



INCUBATION AND FERTILITY RESEARCH GROUP

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Increasing Chicken Eggs Hatchability by Matching

Incubator Humidity to Eggshell Conductance

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ABSTRACT

To achieve maximal hatchability and chick quality, each egg should lose an optimal amount of its mass during incubation as water vapour. Each eggshell has its own porosity because of a fixed conductance for water vapour. According to French and Tullett (1991), eggshell conductance between eggs is highly variable. When incubating all eggs at the same incubator humidity, the eggs with mean eggshell conductance will lose their optimal mass. However, a lot of eggs will lose more or less mass than this optimum because incubator humidity does not match with their conductance for optimal mass loss, resulting in a decreased hatchability.

To address these questions experimentally, three experiments with each 1,800 Hybro[®] eggs (Euribrid, Aarschot, Belgium) were carried out. Before incubation started, all eggs were weighed. During the first four days all eggs were incubated in three incubators (PasReform[®], Zeddum, The Netherlands) at the same conditions of 37.8°C and 55% relative humidity. On the fourth day of incubation, all eggs were weighed again and the percentage loss of mass was calculated as an indicator of eggshell conductance.

Afterwards all eggs were divided in three groups according to eggshell conductance (low, mean and high). 200 eggs of each eggshell conductance group were taken together and those three groups of 600 eggs, each composed of the three conductance groups were incubated in three incubators with different humidity (45%, 55% and 70%) but the same temperature (37.8°C).

On the 18th day of incubation, all eggs were weighed again to calculate their mass loss during incubation and transferred to the hatching baskets. From day 18 onwards, the relative humidity and the temperature was the same for all eggs (37.8°C and 55% RH). After hatching, the non-hatched eggs were collected and examined for fertility.

The results show a different mass loss during incubation in the groups of eggs, depending on their eggshell conductance and the incubator-humidity. For the eggs with high and medium eggshell-porosity, the groups of eggs with an approximate optimal loss of mass had an elevated hatchability in contrast with the groups of eggs with the low eggshell conductance, indicating that other factors than water loss may have become critical for maximal hatchability.

As a conclusion, matching the incubator humidity to eggshell conductance may increase hatchability in eggs with a mean to high eggshell conductance, but this is not possible in eggs with low-conductance eggshells. In the latter eggs, it can be hypothesised that the O₂/CO₂-exchange is the limiting factor rather than the water balance of the growing embryo.

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Genetic Segregation of Growth Rate, Fertility and Sperm Binding in Roosters

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ABSTRACT

The poultry industry has undergone remarkable changes over the last 50 years. These range from application of genetic selection plus crossing-schemes to attainment of production objectives (e.g. Hartman, 1988) to vertical integration of the poultry companies. Furthermore, it has been demonstrated for several species that selection for large body size is negatively associated with reproduction traits (Dunnington and Siegel, 1985). Given the importance placed on meat yield in today's market, it is important to note the negative genetic correlation between breast muscle size and fertility in chickens. This correlation is strong, yet the heritability of breast muscle yield is moderate to high and the heritability of fertility is very low (McDaniel and Craig, 1959; Siegel, 1962). I hypothesised that the negative genetic correlation between breast muscle size and fertility was driven by gene linkage.

I have shown (Barbato, 1999) with lines that were divergently selected for high and low exponential growth rate to 14 days of age (EGR_{14} ; lines 14H or 14L) also diverged significantly in fertility. After only five generations of selection, fertility of the 14L was less than half that of the 14H line. This decline took place precipitously during selection, rather than as a regular, linear decline. This suggested influence of only a few genes. At least a part of the difference in fertility between these lines was due to inability of the majority of sperm to bind to the egg membrane (Barbato *et al.*, 1998). Furthermore, both sperm binding and fertility exhibited marked heterosis (Barbato, 1999), while body and carcass component weights at a fixed age were described almost entirely by additive gene action (Barbato, 1991, 1992b).

To reconcile these observations and test the above hypothesis, a series of F_1 crosses were made between the 14H and 14L lines in the 10th generation of selection (S_{10} ; as in Barbato, 1999). When they reached sexual maturity, the crossing scheme was completed with an F_2 generation using males selected using a two-tail strategy involving fastest and slowest growing 3% of males for each of three growth increments. These were 0–14 days of age (EGR_{14}), 14–42 days of age ($EGR_{14/42}$), and 0–42 days of age (EGR_{42}). When the 48 males reached sexual maturity, semen was collected twice weekly for three months, after which was semen collected and evaluated for sperm binding, using Assay #3 (Barbato *et al.*, 1998), and fertility by insemination of eight unrelated White Leghorn hens per male. For fertility testing, 5×10^7 sperm in 50 μ l were inseminated twice during the first week and once weekly for 3 weeks thereafter. Eggs were collected, set and hatched weekly for the final 3-week period (144–175 eggs/male).

The average EGR_{14} for the high and low tails of the F_2 distribution were 0.1071 ± 0.0041 and 0.0048 ± 0.0067 ln(g) per day ($P < 0.001$). Curiously, while growth rate differed between the two tails of the F_2 generation, there was no difference in percentage of sperm bound or fertility (means were 7.5% and 71%, respectively). However, there clearly were sub-fertile males (*i.e.* $< \frac{1}{2}$ the mean fertility of the entire group) in the F_2 populations. A correlation matrix was constructed using all F_2 individuals ($N = 47$). For % sperm bound and fertility, the r -value was 0.75, but all correlations between sperm binding assay and body weight at 14 or 42 days of age were less than -0.11 (Table 1). Importantly, there was no significant correlation between growth rate during any age increment and fertility-related traits (values less than -0.20). As expected, values for growth traits were significantly correlated (Table 1).

Table 1 Correlation matrix among sperm binding (% sperm bound), fertility (chicks/eggs set * 100%), body weight at 14 and 42 days of age, and exponential growth rate to 14 or 42 days and between 14 and 42 days of age (EGR_{14} , EGR_{42} , $EGR_{14/42}$). All values based on data from 47 roosters

	Sperm binding	Fertility	14 day weight	42 day weight	EGR_{14}	EGR_{42}
Fertility	0.75**					
14 day weight	-0.07	-0.10				
42 d weight	-0.01	-0.05	0.72**			
EGR_{14}	-0.12	-0.19	0.96**	0.72**		
EGR_{42}	-0.11	-0.18	0.69**	0.91**	0.76**	
$EGR_{14/42}$	0.08	0.13	-0.83**	-0.23*	-0.82**	-0.26*

** $P < 0.01$; * $P < 0.05$.

Previously I have suggested (Barbato 1992a, 1999; Barbato *et al.*, 1998) that reduced male fertility was associated with selection for slow EGR₁₄ (and, indirectly, for fast EGR_{14/42}). Based on data summarised herein, these conclusions must now be considered suspect. However, it must also be kept in mind that just as total body growth is non-linear, so is testis growth and maturation. It is possible that genetic differences in fertility were masked by environmental and/or developmental variation, since we only measured fertility at a single age.

It was concluded that fertility and EGR₁₄ are not genetically linked. This conclusion is based on the obvious independent segregation of these two traits in the F₂ generation produced from F₁ crosses of the S₁₀ generation of the 14H and 14L lines. Rather, contrary to the hypothesis, the declining fertility of males in the 14L line is due to genetic drift or founder effect in the establishment of the lines.

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Is There any Evolution of Sex Ratio in Duck Embryos during Incubation?

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ABSTRACT

A common observation in mule duck hatcheries is an unbalanced of sex ratio at hatching (approximately 60% of males and 40% of females). The aim of this study was to assess if this imbalance results from a lower number of females at fertilisation or from higher mortality of female embryos.

Our study was conducted in four different crosses: Pekin ducks, Muscovy ducks, mule and reverse mule ducks. The sex of a minimum of 96 embryos was determined at four different stages of incubation (24 hours, 5 days, second grade cull ducklings and after hatching). Molecular methods were required to determine the sex of the earlier embryos (Day 1 and Day 5). We, therefore, used a polymerase chain reaction (PCR)-based sexing protocol which combined both sex specific and a control reaction in a single tube assay. In older embryos and ducklings gender was determined was conducted macroscopically at hatching. "Second grade, cull" ducklings ($N = 150$) were sexed in an industrial hatchery. The relationship between fertility (assessed by candling after 6 days) and sex ratio at hatching was evaluated on the basis of hatchery results ($N = 2,500,000$ male mule ducks).

Table 1 Sex ratio after 1 day and 5 days of incubation and at hatching

	Peking ducks		Muscovy ducks		Mule ducks		Reverse mule ducks	
	<i>N</i>	%Males	<i>N</i>	%Males	<i>N</i>	%Males	<i>N</i>	%Males
Day 1 embryos	100	53.00	99	46.46	100	55.00	96	47.92
Day 5 embryos	101	55.45	96	48.96	91	49.40	104	55.77
Second grade, cull ducklings	—	—	—	—	150	32.00***	—	Not available
Commercial ducklings	815	47.73	753	49.00	297	58.92**	543	55.43*

Significant departures from 50%: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; All other values were not significant.

Sex ratio was not significantly modified during early embryonic development (day 1 or day 5) irrespective of the breeding cross (Table 1). Our results also confirm the statistically significant modification of the theoretical sex ratio expected at hatch in mule ducks. Only 32% of males were obtained from the 150 second grade, cull mule ducklings. Moreover, as fertility decreases, the percentage of hatched males increases (Figure 1). By contrast, the percentage of males calculated on the basis of fertile eggs is quite constant between 41.84 and 44%. We can conclude that between 83.68 and 88% of the males (alive after one week of incubation) are able to hatch. This result is the same as that obtained in other commercial ducks strains. The percentage of hatched females calculated on the basis of fertile eggs varies between 15 and 26%. This result is very much lower than those obtained in other commercial ducks strains. We can, therefore, conclude that the skewed sex ratio is due to an increase in female late mortality. The role of the W chromosome is still unclear, but we can speculate that the presence of only one copy of the Z chromosome into the female karyotype perhaps makes them more delicate.

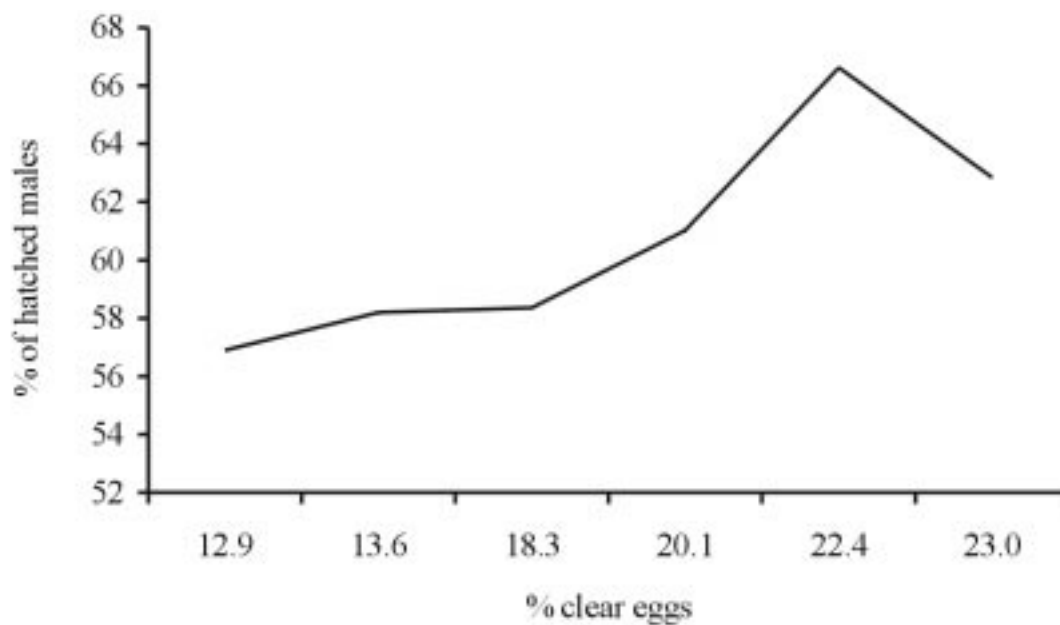


Fig. 1 Sex ratio according to the fertility (assessed by candling after 6 days of incubation). The total effective sample size is 2,500,000 males ducklings.

Molecular and Morphological Differentiation of the Chick

Blastoderm from Different Breeds

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ABSTRACT

The aim of this project is to find embryonic parameters to predict chick quality with respect to the development and functionality of the digestive tract. The underlying idea is the embryonic origin of post-natal health as described for human and domestic animals (Barker, 1995; Boerjan *et al.*, 2000). Also in poultry the relation between nutrition, maternal age and incubation conditions and chick performance is widely known. In this project the embryonic development of two broiler lines, respectively sensitive for the malabsorption syndrome (MAS) and resistant to MAS, was compared.

In the project embryos from two broiler lines A (MAS resistant) and B (MAS sensitive) were collected at different time points from the start to 96 hours of incubation. Line A is a pure breeding line of Cornish origin, fast growing, high meat yield. Line B is a pure breeding line of White Plymouth Rock origin, slower growing, lower meat yield. The development of hypoblast and the expression pattern of the homeobox gene goosecoid (*gsc*) was analysed at 0, 8, 12 and 22 hours of incubation. Furthermore, the development of the head, the heart, the wing and limb buds and the tail structures were analysed at 36, 48, 60, 72 and 80 of incubation (Hamburger and Hamilton, 1951). The effects of maternal age (34–42 weeks) and days of storage (1–5 days) on embryonic development were taken into account in the statistical analysis.

The homeobox gene goosecoid has been shown to be a good parameter for anterior–posterior development (Bachvarova *et al.*, 1998; Van de Pavert *et al.*, 2001). In the chick embryo *gsc* is first expressed in the secondary hypoblast at the posterior side of the embryo (Figure 1). As development proceeds the *gsc* positive secondary hypoblast (Figure 2) is replaced by the *gsc* negative endoblast. In the present study it was shown that the primary hypoblast (*gsc* positive) was completely replaced by the secondary hypoblast after 8 hours of incubation. The level of expression in the hypoblast

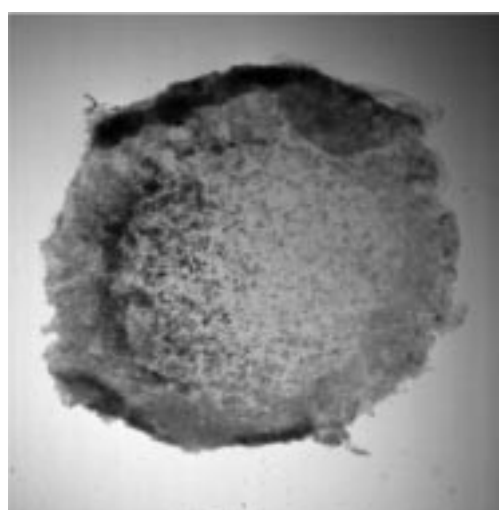


Fig. 1 Whole mount *in-situ* hybridisation of a chick blastoderm (0 h of incubation). Transcripts of the homeobox gene *gsc* are present in the posterior side of the blastoderm (black area at the top of the picture).

