and High resulted in a longer femur (+ 0.9 and +1.2 mm, respectively; $P < 0.001$), tibia (+2.2 and +2.7 mm, respectively; $P < 0.001$), and metatarsus (+1.6 and +1.7 mm, respectively; $P < 0.001$) compared to Very high. Bone length in Very low and Low did not differ from other EST treatments. Tibia diameter was higher for Very high (+0.09 mm; $P = 0.011$) compared to Normal. Femur and metatarsus diameter did not differ between treatments.

Relative weights (expressed as a percentage of YFBM) of the femur and metatarsus were comparable between Normal, High, and Very high. Relative tibia weight was higher for High (+0.05%; $P = 0.013$) than for Normal. Number of hatched chicks was too low for the Very low group to reach statistically significant differences in bone parameters, although large

numerical differences were observed particularly in lower relative tibia and metatarsus weights compared to all other EST treatments.

To conclude, an EST of 37.8ºC (Normal) or 38.6ºC (High) throughout incubation resulted in longer chicks with higher YFBM and longer leg bones than an EST of 39.4ºC (Very high). However, relative bone weights were not different with the exception of relative tibia weight. Whether the differences in bone length found at hatch affect leg health later in life has to be determined in a future experiment.

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## Session: General aspects of incubation

(in order of presentation, speaker underlined)

### Embryonic bone development: comparison between current and mid-80 broiler strains

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Investigation of bone development during incubation shows that between E14 and E17 bone ash content, structural properties and mechanical properties increased significantly, while between E17 and E21 most of those properties remained similar in their levels or even decreased, which might point to a slow-down in bone development in the pre-hatch period (Yair *et al*., 2012). Currently, fast-growing broilers often suffer from leg problems which are associated to their fast growth (Julian, 1998; Angel, 2007). This can be explained by the previously reported inferior properties of bones in fast-growing broilers in comparison to slow-growing strains: increased bone porosity, reduced bone ash content and reduced adjusted (for body weight) mechanical properties (Williams *et al*., 2000; Williams *et al*., 2004). However, there is nothing known about the differences between the strains during incubation.

The aim of this study was to compare leg bone (tibia) properties during last period of incubation of embryos from Cobb 500 and a strain which was not selected since 1986. 100 eggs per strain (Cobb and "1986") were incubated conventionally. Eggs (N=10) were randomly selected on E17, E19 and E21; the tibia and its muscles were dissected from each embryo and weighed. Each tibia was subjected to biomechanical testing using a micromechanical testing device and cortical structure analysis using a high-resolution μCT. Haematoxylin & eosin staining was used to count osteocytes (load-sensing cells). In order to examine bone mineralization of both strains the tissue mineral density (TMD) was examined by μCT and fluorochrome labeling which enables visualization of the calcification rate of the bone. This was done by injection of the fluorochrome calcein on E19, tibia harvesting on E21 and visualization with a fluorescent microscope.

The results show that Cobb broilers had a 12% higher embryo and muscle weights on E21, while bone weight was similar on both groups. In comparison to Cobb, the "1986" showed 9-20% higher tibial-stiffness between E17 and E21, 33% higher tibial cortical area on E21, 100% higher moment-of-inertia on E21, 35% higher osteocyte concentration (per bone area) on E21, 8.9% higher TMD on E21, and higher calcification rate between E19 and E21.

In conclusion, compared to the relatively slow-growing broilers of the 1980's, the bones of contemporary broiler are inferior in terms of lower load-sensing ability (lower osteocyte concentration), reduced structural parameters (cortical area and moment-of-inertia), reduced mineralization rate (calcium incorporation) and mineral density (TMD), and lower mechanical capabilities (stiffness).

The fact that Cobb broilers have inferior embryonic bone properties compare to the slow-growing "1986" broilers may relate to the higher susceptibility of fast-growing broilers to leg problems at older age.

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## Influences of Breeder Age on Energy Utilization and Embryonic Heat Production

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Differences in embryonic heat production (HP) during incubation influence eggshell temperature (EST; Meijerhof and van Beek, 1993), and can therefore affect hatchability and chick quality (Lourens *et al.*, 2005). At a fixed EST, embryos of large eggs produced more heat due to differences in the amount of energy used and converted to yolk free body (YFB; Lourens et al., 2006). As nutrients are obtained from albumen and yolk and this composition is influenced by breeder age and egg size (Nangsuay *et al*., 2011), it can be hypothesized that embryos of eggs originating from different breeder ages and egg sizes utilize energy and produce heat differently. Because egg size normally increases with breeder age, 2 experiments were conducted to investigate particularly the independent effects of breeder age and egg size on energy utilization and embryonic HP.

The experiment I aimed to examine the influence of breeder age and egg size on egg energy content and energy utilization during incubation. A total of 4,800 Ross-308 hatching eggs from 2 breeder flocks (29 and 53 wk of age, or young and old) and within each age 2 egg sizes (57–61 g and 66–70 g, or small and large) were used. Albumen and yolk content, amount of egg energy, YFB wet and dry weight, energy utilization and deposition into YFB and efficiency of converting egg energy into chick body energy ($E_{YFB}$) were measured. The experiment II aimed to examine the influence of breeder age on embryonic HP. A total of 240 Ross-308 hatching eggs of 58-61 g were used from 2 breeder flocks of 29 and 53 wks of age (young and old). In both experiments EST was maintained at 37.8°C. The results of experiment I showed that the amount of yolk relative to albumen was higher in the old flock eggs, and this effect was more pronounced in the large eggs. In the old flock eggs, especially the larger egg size contained more energy as a result of an increase of yolk size. The energy utilization of the embryos was positively related to the yolk size and the amount of energy transferred to YFB was largely determined by the available egg energy. The $E_{YFB}$ was equal for both breeder age and egg size groups. Chick YFB weight of young and old flock eggs was equal, however YFB dry weight increased in chicks from old flock eggs, and was associated with more energy accumulation into YFB. As a consequence, results of experiment II showed that the embryos originating from old flock eggs produced more heat from d 16 of incubation onward than that of the young flock eggs. In conclusion, breeder age (or yolk size) rather than egg size dictates amount of available energy in the eggs.

