

## Oviducal sperm and fertilisation in poultry

Murray R. Bakst

*Germplasm and Gamete Physiology Laboratory, Livestock and Poultry Sciences Institute, Beltsville Area, Agricultural Research Service, USDA, Building 262, Poultry Road, Beltsville, Maryland 20705, USA E-mail: murray.bakst@ars.usda.gov*

### INTRODUCTION

Wordsworth once recited, “The child is father to the man”. In similar sense, the events that transpire in the avian oviduct and give rise to the fertilised ovum will impact the subsequent development of the embryo and hatchling. The oviduct is not only the organ responsible for the formation of the egg but it is intimately involved in such processes as sperm selection, sperm storage, sperm transport, and ultimately fertilisation and early embryonic development.

Whether sperm are present or not, every 25 hours or so, the ovulated yolk is gathered up by the infundibulum and begins its journey through the oviduct (Figure 1) where it accrues albumen in the magnum, the shell membranes in the isthmus, and the hard shell in the uterus [shell gland]. The most caudal oviducal segment, the vagina, serves as a conduit between the uterus and cloaca at oviposition. Oviposition marks the end of the daily ovulatory cycle with the next ovulation, generally within 45 minutes of oviposition, beginning the next ovulatory cycle.



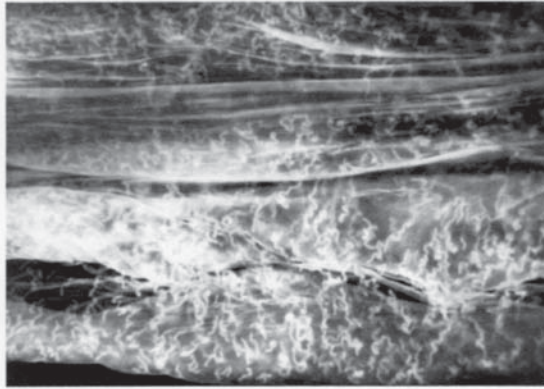
**Figure 1** This turkey oviduct has a hard-shell egg in the uterus. The arrow denotes the utero-vaginal junction (UVJ), the location of the sperm-storage tubules (SST). The funnel-like fimbriated region of the infundibulum is evident (white arrow), as are the coiled magnum and isthmus segments.

If the ovum is to be fertilised, it will take place in the infundibulum. Here sperm interacting with the ovum may have resided in the hen’s sperm-storage tubules (SST) located at the utero-vaginal junction (UVJ) (Figure 1) for a day, or weeks, following insemination. If fertilised, the developing embryo would have rapidly divided (see Fassenko, 2001) and at the time of oviposition, an embryo (blastoderm) of 60,000 cells (domestic fowl) or 30,000 cells (turkey) can be discerned on the surface of the ovum. In this chapter, our current knowledge of regarding the fate of sperm in the oviduct is briefly reviewed, particularly sperm storage at the distal end of the oviduct, and sperm:ovum interaction at the anterior end of the oviduct.

### SPERM SELECTION, STORAGE AND TRANSPORT

Following sperm transfer into the vagina, which is accomplished by either natural mating or artificial insemination, sperm are selected for transport to the UVJ. This is either by some yet to be defined mechanism in the vagina, possibly immunological in nature (Wishart and Horrocks, 2000), or sperm succeed based on their own intrinsic mobility. This question of sperm competition is quite fascinating and has tremendous implications for commercial turkey breeding. For example, it is known that 50–70% of the progeny from hens inseminated with semen pooled from 12–15 toms will be derived from only one or two toms. Not only is this further limiting the gene pool for successive generations, but also it is not known if the toms producing the more “competitive sperm” have the most desirable phenotype.

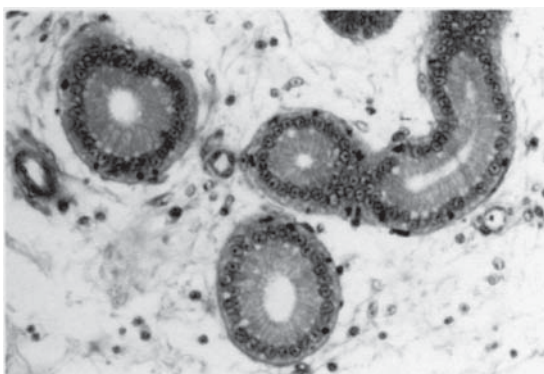
Located in the UVJ are the anatomical structures responsible for oviducal sperm storage, the SSTs (Figures 2, 3, 4 and 5). These are located predominately in the apex of the UVJ mucosal folds and are generally located at the end of short fine folds or groves lined with cilia. Unlike the tubular glands found in the distal infundibulum, magnum, isthmus and uterus, the SSTs are not true glands. Histologically, the columnar cells forming the SST show little evidence of secretory activity (Figures 3,



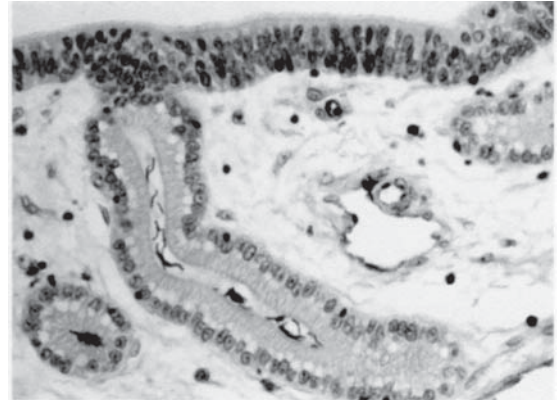
**Figure 2** Sperm-storage tubules are observed in this preparation of mucosa from the UVJ. The SSTs are visualised by directing an intense light source at the level of the specimen and parallel to the stage of a stereomicroscope. Each fold is about 2  $\mu\text{m}$  wide.

4 and 5). From the perspective of the resident sperm, this is good because cyclical secretory activity would flush sperm from the SST lumen. It has been suggested that the SST epithelium may have an absorptive function.

Although inseminated weekly with 200–400 million sperm when in egg production fewer than 2 million sperm are found in the turkey SSTs. This could reflect the highly competitive nature of sperm selection by the vagina or a finite capacity of the SST. How sperm navigate against the abovarian (movement away from the ovary) beat of the cilia lining the vaginal mucosa (Figure 6) and ascend to the UVJ and later to the site of fertilisation, is still speculative. It is now known that intrinsic sperm mobility, as measured by the sperm's ability to successfully penetrate a viscous media, is correlated with sperm fecundity within a male fowl or turkey. This has lead to the suggestion that the vagina orchestrates a very intense sperm selection process (Steele and Wishart, 1992). Dead sperm and sperm enzymatically



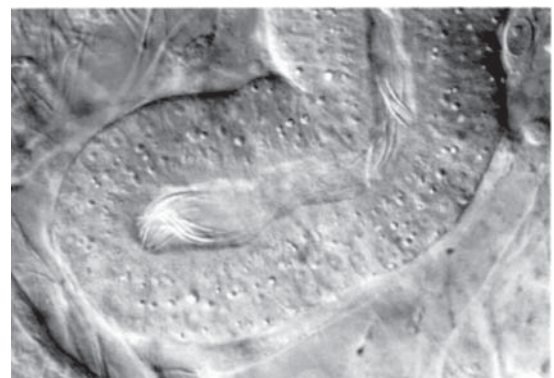
**Figure 3** Histological preparation of individual SSTs reveal a simple columnar, non-secretory, epithelium characterised by a basally located nucleus and a supranuclear lipid droplet. The width of each SST is about 45  $\mu\text{m}$ .