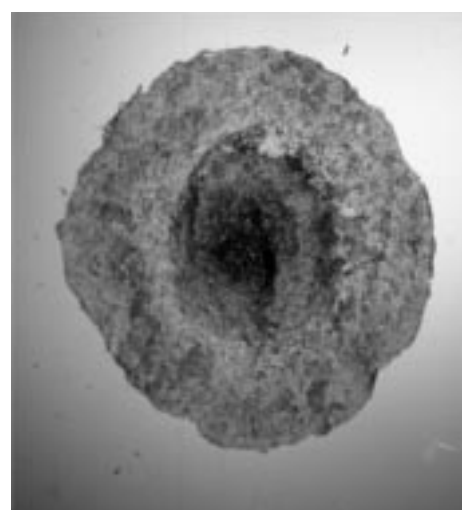


Fig. 2 Whole mount *in-situ* hybridisation of a chick blastoderm (8 h of incubation). Transcripts of the homeobox gene *gsc* are present in the hypoblast (dark area) which has been developed completely.

differed significantly between the lines which might point to differences between the lines in endoderm differentiation. In future studies the expression of other genes which are expressed specifically in the developing digestive tract will be analysed. We also found an interaction between the maternal age and the expression pattern of *gsc* and the development of the hypoblast.

With respect to the morphological development of the different embryonic structures the timing of the development of the tailbud differed between the lines ($P = 0.057$). No differences in timing of the development of the head, the heart, the wing and limb buds was found. However, we found significant effects of egg storage and maternal age on the development of the heart and rotation of the embryo. The implications of this observation for further development and chick quality are not yet known but will be the subject of future research.

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Preliminary Observations on the Electron Microscopy, Histochemistry and Biochemistry of Glycogen in the Sperm Storage Tubules of Fowl Hens

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ABSTRACT

In avian species, fully functional spermatozoa may survive for prolonged periods in specialised portions of the oviduct called sperm storage tubules (SST). To date, however, the basis of how the main biological functions of spermatozoa (e.g. viability, plasma membrane integrity, fertilising potential) are preserved in the SSTs has received only limited attention. Based on a series of observations performed in fowl hens, this study intended to assess how glycogen, present in the cytoplasm of tubular cells in this species, may act as a putative energetic source for spermatozoa in order for them to accomplish fertilisation.

In this preliminary study, we first confirmed the presence of glycogen in the cytoplasm of tubular cells, based on observations conducted both by mean of electron microscopy and histochemistry. From a cellular standpoint, glycogen was observable as quasi-spheric micro-nodules dispatched over the cytoplasm (observed using transmission electron microscopy). Histochemical observations performed in transverse sections of SST indicated a preferential, but non exclusive, accumulation of glycogen along the luminal border of the SST cells.

The quantitative distribution of glycogen in the vaginal, uterovaginal and uterine portions of the oviduct was compared in fowl hens during early (33–35wks; $n_1 = 5$) or late (55–60 wks; $n_2 = 6$) stages of the laying period. Glycogen was hydrolysed (acid hydrolysis) into glucose, itself dosed by enzymatic analysis. Statistical analyses were performed by Fisher test.

The results (Figure 1) showed firstly a significantly higher concentration of glycogen in the SST compared with the vaginal or uterine portions of the oviduct irrespective of age ($P < 0.01$). Secondly, we observed a significantly higher concentration of glycogen in the SSTs of young hens compared with old hens ($P < 0.01$).

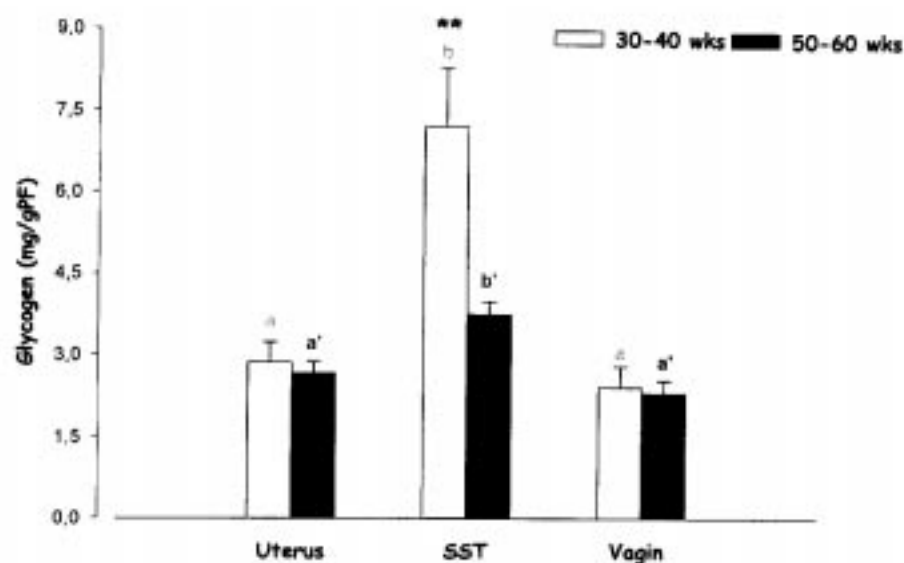


Fig. 1 Age-dependent variations of mean (+ SEM) glycogen concentrations in various oviducal tissues. Significant differences ($P < 0.01$) between oviducal tissues for each age are indicated by different letters. ** indicates significant differences ($P < 0.01$) between ages for each tissue.

These results suggest a possible age-dependent nature of glycogen accumulation (or synthesis?) in the SST of hens. Further research is needed to assess, if any, the various steps of glycogen metabolism into the cytoplasm of tubular cells up to its passage through the cytoplasmic membrane and, possibly, its assimilation by spermatozoa present in the SST lumen.

Lipid Manipulation of Chicken Semen by Dietary Means and its Relation to Fertility: A Review.

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ABSTRACT

Semen is characterised by high levels of long chain polyunsaturated fatty acids (PUFA). Avian and mammalian spermatozoa dramatically differ in their lipid contents as their PUFA belong from, respectively, the $n-6$ and the $n-3$ series of essential fatty acids. The major polyunsaturate in phospholipids of chicken spermatozoa is docosatetraenoic acid (C22:4 $n-6$), which is positively correlated with sperm motility and fertility. The potential of sperm fatty acid dietary manipulation in order to improve male fertility has been studied in the chicken. The effects of diets enriched in $n-3$ and $n-6$ PUFAs have been investigated in different trials considering different oil sources and the rate of oil inclusion. An important "co-factor" of fatty acid manipulation in the feed is the presence of adequate amounts of antioxidant protection. Therefore, vitamin E has been considered as dietary antioxidant and different dietary levels in combination with the experimental oils have been also studied.

The C22:6 $n-3$ and C22:5 $n-3$ content in avian spermatozoa was increased at some extent by supplementing the feed with fish oil (rich in C22:6 $n-3$) and linseed oil (rich in C18:3 $n-3$) respectively. Different dietary levels of C22:6 $n-3$, from 16 to 2% of total fatty acids in total lipids, have been used in few trials to feed chicken breeders. However the

increase in DHA sperm content was always very similar being from 3 to 4 times the control value. The highest proportion (6% of total fatty acids) was recorded in spermatozoa collected from males fed 2% fish oil rich diet (6% C20:5*n*-3 and 4% C22:6*n*-3). The C22:4*n*-6 content was also increased by supplementing the feed with evening primrose oil (rich in C18:3*n*-6) but only if associated with high level of vitamin E (200 mg/kg).

Both *n*-3 and *n*-6 rich diets showed a positive effect on sperm movement during the reproductive period, and an age dependent positive effect on fertility. A significant improvement of forward progressive motility (FPM) was observed in spermatozoa enriched in C22:6*n*-3 (6%) and still with a high level of C22:4*n*-6 (20%) and in spermatozoa enriched in C22:4*n*-6 (26%). Such a sperm fatty acid manipulation was obtained by feeding the males 2% fish and evening primrose oil rich diet respectively. Furthermore, a concomitant increase of vitamin E content in the feed from 40 to 200 mg/kg was associated with a further additive improvement of FPM. The high dietary vitamin E content showed also a positive specific effect on the proportion of motile sperm in the ejaculate whatever the fatty acid composition of the cells. Spermatozoa enriched in 22:5*n*-3, or 22:6*n*-3 or 22:4*n*-6 performed significantly higher fertility values following artificial insemination compared to control spermatozoa; however, such a positive effect was recorded on 39 and 41 weeks of age and was not more present on later ages during the reproductive period.

In conclusion, specific fatty acid and antioxidant requirements are suggested for male breeders to improve the reproductive performance.

Assessment of pH, Osmolarity, Motility and Viability of Houbara Bustard Semen (*Chlamydotis undulata undulata*)

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ABSTRACT

The Houbara bustard (*Chlamydotis undulata undulata*) is considered by the IUCN to be near extinction. Several attempts have been made to breed Houbara bustards in captivity by natural mating (Mendelssohn *et al.*, 1979) and, more successfully, by artificial insemination (St Jalmes *et al.*, 1994). The Emirates Center for Wildlife Propagation (ECWP) in Missouri, Morocco, was established in 1996 with a central goal of breeding the Houbara bustard (*Chlamydotis undulata undulata*) in captivity by artificial means and releasing the surplus progeny into eastern Morocco to reinforce the existing wild population. Currently, the ECWP artificial insemination (AI) program is successfully using both fresh and cryo-preserved semen. However, to maintain high fertility and hatching rates, the study of sperm quality needed further development. The objective of the present work was to enhance our understanding of the quality of Houbara bustard semen based on the assessment of pH, osmolarity and the influence of both on motility and viability.

Semen was routinely collected from 37 male donors (aged between 1 and 15 years). Immediately after collection several quantitative and qualitative aspects of each ejaculate was measured: volume, concentration, pH, viability/morphology and finally motility. Afterward, all ejaculates were centrifuged and the osmolarity of the liquid seminal plasma was assessed. All results obtained are summarised in Table 1.

Table 1 Results of the quantitative and qualitative aspects of spermatozoa from 37 male Houbara bustard (*Chlamydotis undulata undulata*).

	Volume (µl)	Concentration ($\times 10^6$ spermatozoa/ml)	pH	Osmolarity (mOsm/kg)	Motility (%)	(%) Live normal spermatozoa
Mean \pm SE	86 \pm 6	346 \pm 38	8.25 \pm 0.05	338 \pm 1.8	67 \pm 1.8	67 \pm 2
Range	30–180	102–1220	7.24–8.79	320–360	40–80	28–89

The pH of Houbara bustard semen appeared to be slightly alkaline ranging from 7.24 to 8.79. Our results do not show a significant relationship between pH and motility or pH and live normal spermatozoa. In addition, variability in osmolarity has been obtained and ranged between 320 and 360 mOsm/kg. In some cases the variability in osmolarity was significant, but it did not influence viability (live normal spermatozoa) or motility.

Additional studies on Houbara bustard (*Chlamydotis undulata undulata*) semen should be developed to investigate if pH and osmolarity have an influence on both fertility and hatchability.

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Viability, Motility and Ultra-structure of Houbara Bustard Sperm (*Chlamydotis undulata undulata*) Before and After the Freezing Process

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ABSTRACT

Several studies have been undertaken into the cryo-preservation of poultry semen, and a variety of methods have been developed and used successfully. Despite the existence of established procedures, the cryo-preservation of semen from non-domesticated birds has remained limited primarily due to the intense labour needed to routinely collect, freeze and store semen. Recently, an ultra-rapid method of cryo-preservation (pellet method) initially developed for domestic fowl semen (Tselutin *et al.*, 1995) has been successfully applied on Houbara bustard semen (*Chlamydotis undulata undulata*; Hartley *et al.*, 1999).

The present work was conducted at the Emirates Centre for Wildlife Propagation (ECWP) in Missouri, Morocco which has a central goal of breeding and releasing Houbara bustards. This experiments attempted to assess the impact of various stages of the Tselutin freezing/thawing process on the quality of semen: motility, viability, morphology and ultra-structure.

In Experiment 1 semen was routinely collected from 17 males (aged from 1 to 7 years). Immediately after collection, sperm volume and concentration were measured. Samples were then frozen and thawed on the basis of Tselutin's procedure (Tselutin *et al.*, 1995) using DMA (Dimethylacetamide) as a cryo-protectant. The viable/non-viable spermatozoa and motility were examined at various stages of the freezing process: after collection [T_0], stored 30 min at 4°C + DMA for 60 s [T_{31}], and after thawing. Finally, the osmolarity of semen was measured at T_0 and T_{31} (after the equilibration time with DMA).

In Experiment 2, semen was collected, frozen and thawed from five randomly selected males within the 17 used in Experiment 1. At each step of the freezing/thawing procedure (T_0 , T_{31} and thawed semen) aliquots of semen samples were analysed under electron microscopy for their ultrastructure.

The mean volume was 0.11 ± 0.016 ml ranging between 0.06 and 0.25 ml whereas the mean concentration of spermatozoa (spz) was $412 (\pm 76) \times 10^6$ spz/ml ranging between 72 and 1101×10^6 spz/ml. Both motility and viable normal spermatozoa were depressed after equilibration with DMA (Motility: 71% to 58%; Viable normal spz: 68% to 62%; $P < 0.05$). This decline was accompanied by an increase in the osmolarity of the media (T_0 : 391 ± 8 mOsm/kg; T_{31} : 525 ± 7.8 mOsm/kg; $P < 0.05$). Finally, the deleterious effects on both motility and viability appeared to be more drastic with the combination of the freezing and thawing process (motility: 38%; viable normal spz: 40%).

Before the freezing process and after the equilibration with DMA, electron microscopy showed that the spermatozoa were damaged by a distension of the plasmalemma and swelling of the mitochondria. Nuclear decondensation,

bent spermatozoa and disruption of mitochondria were present after the combination of the freezing/thawing process. Damage to the acrosome was only observed in spermatozoa following freezing and thawing. Finally, a significant proportion of intact spermatozoa (40% viable normal spz and 38% motile) were resistant to the freezing/thawing process. Such a resistance should be further investigated on the basis of the biochemistry composition (proteins, phospholipids) of Houbara bustard spermatozoa.