Higher breeder age and larger yolk size leads to more energy used and deposit in YFB and consequently higher embryonic HP.

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## Effects of mechanical impacts on hatchability of Broiler breeders

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Aims and Goals
The negative effect of transport on hatchability is known widely and customers are warned about the importance of good transporting conditions by representatives of incubator manufacturers and breeding companies but no measurements have been done so far to determine the threshold for mechanical impacts. Egg shipments under field conditions can be equipped with Tinytag® high sensitivity shock and vibration loggers to monitor transport conditions. The idea is to use a device for simulate the transport conditions in a better controlled and repeatable manner. Hence, the aim of these initial trials was to determine if the testing device (Crazy Fit Massage machine – CFM machine) was able to replicate and model the mechanical impacts experienced during transport when applying the same level of shaking and vibration as the eggs experience during transport.

Material and methods
Trials 1 and 2 were 2100 eggs in each of the control and trial groups. In Trial 3, there were 1350 eggs in each of the control and treated groups. The CFM is a vibration machine with a two dimensional vibration plate and it can be set for different levels of vibration between 0-30 Hz. Treated eggs were placed on Keyes trays then placed onto the CFM machine in subunits (150 eggs) and received single 10 minute vibration treatment. In Trials 1 and 2, the eggs were treated with periodically changing vibration in a range between 10-30 HZ. In Trial 3, trial eggs were divided into two groups. Eggs in the first trial group received a constant 20 Hz, while in the second trial group the eggs received 30 Hz constant vibration. Treatments and data collection were performed on the individual subunits. SPSS software was used to analyze the data statistically.

Results and Discussion
Hatchability (%) decreased significantly due the treatment in Trial No.3 ($80.75^{a}\pm1.39$ vs. $76.80^{b}\pm2.97$ vs. $64.89^{c}\pm4.27$). The observed loss in hatchability was mainly due to the increased level of embryo mortality in early-dead stage which is in accordance with the field observations following transportation of eggs. The difference in early-dead percentages was statistically significant in Trial 2 ($9.00^{a}\pm2.97$ vs. $21.68^{b}\pm5.29$) and in the 30 Hz treated

group in Trial 3 (0.55 [a]±0.59 vs. 1.85 [bc]±0.77). The significant percentage increase (0.56 [a]±0.70 vs.1.68 [c]±0.88) in mid-term dead embryos in Trial 3 between the untreated eggs and the 30 Hz treated group was unexpected. There was no significant difference in late-dead embryo levels in any of the trials which is in accordance with the field experience after transportation of eggs. The significantly higher % of malformations in Trial 1 (1.12 [a]±0.63 vs. 2.28[b]±0.97) and 3 (0.55 [a]±0.59 vs. 1.85 [bc]±0.77) vs untreated eggs might be due to the short resting period between the treatment and the incubation of the eggs. This will be the subject for further trials as well as the significantly increased % of malpositions in Trial 2 (22 [a]±1.12 vs. 4.21 [b]±1.51) when compared with untreated eggs.

The trials confirmed that the CFM Machine was able to create mechanical impacts that were repeatable in order to set up statistically reliable trials on hatching eggs.The long-term plan is to determine whether the g–force or the vibration pattern has the bigger impact and determine the threshold for the more critical parameter for hatching egg transport on the field.

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## Double and single-yolked duck egg dimensions and contents: the importance of yolk size and position for albumen secretion

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The weight, dimensions (length, width) and contents (yolk, albumen, shell) of double-yolked (DY) and single-yolked (SY) duck eggs were measured and compared. Yolk position was recorded in DY eggs and the yolk closer to the airspace was termed Yolk 1.

DY eggs were 21.5 % heavier, 11.7 % longer and 4 % wider (all $p<0.001$). On average, DY eggs had more albumen and shell than SY eggs (both $p<0.001$), but formed a significantly smaller proportion of egg weight when compared to SY eggs (both $p<0.001$). In DY eggs, the two yolks differed on average by 0.3 g (range=0.2 - 5.2 g), which was significant when compared by size ($p<0.001$). However, when yolks were compared by position, the difference in weight was not significant ($p=0.144$). The individual yolks in DY eggs were significantly lighter than that of a SY egg ($p<0.001$), but their combined weight was significantly heavier than a SY egg yolk ($p<0.001$).

Following the procedures of Deeming (2011), the albumen weight of DY eggs was predicted on the basis of SY yolk weight to examine how much additional albumen is secreted with Yolk 2 in DY eggs. On average, the additional albumen for Yolk 2 in DY eggs weighed 12.2 g and was 24.5 % of the albumen weight predicted on the basis of SY yolk weight, which is comparable to the 33.7 % additional albumen secreted in DY pheasant eggs (Deeming, 2011). Thus DY eggs have more albumen than a SY egg of similar weight. This is attributed to the more stimulation of the magnum wall by the two yolks.