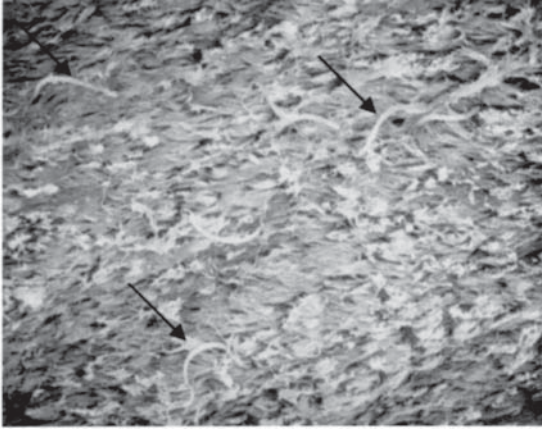
**Figure 4** Histological preparation of individual SSTs. Sperm are observed residing in the SSTs and the ciliated pseudostratified columnar epithelium lining the UVJ surface mucosa can be seen. Width of each SST is about 45  $\mu\text{m}$ .

treated to remove specific surface molecules fail to ascend to the UVJ. Conversely, highly mobile sperm, which have been found to come from the most fecund males, out compete the less mobile sperm and reach the UVJ and populate the SSTs (Donoghue, 1999).

The importance of the SSTs in the strategy of avian reproduction cannot be underestimated. Synchronisation of ovulation and the presence of sperm at the upper infundibulum is of singular importance to assure fertilisation. Birds do not have an oestrus cycle, and consequently there is no signalling of the female to the male that ovulation is imminent and so copulation is a priority. Birds overcome this apparent obstacle by having the capacity to store sperm in the SSTs. With all avian species studied to date, oviducal sperm storage within the SST assures the following: (1) eliminates the need for synchronisation of copulation with ovulation; (2) minimises the need for sperm transfer for the production of fertile eggs over one or more clutches; (3) provides a reservoir for “selected” sperm; and (4) affords



**Figure 5** The distal end of a SST is viewed by differential interference contrast microscopy. Using this technique, specimens can be viewed unfixed and sperm are observed slowly oscillating in many SSTs. The width of the SST is about 45  $\mu\text{m}$ .



**Figure 6** This scanning electron micrograph shows sperm on the cilia lining the vaginal mucosa. Note that the orientation of the sperm heads is in the opposite direction of the cilia beat.

protection to sperm during the daily ovulatory cycle. For many years there has been considerable research effort to attempt to understand how sperm survive within the SST. It is assumed that sperm residing in the SST are metabolically quiescent, motility suppressed, and that the plasmalemma and acrosomal membranes are stabilised. Whether resident sperm release signalling molecules to the surrounding SST epithelial cells (paracrine signaling) is also unknown. Possibly carbohydrate and lipid materials are transported to resident sperm. Carbonic anhydrase concentration, as well as zinc concentration in and around the SST epithelium, varies between the turkey, fowl, and quail (Holm *et al.*, 1996).

The actual mechanisms of sperm release from the SST have been speculative and include a passive escape of resident sperm while the hen is in egg production, to a squeezing of the sperm when the egg mass passes through the UVJ. Whatever the mechanism is, it has to be assumed that the quiescent sperm in the SST are activated upon leaving the SST, or when in the vicinity or contact the ovum.

Recently published observations (Freedman *et al.*, 2001) revealed that the SSTs are innervated and that actin is present in the apical cytoplasm (terminal web) of the SST epithelial cells. The observation that nerve fibres are associated with individual SST suggests the possibility that a neural mechanism may be involved in the release of sperm from the SST. The actin-rich terminal web in the SST epithelium may be involved in a contraction, or a series of contractions of the SST, thereby expelling sperm closest to the SST opening. Speculating further, cholinergic neurotransmitters liberated from nerve terminals adjacent to SST may alter resident sperm plasmalemma permeability to calcium ions ( $\text{Ca}^{2+}$ ). Given the crucial role of intracellular  $\text{Ca}^{2+}$  in avian sperm motility (Ashizawa *et al.*, 1992), cholinergic induced influx of  $\text{Ca}^{2+}$  may reac-

tivate motility of some sperm cells leading to their rapid escape from the SSTs. It has been proposed above that there is a reversible suppression of sperm motility and metabolism, as well as a stabilisation of the sperm plasmalemma and acrosomal enzymes during residency in the SST, an inactive or “decapacitated” state (Bakst *et al.*, 1994). An acetylcholine-induced influx of  $\text{Ca}^{2+}$  into the SST lumen may represent a form of sperm activation (capacitation?).

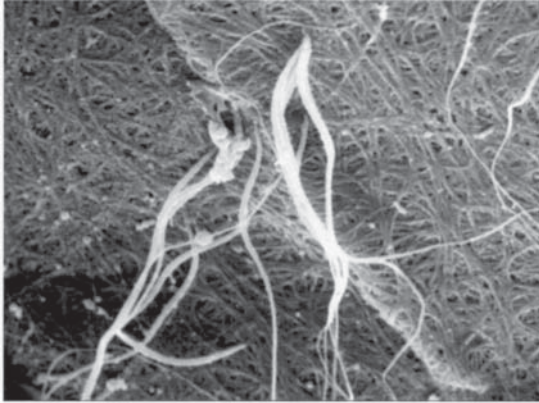
To fertilise a nearly daily succession of ova, sperm are slowly but continuously released from the SST and ascend to the site of fertilisation, the infundibulum, which is the most anterior segment of the oviduct. Here the sperm may accumulate in very small numbers. Given the attributes of the UVJ SSTs (see above), and the paucity of sperm at the infundibulum at any one time, it can be debated whether (Bakst, 2001) whether the infundibulum should be considered a secondary sperm storage site.

To conclude here, an understanding of the biological mechanisms regulating oviducal sperm transport and storage will provide the foundation for improvements in the efficiency of poultry breeding and aid in the development of improved technologies for the propagation of other domestic and feral birds and the preservation of their germplasm.

#### SPERM: OÖCYTE INTERACTION

One should not begin a discussion on fertilisation without briefly describing gamete structure and the function of the organelles in the process of fertilisation. Briefly, galliform sperm are elongated and widest ( $0.6\ \mu\text{m}$ ) at the distal head region (Figures 6 and 7). The head consists of an elongated nucleus capped with an acrosome. The acrosome contains hydrolytic enzymes that when released at the time of the acrosome reaction, digest a path through the peri-vitelline layer (PL), the acellular investment around the hen’s ovum. The tail of the sperm consists of paired centrioles from which the flagellar portion of the tail is derived and the midpiece, a segment containing an array of mitochondria. Surrounding the sperm is the plasmalemma.