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Changes in Cardiac Energy Metabolism during the Plateau Stage in Oxygen Consumption of the Turkey Embryo

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ABSTRACT

Diffusion of oxygen through the eggshell pores of turkey eggs increases exponentially until the 25th and 26th days of incubation. At that time the oxygen requirement of the developing tissue mass exceeds the diffusion characteristics of the eggshell. The embryo has prepared for this eventuality throughout its life by storing tissue glycogen to survive hypoxia. The heart is unique in avian species in that it has no gluconeogenic ability and a very limited storage capacity (Pearce and Brown, 1971). The liver synthesises glucose for cardiac tissue and thyroid hormones are responsible for its transport to the heart (Nobukuni *et al.*, 1989). The hypothesis proposed for this study was that incubation temperature and ambient oxygen partial pressures may influence these physiological processes.

In Experiment 1, fertilised turkey eggs were incubated using standard procedures until the 25th day of incubation when they were randomly assigned to four cabinets operating at 36°, 37°, 38° or 39°C. In Experiment 2, all procedures were identical, but the treatments were 130, 145, 160 and 175 (mm Hg) of oxygen partial pressure in the cabinets. Cardiac tissues were collected at pipping and hatching and analysed for glycogen and lactate content.

Higher temperatures decreased glycogen in cardiac tissues with no effect on lactate. Lower oxygen tensions increased glycogen similarly. The temperature and oxygen treatments that influenced the processes to the greatest degree were used in a third experiment in a 2x2 factorial arrangement of treatments. The temperature and oxygen treatments displayed interactions for both glycogen and lactate (Table 1).

Lower temperatures and higher oxygen concentrations resulted in more cardiac glycogen than lactate, but in the presence of low oxygen concentrations, temperature had no effect. The high temperature and high oxygen treatment resulted in the lowest levels of glycogen and lactate. It was concluded that lower incubation temperatures and higher

Table 1 Cardiac glycogen to lactate ratios in chick embryos exposed to high or low temperatures and high and low oxygen tensions at the plateau stage in oxygen consumption

Temperature (°C)	Oxygen (mmHg)	Day of incubation	
		27	28
36.0	130	2.53 ^b	1.77 ^{cd}
	175	3.64 ^a	1.59 ^{cde}
39.0	130	2.62 ^b	1.44 ^{de}
	175	2.01 ^c	1.07 ^e

^{a,b,c,d,e} Interaction means followed by a different superscript differ significantly ($P \leq 0.05$).

oxygen tensions interact to affect cardiac physiology in turkey embryos at the plateau stage in oxygen consumption.

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Relationships Between Egg Mass and Attentiveness during Avian Incubation in Nests

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ABSTRACT

Contact incubation is not a full-time occupation for many species of bird. Attentiveness, as a percentage of time, can vary between Orders of birds and even for closely related species. In many birds (in ~50% of all Families) both genders share incubation. In ~37.5% of families, it is female alone which incubates compared with ~6% of families where the male does all of the incubation duties. In most Orders attentiveness averages over 85% with only the Apodiformes (swifts) and Passeriformes (songbirds) having attentiveness of around 75–80%. This analysis aimed to investigate whether there were factors underlying the pattern of attentiveness in birds as a group.

The widest range in %attentiveness is exhibited by female-only species (45–100%) with a mode around 75–80% although there were more examples of higher attentiveness than lower values. Male-only incubation was mainly over 90% with attentiveness of less than 80% occurring in a few species of waders (Charadriiformes). In birds exhibiting shared incubation, attentiveness ranged from 60 to 100% but most species were above 85%. There was a relationship with the degree of hatchling maturity. In species exhibiting shared incubation, the pattern of distribution for %attentiveness was largely similar in altricial and precocial species but for female-only incubation altricial species had a range from 45 to 100% compared with 75 to 100% for precocial species.

Drent (1975) showed that egg mass could be a factor in determining attentiveness patterns: large herring gull (*Larus argentatus*) eggs require a lot of energy to warm up and so need to be attended almost continuously, whereas small blue tit eggs (*Parus caeruleus*) warmed much faster and could be left for longer whilst the female foraged. This idea has received little attention so I have examined it further. Attentiveness data were extracted from the literature for 451 species from 24 Orders of bird together with (where possible) initial egg mass and clutch size (Deeming, 2001). For egg mass below 1 g attentiveness was between 60 and 80% compared with 45 and 100% for eggs weighing between 1 and 10 g. Between 10 and 100 g attentiveness was between 80 and 100% and between 90 and 100% for eggs weighing up to 1 kg. Above this attentiveness was 100%. For all species exhibiting female-only (and male-only) incubation, attentiveness averaged 75% up to 5 g rising to 85% at 10 g and above. At 200 g or more attentiveness was over 90%. For shared incubation attentiveness was always above 90% irrespective of egg mass. A similar pattern was observed when total clutch mass was taken into account.

In single-gender incubation small egg size appears to allow a wide range of incubation strategies as indicated by the range of attentiveness. As egg mass increases the behaviour of the bird seems to be more constrained by the requirements of the egg. High attentiveness in species with small eggs and exhibiting shared incubation may be related to prevailing environmental conditions (e.g. low ambient temperature) which require that both parents are around to maintain egg temperature. This is also exhibited by some species with large eggs, e.g. kiwis (Deeming, 2002).

It is known that the thermal characteristics of eggs are related to egg mass with larger eggs taking longer to warm and cool (Turner, 1987). Turner (1994) suggested that thermal impedance was important in contact incubation as it took into account the time-sequence of warming events. It was shown that efficient heating occurred when impedance approximated to thermal resistance. This idea was tested for eggs of different masses and it was predicted that as egg

mass decreases then the time period required to achieve low impedance heating gets shorter. Therefore, the incubation sessions of small eggs should be shorter than those for larger eggs. Examination of the length of the incubation sessions and recesses for female-only incubation in hummingbirds, songbirds, galliformes and waterfowl confirmed this prediction. As attentiveness increased with egg mass this was related to a more rapidly increasing session length rather than shorter recess length.

As in many other aspects of incubation, egg mass appears to be important in being an underlying factor determining the incubation pattern of parent birds. This work may help to shift the emphasis of incubation research from examining the constraints on incubation and foraging in small birds to greater investigation of why species with large eggs have higher attentiveness.

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Incubation Patterns in a Blackbird (*Turdus merula*) Nest

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ABSTRACT

Incubation is not continuous in many species of bird (Deeming, 2002). In Passerines, attentiveness on eggs ranges from 100% down to ~50% of daylight hours and can involve either both parents or the female alone. Factors affecting the degree of attentiveness include egg mass, ambient temperature and adult metabolism (Deeming, 2002). This study examined the pattern of attentiveness at a blackbird (*Turdus merula*) nest located in a suburban garden in Wallingford, England. The goal of the study was to investigate the factors affecting attentiveness, in particular whether the length of an incubation recess was correlated with the length of the preceding or following incubation session.

The nest was located at approximately 1.7 m above the ground in an ivy (*Hedera helix*) bush. Clutch size was three eggs laid during the third week of March 2001. Bird behaviour was monitored using video surveillance of the general nest site for a total of eight hours on each day for nine days leading up to and including the first day that a chick was observed in the nest. Activity at the nest site was relatively rare and so video recordings were used to allow real time events to be speeded up. The time (to the nearest minute) that the female and male birds departed from, and arrived at, the nest site were noted to allow determination of the length of the incubation session (time at the nest, presumably on eggs) and the length of the incubation recess (time away from the nest). This data allowed calculation of the incubation cycle (one recess + one session) and attentiveness (total time on the nest as a percentage of the total time observed for complete cycles). Recording started with the first observed departure or arrival of a bird. Incubation behaviour was correlated with maximum daytime temperature ($^{\circ}\text{C}$), humidity (%RH) and daily rainfall (mm) recorded at the Radcliffe Meteorological Station, Oxford (grid reference SP509072) some 10 miles from Wallingford.

The female carried out the bulk of the incubation duties with an average attentiveness at the nest of 85.2% ($N = 9$ days; SEM=1.6) although the amount of attentiveness varied from day to day (Figure 1). There was a significant negative correlation between the maximum temperature recorded each day and the %attentiveness of the female ($r_7 = -0.837$, $P < 0.01$) but no significant correlation with rainfall or relative humidity. Mean daily attentiveness by the male was 3.2% (0.9) with most time spent at the nest occurring during periods of high attentiveness by the female (Figure 1). There was no significant correlation of male attentiveness with any of the climatic measurements. Mean total attendance (female + male) at the nest was 88.4% (2.0) and this was significantly correlated with the maximum temperature recorded each day ($r_7 = -0.866$, $P < 0.01$) but not with rainfall or relative humidity.

An incubation session was deemed to begin at the start of an incubation recess and the mean daily cycle was 48.3 min (1.6). One-way ANOVA showed that there was no significant effect of the day of incubation (Figure 2). Simi-

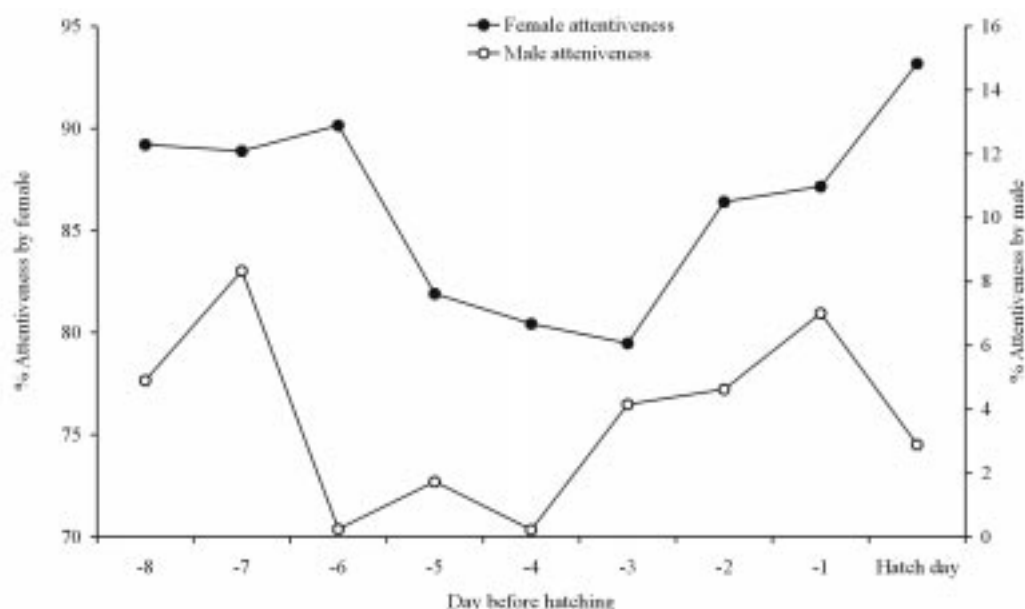


Fig. 1 The pattern of mean daily %attentiveness observed for the female and male blackbirds.

larly, day of incubation had no significant effect on the length of the sessions, which was highly variable (range 7–199 min). By contrast, day of incubation had a significant (one-way ANOVA, $P = 0.001$) effect on the length of the recesses. On a daily basis %attentiveness by the female was negatively correlated with the mean length of the incubation recess ($r_7 = -0.963$, $P < 0.001$) but was not significantly correlated with the length of the sessions. Mean recess length was significantly positively correlated with the maximum temperature recorded each day ($r_7 = -0.805$, $P < 0.01$).

There were no significant correlations between the length of the session before, or after, a recess. %Attentiveness calculated on the basis of a cycle starting with a recess was not significantly different from that calculated from a cycle calculated starting with a session (Two-sample t -test, $P > 0.05$).

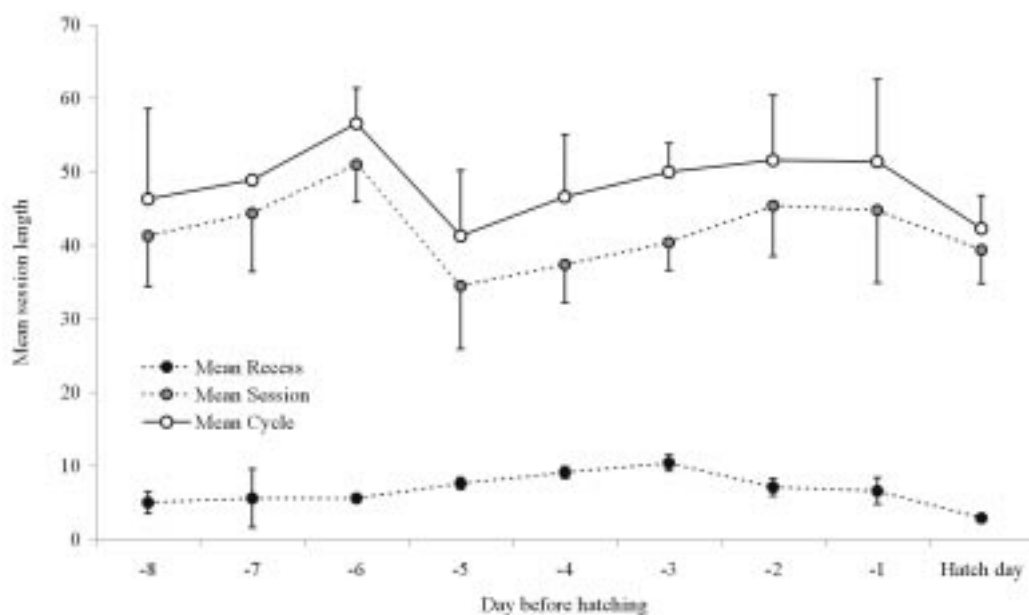


Fig. 2 Mean daily lengths of the incubation sessions and recesses for the female blackbird together with the mean length of the incubation cycle (session + recess). Error bars are SEM.

Although this study was limited by observations on one pair of birds it does challenge the view that incubation in the blackbird is carried out solely by the female (Gurr, 1954; Snow, 1958). The male bird may not contribute very much to the incubation process but his presence is associated with periods of high attendance by the female. At averages of 6.6–7.4 minutes the length of the recesses reported by Gurr (1954) were similar to those reported here (6.7 ± 0.7) but session length (42.1 ± 1.6) was much shorter in the present study than the 65.2–79.4 min reported by Gurr. Hence, mean %attentiveness (88.5–91.8%) was higher in the blackbirds studied by Gurr (1954).