In DY eggs, the heavier yolk is regarded as the first in the ovulation sequence and the second yolk following it considered to be ovulated prematurely (Conrad and Warren, 1940; Deeming, 2011). Here, using the yolk position data, Yolk 1 was heavier in 62.5 % of DY eggs, supporting the view that the yolk closer to the airspace was ovulated first. Thus, DY eggs were divided into two groups based on yolk weight. In Group A, Yolk 1 was heavier. In Group B, Yolk 2 was heavier. Significantly more albumen was found in Group B (Group A: 51.37 % vs. Group B: 53.29 % albumen; $p=0.031$) supporting the mechanical stimulation hypothesis, with the larger Yolk 2 stimulating the secretion of additional albumen by the magnum wall.

This supports the finding of Deeming (2011) that DY pheasant eggs have a higher proportion of albumen and the additional stimulation of the magnum wall by the two descending yolks was suggested. Longer egg formation time is associated with increased egg contents (reviewed in Shanawany, 1990), which can be a contributing factor too. However, here we found that the size of the yolk and sequence of ovulation is important for albumen secretion.

It is suggested that work with DY eggs could provide a useful non-invasive tool to examine the mechanisms underlying albumen secretion.

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## The innate immunity of the egg and its regulation, related to the hen environment and to egg storage

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Abstract to follow.

## Poster session
(in alphabetical order of first author)

### Sperm ultrastructure in goshawk (*Accipiter gentilis*) and preliminary results on semen traits and sperm sensibility to cryopreservation

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In order to protect endangered bird species two main proposals for conservation were formulated*: in situ* (Hanks, 2001), which provides for the protection of habitat to protect species at its internal, and *ex-situ (*Blesbois *et al.,* 2007*),* which relies on breeding and propagation in captivity. In this second strategy, the biotechnology of reproduction, in particular the cryopreservation of semen and artificial insemination, plays a fundamental role because it allows the increase in the number of individuals and the maintenance of genetic variability. The freezing of the semen is a highly specific process and so far it has been studied and partially achieved for most of avian species of economic interest. For the wild ones instead, it is necessary to develop further techniques to be able to expand the use. This paper reports the findings of the first study undertaken to describe the morphology and ultrastructure of spermatozoa of the goshawk, considered "Vulnerable" species in the European Red List, and the results of the first attempt of cryopreservation of its semen. The semen was collected weekly during the breeding season from three specimens of different ages, reared with the cooperative method. This technique requires that the animals, kept in close contact with the man, socialize and express sexual behavior toward the human being until to attempt to mate with it (Gee *et al.*, 2004). The collected samples were examined to assess various parameters such as semen volume and sperm concentration, ultrastructure, vitality and motility. The samples with the best traits were processed for the cryopreservation adopting a protocol in pellets (Castillo *et al.*, 2011). The spermatozoon is a long and slender cell, consisting of a sickle-shaped head (Figs. 1, 2) and a long tail (Fig. 1). In the apical part of the head, the acrosomal complex consists of an acrosome vesicle and a *perforatorium* with a characteristic thickening at the point where the nucleus is raised to form the nuclear *rostrum* (Figs. 4, 6, 7, 8). The transitional region between the midpiece and principal piece of the tail is defined by the *annulus* (Figs. 3, 5). Characteristic structures of the tail are a manchette of four mitochondria in the midpiece (Figs. 5, 9, 10) and nine dense fibers in the principal piece of the tail (Fig. 10) surrounding the classic "9+2" model of axoneme (Fig. 11). Regarding the qualitative characteristics of fresh semen, sperm concentration ranged from a minimum value of $2.3 \times 10^5$ to a maximum value of $1.0 \times 10^6$ cell/ml, more than 80% of spermatozoa were morphologically normal, mobile and vital; after the entire cryopreservation process about 20% were motile and 35% were viable. The morphological observations confirm the similarity of the goshawk sperm structure to the one of non-passerine birds. This study, although based on a small number of samples, enabled us to test the feasibility of hawk semen to be cryopreserved. This promising outcome could be a starting point to develop a suitable freezing/thawing protocol for this species and further evaluation *in vivo* of the thawed semen.

*Acknowledgements*
*The authors thank Daniele Arcioni for his availability and cooperation during the study phase of the captive breeding, and Enzo Arcioni for having made available the knowledge gained during his experience of years on breeding of birds of prey.*