The megalecithal ovum (large yolky type; see Fasenko, 2001) at ovulation consists of a vast vegetal region, characterised by yellow yolk, and the 3–4 mm diameter germinal disc. The germinal disc contains white yolk spheres and cell organelles. In addition to organelles, the oolemma (the plasmalemma of the ovum) in the germinal disc has a dense array of microvilli which project into the peri-vitelline space between the underside of the PL and the ovum (Figure 8). Microvilli and the intact oolemma are unique to the germinal disc as the oolemma is

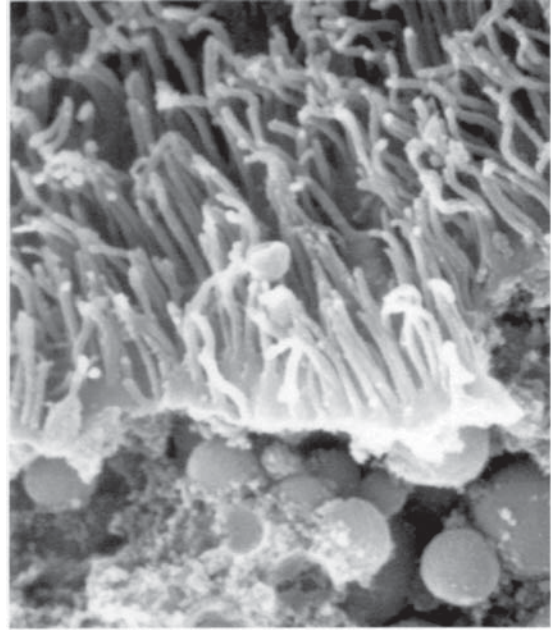


**Figure 7** A scanning electron micrograph reveals a cluster of sperm and some single sperm on the surface of the peri-vitelline layer (PL). The PL was isolated from a recently ovulated ovum and co-cultured with sperm before fixation.

discontinuous in the vegetal region and the yolk abuts directly to the underside of the PL.

It is assumed that within a very short time window, possibly minutes, one or dozens of sperm make contact with the surface of the recently ovulated ovum, certainly before any secretions from the infundibulum begin to envelop the ovum and mask the surface of the PL. The PL contains constitutive proteins as well as glycoproteins that are involved in sperm recognition and initiation of the acrosome reaction. Sperm appear to bind to the PL (Figure 7) and may undergo an acrosome reaction. In a process which transpires predominantly on the PL overlying the germinal disc, sperm undergo the acrosome reaction, release one or more hydrolytic enzymes that digest the fibrous reticulum of the PL and transforms it into a fibrillar mesh which sperm can penetrate (Figure 9). It has been suggested by Wishart and Horrocks (2000) that factors associated with the vegetal region (yellow yolk) may inhibit or some way deter sperm from penetrating its overlying PL. Bakst and Howarth (1976) suggested that sperm receptors may be associated with the cytoplasmic extensions elaborated by the oolemma at the germinal disc. Sperm in the perivitelline space appear to be engulfed by the microvilli and this intimate association leads to fusion of the inner acrosomal membrane and the oolemma. Subsequently the sperm are incorporated into the vitellus (the ovum). There appears to be no limit as to the number of sperm that can hydrolyse the PL as there is no block to polyspermy other than the accretion of oviducal secretions. Conversely, incorporation of the sperm into the ovum by fusion with the oolemma may have a physiological limit.

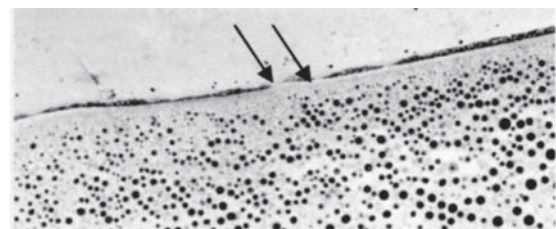
In the germinal disc, the sperm nucleus undergoes a transformation from a highly condensed elongated organelle to a spherical pronucleus. While several



**Figure 8** The dense array of microvilli observed in this scanning electron micrograph is restricted to the germinal disc region. The microvilli, which are about 1.5  $\mu\text{m}$  in height, are thought to assist in the incorporation of the sperm into the vitellus (ovum). White yolk spheres are shown at bottom of the picture.

sperm nuclei may undergo this transformation only one aligns closely apposed to the female pronucleus in the centre of the germinal disc and completes syngamy, the reconstitution of the diploid number of chromosomes, to form the zygote. Supernumerary sperm, those sperm not involved in syngamy, also undergo nuclear decondensation and some at least form pronuclei within an hour following ovulation. Within 4 hours of ovulation centrally located apposed male and female pronuclei, as well as some of the more peripherally located sperm pronuclei, undergo mitosis initiating the first cleavage of the zygote.

Little is known of the events that trigger sperm nuclear decondensation and the onset of embryogenesis. Most likely maturation-promoting factor (MPF), which is found in mammalian and amphibian oocytes and is thought to bring about both germinal vesicle



**Figure 9** A histological radial section of the germinal disc showing areas of the peri-vitelline layer (PL) that have been hydrolysed by sperm (arrows). Dots are yolk spheres. The intact PL is about 2  $\mu\text{m}$  thick.

breakdown (onset of nuclear maturation) and resumption of meiosis in the pre-ovulatory oöcyte and sperm nuclear decondensation in the fertilised ovum, is found in the germinal disc. Recently, Dong *et al.* (2000) reported another factor carried by sperm into the germinal disc that can initiate the  $\text{Ca}^{2+}$  wave within the germinal disc, activating the ovum and triggering certain events that lead to zygote formation. In species where fertilisation is monospermic, this  $\text{Ca}^{2+}$  wave triggers a block to polyspermy. In birds, polyspermy is normal, and therefore, from a teleological perspective, polyspermy could be viewed as a means to assure that the 3–4 mm diameter germinal disc is “activated”.

## CONCLUSION

To conclude, there is much we do not know about events transpiring in the oviduct leading fertilisation and initiation of development. Bakst *et al.* (1994) and Bakst (1998) provide more comprehensive reviews of the fate of sperm in the oviduct. For a review describing the current state of knowledge of the process of fertilisation in birds, the chapter by Wishart and Horrocks (2000) where current work in this area is summarised is strongly recommended.

## REFERENCES

- Ashizawa, K., Hashiguchi, A. and Tsuzuki, Y. (1992) Intracellular free Ca concentration in fowl spermatozoa and its relationship to motility and respiration in spermatozoa. *J. Reprod. Fert.*, **96**, 395–405.
- Bakst, M.R. (1998) Structure of the avian oviduct with emphasis on sperm storage in poultry. *J. Exp. Zool.*, **282**, 618–626.
- Bakst, M.R. (2001) Oviducal sperm storage in turkeys (*Meleagris gallopavo*): the infundibulum as a secondary sperm storage site, or is it? In: *Proceeding of the XVIIIth (New) International Congress of Zoology*, Athens, Greece.
- Bakst, M.R. and Howarth, B. (1976) Hydrolysis of the hen's perivitelline layer by cock sperm *in vitro*. *Biol. Reprod.*, **17**, 370–379.
- Bakst, M.R., Wishart, G. and Brillard, J.P. (1994) Oviducal sperm selection, transport, and storage in poultry. *Poult. Sci. Rev.*, **5**, 117–143.
- Dong, J., Tang, T. and Sun, F. (2000) *Xenopus* and chicken sperm contain a cytosolic soluble proetin factor which can trigger calcium oscillations in mouse eggs. *Biochem. Biophys. Res. Comm.*, **268**, 947–951.
- Donoghue, A.M. (1999) Prospective approaches to avoid future flock fertility problems: Predictive assessment of sperm function traits in poultry. *Poult. Sci.*, **78**, 437–443.
- Fasenko, G.M. (2001) The first forty-eight hours of embryonic life: Development before and after oviposition. In: *Perspectives in Fertilisation and Embryonic Development in Poultry*, pp. 7–14. Ratite Conference Books, Wallingford. Reprinted in 2009: *Avian Biol. Res.*, **2**, 7–13.
- Freedman, S., Akuffo, V. and Bakst, M.R. (2001) New evidence for the innervation of the sperm storage tubules in the turkey (*Meleagris gallopavo*). *Reproduction*, **121**, 809–814.
- Holm, L., Ridderstrale, Y. and Knutsson, P. (1996) Localisation of carbonic anhydrase in the sperm storing regions of the domestic hen oviduct. *Acta Anatom.*, **156**, 253–260.
- Steele, M.G. and Wishart, G.J. (1992) A vaginal sperm-selection mechanism recognises sperm surface proteins. In: *Proceedings of the 12th International Congress on Animal Reproduction*, pp. 1593–1595. The Hague, The Netherlands.
- Wishart, G.J. and Horrocks, A.J. (2000) Fertilization in birds. In: *Fertilization in Protozoa and Metazoan Animals: Cellular and Molecular Aspects*, Tarín, J.J. and Cano, A. (eds.), pp. 193–222. Springer-Verlag, New York.