Lengths of the sessions and recesses were not correlated suggesting that time spent on eggs did not influence the time spent away from the nest, and *vice versa*. Rather it seemed that the prevailing temperature affected the length of the recesses but not the sessions and as such this may be related to the cooling rate of the eggs (Deeming, 2002). Gurr (1954) reported that the weather had little effect on the incubation behaviour of one pair of blackbirds although this nest was well protected within foliage. However, in the present study there was a clear influence of ambient temperature on the incubation behaviour of the female. High levels of attentiveness are also matched by an increase in attentiveness in the males. A similar response to climate is observed in kiwis (*Apteryx* spp.) incubating at altitude (McLennan and McCann, 1989). Females supplement the normal incubation activity of males so that the overall attentiveness at the nest is equal to kiwis nesting in more equable climates on the coast. Whether the patterns of single-gender incubation can be modified by prevailing climatic conditions in other birds remains unclear and is certainly worthy of more investigation.

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Attentiveness and Turning Patterns during Incubation in a Houbara Bustard (*Chlamydotis undulata macqueenii*) Nest

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ABSTRACT

The Houbara bustard (*Chlamydotis undulata*) is a native of dry habitats in Northern Africa, Arabia and Central Asia. Severely threatened in many parts of its range it is the subject of captive breeding programmes in Saudi Arabia, Abu Dhabi and Morocco often with the intention of re-introduction in the wild. Captive breeding relies on artificial insemination, which can produce high fertility, and on incubation of eggs in incubators, which has not been as successful as hoped (Hémon *et al.*, 2000). Relatively little is known about the natural incubation behaviour in Houbara except for one study by Schulz *et al.* (1991) who recorded % attentiveness on the nest, rate of egg turning, and temperature of eggs from one captive bird incubating dummy eggs containing temperature probes. The present study recorded behavioural data from a second incubating female captive Houbara in order to confirm some of the previous observations.

Videotapes of time-lapse surveillance of a female Houbara incubating her eggs in 1992 were observed to record the times that the bird left or arrived at the nest, and the times that egg turning was observed. Attentiveness (%) during daylight hours and turning rates (turns/h) were calculated. Recording sessions started a day before full incubation was started and continued through to day 13 of incubation. Thereafter, loss of tapes meant that data could only be collected for day 17 of incubation. The complete incubation period is 21–22 days.

Attentiveness was only 34.4% of daylight hours and turning rate was 0.71 turns/h for the first day when there was only one egg in the nest. By day 1, when two eggs were present, attentiveness was over 90%. Thereafter there was a general slow decline in attentiveness as incubation proceeded and levels of less than 86% were recorded in the second half of incubation. During daylight hours absences from the nest (incubation recesses) averaged 3.3 per day and averaged 71.3 minutes. Mean recess length was less than 10 minutes during the morning but during the afternoon recesses were longer, peaking at over 30 minutes between 16:00–17:00^h (Figure 1). During the heat of midday the bird almost never left the nest.

Mean turning rates each day were variable but averaged 0.65 turns/h over the entire observation period ($N = 14$ days). Again the behaviour of the bird was affected by the time of the day. Turning rates were much higher during the morning (08:00–13:00 h) than during the afternoon (Figure 2). These rates were recorded as events when the bird used her bill for changes of egg position. However, the act of resettling on the nest after an incubation recess often caused a change in egg position and it is possible that resettling also constituted a turning event. If these events are also included then the average rate of egg turning rose to 0.91 turns/h.

It was not possible to record behaviour during the night hours. It can only be assumed that attentiveness is 100% during this time. One tape did show the enclosure during the night and lit by moonlight. The bird did sit for the time observed and rose twice to turn the eggs but these few hours of recording were insufficient to make any conclusions about behaviour.

These observations are similar to those reported by Schulz *et al.* (1991). Attentiveness of the female in this study averaged 87.1% of the entire 24-hour period, which represented an average of 185 minutes off the nest per day. This could mean that average attentiveness during the daylight was 76.3%. Attentiveness was highest during days 4–7 of incubation and then progressively declined towards the end of incubation. The pattern of incubation recesses was similar to the present study but the lengths during the afternoon were much longer in the study by Schulz *et al.* (1991). It is unclear what factors determine attentiveness in Houbara but behaviours observed at different times of the day suggest that the prevailing ambient temperature may be very important.

Rates of egg turning recorded by Schulz *et al.* (1991) were 0.82 turns per hour with the highest rates of turning again during the morning. This value included times when the female returned to the nest after bouts of feeding but it was not stated whether these events involved moving the eggs with the bill.

The data collected during from these two studies of incubation in Houbara are comparable. What is unclear is how incubation under captive conditions, with its element of human interference, differs from that in the wild situation.

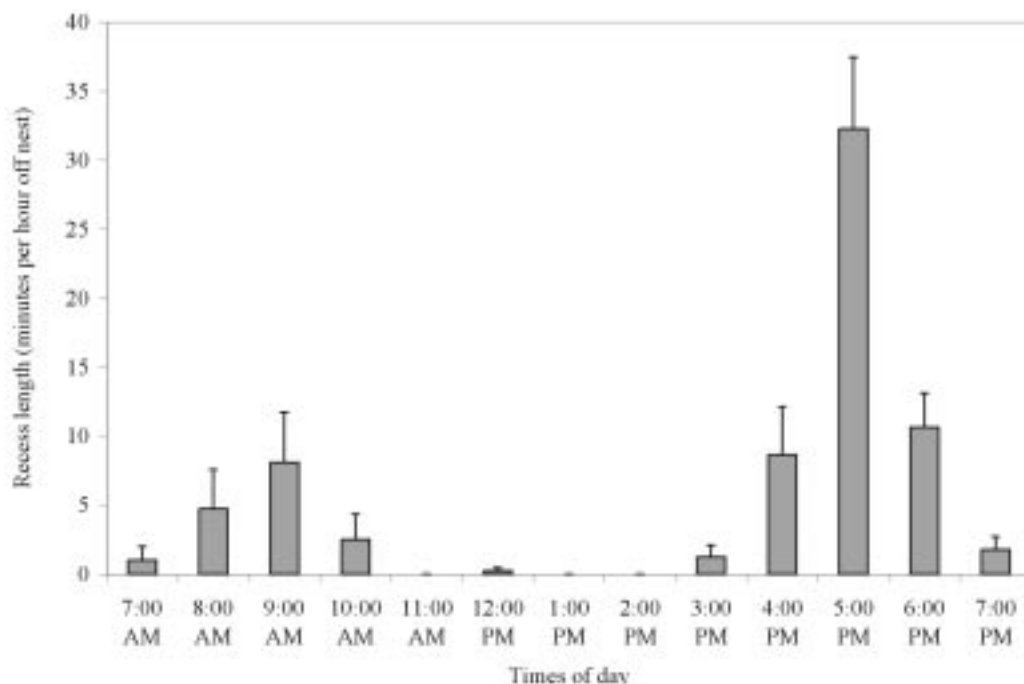


Fig. 1 Mean recess length (+ SEM) for the Houbara female at different times during daylight hours.

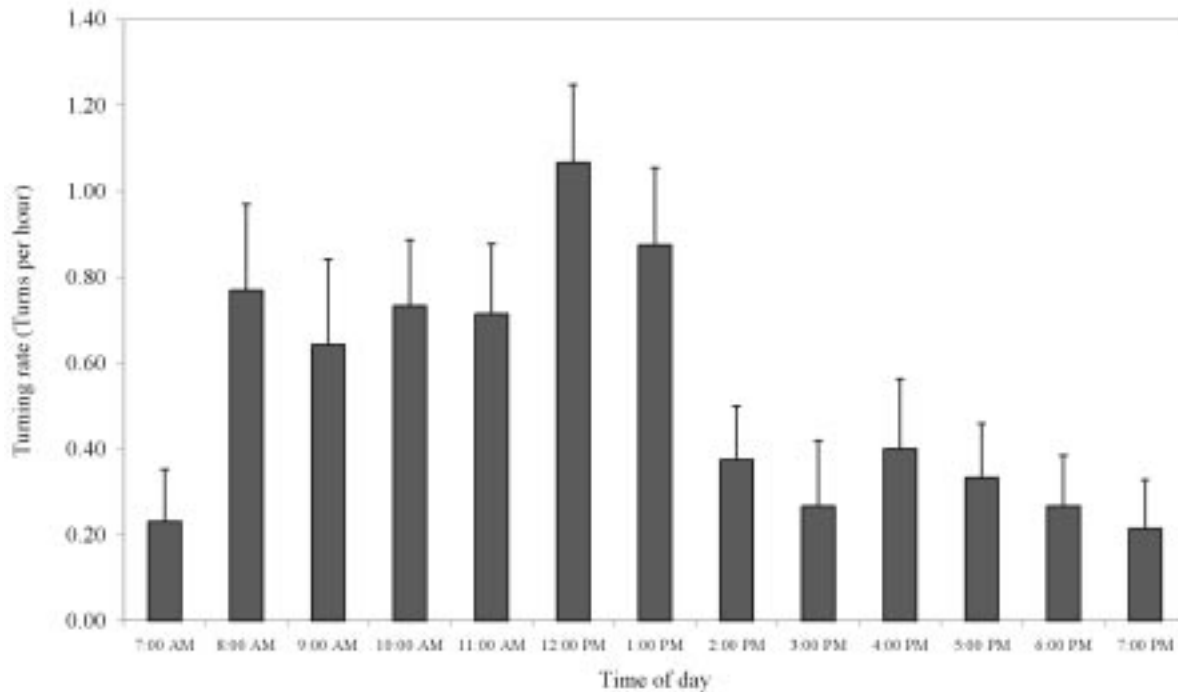


Fig. 2 Mean turning rate (+ SEM) for the Houbara female at different times during daylight hours.

Future studies should attempt to monitor incubation in the natural environment in order to determine how the environment affects incubation behaviour and to investigate how lack of human disturbance affects aspects of incubation, such as egg turning. Such information could prove useful in improving the techniques employed during artificial incubation.

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Temperatures in a Tunnel Incubator – a Long Term Study

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ABSTRACT

The environment within a commercial incubator is determined not only by the machine but also by the eggs within the machine. From about midway through the incubation period the embryo within the egg starts to generate significant quantities of metabolic heat. The objective of the present study was to investigate the effects of the embryo heat production on the incubation temperature within a turkey tunnel incubator (*Jamesway Big J*) over a 6-month period.

The principle of a multi-stage tunnel machine is to pass air through the eggs at the end of incubation towards the eggs at the start of the incubation process. The metabolic heat generated by the turkey eggs in week 3 and 4 of incubation is used to heat the eggs in week 1 and 2 of incubation. Eggs enter the machine at one end and move one position through the machine each week until they are transferred out of the machine and into hatchers at 25 days.

This study monitored temperatures within a turkey tunnel incubator using thermistors connected to a data-logger. Over a 6-month period, temperature readings were taken hourly at three locations within the incubator (Figure 1): A) next to the machine control thermostat; B) where the air entered the mass of eggs; and C) where the air exited the mass of eggs. The set made into the machine each week always consisted of a completely full trolley of eggs (7,800 eggs) from one breeder flock. For each set the mean egg weight, fertility and hatchability was recorded. For one incubation, temperatures were also monitored amongst the eggs on one trolley through the complete incubation cycle.

An example of the temperatures recorded within the tunnel incubator over a two-month period is shown in Figure 2. Temperatures recorded next to the machine thermostat (A) and where the air entered the egg mass (B) was relatively constant through incubation. The decline in daily temperature seen every seven days was due to cold eggs being set into the incubator. Mean temperature at the Location A was 98.7°F and at Location B it was 98.9°F.

The temperature recorded where the air exited the eggs (C) was much warmer (mean 99.7°F) and more variable than those recorded at the other locations. The temperature was higher at position C because of the heat picked up by the moving air as it passes over eggs in the third and fourth week of incubation. Temperatures monitored amongst eggs as they passed through the incubator showed that the temperature measured at location C more closely corresponded

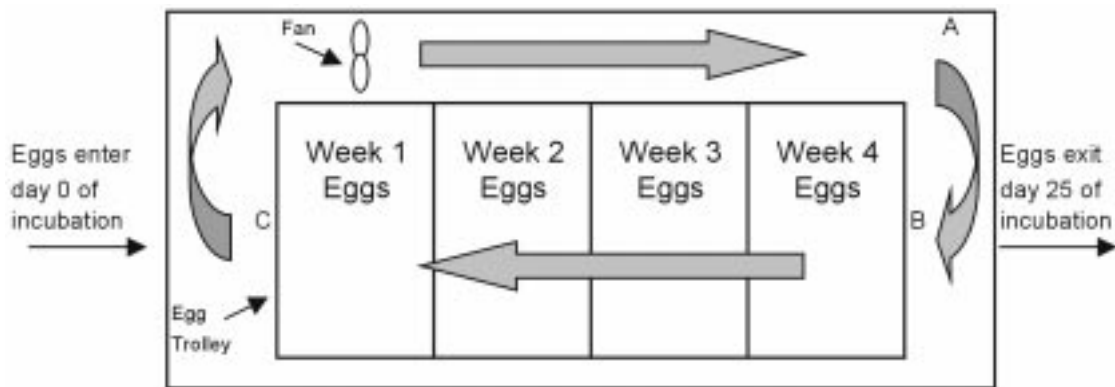


Fig. 1 Diagram of a turkey tunnel incubator showing the location of eggs at each stage of incubation and the direction of airflow within the machine.

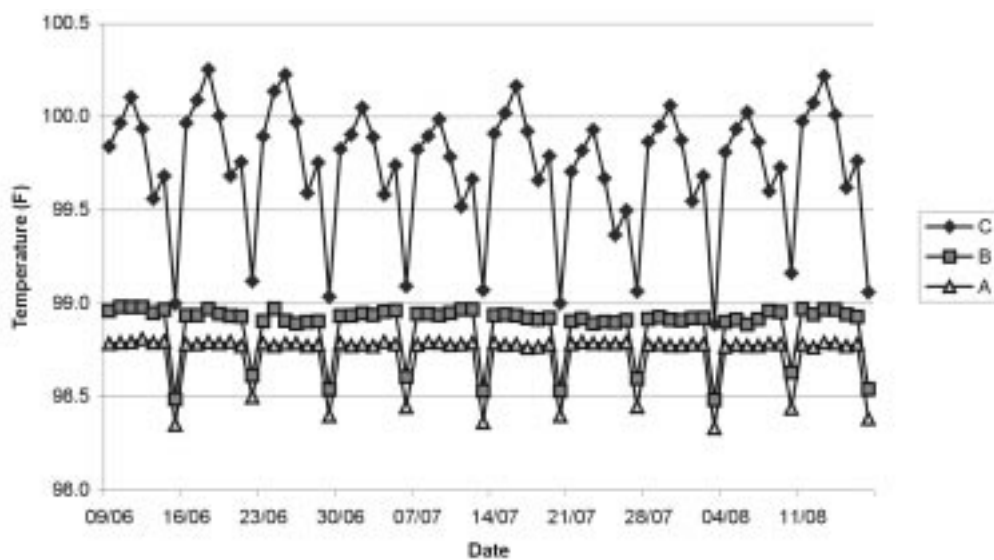


Fig. 2 Mean daily temperatures recorded at three locations (see Fig. 1) over a two-month period within a tunnel incubator.

to the temperature around the eggs than temperatures measured at locations A and B. The weekly pattern of temperature change observed at location C was caused by the setting of eggs (coolest point) and then the transfer of eggs out of the machine four days later.