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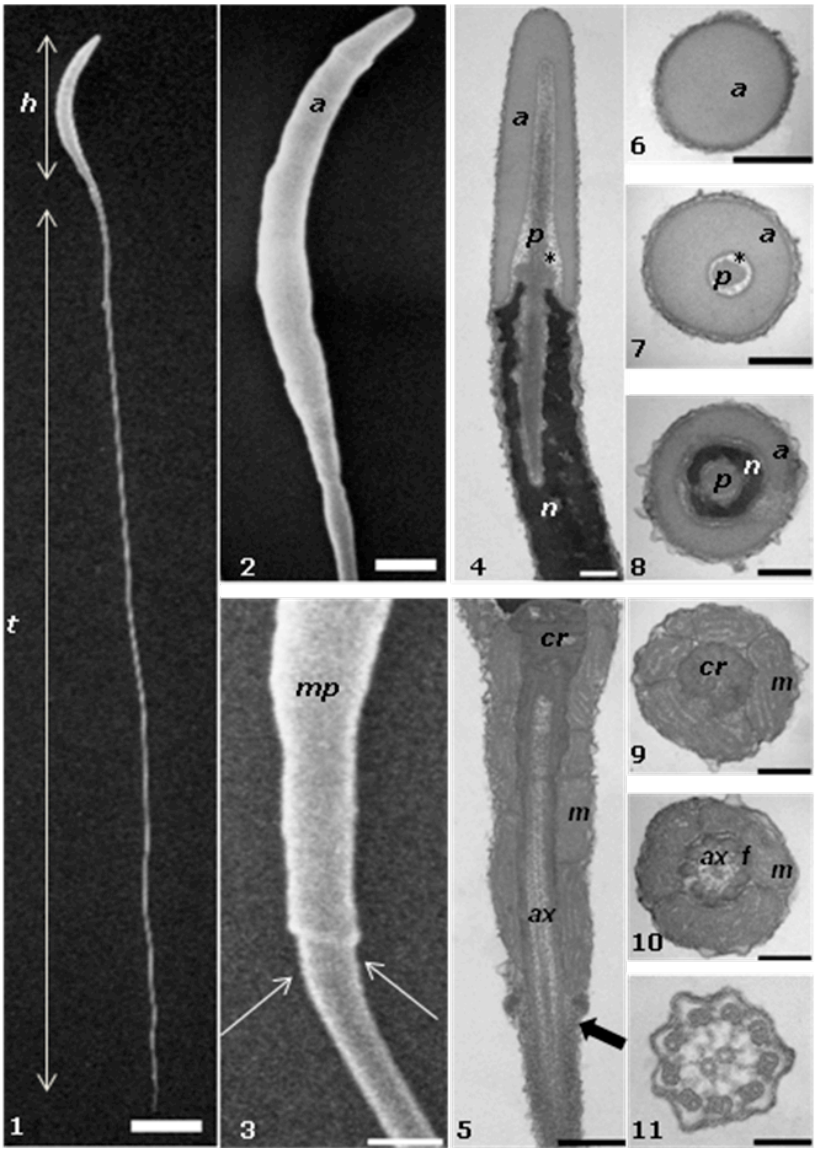
Figs. 1-3 SEM images of a goshawk spermatozoon.
Fig. 1: general view of spermatozoon.
Fig. 2: sperm head (scale bar = 1 µm).
Fig. 3: detail of the midpiece and the principal piece of sperm tail (scale bar = 500 nm).
Figs 4-5: TEM micrographs of longitudinal sections of the sperm.
Fig. 4: acrosome and post-acrosomal regions (scale bar = 250 nm).
Fig. 5: midpiece and principal piece of the tail defined by *annulus* (scale bar = 500 nm).
Figs. 6-8: transversal sections of the acrosomal region at different levels from apical to basal part (scale bars = 250 nm).
Figs 9-10: transversal sections of the midpiece (scale bar = 250 nm).
Fig. 11: transversal section of the terminal part of the tail with "9 + 2" model axoneme (scale bar = 100 nm).

*h:* head; *t:* tail*; a:* acrosome*; mp:* midpiece; *p:perforatorium; asterisk: subacrosomal material; n*: nucleus; arrow: *annulus*; *cr*: centriolar region; *m*: mitochondria; *f*: dense fibers.



## Morphological and ultrastructural characteristics of pheasant spermatozoa

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Among the poultry species of economic interest as food source, the normal cytomorphology and the fine structure of the spermatozoon has been determined in chicken, turkey, quail, guinea fowl, ostrich and drake. Similarities, but also evident differences, have been observed in the male gamete from these species as well as between the three domestic exponents of the same family (Phasianidae). The common pheasant (*Phasianus colchicus mongolicus*) is representative of another important genus of this family. Nevertheless, little information exists regarding the morphometry of its spermatozoon (Ducci *et al.*, 1998; Immler *et al.*, 2007). Besides, there are no studies reporting its three-dimensional and ultrastructural features. Therefore, aim of this paper was to obtain a close-up view of the common pheasant spermatozoon morphology by the use of the scanning (SEM) and the transmission (TEM) electron microscopes.

Spermatozoa examined by SEM or TEM were from semen samples collected by dorso-abdominal massage method and pooled from 12 males all from their first reproductive season. For electron microscopy analysis, conventional procedures for sample preparation were applied.

The pheasant spermatozoon like other avian species, is a filiform, long (77.74 ± 2.78 µm) and slender (0.62 ± 0.07 µm) cell (Figs. 1, 2). It consists of a head of 10.87 ± 0.69 µm followed by a short midpiece (Fig. 3) and a long tail characterized by a tapered end (Fig. 1A). The sperm head is composed by the acrosomal region (mean length of 2.20 ± 0.10 µm; Figs. 1, 2, 4, 7, 8, 9) and the nucleus (Figs. 1, 2, 4, 5, 9, 10). At the anterior end of the nucleus, the acrosomal cap overlaps a prominent *perforatorium* (Figs. 4, 8, 9), inserted in an endonuclear channel. A granular acrosomal material (Figs. 4, 8) surrounds the *perforatorium*. The tail (total mean length 61.35 ± 3.28 µm) is composed of an intermediate, a main and a terminal part. The midpiece (mean length of 4.23 ± 0.35 µm), the transitional region between the sperm head and the principal piece of the tail, is defined for the *annulus* (Fig. 3) at its distal end. The midpiece is characterized by a manchette of four mitochondria (Figs. 5, 6, 11). The longitudinal section of the midpiece shows the mitochondrial sheath and six rows of mitochondria (Fig. 11). The axoneme originates from the centriolar region (Figs. 5-11) and it has the classic "9+2" model, with 9 doublets of external and a pair of central microtubules (Fig. 12).

Morphometric data obtained from this study are slightly dissimilar from those reported in literature for the *Phasianus colchicus* and for *Phasianus versicolor*. The use of the electron microscopy technology permitted to obtain more precise measurements, thus spermatozoa are reported slightly smaller. Within the *Phasianidae* family, the general shape of pheasant spermatozoon is most like that of chicken and turkey.