# The first 48 hours of embryonic life: Development before and after oviposition

Gaylene M. Fasenko

Alberta Hatching Egg Producers Hatchery Poultry Research Centre, Edmonton Research Station, F1, University of Alberta, Edmonton, Alberta, T6G 2P5, Canada E-mail: Gaylene.Fasenko@ualberta.ca

## INTRODUCTION

In Eutherian mammals, the embryo develops in a uterus where it receives nourishment, eliminates waste products, and exchanges gases through a placenta attached to the uterine wall of the mother. The evolution of the placenta has eliminated the need for large yolk reserves in eggs of these species. Eggs of Eutherian mammals are classified as microlecithal (small + yolk). In birds, where the embryo grows in a cleidoic (closed) environment (Needham, 1931) and there is no physical attachment to the hen once the egg is laid. In this system there is a need for large amounts of yolk reserves in the egg to support embryonic development. As a result of the large amount of yolk in megalecithal bird eggs, the general structure of the oöcyte (unfertilised egg consisting of yolk plus maternal chromosomes) is also different. The yolk of bird eggs is concentrated on one side (vegetal pole) while the active cytoplasm containing the maternal chromosomes, is located in an area called the blastodisc, at the other side (animal pole). Eggs that have an asymmetric distribution of yolk are also referred to as being telolecithal, while homolecithal eggs (*i.e.* most mammals) have a more uniform distribution of yolk. The blastodisc is whitish in colour and is approximately 2–3 mm in diameter (Eyal-Giladi, 1991).

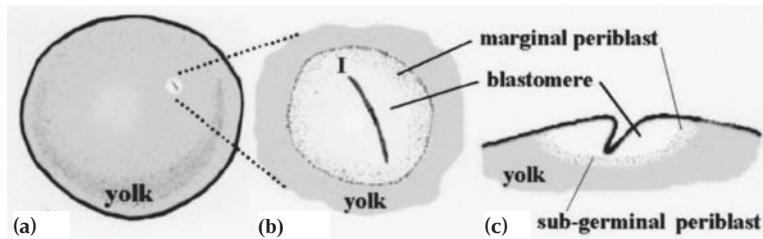
During fertilisation, the female (oöcyte) and male (spermatozoon) gametes each having a haploid set of chromosomes, come together. The union of the two sex cells produces a zygote restoring the full complement of chromosomes distinctive of the species (diploid number), and providing the driving force behind the initiation of development. After fertilisation, mitosis (cell division) involves cleavage of the zygote as the first step in producing a multi-cellular embryo. During mitosis, a single parent cell divides to produce two genetically identical cells having the same chromosome number as the parent cell. The distribution of the yolk in the egg dictates the type of cleavage that occurs. Mammalian cells undergo total (holoblastic) cleavage of yolk and active cytoplasm to

produce cells of about the same size. The large amount of yolk found in telolecithal bird eggs makes it physically impossible for holoblastic cleavage to occur. Instead, cell division in the zygote of birds is meroblastic (partial), so that only the cells at the animal pole, and not the yolk, divide.

Embryonic development in vertebrate animals can be defined as the maturational changes experienced by an individual from the point immediately after fertilisation (union of the egg and sperm) to the point in time when the animal hatches or is born. In this chapter, description of embryonic development in the domestic fowl will be limited to approximately the first 48 hours immediately following fertilisation. The process of differentiation of embryonic cells is described by Bellairs (2001). Much of this chapter is based upon the embryonic developmental stages outlined for the domestic fowl by Hamburger and Hamilton (1951) [abbreviated to HH Arabic numerals], Eyal-Giladi and Kochav (1976) [abbreviated to EG-K Roman numerals] and Kochav *et al.* (1980). Gupta and Bakst (1993) and Bakst *et al.* (1997) provide a detailed description of early embryonic development of the domestic turkey. Terminology is as recommended by Baumel *et al.* (1993).

## EGG FORMATION AND EMBRYONIC DEVELOPMENT WITHIN THE OVIDUCT

The incubation period of a domestic fowl egg from laying to hatch is about 21 days. What is often overlooked is the fact that during the approximate 26 hours it takes for an egg to form in the oviduct of the hen, the first stages of embryonic development are occurring. The embryonic developmental period while the egg is within the oviduct of the hen makes up approximately 5% of the total developmental time required for a chick to hatch. It should be noted, however, that the duration of egg formation and the stages of embryonic development most common at each segment of the oviduct may vary between individual birds of the same age and strain, or between



**Figure 1** Diagrammatic representation of the first mitotic division in a domestic fowl embryo. (a) View of entire ovum showing the embryo (embryo diameter is approximately 1/15 to 1/20 of the diameter of the ovum). (b) Enlargement of the animal pole area of the ovum showing a view of the entire embryo with the first cleavage furrow producing two cells (blastomeres). (c) Cross sectional view of the first cleavage furrow. The cleavage furrow does not extend down through the entire depth of the embryo, thus the two blastomeres are not entirely delineated from the yolk. The cytoplasm of the cells and the yolk mix in an area termed the periblast (Bellairs *et al.*, 1978). The peripheral periblast is called the marginal periblast, while the periblast beneath the embryo is termed the sub-germinal periblast.

Diagram adapted from Barrett *et al.* (1985) and Etches (1996).

birds of varying strains and ages. These processes are summarised here.

#### Fertilisation and extrusion of the second polar body

In birds, egg formation occurs as the yolk passes through five portions of the oviduct (infundibulum, magnum, isthmus, uterus [shell gland], and vagina) and coincides with early developmental processes of the embryo. After ovulation into the body cavity, the yolk (secondary oöcyte) enters the first portion of the oviduct (the infundibulum). Fertilisation takes place, assuming sperm are present, during the 15 to 30 minutes the oöcyte stays in the infundibulum (see Bakst, 2001). Within an hour after fertilisation of the oöcyte, the second polar body (discarded nucleus) is extruded from the oöcyte thereby completing the second meiotic division (Olsen and Fraps, 1944). During meiotic division a single parent cell produces four genetically identical cells having half the chromosome number of the parent cell. The fertilised yolk (oöcyte) is now called an ovum. Meiosis is completed during the two to three hour movement of the ovum through the magnum, where albumen proteins are deposited.