How much air temperature increased as it passed through the eggs varied from hatch to hatch (Figure 2). Using measured values of metabolic heat production of turkey eggs (Dietz *et al.*, 1998), egg mass and fertility for each hatch it was possible to estimate the amount of metabolic heat produced by all the eggs within the machine for each day over the whole 6-month study period. The rise in air temperature ($^{\circ}\text{F}$) between locations B and C was found to be significantly correlated ($r^2 = 0.7233$, $n = 24$ weekly cycles, $P < 0.01$) with the estimated total embryo heat production (kW) within the incubator. The regression equation showed that for every 1 kW of embryo heat production the temperature gradient increased by 0.9°F .

The study shows that the actual temperature experienced by a turkey egg in a tunnel incubator is dependent on the metabolic heat output of the eggs within the machine which in turn is dependent on egg mass and fertility.

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Incubation Investment Increases Offspring Fecundity

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ABSTRACT

Early development is a vitally important stage in an organism's life-cycle, as perturbations in the rearing conditions even in the early stages of development can result in long-lasting effects on the phenotype (Lindström, 1999). For birds the rearing conditions during embryonic development are determined by the parents' incubation behaviour. In groups other than birds, for example, reptiles and mammals, the conditions during embryonic development have been shown to affect the phenotype of offspring but very little is known from birds.

Changes in the parents' incubation behaviour might result from differences in parental body condition and affect the embryo's rearing conditions, with potential effects on the offspring's phenotype later in life. This is known as a maternal effect, an effect of the phenotype of the parents on the phenotype of the offspring, independent of genetic effects. Here we tested the hypothesis that (1) zebra finches (*Taeniopygia guttata*) of different body condition would adopt different incubation strategies, and (2) that different incubation strategies affect chick phenotype.

We manipulated the pre-breeding body condition of zebra finches using two diets of different protein contents fed to the birds for four weeks before laying. All females were manipulated to make a similar investment into clutch production so that the differences in body condition persisted into incubation. All birds were kept under identical conditions during incubation and eggs were randomly distributed between the treatment groups. During incubation we recorded body mass, and incubation behaviour of the parents was observed using infrared video cameras.

We found that birds from the two treatments adopted different incubation strategies. The same pattern of behaviour was observed in both sexes, but was more pronounced in females as they were responsible for the majority of incubation. During the first half of the incubation period females from the protein-rich diet group lost weight at a faster rate than those from the low protein group. Incubation bout length increased with progressing incubation period in both groups, but the increase occurred later in the protein-poor group. Total attentiveness also increased, but this did not differ between the treatment groups. We suggest that females in better condition adopt a possibly advantageous incubation pattern from the beginning whereas females in poorer condition delay the onset of high investment into incubation, perhaps allowing them to recoup their energy levels (Wiebe and Martin, 2000).

The differences in incubation strategy between the treatment groups had a marked effect on the fecundity of the daughters. Female offspring who had been incubated by foster parents that had received a high quality diet laid on average twice as many eggs in their first breeding attempt than those incubated by low quality foster parents. Hence small differences in the early embryonic development had a marked effect on an important life-history trait in zebra finches.

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Wiebe, K.L. and Martin, K. 2000. The use of incubation behaviour to adjust avian reproductive costs after egg laying. *Behav. Ecol. Sociobiol.*, **48**, 463–470.

Functional Aspects of Bird Nests

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ABSTRACT

Birds are remarkable, although not unique, among vertebrates in building architecturally sophisticated nests that have no other purpose than the care of the young. Birds are also the only vertebrate group with no species that exhibit viviparity. The association between eggs and nests probably goes back to the evolutionary origins of birds. The incubation process has therefore to a degree been influenced by the nest and, of course, *vice versa*.

Bird nests are often species specific in their structure and composition. However, systematic examination of them shows that there are few architectural variations and that the nest of any species is generally composed of only a small number of quite specific materials. The problem now is to understand what they are chosen for.

Up to four zones can be distinguished in nests, although not all are present in all nests. These zones are *attachment*, *outer layer*, *structural layer*, and *lining layer*. Attachment materials are concerned solely with holding the nest in position. For nests positioned above ground, a structural layer is required to give the nest rigidity and integrity. This may be covered on the outside by a thin layer of different materials that generally change the appearance of the nest. Inside the structural layer there may be other materials forming a lining to the nest cup.

A nest protects the brood from the physical and biological hazards that surround it. It may provide climatic control of temperature and humidity of the incubation environment; it may provide protection from predation through concealment, inaccessibility, or the mechanical properties of the nest. The nest may also contain devices for limiting the risk of disease or ectoparasitism to the chicks. Differentiation of the nest into zones can be understood as the distribution of different functions to different zones. However, materials in one zone may compromise the effectiveness of materials in another. We should therefore expect trade-offs between the use of materials concerned with different functions.

Although at present there is little experimental evidence to test functional-design explanations, it is time to identify what could and should be tested. In the context of incubation two particular trade-offs are interesting. Selection for better nest insulation could favour larger nest sizes, while selection and for better nest concealment could favour those that are smaller. Even considering control of nest cup temperature alone, some evidence suggests that, because the thermal insulation provided by a material is drastically reduced by water penetration, a material with only moderate insulating properties may be optimal in certain environments because it dries out quickly.

The functional roles of the nest vary in their extent between species. The nests of some ground nesting species, because of their location, require no structural materials to hold them together and, as the chicks are precocial, the nests have no role beyond the protection of the eggs. Nests with these more limited roles offer an opportunity of experimental investigation of functional design using a simplified system, and so provide a stepping stone to understanding the biology of more complex nest designs.

Chick Length Uniformity Profiles as a Field Measurement of Chick Quality?

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ABSTRACT

As the poultry industry changes to high-yield type birds, it becomes more difficult to reach hatchability of fertile eggs and one-week mortality goals. The problem is seasonal, generally being worse in the winter. In the field, generally complaints are about small, dehydrated birds that die in the first week, especially from the youngest breeder flocks. The hatchery will see increased late dead, especially in the oldest breeder flocks. The problem is driven by the fact that the embryo of today has changed just as much as the weight and feed conversion performance of the broiler has changed. The embryo brings a different physiology to the incubation equation. The incubation equipment design has not changed to meet the needs of the high-yield type embryos. The eggs from the youngest and the oldest breeder flocks are the eggs that bring the most extreme changes to the incubation equation. Consequently, the losses are most evident in eggs from the non-prime flocks.

Wineland *et al.* (2000) reported that the incubation environment impacts upon the development of the chick. With high setter and high hatcher temperatures, the heart is smaller and the chick without yolk is smaller. It was also demonstrated that there was greater residual yolk with higher setter and hatcher temperatures. The optimally incubated birds are able to direct resources to organ development and growth. Those in less than optimum incubation conditions must utilise resources to survive not develop and grow.

Embryo growth relationships exist for egg weight, eggshell conductance, and length of the incubation period across species. Christensen *et al.* (2001) showed that these relationships are crucial to incubation within a species to improve embryo survival and poult quality. Turkey eggs produced at 33 weeks of age hatched better at shorter incubation periods, eggs produced at 43 weeks of age hatched equally well at both incubation periods, and eggs produced at 54 weeks of age hatched better at longer incubation periods. The poults also lived better with longer incubation periods.

Based on the above research and field observations of growth restriction related to incubation, crown to rump measurements chicks were used to develop uniformity profiles in various areas of the egg mass. In this database, I measured all of the chicks hatched in at least three trays per profile. The trays came from three different areas of the hatcher: relatively good airflow, relatively cool areas of the hatcher, and relatively hot areas of the hatcher. In some cases there are up to eight trays per profile, but each has a minimum of three trays. There are 32 profiles in the database at this time. Generally chicks from an old, prime, and a young breeder flock are sampled.

The chick length uniformity profile has show the following trends to date: (1) Chick length increases with breeder flock age; (2) Chick length in multi-stage incubation in the US is larger in the summer than in the spring; (3) Preliminary data suggests that chick length in single stage incubation is larger than in multi-stage incubation; (4) The shortest chick length is found in hatcheries with the greatest one-week mortality problems and hatchery problems; (5) Chick length varies based on location within the hatcher and this is predictable within machine types; (6) The length of chicks hatched from the oldest breeder flocks is usually less then the prime flocks in the same hatchery; (7) The measurement appears to be valid for poults also and (8) Chick length can be used as a field diagnostic tool. When the chicks that die in the first week are the same size as the average 18 day embryo, the problem is in the incubation of the embryo.

In the interest of correlation between individuals, I have recently begun to use a tip of beak to tip of toe measurement. In my early comparisons between the two measurements, I find that the crown to rump measurement is more sensitive of chick quality, but the tip of beak to tip of toe is more repeatable between different people. The chick length uniformity profile may be a very useful tool to monitor chick quality over time. Once the incubation quality and chick performance relationships can be evaluated seasonally, economic justifications for upgrades of hatchery equipment based on performance costs can be made.

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Chick Quality, the Result of Maximising Embryonic Metabolism

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ABSTRACT

The definition for chick quality has traditionally been hatchability and three day mortality. In recent studies (Gladys *et al.*, 2000; Hulet *et al.*, 2000; Lourens and van Middelkoop, 2000; Wineland *et al.*, 2000), hatchery performance has been linked with improved post-hatch performance and with improved physical chick characteristics. It has also been found that the high-yielding broilers have a higher potential heat production than has been previously reported. In commercial incubators, design problems and increased heat production from high meat-yielding strains have been associated with decreased hatchability. The problems associated with high temperatures either in the setter or the hatcher have included: by brain lesions, shortened hatch time, decreased hatchability, plump chicks, unhealed navels in hatch residue and decreased growth and/or greater feed efficiency after hatch.

Many different factors affect the setter's internal environment that changes what would be optimal for the eggs to hatch such as: egg weight, breeder strain, storage time of eggs, stage of incubation, *etc.* For example, French (1996) stated that as egg mass increases, thermal conductance does not increase proportionally. Therefore, larger eggs should have greater difficulty losing metabolic heat produced by the embryo. In an earlier paper, Wyatt *et al.* (1985), found influences on fertility and hatchability due to strain, age of breeder hen and egg size as critical factors in hatch of chicks. Physical conditions as well as design of the incubator can also influence thermal conductivity. Meijerhof and van Beek (1993) considered that despite different physical characteristics of the eggs, if conditions were adjusted to optimise hatchability by maximising the metabolism or heat production of the embryos. Other results would be a more fully developed and mature chick hatched and hence, improved chick quality.

Two studies were conducted to examine the hatchability and heat production of high-yielding broilers in a single stage setter. Eggs from the same age breeder flock and strain were set in *Hatch Tech* incubators and either adjusted for temperature daily after five days of incubation against a single stage incubation set that was placed on a pre-set pattern of temperature, humidity, and ventilation for the 18 days of incubation. Information on the temperature, humidity, eggshell temperature, air temperature, CO₂ production, and heat production by the embryos was logged on a daily basis. Test and control eggs (115,400 each, two setters) were tested in two separate studies. Improvement in hatchability (~2%) and increased heat production were found by optimising the heat production or metabolism of the developing embryos by measuring carbon dioxide output.

This is one of the first studies where conditions of the setter were changed in real time (daily) according to metabolic activity of the embryos to improve hatchability. More information is needed to determine if the improved hatchability also translates into improved growth, efficiency or physical characteristics (size, immune capacity, intestinal characteristics, *etc.*) that could determine or define improved chick quality. This information could give hatchery managers tools to manage hatchability of eggs in single stage incubators.

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Influence of Vibrations and Eggshell Opening on the Chicken Embryo Development in Windowed Eggs

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ABSTRACT

Manipulation of the germinal disc or early embryos and further incubation requires that the shell and membranes are opened to get access to the embryo inside the egg. However, opening eggs results in a heavily reduced hatchability although the embryos seem to develop properly immediately after treatment up to about 5 to 6 days of incubation (Speksnijder and Ivarie, 2000). Previously, our own experiments on eggs windowed before incubation led to a complete loss of opened eggs by day 11 of incubation. The embryos died between day 8 to 11 of incubation after normal development to embryonic day 6 or 7. The chorio-allantoic membrane did not expand completely to the sharp end of the egg and albumen formed a highly viscous compound around the egg opening.

In this study single parameters of the opening procedure were evaluated separately. Opening the eggshell was performed by cutting the eggshell with a high speed drill with the shell membranes being removed manually in a second step. Vibrations caused by drilling were evaluated by sound analysis directly on the shell. The resulting sonagram provided data on the characteristic frequencies and energy parameters over time. These signals were used for simulation experiments with variations of frequency and amplitude on intact eggs to evaluate the influence of vibrations separately from the opening of the eggshells. Neither simulating the noise of the drill, nor application of noise at the maximum amplitude or twice the frequency had any significant effect on hatchability.

A second series of experiments was performed to remove the eggshells, with the shell membranes still intact on top of the window. The pattern of embryonic mortality and hatchability were unaffected by the treatment (Table 1).