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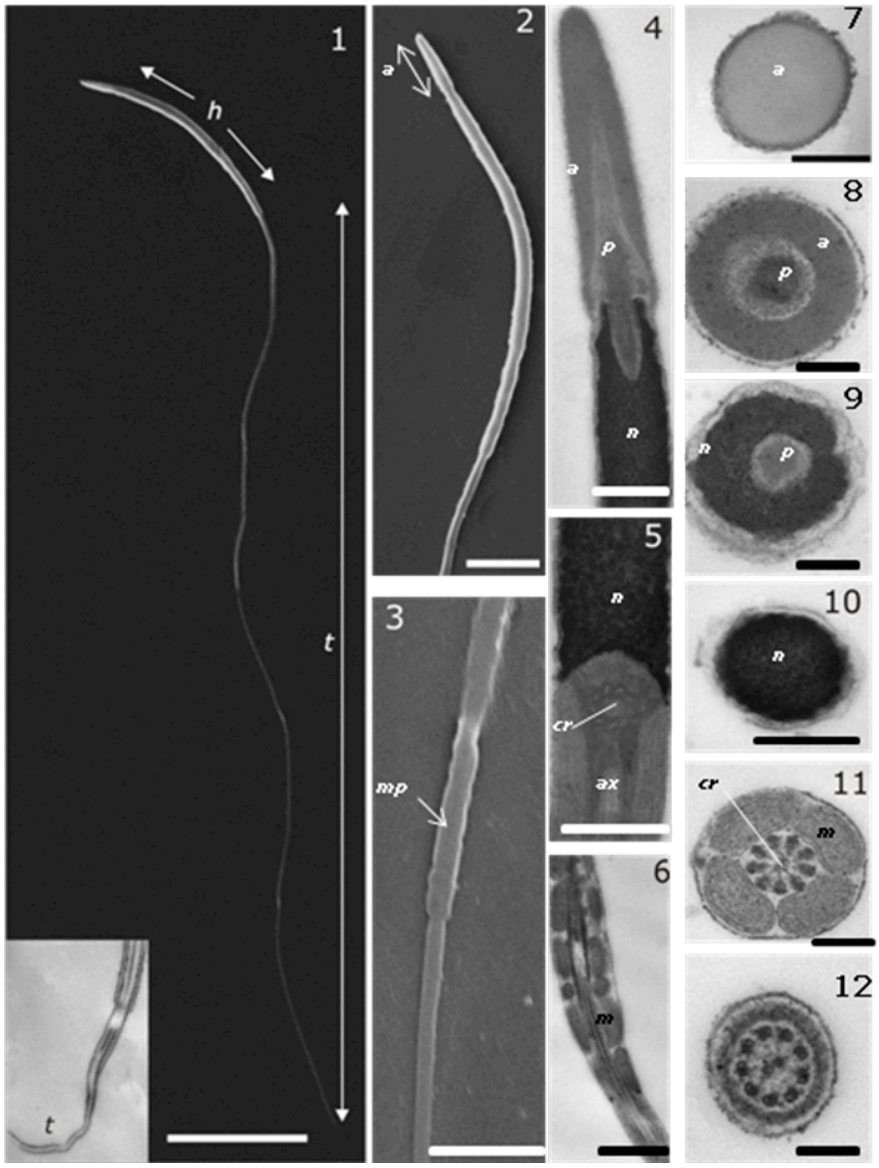


Figs. 1-3 SEM images of the common pheasant spermatozoon.
Fig. 1: general view of spermatozoon (scale bar = 10 µm), (1A) TEM micrograph of the terminal part of the tail.
Fig. 2: acrosome and sperm head (scale bar = 1.5 µm).
Fig. 3: part of the tail with the midpiece (scale bar = 1 µm).
Figs. 4-6: TEM images of longitudinal sections of the acrosomal region (Fig. 4: scale bar = 0.5 µm), the centriolar region (Fig. 5: scale bar = 500 nm) and a portion of the midpiece and the principal piece of the tail (Fig. 6: scale bar = 1 µm).
Figs. 7-9: transversal sections of the acrosomal region (scale bars = 200 nm).
Fig. 10: transversal section of the nucleus (scale bar = 500 nm).
Figs. 11-12: transversal sections of the midpiece (Fig. 11) and the principal piece with "9 + 2" model axoneme (Fig. 12) (scale bars = 200 nm).

*h:* head; *t:* tail*; a:* acrosome; *mp:* midpiece; *p:* perforatorium; *n*: nucleus; arrow: *annulus; cr*: centriolar region; *m*: mitochondria; *ax*: axoneme

## Proteins secreted by adipose tissue affect hen ovarian cells

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Introduction
Adipose tissue has been initially described as an energy reservoir. However, increasing evidences support that adipose tissue may also serve as an endocrine organ by secreting many proteins called adipokines involved in multiple physiological processes and pathophysiology of obesity-related metabolic disorders. Chemerin and visfatin are two novel adipokines mainly secreted by visceral adipose tissue. Chemerin or RARRES 2 (Retinoic Acid Receptor Responder protein 2) acts through two receptors, ChemR23 or CMKLR1 (Chemokine-like receptor 1) and CCRL2 (chemokine (C-C motif) receptor like 2). The receptor of visfatin is still unknown. In chicken, chemerin has never been studied whatever the tissue. At the opposite, there is evidence showing that visfatin is expressed in various tissues of chicken and it is likely involved in the regulation of muscle growth and metabolism (Krzysik-Walker *et al*., 2008), food intake, and testicular functions (Cline *et al*., 2008; Ocón-Grove *et al*., 2010). However, the expression and the role of visfatin in hen ovarian cells are totally unknown.