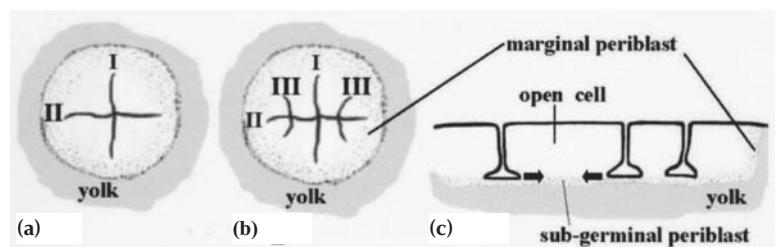
#### Cleavage of embryonic cells (EG-K Stages I to VI)

The inner and outer shell membranes are deposited during the 60 to 75 minute

interval that the ovum takes to travel through the isthmus. While the ovum is entering the uterus, approximately three to five hours after fertilisation, the first cleavage furrow develops (Olsen, 1942). Cleavage of the zygote (the fertilised single cell) involves repeated mitotic divisions to produce an embryo with thousands of genetically identical cells. As mentioned previously, the large amount of yolk contained in the ovum of telolecithal eggs makes it impossible for division of the yolk to occur and therefore the cleavage is restricted to the active cytoplasm located at the animal pole (Figure 1).

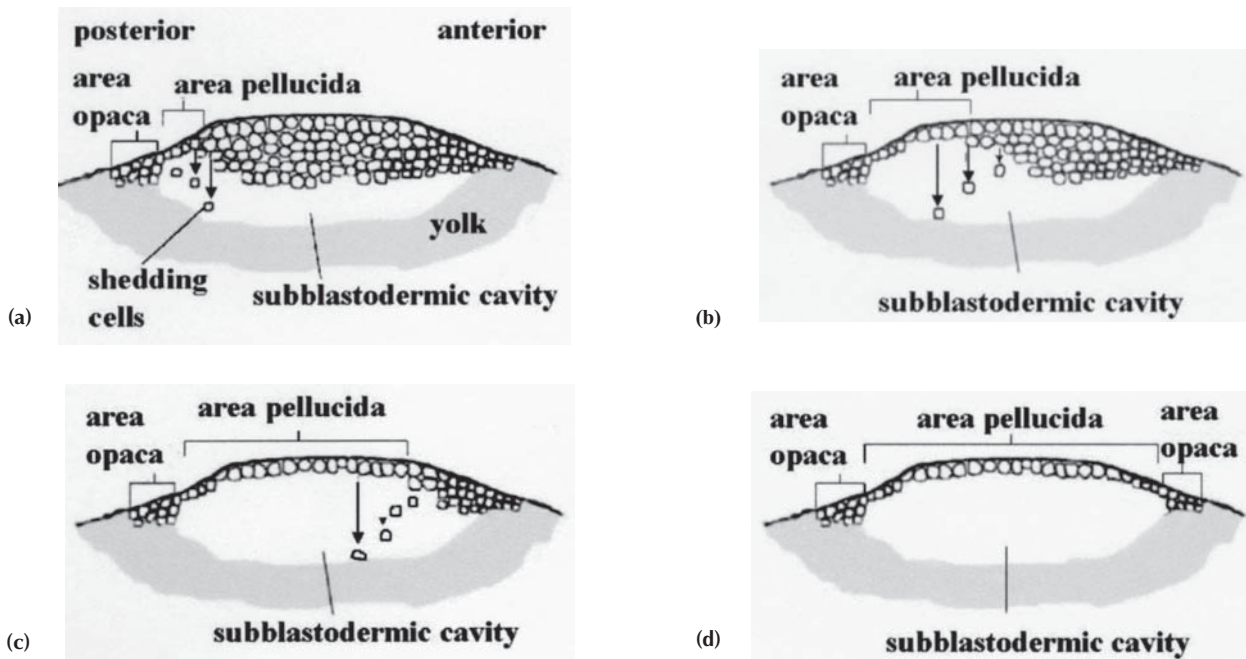
Shell deposition occurs in the uterus [shell gland] over the next 18–21 hours. It is likely that the second cleavage (Figure 2A) through to the sixth cleavages occur during the first two hours that the ovum is in the uterus (Eyal-Giladi, 1991). At the 8-cell

stage of development (Figure 2B) the blastomeres are not completely surrounded by the cell membrane, and the cell contents are therefore still in contact with the yolk (Figure 2C); blastomeres of this nature are described as “open cells” (Bellairs *et al.*, 1978). Only after two hours in the uterus do the central cells separate from the yolk when the deep ends of the cleavage furrows gradually merge together horizontally to form a fluid filled sub-blastodermic cavity (Figure 2C; Eyal-Giladi, 1984). The formation of the sub-blastodermic cavity is complete approximately 6 hours later (Kochav *et al.* 1980). At this point horizontal as well as vertical cell cleavage occurs (Eyal-Giladi, 1991).



**Figure 2** Diagrammatic representation of the second and third mitotic divisions in a domestic fowl embryo. (a) Enlargement of the animal pole of the ovum showing a view of the entire embryo and the first (I) and second (II) cleavage furrows (4-cell stage). (b) Enlargement of the animal pole showing a view of the entire embryo and the first, second, and third (III) cleavage furrows (8-cell stage). (c) Longitudinal cross sectional view of an 8-cell embryo. At this stage of development the cytoplasm of the blastomeres is still in contact with the yolk and the blastomeres are described as “open” cells. The arrows indicate the gradual horizontal merging of the deep ends of the cleavage furrows. These isolated spaces eventually combine to form the fluid filled sub-blastodermic cavity (Eyal-Giladi, 1984).

Diagram adapted from Romanoff (1960) and Bellairs *et al.* (1978).



**Figure 3** Diagrammatic representation of the stages of embryonic development in the domestic fowl associated with the formation of the *area pellucida*. (a) Longitudinal cross sectional view through a blastoderm at Eyal-Giladi and Kochav (EG-K) (1976) Stage VII. At this stage cell shedding has begun at the future posterior end of the blastoderm. (b) Longitudinal cross sectional view through a blastoderm at EG-K Stage VIII. Cell shedding continues in a postero-anterior fashion and reaches the centre of the blastoderm. (c) Longitudinal cross sectional view through a blastoderm at EG-K Stage IX. The blastoderm is between one to three cell layers thick. (d) Longitudinal cross sectional view through a blastoderm at EG-K Stage X. At this juncture all the blastodermal cells in the lower layer have been shed into the sub-blastodermic cavity. The result is a blastoderm that has a clearly demarcated central area that is translucent (*area pellucida*) surrounded by an opaque ring of cells that are still attached to the yolk (*area opaca*). Diagrams adapted from photomicrographs from Kochav *et al.* (1980).

#### Formation of the *area pellucida* (EG-K Stages VII-X)

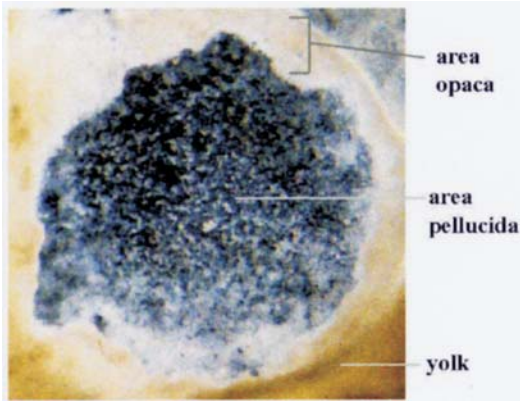
Approximately 11 hours after the presence of the first cleavage furrow, at the end of the cleavage period, the embryo, now called a blastoderm, is a circular disc about five to six cells thick in the centre but tapering out to one to two at the periphery (Figure 3A; Bellairs, 1971). The formation of the *area pellucida* marks the first morphogenetic event in the development of the domestic fowl embryo. This stage of development (EG-K VII) marks the initiation of cellular shedding from the central area of the blastoderm, which occurs over the next 8–9 hours while the ovum is still in the uterus. The shed cells fall into the anterior portion of the sub-blastodermic cavity and likely disintegrate merging with the yolk. The most ventrally located cells in the embryo next to the sub-blastodermic cavity continue to shed over the next 8–9 hours such that at the time the egg is laid, the blastoderm consists of a central area which is one cell layer thick, with a peripheral area that is several layers thick and is attached to the yolk (Figure 3D). The cell shedding starts at the future posterior side of the embryo and moves towards the future anterior side (for a review of axis determination in the avian embryo see Eyal-Giladi, 1991; Khaner, 1993; and Bellairs, 2001). The

translucent central area of the blastoderm is called the *area pellucida*, while the peripheral “ring” of cells that are still in contact with the yolk, is termed the *area opaca*. It is the epiblast cells that compose the *area pellucida*, which eventually form all the structures in the embryo proper.