Table 1 Hatchability and incubation losses after erosion of eggshell and in untreated controls. $\chi^2 = 0.127$; FG = 1; $P = 0.721$; Fisher's $P = 0.423$

Fertile eggs set	Dead at 8 days (% of fertile eggs)	Losses between 8 and 18 days (% of fertile eggs)	Losses between 19 and 21 days (% of fertile eggs)	Hatched chicks (% of fertile eggs)
Controls ($N = 122$)	9 (7.4)	7 (5.7)	5 (4.1)	101 (82.2)
Erosion of eggshell ($N = 132$)	9 (6.8)	10 (7.6)	6 (4.5)	107 (81.1)

Whether in the type or number of early or late embryonic deaths, or in the spreading of the chorio-allantoic membrane, no differences between the treatments were seen in either experiment. It was clear that continuous vibrations on the eggshell before incubation were not responsible for the reduction in hatchability. Furthermore, opening the eggshell did not appear to cause the severe distortions of the growing chorio-allantoic membrane. Although the completeness of the two shell membranes were a high enough barrier to prevent infections, uncontrolled changes in the albumen might cause agglutination at the window opening and prevent spreading of the chorio-allantoic-membrane. The selective removal of the shell and distinctive puncture through the shell membrane avoiding direct contact of albumen with the environment might allow for cell sampling without reducing hatchability.

Sex Ratio of the First Twenty Eggs in Layers

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ABSTRACT

Sexual reproduction ensures the evolutionary advantage of recombination of genetic information and thereby increases the adaptability to changing environmental conditions. In avian species, the female has the heteromorphic chromosomes (ZW) and the sex of the progeny is determined by the sex chromosome constitution inside the ovulated egg before fertilisation. Thus to skew the sex ratio from a typical 50:50 male to female might be established by the female before the first meiotic division when one of the two sex chromosomes is directed into the first polar body. It has been shown by Dijkstra *et al.* (1990), Hardy (1997), and Ellegren *et al.* (2000) that birds can influence the gender of their offspring.

In this study, the sex was investigated in the first 20 eggs of 40 layer parents to investigate whether chickens might be able to influence the sex ratio of their progeny. Sex was determined in chicks by morphological inspection and in all eggs with dead embryos before differentiation of gonads by polymerase chain reaction (PCR) of the W-specific repetitive Xho1 sequence. Preparations of 72% of the unfertilised eggs were successfully investigated for their sex chromosome constitution by PCR.

For the first 10 eggs of the hens ($N = 345$) the sex ratio was significantly skewed in favour of males (57.7% males; Figure 1). From egg 11 to 20 of each hen ($N = 363$) the sex ratio was more equal (Figure 1). For the first 10 eggs the percentage of males in dead embryos (59.3%) was significantly higher than that of females (40.7%). These differences were caused predominately by an increase in 'blastoderms without embryo' (BWE) in males (Figure 2). In successfully analysed unfertilised eggs 68% did not carry the W chromosome and could have only been fertilised to become male.

These results suggest that the female chicken can affect the sex ratio of the progeny to maximise its reproductive success in the next generation. Further investigations will evaluate the repeatability of this result and investigate the influences of the genetic background and rearing environment of the birds on the sex ratio.

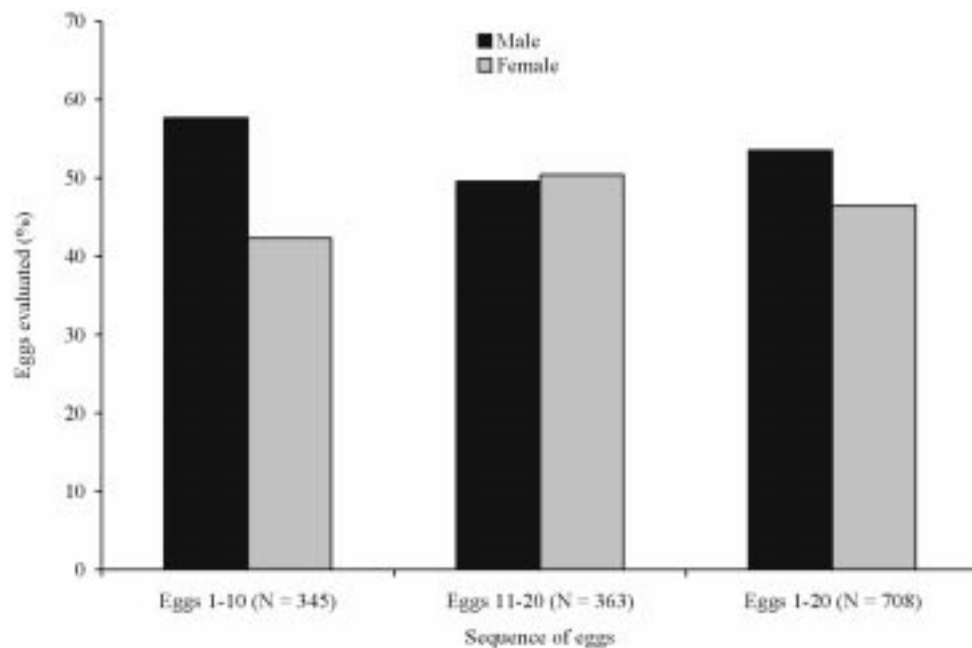


Fig. 1 Sex ratio in the first 20 eggs of 40 layer parents (708 eggs evaluated, 88.5 % of eggs laid) $\chi^2 = 4.6$, $P = 0.03$.

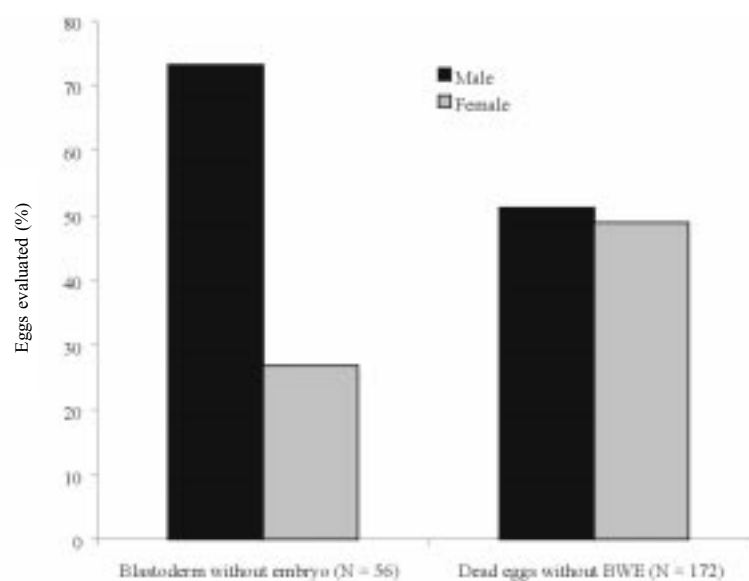


Fig. 2 Sex ratio of died chicken embryos within the first 20 eggs of the laying cycle ($N = 40$ hens) $\chi^2 = 8.4$, $P = 0.0038$, Fisher's $P = 0.0027$.

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Effect of Prolonged Incubation Time on Broiler Performance and Yield

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ABSTRACT

An experiment was conducted to test the hypothesis that prolonged incubation time increases broiler growth and slaughter yield. The hypothesis was tested under the precondition that eggshell temperature during incubation did not fluctuate by 0.5°F away from 100.0°F.

A total of 1800 hatching eggs from a 43 week-old Ross 508 breeder flock were collected and transported to the Spelderholt Research Hatchery on the same day. The eggs were split randomly across six groups of 300 eggs and placed at 24 incubator trays. Between every other tray, one empty tray was removed, and on each tray (150 egg places), only 75 eggs were set at every other egg place, to ensure maximum airflow around all eggs during incubation. Thermistors were attached to eggshells of 18 different eggs equally split across the six groups of eggs. Eggs were disinfected with formalin, and stored at 20°C room temperature until the next morning when incubation was started. Eggshell temperature was set at 100.0°F and measured and read every four hours. Machine operating temperature was adjusted accordingly to achieve or maintain the desired eggshell temperature, as described in Lourens and van Middelkoop (2000).

Eggs were set at different times in the six different groups according to the expected hatch periods (HP) and hatcher holding times (HHT) (Figure 1). HP's were: "early" – between 468 and 480 h; "mid" – between 480 and

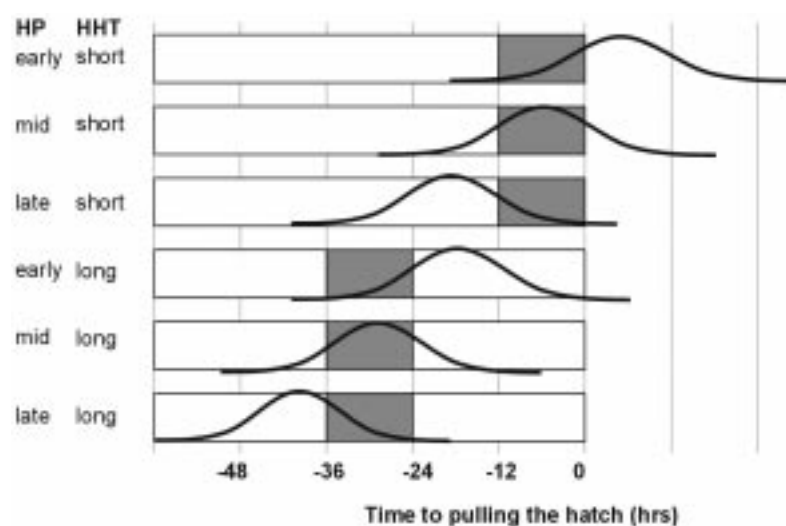


Fig. 1 Experimental 3×2 split plot design with three levels of HP (early, mid and late, shown as grey area) and two levels of HHT (short and long). The theoretical hatch curves are plotted as solid lines. At zero hours, all chicks were removed from the hatcher.

492 h; or “late” – between 492 and 504 h. HHT’s were: “short” – dry chicks were removed during the period up to 12 h after each HP; or “long”: dry chicks were removed during the period 24–36 h after each HP. Chicks that hatched before or after these designated HP’s were removed and discarded from the trial.

Chicks were vent sexed and 288 male and 288 female chicks were separately placed in 48 floor pens (12 chicks per pen; 16 chicks per m^2) within 2 hours from the moment of pulling the hatch. All birds were offered feed and water *ad libitum* and received continuous lighting the first two days, after day three an intermitting lighting scheme (18L:6D) was applied. Vaccination for Newcastle disease and infectious bronchitis were conducted at the first day in the broiler house, then immunisation for Newcastle disease followed 21 days later. All birds were raised at 34°C for the first day. The temperature was decreased daily till 19°C on day 29. From day 29 onwards, the temperature remained constant at 19°C . At 0, 10, 33 and 40 days of age, chicks and residual feed were weighed by pen. The feed conversion rate (FCR) for each pen was calculated using total feed consumption and the total weight gain for the period and was corrected for the feed consumed by the birds that died. After weighing at day 40, 10 birds per pen were slaughtered for carcass analysis. Data were arranged for a statistical analysis as a $2 \times 3 \times 2$ factorial arrangement of treatments. The statistical models for body weight, feed intake, FCR and slaughter yield included the effect of gender, HP and HHT and all possible interactions. All analyses were conducted using the General Linear Model (GLM) procedures of SAS[®].

Egg weight at setting and transfer, and chick weights and FCR at days 1, 10, 33 and 40 by gender, HP and HHT are in Table 1. Initial and transfer egg weights did not differ between HP or HHT. Chicks that hatched early were the heaviest

Table 1 Egg weight, chick weight and FCR by gender, hatch period (HP) and hatcher holding time (HHT)

	Egg weight (g)		Chick weight (g)				FCR 1800 g
	Setting	Transfer	Day 1	Day 10	Day 33	Day 40	
Gender							
Males	–	–	43.4	241.1	1713.8 ^a	2233.3 ^a	1.457 ^b
Females	–	–	42.8	240.3	1518.3 ^b	1949.0 ^b	1.683 ^a
HP							
Early	61.9	56.0	43.6 ^a	229.1 ^b	1569.8 ^b	2033.2 ^b	1.619 ^a
Mid	61.7	55.8	43.2 ^a	247.0 ^a	1637.2 ^a	2121.1 ^a	1.536 ^b
Late	61.7	55.9	42.4 ^b	246.3 ^a	1623.2 ^a	2092.5 ^a	1.575 ^{ab}
HHT							
Short	61.9	55.8	44.7 ^a	233.3 ^b	1592.0 ^b	2058.9 ^b	1.602 ^a
Long	61.7	56.0	41.4 ^b	248.3 ^a	1628.3 ^a	2106.2 ^a	1.551 ^b

^{a,b}Within columns means with no common letters differ significantly ($P < 0.05$).

Table 2 Broiler yield by gender, hatch period (HP) and hatcher holding time (HHT)

	Carcass		Wings		Legs		Filet	
	(g)	(%) ¹	(g)	(%) ²	(g)	(%) ²	(g)	(%) ²
Gender								
Males	1516.7 ^a	67.9 ^a	166.0 ^a	11.0	538.4 ^a	35.5 ^a	399.1 ^a	26.3 ^b
Females	1329.7 ^b	68.2 ^b	146.0 ^b	11.0	459.2 ^b	34.5 ^b	356.7 ^b	26.8 ^a
HT								
Early	1381.0 ^b	67.9 ^{cd}	152.2 ^b	11.0	483.4 ^b	35.0	366.3 ^b	26.5
Mid	1431.5 ^a	67.5 ^d	156.1 ^a	10.9	501.7 ^a	35.0	381.0 ^a	26.6
Late	1438.1 ^a	68.7 ^c	157.8 ^a	11.0	503.0 ^a	35.0	382.1 ^a	26.6
HHT								
Short	1402.5 ^b	68.1 ^a	153.5 ^b	11.0	490.7 ^b	35.0	371.2 ^b	26.5
Long	1429.5 ^a	67.9 ^b	157.0 ^a	11.0	500.9 ^a	35.0	381.2 ^a	26.7

¹Percentage of live body weight; ²Percentage of carcass weight; Within columns means with different letters differ significantly (^{a,b} $P < 0.05$; ^{c,d} $P < 0.10$).

at placement, but after 10 days body weights were lower compared to mid or late hatched chicks. At 40 days, early hatched chicks weighed on average 87.9g less compared to mid hatched chicks and 59.3g less compared to late hatched chicks.

A 24 hours longer HHT decreased chick weight by 3.3g, but from 10 days onwards, body weights of long held chicks were 15g heavier compared to short held chicks. At 40 days, body weights differed by 47.3g. At 40 days, differences in body weight, carcass weight and filet weight paralleled differences in live bodyweight at 10 days (Tables 1 and 2).

FCR was lowest for mid-hatched chicks and differed 8.3 points from chicks that hatched early. A long HHT reduced FCR by 5.5 points. First week mortality (0.15%) and total mortality (1.05%) did not differ between HP or HHT.