Methods and Results
Here, we have investigated the presence and the function of visfatin and chemerin in different ovarian cells in the chicken *Gallus gallus* species. The messengers and proteins of these two adipokines as well as those of CMKLR1 and CCRL2 have been found in the different ovarian cells (theca and granulosa cells of F1, F2, F3/4 follicles) by RT-PCR and Western-Blot, respectively. By qRT-PCR we observed that the expression of these adipokines and their receptors varied in theca and granulosa cells during the follicular maturation. The addition of recombinant visfatin in primary granulosa cell inhibits basal and IGF-1 induced progesterone secretion whatever the follicular stage. This was associated to an inhibition of the MAPK ERK½ signaling pathway from 30 minutes of visfatin incubation whereas the AKT signaling pathway was stimulated after one hour of stimulation. In order to better identify connection between hormones secreted by adipose tissue and their impact on ovarian tissue, we have developed a dynamic co-culture of adipocytes and granulosa cells allowing to simulate the vascular flow between each tissue as in living organisms. Hence, the flow enriched the culture medium by adipocytes secretion (fatty acids, hormones) and irrigates granulosa cells with secreted factors.

Conclusion
In conclusion, chemerin and visfatin are present in chicken ovarian cells. Visfatin is active and can modulate progesterone secretion in hen primary granulosa cells. In parallel, we study the impact of adipocytes secretion (fatty acids and hormones) to granulosa cells in a dynamic environment controlled and more precisely consequences of in vitro proliferation, lipid metabolism and ovarian cell maturation.

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## Freezability of chicken immature germ cells in their seminiferous tubules

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Introduction
Male germ cell production takes place within the avascular seminiferous tubules of the testis. Specialized Sertoli cells are tighly linked between them and create a "blood-testis" barrier that restricts passage of substances from the systemic circulation and creates a specific environment for germ cells. In 1993, the Steinberger team published a two-compartment system for culture of rat testicular cells which can simulate, to some degree, the normal physiologic conditions. It could also restrict the passage of substances between the two compartments, in analogy to the blood-testis barrier.

One of the goals of a part of a French national project (post-Grenelle Environment Forum), was to establish an in vitro culture system for germ cells and somatic cells from chicken testis. We have developed a similar model as the Steinberger model (Steinberger *et a*l., 1993 ; Staub *et al*., 2000) and made improvements in order to culture frozen/thawed chicken testicular cells. We have evaluated the sensitivity against oestrogen-like substances also known to be endocrine disruptors such as the ethinylestradiol (EE2). The chicken culture model was compared to previous studies using rodent and human system.

Methods
Seminiferous cells were prepared from immature chickens (6 weeks-old). Seminiferous tubules at this stage contained mainly mitotic and meiotic germ cells (spermatogonia and spermatocytes). These cells were cultured in inserts at the apical compartment for 96 hours or frozen to culture later.

Results
In our culture system, we have shown that 0.6 ng / ml EE2 (about 15 fold the concentration measured in output wastewater; [<0.001 to 0.04 ng/ml], (Snyder *et al*., 2001) is enough to increase by nearly 40% the percentage of immature germ cells in culture. A 20% increase in the population of germ cells was observed at 0.1 ng/ml EE2 (2-fold higher compared to the concentration measured at the output waste water).
We have shown that frozen and thawed seminiferous tubules are able to proliferate (2-fold lower compared to fresh cells) and were still sensitive to reproductive hormones (follicle stimulating hormone). However, at the beginning of culture, there were 3 times more dead cells with frozen/thawed cells when compared to fresh cells. The percentage of post-thawing immature germ cells incorporating BrDU was 2.5 fold lower than fresh germ cells. Despite the reduction in the number of alive and proliferative cells after a freeze/thaw step, seminiferous cells maintain their sensitivity to EE2 at a similar level as fresh cells.

Conclusion
The rapidity of response and high sensitivity of avian cell culture is similar or even greater than those observed in rat. The development of freezing protocols allows the very promising development of test kits (a cryotube containing chicken germ and somatic testicular cells, a plate with inserts). Therefore, in this model, a "non-expert" technician could assess the harmfulness of chemicals (industrial / drugs / contamination) on fertility criteria of birds (wild-birds and poultry) in a highly reproducible manner.

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## The effects of microclimate on day-old chicks during transportation from hatchery to a rearing farm

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Quality is an important parameter which determines the growth and performance of day-old broiler chick (DOC) throughout its lifespan. There are several factors known to reduce the quality of DOC, climatic factors being one. It is a common practice in the Netherlands to transport DOC's from the hatcheries to the rearing farms. The outside climatic factors vary during the transport, thus being able to control and maintain a stable internal climatic environment is important. This research study concentrates on changes in the microenvironment and its effect on DOC's during road transportation. The research hypothesis is that during transportation the microclimate temperature and relative humidity does not move outside the lower ($30^{o}$ C) and upper ($<36^{o}$ C) critical temperature thresholds, and so the DOC's do not endure thermal stress during road transportation. The objective of my research is to study the thermoregulatory effects due to variation in temperature and humidity in the chick. Therefore, in this study, the changes in the core body temperature, heat loss or gain, mortality and behavioral responses in the chicks will be analyzed. The expectation is that the results of this study will show that DOC quality and welfare is not compromised during road transportation.

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## Chicken embryo as a model in studies on the influence of electromagnetic fields on the embryogenesis process.