#### POST-OVIPOSITIONAL EMBRYONIC DEVELOPMENT

By the time the egg is laid, the embryo consists of 40,000 to 60,000 cells. The most common stage of embryonic development at oviposition is EG-K Stage X (Figure 4). The catalyst that initiates embryonic development once the egg is laid is the environmental temperature to which the egg is exposed. Artificial incubation of eggs in commercial incubators takes place at temperatures between 37.0–38.0°C. Embryonic developmental rate may vary due to a variety of genetic and environmental factors, however, the approximate number of incubation hours required to reach the most prevalent stage of development are shown based on an recent experiment conducted by the author.



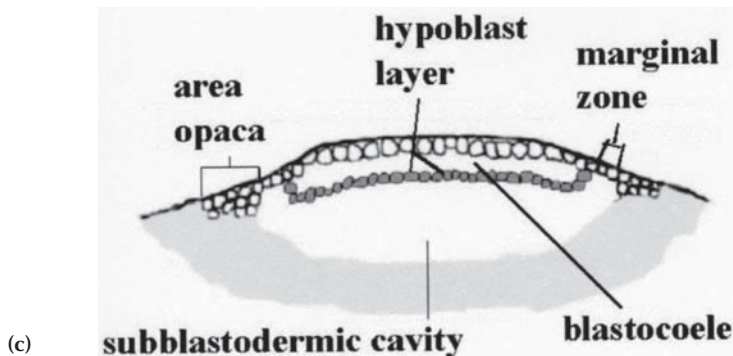
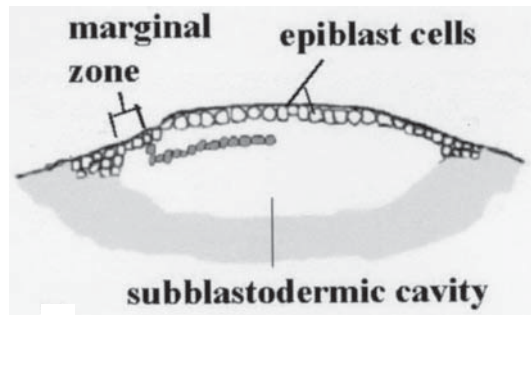
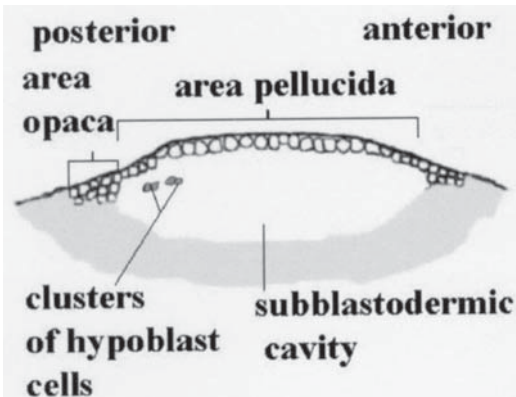


**Figure 4** Photomicrograph of an Eyal-Giladi and Kochav (1976) Stage X domestic fowl blastoderm showing a central translucent disc, the *area pellucida*, surrounded by an opaque ring, the *area opaca*, surrounding the disc. This is the most common stage of embryonic development at the time of oviposition. Print magnification is 16X.

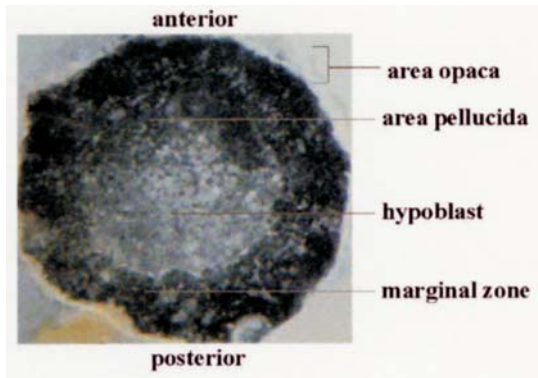
**Hypoblast formation (EG-K Stages XI-XIII)**

Stage XI embryos are most prevalent between three and six hours of incubation. At this stage of develop-

ment (EG-K XI) a sickle-shaped cluster of cells (Koller's sickle) appears at the posterior side of the *area opaca* (Figure 5A). The origin of these cells has been a topic of debate (Stern, 1990; Eyal-Giladi, 1991, 1992). This event marks the beginning of the first stage of gastrulation of the chick embryo, the formation of the hypoblast and epiblast. Gastrulation is defined as the period during embryonic development when cellular movements in the embryo transform the single cell layered embryo into an embryo with two or three distinct layers of cells. Between six and eight hours of incubation, the hypoblast cells have migrated approximately half way across the lower surface of the *area pellucida* (EG-K Stage XII; Figure 5B and Figure 6). By nine to ten hours of incubation, the formation of the hypoblast is complete such that the hypoblast forms a continuous disc of cells on the underside of the blastoderm (EG-K Stage XIII; Figure 5C). Two to three hours later, the blastoderm has formed a bridge of cells between the hypoblast and the *area opaca* (EG-K Stage XIV). The sole goal of the hypoblast is to induce primitive streak formation in the epiblast layer.



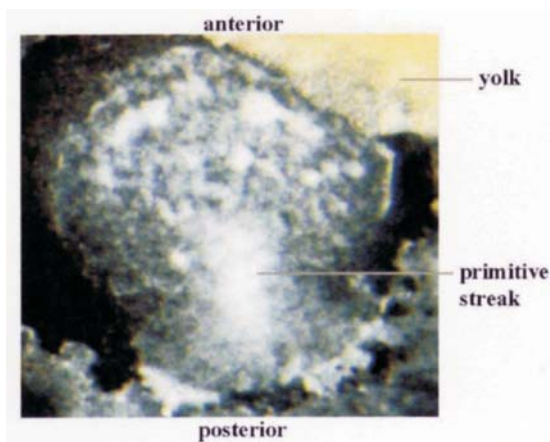
**Figure 5** Diagrammatic representation of the stages of embryonic development in the domestic fowl associated with hypoblast formation. (a) Longitudinal cross sectional view through a blastoderm at Eyal-Giladi and Kochav [EG-K] (1976) Stage XI. At this stage clusters of hypoblast cells line approximately one third of the posterior portion of the blastoderm. (b) Longitudinal cross sectional view through a blastoderm at EG-K Stage XII. Hypoblast formation continues in an anterior direction and now occupies half of the under surface of the blastoderm. (c) Longitudinal cross sectional view through a blastoderm at EG-K Stage XIII. Hypoblast formation is complete and covers the central portion of the *area pellucida*. A hypoblast-free ring of *area pellucida* called the marginal zone borders the hypoblast. Diagrams adapted from photomicrographs from Kochav *et al.* (1980).