These results indicate that if the process of incubation is prolonged by at least one day, we make better use of the growth potential of the current high yielding broilers. Hager and Beane (1982) and Wyatt *et al.* (1985) found beneficial effects of early, mid and late collection procedures on chick performance. Probably chicks that are forced to hatch early because of, for example, high incubation temperatures benefit from early collection procedures. We would suggest that if incubation egg temperatures are controlled precisely at $100.0 \pm 0.5^\circ\text{F}$ during the process of incubation, chicks are better off inside a temperature-controlled environment. A hatcher environment matches the needs during the first few days of life of neonatal chicks more closely than for example sub-optimal transport or broiler house conditions.

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Effect of Dietary Supplementation with Sunflower and Linseed Oils on Fertility and Semen Quality in Pheasants

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ABSTRACT

Changes in semen quality (concentration and motility) have sometimes been observed by dietary oil supplementation. Fertility results seem to be contradictory modified by dietary lipid supplementation on chicken males (Blesbois *et al.*, 1997, Cerolini *et al.*, 1998). The aim of the present experiment was to study the effect of dietary inclusion of oils from oleaginous seeds on fertilising ability and characteristics of semen in a nearly wild poultry species, the pheasant.

Table 1 Fertility (%) from set eggs of semen collected from pheasants on the two diets

	Phase 1 [†]		Phase 2 [‡]	
	Sunflower oil	Linseed oil	Sunflower oil	Linseed oil
Days from AI				
2nd + 3rd	84.38 ^{ab}	79.03 ^{ab}	85.46 ^a	86.05 ^a
4th + 5th	86.96 ^a	90.14 ^a	85.71 ^a	80.56 ^a
6th + 7th	85.92 ^a	79.45 ^{ab}	81.25 ^a	70.97 ^{ab}
8th + 9th	73.85 ^{ab}	70.97 ^b	71.88 ^a	41.94 ^{cd*}
10th + 11th	74.58 ^{ab}	73.02 ^b	67.74 ^a	46.88 ^{bc}
12th + 13th	68.92 ^b	62.90 ^{bc}	37.84 ^b	53.13 ^{bc}
14th + 15th	50.00 ^c	46.15 ^c	23.08 ^b	16.00 ^d
Days interval from AI				
2–7	85.78 ^A	83.01 ^A	84.06 ^A	80.00 ^A
8–15	66.79 ^B	62.85 ^B	50.79 ^B	40.83 ^B

[†]Results pooled from the single AIs performed at 43rd and 45th week; [‡]Results pooled from the single AIs performed at 47th and 49th week. Comparison between diets for birds of the same phase: * $P < 0.05$. Values in the same column followed by different superscripts are significantly different (a,b,c,d: $P < 0.05$, A,B: $P < 0.01$).

Forty-six pheasant males (*Phasianus colchicus mongolicus*), were milked weekly throughout the reproductive season (March–July), and fed two experimental diets (metabolisable energy of 12.28 MJ/kg, crude protein of 18.2%, total fat 7.7%) containing either 4% sunflower oil or 4% linseed oil from 40 weeks of age (23 birds/diet). At 43 (11 May), 45, 47 and 49 weeks of age ejaculates from males on each diet were diluted 1:1 with a commercial extender, pooled and used to assess fertilising ability *in vivo* by artificially inseminating 48 laying pheasant hens. Males and females were the same age. Females were inseminated with a single dose of spermatozoa (approximately 100×10^6) in late afternoon. Egg fertility was recorded thereafter. At 46 and 49 weeks of age collected semen was evaluated in volume (by weight), concentration (by hemocytometer procedure) and live spermatozoa (eosin/nigrosin staining). Statistical comparisons were performed using analysis of variance and χ^2 test with respect to percentage data.

Egg fertility results from the data of two consecutive days and over 2- to 7-day or 8- to 15-day intervals from AI are shown in the Table 1. Values and trends of fertility were very similar in the treatments at any phase. However, the persistence of fertility in relation to the time after AI was a little greater for the males fed on the sunflower oil supplemented diet in respect to the males on the other diet: the peak of fertility of semen from pheasants on the *n*-6 polyunsaturates rich oil lasted 2 days longer at any phase (phase 1: 6–7 days versus 4–5 days; phase 2: 4–5 days versus 2–3 days). These data agree with the findings of Cerolini *et al.* (1997, 1998) which indicate C20-22*n*-6 polyunsaturates positively correlated with fertility recorded the 2nd week after AI. They supposed those PUFAs playing a role in maintaining the survival of spermatozoa in female tract.

Dietary oil supplementation showed a significant effect on percentage of live spermatozoa at 49 weeks of age (Table 2). The proportion of live cells in semen samples was significantly enhanced in the linseed oil supplemented group compared to sunflower oil group. It seems the *n*-3 polyunsaturates rich oil slows down the age-related loss of vitality of spermatozoa.

The results suggest that the dietary inclusion of sunflower or linseed oil does not affect fertilising ability of semen although the persistence of fertility is slightly extended by *n*-6 PUFA rich oil. Moreover, although linseed oil appears to sustain the decline of live spermatozoa in pheasant semen with advancing age, lipid source had little effect on the considered semen characteristics.

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Table 2 Characteristics of semen from pheasants on the two diets at 46 and 49 weeks of age

Semen characteristic	46 weeks		49 weeks	
	Sunflower oil	Linseed oil	Sunflower oil	Linseed oil
Volume (μ l)	176.8	149.1	169.2	157.9
Concentration (10^9 cells/ml)	6.82	6.68	6.93	6.33
Live spermatozoa (%)	93.25 ^A	93.20	86.60 ^B	92.20 ^{**}

Comparison between diets for birds of the same age: $**P < 0.01$. Comparison between ages for birds on the same diet: A,B: $P < 0.01$.

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Study of Functional Changes in Early Embryogenesis Depending on Consumption by the Hens of Retinol and Tocopherol

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ABSTRACT

The biological quality of eggs depends on many factors, from a feeding level, conditions of the contents, and physiological condition of the organism of the poultry. Thus fat-soluble vitamins are considered as regulators in the processes of an exchange of substances in living organisms. Both the lack and superfluous of vitamin A and E, results in various infringements in development embryos of poultry.

In this connection, the purpose of our research was to study embryonic features during development of chickens that depend on A and E vitamin maintenance of the hens. We have determined the influence of excessive doses of vitamins on morphological and biochemical parameters of quality eggs. Thus it has been established, that the high contents of vitamin A in a diet of the hens reduced fibre and yolk index. A similar law was established at sharing 30 × dose retinol and 50 × dose tocopherol.

To 10 and 30—the multiple dose of vitamin A in a combination with 50 × dose of vitamin E probably reduced the carotenoid content in the yolk of eggs. The significant deviations in germ development early in the prenatal period are established by feeding the hens these doses of vitamins. At 7 days of incubation, embryos exhibited significant changes—hydropic dystrophy in a liver and kidneys. Besides the increased quantity of vitamins reduced hatchability up to 63.2 % ($P < 0.05$) and hatched chicks up to 51.7 % ($P < 0.01$; Table 1). Thus the quantity of embryos in the beginning ('ringed yolk'), in the middle ('standstill') and in the end ('strangled embryos') incubation was increased. On opening, chickens in these eggs had livers with reddish colour and covered with a thin green paste. This can be used to establish the reasons of destruction of chickens during the first 7 days of cultivation. These data can be used to establish the anti-stressful additives for a poultry of parental herd.

Table 1 The results of the incubation depending on A- and E-vitamin security of the hens

	The additives of vitamins in forage to the hens		Incubation waste (%)			Hatchability (%)	Youngster output (%)
	A (mln. IO/t)	E (g/t)	Ringed yolk	Standstill	Strangled embryos		
1	10	10	5.1	9.7	9.0	76.2	74.2
2	100	10	7.1*	8.9	10.8	73.2	70.2*
3	300	10	11.6**	10.7	14.5*	63.2*	51.7**
4	10	100	6.1	9.5	5.4	79.0	76.9
5	10	500	6.0	9.8	8.8	75.4	70.9
6	40	40	3.9*	7.6*	5.7*	81.5*	78.6*
7	300	500	6.8	9.8	10.2	73.2	69.5*

Difference is probable at: * $P < 0.05$; ** $P < 0.01$.

Population of Spermatozoa in the Peri-vitelline Layer of Eggs Laid by Four Different Cross-breedings of Ducks

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ABSTRACT

Mule ducks are produced by artificial insemination of a Pekin dam with sperm from a Muscovy drake. Average fertility rates in this crossbreeding are generally below those found in pure breeds and the maximum duration of fertility is shorter (5.5 days *versus* 7.7 days).

In order to better understand the rationale for pre-cited differences, an experiment was carried out to measure the population of spermatozoa trapped in the perivitelline (PV) layer of eggs laid by females from four cross-breedings: (1) ♀ Pekin X ♂ Pekin; (2) ♀ Pekin X ♂ Muscovy; (3) ♀ Muscovy X ♂ Pekin; and (4) ♀ Muscovy X ♂ Muscovy. Following a single artificial insemination (AI) of 200×10^6 spermatozoa/hen (pooled semen samples from 5–6 males), eggs were collected for 10 days from the 2nd day post-AI. Eggs were incubated for 20 hours in order to assess early fertility and a total of 8 eggs/day/cross-breeding were then treated to determine the population of PV sperm.

Despite a high intra-cross-breeding variability of PV sperm within a given day ($60\% < CV < 200\%$), the population of PV sperm/egg was significantly higher in eggs from Pekin compared with Muscovy hens on Days 2 and 4 after AI. The highest sperm population was generally observed on Day 2 post-AI with an exception for eggs from the Muscovy X Muscovy cross on Day 3. Sperm population at maximum represented 0.08, 0.02, 0.05 and 0.04 % of the inseminated dose in ♀ Pekin X ♂ Pekin, ♀ Pekin X ♂ Muscovy, ♀ Muscovy X ♂ Pekin, ♀ Muscovy X ♂ Muscovy crossbreeding respectively.

Following the day with maximum PV sperm, the population of PV spermatozoa decreased dramatically (*i.e.* by 30–80% from day to day) in all crosses reaching near zero values by Day 5 post-AI in the ♀ Pekin X ♂ Muscovy breed and on days 7–8 in the three other breeds. For a given day post-AI, the ♀ Pekin X ♂ Muscovy cross was found to have the lowest population of PV sperm and, also, the lowest level of fertility. Additionally, it appeared that the population of PV sperm issued from Pekin drakes was lower in eggs laid by Muscovy ducks than in eggs laid by Pekin ducks. In females inseminated with semen from Muscovy drakes, the populations of PV sperm were comparable irrespective of the female origin. In conclusion, these observations indicate that selection of Muscovy sperm by the oviduct is similar irrespective of the genetic origin of females.

Albumen Quality, CO₂ Pressure in Air Cell and Chick Performance Parameters as Affected by Flock Age, Egg Storage Length and Turning Duration during Incubation

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ABSTRACT

Incubating egg characteristics are linked to the age of broiler breeders and/or storage conditions. Their interactions with incubation conditions can affect embryonic development during incubation and chick production parameters. Egg turning during artificial incubation is necessary to allow the embryo to utilise albumen protein (Deeming, 1989). Therefore, this study aimed to

investigate the effects of strain, age of breeders, egg storage time and turning duration during incubation on albumen Haugh unit (HU) and pH, CO₂ pressure (*p*CO₂) in air cell, embryonic mortality, hatchability and chick quality.

A total of 8,400 incubating eggs produced by two commercial flocks of Cobb and Ross broiler breeders and stored for 18, 13, 8 and 3 days were used during four incubation settings corresponding to the breeders age of 35, 45, 52 and 62 weeks. Samples of eggs were broken to measure albumen HU and pH before the beginning of incubation. The eggs were incubated at 37.6°C, 55% relative humidity and turning once an hour during the first 12, 15 or 18 days of incubation. On day 18 of incubation, samples of incubated eggs with an evidence of living embryos were used to measure *p*CO₂ in air cell on day 18. Dead embryos during incubation were classified as early or late dead embryos. Samples of hatched chicks of good quality were weighed at the end of incubation and again after 7 days of rearing.

Longer storage length resulted in increasing albumen pH, early and late embryonic mortality and day-old chick weights ($P < 0.001$) and decreasing in HU, hatchability, 7 days old chick weights and *p*CO₂ ($P < 0.001$).

Increasing age of breeders was followed by decreasing HU, hatchability and percentage of chicks of good quality and by increasing embryonic late mortality, chick weights ($P < 0.001$). The lowest *p*CO₂ was obtained when the breeders were 45 weeks of age in both strains.

Turning the eggs of old breeders during the first 15 days of incubation resulted in lower hatchability compared with turning during the first 12 or 18 days of incubation. In eggs from old breeders, the percentage of chicks of good quality increased with the turning duration in both strains ($P = 0.01$). For Cobb eggs, day-old chick weights in eggs subjected to turning during the first 12 days of incubation were smaller than those in eggs subjected to turning during the first 15 or 18 days of incubation ($P < 0.001$). However, 7 days old chick weights decreased significantly as the turning duration increased for Cobb eggs at all ages ($P < 0.001$) and for Ross eggs from flocks at 52 and 62 weeks of age ($P < 0.001$).

It is concluded that fresh eggs with high HU value may be stored longer than those with low values of HU. The eggs of young breeders may be turned during the first 12 days of incubation while those of the old breeders until day 18 of incubation. The strain, the age of breeders and the egg storage length may be considered in order to design differential incubation conditions in order to improve production parameters.

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Relationship Between Eggshell Temperature and Incubator

Temperature

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ABSTRACT

Maintenance of the optimum incubation temperature (37.2–37.8°C) is a critical factor in determining hatchability of eggs of an incubator. The temperature around the embryo depends on three factors: (1) the air temperature of the incubator; (2) the transport of heat between egg and environment; and (3) the time-variable heat production of the embryo. In commercial incubators, the transport of heat between the egg and its environment is convection. The objective of this research was to use infrared thermography to quantify the eggshell temperature and analyse the resulting eggshell temperatures in relation to the micro environment in an incubator. Thermocouples and infrared thermography, a non-intrusive temperature measurement, were used to study the heat transfer from the embryo to its environment in a laboratory incubator test installation.

From about 100 minutes after the start of the heating at the beginning of the incubation process, eggshell temperatures reached 37°C (Figure 1). It was clear that the eggs more towards the edges, warmed up more quickly than eggs located more towards the inside. This may be due to a better flow of conditioned air around the egg more towards the edges. Although the set point of air temperature was 37.7°C, the eggshell temperatures at day 17 varied from 37.15°C to 39.06°C (Figure 1). Regardless of these large eggshell temperature gradients, 222 chickens hatched from the total number of 300 incubated eggs.