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During the course of evolution, living organisms have developed in the constant presence of natural electromagnetic fields (EMF). Today, artificial electromagnetic fields which are a consequence of human activities begin to play a considerable role in shaping the Earth's electromagnetic environment. Due to the rate and specific characteristics of development and the well-understood process of embryogenesis, chick embryo is frequently used as a model in different kinds of biological research, including studies investigating the effect of EMF on living organisms. Therefore, this study attempted to determine the effects of the 1800 MHz and 50 Hz electromagnetic field on chicken embryogenesis. Hatching eggs of Ross 308 line (n = 150) were used in the experiment. The eggs were randomly divided into three equal groups and incubated under standard conditions in a laboratory incubator. Group I was incubated in control conditions, i.e. in the incubator without an EM field generator. Group II - chicken embryos were subjected to continuous exposure to electromagnetic fields 50 Hz for all of the period of incubation. Group III - chicken embryos were exposed to 1800 MHz electromagnetic fields 10 times per day for 4 min throughout the whole period of incubation. The mortality of embryos and the time-course of hatching were determined as well as thyroxine ($T_4$) and triiodothyronine ($T_3$) concentrations in the plasma of newly-hatched chicks. The results showed that exposure of chicken embryos to EMF markedly increased $T_4$ and $T_3$ levels in group II, while in group III hormone concentrations significantly decreased. In both groups EMF frequency used significantly accelerated the time of hatching. In II group (50 Hz) it was 7,2 hours and in III group (1800 MHz) it was 24 hours earlier as compared to the control group. However, percent of hatched chicks in experimental groups with the additional EMF (87.4% and 86.9% for Group II and III, respectively ) was close to the control group (87.2%).

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## Effect of incubation temperature on antioxidant enzyme activities of broilers exposed to cooler rearing temperatures

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This experiment was designed to investigate the effect of cooler incubation temperature on antioxidant enzyme activities of broilers exposed to cooler rearing temperatures. A total of 900 eggs were obtained from a Ross 308 broiler breeder flock. Eggs were randomly assigned to two incubation temperature treatments: eggs were incubated at 37.6℃ (Inc-control) or exposed to 36.6℃ for 6 h each day from 10 to 18 d of incubation (Inc-cooler). On day of hatch, chicks from each incubation temperature were randomly distributed to 18 environmentally controlled pens and divided into three treatments: *Control temperature* (**Cont**): standard brooding and rearing temperatures were use. *Cooler temperature* (**CT**): standard brooding temperatures and from 22 to 24 d of rearing period chicks were exposed to 17±1°C. *Conditioning+cooler temperature* (**Cond+CT**): standard brooding temperatures, except chicks were exposed to 17±1°C for 6 h on d 5 and from 22 to 24 d of rearing period chicks were exposed to 17±1°C. At 23 d (one d after cold stress), 12 chicks were randomly selected from each group and liver malondialdehyde (MDA), catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) levels were determined.

There was no effect of incubation temperature on liver MDA, SOD and CAT activities of broilers on d 23. Cold stress elevated the level of lipid peroxidation as reflected by higher MDA for CT broilers than Cont, however, liver MDA activation of Cond+CT was similar to Cont. Lower SOD activities of broilers from both Cond+CT and CT suggested that hepatic damage that was due to lipid peroxidation was aggravated. Higher CAT activities obtained for CT compare to Cont and Cond+CT may be considered as a protective mechanism against free radical production and lipid peroxidation.

A significant incubation temperature X rearing temperature interaction for GSH-Px and GR levels showed that under CT conditions, there was a significant increase in the liver GSH-Px and GR levels of broilers from eggs Inc-cooler compare to broilers from eggs Inc-control. These results indicated that Inc-cooler inhibited cold induced oxidative stress by increasing GSH-Px and GR activities in liver of broilers under CT.

## Session: Workshop - Egg quality

(in order of presentation, speaker underlined)

### Ron Board – a lifetime's contribution to egg science and so much more besides.

**Nick Sparks**

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Professor Ronald G. Board, who died on the 12 March 2013, will be remembered by the many with whom he collaborated, or those who had the privilege to be trained by him, as a man with an infectious passion for his science. Although he will be known by the IFRG for his many papers on studies related to eggshells, Ron started his research career as a microbiologist. In the mid-sixties, having obtained his PhD from the University of Edinburgh, he went on to study in America and to publish what were to become key papers on the factors affecting the course of the microbial infection in birds' eggs. Returning from America, Ron secured a post at the University of Bath, becoming one of its original academics. Ron was quick to identify the potential of materials science and in particular, the then rapidly developing field of electron microscopy, as a way of advancing not only the understanding of the egg's antimicrobial defence systems but also the mechanisms that made an eggshell so perfectly suited as a vessel for a developing embryo. This interest led directly, in 1976, to Ron organising the first meeting of what was to become the Incubation Research Group (now the Incubation and Fertility Research Group (WPSA Working group 6 (Reproduction))). Working with postgraduate students such as Steve Tullett and Nick French, Ron collaborated with material scientists to describe the biomineralisation of the eggshell and to relate the formation of structures within and on the shell, such as the pore canals and the cuticle, to their biological function in the context of both the naturally and the artificially incubated egg. It was this research that led to collaborations with scientists such as Herman Rahn and his colleagues. Ron was a gifted and exceptional scientist, who was recognised internationally as a meat and egg microbiologist as well as an expert on eggshell structure. More importantly perhaps, was his ability to get the best from his students and enthuse them in turn with a passion for the subjects which he made his life's work.

### The potential of novel sensor technologies and data processing techniques for noninvasive egg quality assessment

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The development of fast, accurate and noninvasive sensor technologies has spurred during the last decades, and many of them offer great potential to assess the quality of eggs. This talk aims at reviewing those techniques that are successfully applied to the different aspects of egg quality, such as shell and albumen quality. Special attention will be paid on their application to incubation eggs where parameters of interest are (amongst others) the growth and the sex of the embryo. Given the large amount of data that are generated by modern sensors, also the importance of a good data processing strategy will be briefly demonstrated.