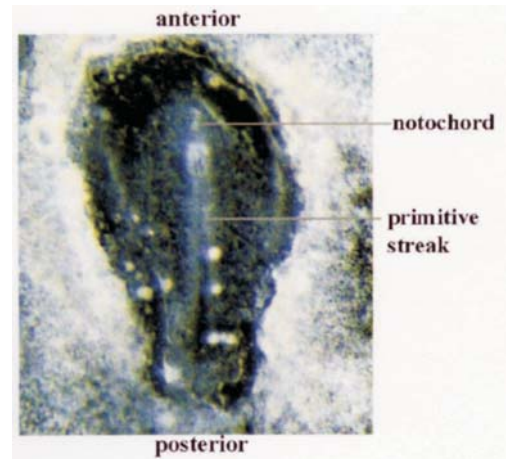
**Figure 6** Photomicrograph of the ventral view of a Eyal-Giladi and Kochav (1976) Stage XII domestic fowl embryo after approximately six to eight hours of incubation. At this stage the hypoblast cells have migrated approximately half ways across the lower surface of the *area pellucida*. The marginal zone between the hypoblast and the *area opaca* is evident. Print magnification is 16X.

### Primitive streak formation

The remaining embryonic developmental stages through to hatching have been described by Hamburger and Hamilton (1951). By approximately 12 hours of incubation, hypoblast formation is complete and primitive streak formation is initiated. Formation of the primitive streak is the second phase of gastrulation. The presence of a primitive streak is first noted at about 12 hours of incubation (HH Stage 2), and by 13–15 hours of incubation the primitive streak extends to approximately the centre of the *area pellucida* (Figure 7). As the primitive streak is formed, the epiblast cells on the surface of the blastoderm migrate to the groove in the primitive streak and ingress into the interior of the embryo (for a comprehensive review see Bellairs, 1986, 2001). As



**Figure 7** Photomicrograph of the ventral view of a Hamburger and Hamilton (1951) Stage 3 chick embryo after approximately 13–15 hours of incubation. At this stage the primitive streak development has progressed in a postero-anterior direction to approximately the centre of the *area pellucida*. Print magnification is 16X.



**Figure 8** Photomicrograph of the ventral view of a Hamburger and Hamilton (1951) Stage 5 chick embryo after approximately 21–24 hours of incubation. At this stage the primitive streak is regressing and the notochord is developing from the anterior end of the primitive streak in an antero-posterior direction.

the epiblast cells invade the interior of the blastoderm they spread out laterally and form two new cell layers, the mesoderm and endoderm. Epiblast cells remaining on the surface of the embryo form ectodermal tissue. It is from these three cell layers that all the structures of the embryo, and ultimately, the chick, are formed. As the embryo nears 24 hours of incubation the primitive streak begins to regress (Figure 8) and is replaced by a notochord that, later in development in vertebrates, becomes part of the vertebral column.

### FACTORS AFFECTING EMBRYONIC DEVELOPMENT AT OVIPOSITION

Although it is often stated that EG-K Stage X is the commonest stage of embryonic development at oviposition, there is considerable variation in developmental stages at lay. One of the factors that have been determined to influence embryonic development at the time of lay is sequence position. In domestic fowl, egg laying is described in terms of sequences rather than “clutches” observed with egg laying in wild birds. A sequence is defined as one or more consecutive days of egg laying separated by a pause day on which no egg is laid. First-of-sequence eggs are laid after a pause day and take about 40 hours to be laid from the time of the previous ovulation versus 26 hours for eggs laid on consecutive days. Therefore, the mature follicle of a first-of-sequence egg stays in the ovary for about 14 hours longer. Bernier *et al.* (1951) established that the first and last eggs in a sequence contain more advanced embryos than eggs intermediate in a sequence. Fassenko *et al.* (1992a) showed that

embryos of first-of-sequence eggs were significantly more developed than embryos from subsequent eggs in a sequence and that embryonic viability was lower in embryos from first-of-sequence eggs. The extended period that the follicle of a first-of-sequence egg remains in the ovary means that there may be ageing effects on the follicle that contribute to an increase in development and a decrease in embryonic viability. It has also been established that that blastoderm area increases as birds age (Mather and Laughlin, 1979). This may be related to sequence position, as older hens lay shorter sequences and thus have a higher percentage of first of sequence eggs (Robinson *et al.*, 1990, 1991). Mather and Laughlin (1979) hypothesised that the advanced embryonic development in older birds could also be due to the egg staying in the oviduct for a longer period of time, either because of a delayed rate of egg passage or because of a longer oviduct.

In an effort to manage hatching egg numbers, fertile hatching eggs are regularly stored at cool temperatures for usually a few days although storage can extend to a few weeks. It is usually assumed that below a temperature of 20–21°C (physiological zero) embryonic development is arrested. Using the Eyal-Giladi and Kochav (1976) classifications of embryonic development, Fasenko *et al.* (1992b) confirmed that embryonic development is arrested when hatching eggs are held at 14°C. However, management factors such as frequency of egg collection and method of egg storage can influence embryonic development between oviposition and egg storage by increasing the amount of time required for the internal egg temperature to cool below physiological zero (Fasenko *et al.*, 1991).

#### **FACTORS AFFECTING EMBRYONIC DEVELOPMENT DURING THE FIRST 24 HOURS OF INCUBATION**

One of the major factors in the hatching egg industry that negatively affects post-ovipositional embryonic development is storing eggs longer than one week. It is well established that the amount of incubation time required for a chick to hatch is lengthened when hatching eggs are stored for extended periods (Kosin and Konishi, 1973; Mather and Laughlin, 1976, 1977). The hypotheses put forth to explain this were that (1) the initiation of embryonic development is delayed, and/or (2) that embryonic development proceeds at a slower rate, in embryos from eggs stored for long periods. Unpublished data recently collected by the author verified that eggs stored for 14 days had embryos in which chronological age lagged behind the biological age. A comprehensive exam-

ination of development during the first 24 hours by the author showed that not all embryos reacted in the same manner to storage. Some embryos of 14 day stored eggs did not initiate development once incubation temperatures were provided, while other embryos began development, but at a slower rate. It was also shown that the metabolism of embryos, as measured indirectly by embryonic CO<sub>2</sub> output, proceeds at a slower rate. Perhaps the most significant finding from the study was that the development of some embryos exposed to 14 days of storage was not affected. This fact may provide the basis for future studies aimed at genetically selecting for factors that allow these embryos to withstand the negative effects of egg storage.

Arora and Kosin (1968) provided an indication of the negative physiological effects of long term storage on embryonic cell viability. In this study it was shown that the number of embryonic cells with necrotic nuclei increased as storage duration increased. A reduction in viable cell numbers may be the reason behind the observation made by Mather and Laughlin (1979) that there is shrinkage of the blastoderm when eggs are stored for 7 or 14 days. In this study they also showed that there was an increase in malformed embryos with storage, especially in eggs from young and old birds. Recent research has established that apoptosis (programmed cell death) increases when embryos are stored for 14 days at 12°C (Bloom *et al.*, 1998). It is the hypothesis of this author that long term exposure of avian embryos to storage below physiological zero may increase the number of necrotic (dead) or apoptotic cells relative to viable embryonic cells. This may inhibit initiation of embryonic development and/or impede normal development thus resulting in a higher percentage of embryonic abnormality and mortality, as there may be an optimum number of viable embryonic cells required for normal growth and development. Research currently underway by the author and colleagues aims to elucidate the relationship between long term storage and the incidence of necrotic and apoptotic cells.