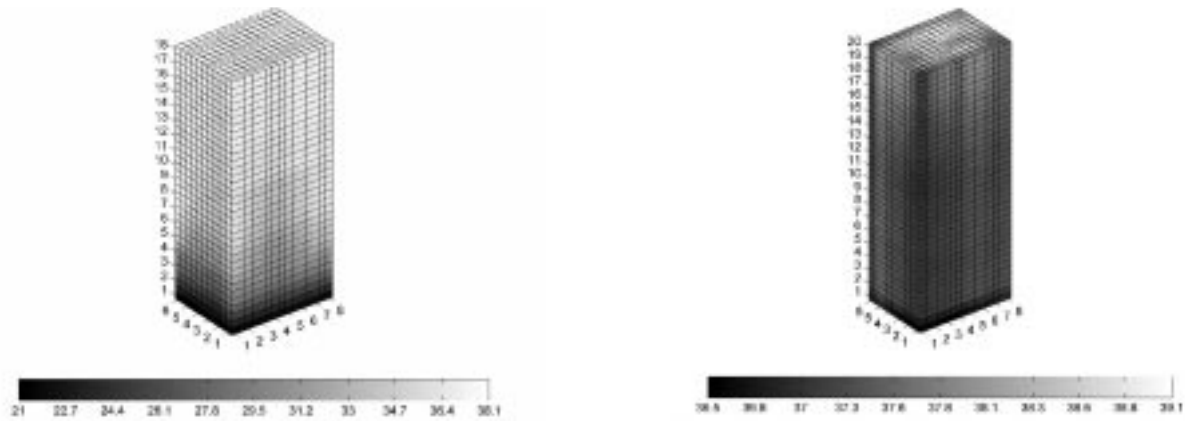


Fig. 1 Spatio-temporal gradients of the eggshell temperature for (1) the first 170 minutes of the incubation process (left), and (2) on day 17 of incubation (right). Both images are of the front view of the egg stack. Note different scales.

The mean eggshell and air temperature was recorded for the eggs located in the bottom egg tray throughout incubation (Figure 2). From day 2 to 6, the mean air temperature was 37.60°C, and the mean eggshell temperature was 37.58°C. This difference is due to the fact that the eggs are losing water but not generating significant amounts of metabolic heat at this time. The heat production at this stage was –10 mW per egg (Romijn and Lokhorst, 1960). From day 16 to 17, the mean air temperature was 37.95°C yet the mean eggshell temperature was 38.25°C. At this time in the incubation process each egg produced about 115 mW of heat (Romijn and Lokhorst, 1960).

From the surface area of the eggshell, determined from measurements of the length of the egg and the maximum breadth (Narushin, 2001), the heat production and the temperature difference between eggshell and air, the convection coefficient was quantified. At the beginning of the incubation process the convection coefficient was 71 W/(m²°C) whereas at the end of the incubation it was 57 W/(m²°C). The mean value for the convection coefficient was 64 W/(m²°C).

Although the air temperature was controlled at 37.7°C, the measured eggshell temperatures showed large spatio-temporal gradients due to imperfectly mixing of air in the incubator.

Narushin, V.G. 2001. Shape geometry of the avian egg. *J. Agric. Engng. Res.*, 8.

Romijn, C. and Lokhorst, W. 1960. Foetal heat production in the fowl. *J. Physiol.*, **150**, 239–249.

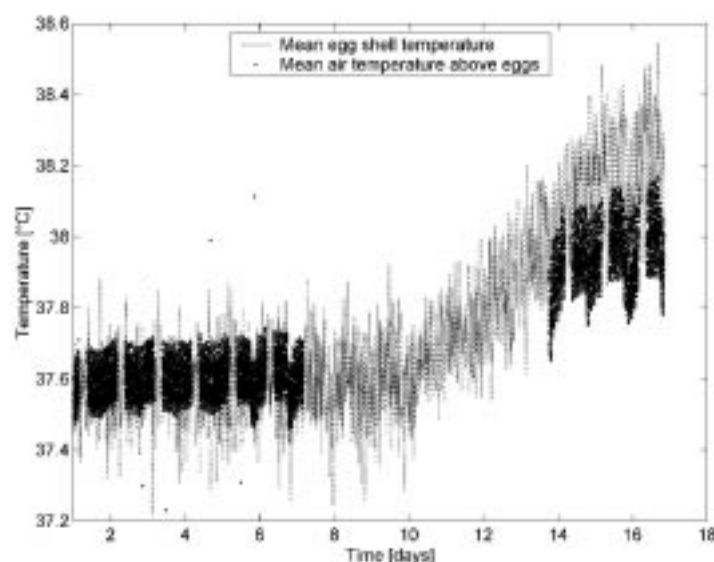


Fig. 2 Mean eggshell and the mean air temperature in time for the eggs located in the bottom egg tray.

Effect of Temperature and Oxygen upon Embryos during the Plateau Stage

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ABSTRACT

The incubation temperature and the oxygen partial pressures that embryos are subjected to during the plateau stage of development could alter the metabolism and affect organ development. Investigators have suggested that an orderly maturation and growth of the organ systems by the time of hatch may impact performance of the hatchling in the grow-out house. A number of investigators (French, 2000; Givisiez *et al.*, 2001) have demonstrated the effects of temperature upon hatchability, and organ growth. The effect that incubation temperature has upon oxygen consumption of the embryo has also been investigated (Hulet and Meijerhof, 2001).

The objective of this three-part study was to investigate the metabolism of the heart and intestinal maturation in broiler breeder embryos exposed to different temperatures and oxygen concentrations. Fertile eggs were obtained from flocks when 50 to 55 weeks of age. Experiment 1 was conducted to examine affects of different oxygen exposures from day 17 to 21 of incubation: oxygen partial pressures of 130, 145, 160 (normal air), and 175 (mm Hg). Experiment 2 was conducted to examine effects of cabinet temperature of 36°, 37°, 38°, and 39°C. During Experiment 2 the relative humidity was adjusted depending upon temperature treatment to obtain the same water vapour pressure in the cabinets, while the partial pressure of CO₂ was maintained at less than 0.7 mm Hg. The third experiment was a 2 by 2 factorial arranged experiment using cabinet temperatures of 36° and 39° with oxygen concentrations of 140 and 170 mm Hg. Intestine (jejunum), heart and liver tissues were collected from externally pipped and hatched embryos. Tissues were weighed and heart glycogen, liver glycogen, blood glucose, jejunal weight and jejunal maltase and alkaline phosphatase activity were determined.

Reducing oxygen partial pressures (130 and 145 mm Hg) decreased heart weights with no changes in total heart glycogen (Table 1). High oxygen partial pressure (175 mm Hg) increased jejunal maltase activity compared to all other treatments (Table 1). Elevating incubation temperatures depressed heart weight and heart glycogen (Table 2). The factorial arrangement (Table 3) demonstrated that jejunal maltase was significantly elevated when embryos are exposed to lower cabinet temperatures.

Table 1 Cardiac glycogen of hatched chicks subjected to four different oxygen partial pressures ($n = 10/\text{treatment}$)

Oxygen Partial Pressure	Heart weight (g)	Heart glycogen (mg)
130 mm Hg	0.2794 ^b	0.42
145 mm Hg	0.2739 ^b	0.39
160 mm Hg	0.3307 ^a	0.31
175 mm Hg	0.3107 ^a	0.42

Means followed by a different superscript differ significantly ($P < 0.05$).

Table 2 Cardiac glycogen of hatched chicks subjected to four different temperatures ($n = 10/\text{treatment}$)

Temperature (°C)	Heart weight (g)	Heart glycogen (mg)
36	0.3596 ^a	0.63 ^a
37	0.3678 ^a	0.51 ^{ab}
38	0.3367 ^{ab}	0.45 ^b
39	0.3110 ^b	0.39 ^b

Means followed by a different superscript differ significantly ($P \leq 0.05$).

Table 3 Jejunum intestine maltase activity (1 mol glucose/h/mg jejunum) of hatched chicks subjected to two temperatures and two different oxygen partial pressures ($n = 10/\text{treatment}$)

Temperature (°C)/ oxygen partial pressure	140 mm Hg	170 mm Hg	
36	0.363	0.367	0.365 ^a
39	0.189	0.139	0.163 ^b
	0.275	0.253	

Means followed by a different superscript differ significantly ($P \leq 0.05$).

There were effects of temperature and oxygen on heart and intestinal physiology. These data suggest that high incubation temperatures and low oxygen partial pressures reduce the rate of heart growth. Lower incubation temperatures possibly assist to conserve heart glycogen while oxygen concentration had no effect. Lower incubation temperatures demonstrated enhanced maltase activity for the hatched chick. The increased jejunal maltase activity may suggest that reduced incubation temperatures enhance intestinal functionality.

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Progeny from Cryopreserved Golden Eagle Spermatozoa

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ABSTRACT

Successful production of progeny of non-domestic avian species from cryopreserved spermatozoa has been limited to a few species (see Wishart, 2000), based on interest in their conservation, the availability of captive birds and the expertise required for semen collection and artificial insemination (AI). Falconers have developed techniques for semen collection and AI in several raptors and progeny have been produced from cryopreserved semen of American kestrels (Brock and Bird, 1991) and peregrine falcons (Parkes *et al.*, 1996; Samour, 1987; J. Blanco, personal communications). Here we present the first published account of progeny from cryopreserved eagle spermatozoa.

One of the problems of cryopreservation of semen from non-domestic birds has been the need to use sophisticated laboratory equipment under non-laboratory conditions. Therefore, we have sought to utilise 'field methods', the simplest of which involves freezing sperm as pellets formed by dropping diluted semen directly into liquid nitrogen (Tselutin *et al.*, 1995). Whilst this has been successful for some species such as the Houbara bustard (Hartley *et al.*, 1999), we had been unable to obtain progeny from raptors with this fast-cooling method. Recently, it was shown that spermatozoa from eagles and falcons are particularly resistant to the hyperosmotic conditions that occur during cryopreservation (Blanco *et al.*, 2000). We therefore investigated cryopreservation of golden eagle spermatozoa at slow cooling rates. This was achieved using a simple freezing apparatus, 'Mr Frosty' (Nalgene[®]), placed in a domestic freezer at -20°C . 'Mr Frosty' is a plastic container that is designed, when placed in a -80°C freezer, to cool vials of cultured cells within its isopropanol-jacketed chamber at a rate of -1°C per min.

The sperm donor was a 5-year-old golden eagle. Approximately 40 μl semen was collected, twice-daily, by voluntary deposition onto a polythene-gloved hand and then transferred to a capillary tube. After cooling to 5°C in a refrigerator, the semen was diluted with the same volume of a glutamate-based solution (Lake and Stewart, 1978) in a small cryovial. Samples were equilibrated at 5°C for 15 min and then mixed with the same volume of diluent, containing 18% dimethylacetamide. These were inserted into a 'Mr Frosty', also equilibrated at 5°C , and then placed inside a domestic freezer for 1.5–2 hours, after which the cryovials were removed from 'Mr Frosty' and plunged into liquid

nitrogen. Alternatively, samples were dropped into liquid nitrogen to form pellets (Tselutin *et al.*, 1995), which were then placed into cryovials. All samples were stored in liquid nitrogen and were thawed by dipping cryovials into a bath of warm ($\sim 37^{\circ}\text{C}$) water. The proportion of motile spermatozoa was estimated by manual assessment of individual spermatozoa in replayed video-recordings and the proportion of live spermatozoa were estimated after staining with nigrosin/eosin (Hartley *et al.*, 1999). Samples frozen in 'Mr Frosty' were inseminated into a 8-year-old Steppe eagle by transferring the thawed semen into a $100\ \mu\text{L}$ capillary tube and blowing the contents directly into the cloaca, after stimulating the eagle for copulation. Inseminations were performed twice daily for 5 days before lay and 3 times daily for 2 days after the laying of each egg—which occurred at 3-day intervals. Eggs were artificially incubated in a forced air incubator at 37°C , with a calculated average weight loss of 13%.

The cooling rate for a samples contained within 'Mr Frosty' is shown in Figure 1. This was logarithmic, with rates ranging from $-0.3^{\circ}\text{C}/\text{min}$ between 5 and -5°C to $-0.08^{\circ}\text{C}/\text{min}$ between 12 and 14°C . Nucleation occurred at $-9.6 \pm 2.5^{\circ}\text{C}$, 70 ± 11 min after placing in freezer (mean of four samples). Sample volume and sperm concentration were typically $24 \pm 5\ \mu\text{L}$ and 183 ± 112 million sperm/ml, respectively ($N = 4$). Fresh samples showed $39 \pm 4\%$ motile spermatozoa and $87 \pm 3\%$ 'live' spermatozoa ($N = 3$), whilst in cryopreserved samples, these parameters were reduced to $11 \pm 8\%$ and $35 \pm 14\%$, respectively ($N = 4$). Samples frozen as pellets had 3.1% motile and 4.1% live spermatozoa (mean of two estimations). A total of five eggs were laid by the female eagle during a period of 14 days. All were fertile. Four eggs hatched (see Figure 2) at an average weight of 68 g after 39 days incubation; one embryo died at 36 days due to malpositioning at the time of pipping.

We thank Maurice Lindsay and Don Downie for skilled assistance and are very grateful to Nalgene (Europe) Ltd for the gift of 'Mr Frosty' and to DCP Microdevelopments Ltd for fabricating the temperature sensors.

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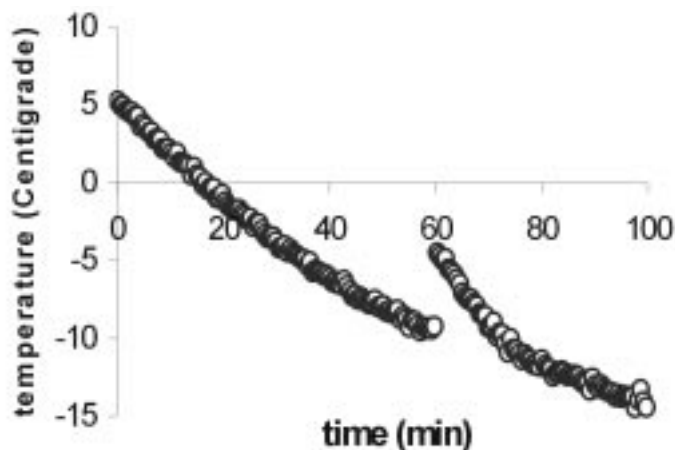


Fig. 1 The cooling rates within 'Mr Frosty' held at -20°C .



Fig. 2 'Thor' at 8 days.