## Quality criteria of layer eggs with focus on optimum incubation results

**A. Förster, D. Cavero, and R. Schulte-Drüggelte**

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Breeding programs for commercial layers pay attention to a big range of egg quality traits in order to optimise the revenue of egg producers and to satisfy the requirements of table egg consumers. However, layer eggs are not only used for human consumption, but also for hatching chicks. Therefore, a good hatchability and a reduced spread of hatch are important selection goals in a breeding program for improving the number and the quality of chicks produced by parent stock. Furthermore, the impact of egg quality traits on hatchability is also measured and taken into account (Förster and Flock, 1996).

There are many factors that affect hatchability, for example the environmental conditions during egg collection and egg handling and specially the configuration of the incubation process will have a great impact on the hatchability results. Furthermore, egg characteristics greatly influence the process of incubation and are responsible for its success (Narushin and Romanov, 2002). While some egg quality parameter affecting hatchability can hardly be influenced by the management of a breeder flock, others can. The aim of this paper is to review the latter and derive general recommendations for layer breeder management with special focus on egg weight and egg shell quality.

Table 1 shows the genetic correlations of egg weight and shell strength and with fertility[1], hatch of set eggs (HoS) and hatch of viable embryos (HoV). A predisposition for high egg weight is related to low hatchability whereas one for high shell strength is related to high hatchability. There is not only a genetic correlation between egg weight and hatchability, but also a phenotypic one. Eggs between 55 and 60 grams do hatch better than heavier eggs, but also very light eggs show reduced hatchability.

**Table 1: Estimated genetic correlations between reproductive traits and egg quality traits in two white egg layer strains**

| Trait | Egg weight | Shell strength |
|---|---|---|
| **Line C** | | |
| **Fertility** | -0.08 | +0.06 |
| **HoS** | -0.43 | +0.19 |
| **HoV** | -0.46 | +0.22 |
| **Line D** | | |
| **Fertility** | -0.18 | +0.10 |
| **HoS** | -0.48 | +0.27 |
| **HoV** | -0.52 | +0.29 |

*Adapted from Cavero et al. (2011)*

Table 2 shows the results of a hatchability test performed with two brown egg laying strains. Before egg setting, different egg quality traits were measured including the dynamic stiffness of the egg shell. Dynamic stiffness allows the identification of eggs with very small hairline cracks, which are not visible by eye. Although the egg weight loss of the cracked eggs during incubation was not excessively high, the hatchability was clearly reduced.

The test results suggest that egg shell quality is crucial for optimum incubation results. The management and feeding of layer breeders should be designed to support shell quality by an adequate calcium supply and a healthy liver. In order to achieve optimum hatchability the average egg weight below 60 grams should be targeted.

[1] Fertility was determined by candling. Thus, no difference was made between true infertile eggs and early embryonic mortality.

Special attention should be also paid to all hatching egg handling procedures to avoid cracked eggs.

**Table 2: Early + mid embryo mortality (%), Egg weight loss during incubation (%) and Hatch of viable embryos (%) of cracked and normal eggs of two brown egg layer strains**

| | Line 1 | | Line 2 | |
|---|---|---|---|---|
| | **Normal (95.7%)** | **Crack (4.3%)** | **Normal (97.9%)** | **Crack (2.1%)** |
| **Embryo mortality (< day 18)** | 6.0% | 12.5% | 7.5% | 32% |
| **Hatch of viable embryos** | 77.7% | 57.0% | 85.1% | 63.2% |
| **Egg weight loss until day 15** | 8.8% | 10.8% | 9.8% | 12.7% |

** The differences between Normal and Crack eggs were significant (P<0.001) for the three presented traits in both lines. The procedures GENMOD (for embryo mortality and Hatch of Viable) and GLM (for egg weight loss) were used for the analysis (SAS 9.3).*

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## Egg Quality and Hatchability in Broiler Breeders

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Egg quality is important for hatchability in broiler parent stock, as it is in strains producing table eggs (Narushin and Romanov, 2002). However, for broiler breeders, there has not been the long term selection for egg shell strength and internal quality necessary for eggs which are to be transported and stored as part of the human food chain. The selection focus for broiler breeders has been more on the quality of the egg as an incubation chamber. Specific gravity (correlated with shell thickness), egg weight loss during incubation (porosity) and albumen thickness all have moderate heritability and a clear genetic correlation with hatchability (Wolc *et al.* 2010). In a broiler breeding programme, the aim is to keep the egg quality traits within specified limits, rather than push for major change.
In commercial broiler production systems, appropriate day-to-day management of the breeding stock on the farm, of egg selection and handling process during egg transport and storage and of incubation conditions are all needed to achieve optimal hatchability and chick quality. The timing of feed and light changes to control sexual maturity, mineral balance, feed allowances and feeding times, and freedom from respiratory disease are all important in producing eggs with sufficient shell quality for structural integrity and for incubation. One

difficulty when trying to resolve problems with egg quality in broiler breeder flocks is that there is very rarely any routine measurement of egg quality traits in commercial breeder operations. Feedback about flock problems tends to be based on numbers of rejected eggs or a subjective visual assessment.

While most broiler parent stock producers recognise that eggshell thickness is important in order to avoid cracks and breakage of the eggs, the impact of poor shell quality on the water balance of the eggs during incubation is often neglected. This is unfortunate, because much of the adverse impact of thin egg shells on hatch and chick quality can be mitigated by adjusting humidity levels in the setter.

The relatively porous shells of broiler breeder eggs help support a higher embryonic metabolic rate than is seen in commercial layer lines (Janke *et al* 2004). Managed correctly, modern incubators have sufficient ventilation and cooling capacity to keep broiler embryos within the optimal temperature range. However, broiler hatching eggs will place more demands on the cooling system of the hatcher and tend to need less incubation time than similarly sized layer hatching eggs.

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