#### **CONCLUSIONS**

The objective of this chapter was to provide a summary of the stages of chick embryonic development during the 24 hours before and after oviposition, and to discuss some of the major factors affecting this period of development. The information provided was not meant to be comprehensive, but was meant to give readers that are unfamiliar with early stages of avian embryonic development an appreciation for this area of study. Although the development in the

oviduct and during the first 24 hours of incubation only make up about 9% of the total developmental period of the embryo, these early stages are critical for successful development of the embryo and hatching of the chick. A general understanding of early embryonic development may also aid hatchery managers in problem solving fertility and hatchability issues in domestic fowl.

## REFERENCES

- Arora, K.L. and Kosin, I.L. (1968) The response of the early chicken embryo to pre-incubation temperature as evidenced from its gross morphology and mitotic pattern. *Physiol. Zool.*, **41**, 104–112.
- Bakst, M.R. (2001) Oviducal sperm and fertilisation in poultry. In: *Perspectives in Fertilisation and Embryonic Development in Poultry*, Baggott, G.K., Bakst, M.R., Bellairs, R., Christensen, V.L., Fasenken, G.M. and Starck, J.M. (eds.), pp. 1–6. Ratite Conference Books, Wallingford. Reprinted in 2009: *Avian Biol. Res.*, **2**, 1–5.
- Bakst, M.R., Gupta, S.K. and Akuffo, V. (1997) Comparative development of the turkey and chicken embryo from cleavage through hypoblast formation. *Poult. Sci.*, **76**, 83–90.
- Baumel, J.J., King, A.S., Breazile, J.E., Evans, H.E. and Vanden Berge, J.C. (1993) *Handbook of Avian Anatomy: Nomina Anatomica Avium*, 2nd Edition. Nuttall Ornithological Club, Cambridge, Massachusetts.
- Barrett, J.M., Abramoff, P., Kumaran, A.K. and Millington, W.F. (1985) *Biology*. Prentice-Hall, Englewood Cliffs, New Jersey.
- Bellairs, R. (1971). *Developmental Processes in Higher Vertebrates*. Logos Press Ltd. Great Britain.
- Bellairs, R. (1986). The primitive streak. *Anat. Embryol.*, **174**, 1–14.
- Bellairs, R. (2001) Some critical events in the differentiation of the avian embryo. In: *Perspectives in Fertilisation and Embryonic Development in Poultry*, Baggott, G.K., Bakst, M.R., Bellairs, R., Christensen, V.L., Fasenken, G.M. and Starck, J.M. (eds.), pp. 15–22. Ratite Conference Books, Wallingford. Reprinted in 2009: *Avian Biol. Res.*, **2**, 15–20.
- Bellairs, R., Lorenz, F.W. and Dunlap, T. (1978) Cleavage in the chick embryo. *J. Embryol. Exp. Morph.*, **43**, 55–69.
- Bernier, P.E., Taylor, L.W. and Gunns, C.A. (1951) The relative effects of inbreeding and outbreeding on reproduction in the domestic fowl. *Hilgardia*, **20**, 529–628.
- Bloom, S.E., Muscarella, D.E., Lee, M.Y. and Rachlinski, M. (1998) Cell death in the avian blastoderm: resistance to stress-induced apoptosis and expression of anti-apoptotic genes. *Cell Death Different.*, **5**, 529–538.
- Etches, R.J. (1996) *Reproduction in Poultry*. CAB International, Wallingford, UK.
- Eyal-Giladi, H. (1984) The gradual establishment of cell commitments during the early stages of chick development. *Cell Different.*, **14**, 245–255.
- Eyal-Giladi, H. (1991) The early embryonic development of the chick, as an epigenetic process. *Crit. Rev. Poult. Biol.*, **3**, 143–166.
- Eyal-Giladi, H. (1992) The posterior section of the chicks area pellucida and its involvement in hypoblast and primitive streak formation. *Development*, **116**, 819–830.
- Eyal-Giladi, H. and Kochav, S. (1976). From cleavage to primitive streak formation: A complementary normal table and a new look at the first stages of the development of the chick. I. General Morphology. *Develop. Biol.*, **49**, 321–337.
- Fasenken, G.M., Robinson, F.E., Armstrong, J.G., Church, J.S. and Hardin, R.T. (1991) Variability in preincubation embryo development in domestic fowl. 1. Effects of nest holding time and method of egg storage. *Poult. Sci.*, **70**, 1876–1881.
- Fasenken, G.M., Hardin, R.T., Robinson, F.E. and Wilson, J.L. (1992a) Relationship of hen age and egg sequence position with fertility, hatchability, viability, and preincubation embryonic development in broiler breeders. *Poult. Sci.*, **71**, 1374–1383.
- Fasenken, G.M., Robinson, F.E. and Hardin, R.T. (1992b) Variability in preincubation embryonic development in domestic fowl. 2. Effects of duration of egg storage period. *Poult. Sci.*, **71**, 2129–2132.
- Gupta, S.K. and Bakst, M.R. (1993) Turkey embryo staging from cleavage through hypoblast formation. *J. Morph.*, **217**, 313–325.
- Hamburger, V. and Hamilton, H.L. (1951) A series of normal stages in the development of the chick embryo. *J. Morph.*, **88**, 49–92. Reprinted (1992) *Develop. Dynamics*, **195**, 231–272.
- Khaner, O. (1993) Axis determination in the avian embryo. *Curr. Top. Develop. Biol.*, **28**, 155–180.
- Kochav, S., Ginsburg, M. and Eyal-Giladi, H. (1980) From cleavage to primitive streak formation: A complementary normal table and a new look at the first stages of the development of the chick. II. Microscopic Anatomy and Cell Population Dynamics. *Develop. Biol.*, **79**, 296–308.
- Kosin, I.L. and Konishi, T. (1973) Preincubation storage conditions and their effect on the subsequent livability of chicken embryos: exogenous CO<sub>2</sub>, plastic bags and extending the holding periods as factors. *Poult. Sci.*, **52**, 296–302.
- Mather, C.M. and Laughlin, K.F. (1976) Storage of hatching eggs: The effect on total incubation period. *Brit. Poult. Sci.*, **17**, 471–479.
- Mather, C.M. and Laughlin, K.F. (1976) Storage of hatching eggs: The effect on early embryonic development. *Brit. Poult. Sci.* (1977), **18**, 597–603.
- Mather, C.M. and Laughlin, K.F. (1979) Storage of hatching eggs. The interaction between parental age and early embryonic development. *Brit. Poult. Sci.*, **20**, 595–604.
- Needham, J. (1931) *Chemical Embryology*. Cambridge.
- Olsen, M.W. (1942) Maturation, fertilization and early cleavage in the hen's egg. *J. Morph.*, **70**, 513–533.
- Olsen, M.W. and Fraps, R.M. (1944) Maturation, fertilization and early cleavage of the egg of the domestic turkey. *J. Morph.*, **74**, 297–309.
- Robinson, F.E., Hardin, R.T. and Robblee, A.R. (1990) Reproductive senescence in domestic fowl: Effects on egg production, sequence length and inter-sequence pause length. *Brit. Poult. Sci.*, **31**, 871–879.
- Robinson, F.E., Robinson, N.A. and Scott, T.A. (1991) Reproductive performance, growth rate and body composition of full-fed versus feed-restricted broiler breeder hens. *Can. J. Anim. Sci.*, **71**, 549–556.
- Romanoff, A.L. (1960) *The Avian Embryo*. MacMillan, New York.
- Stern, C.D. (1990) The marginal zone and its contribution to the hypoblast and primitive streak of the embryo. *Develop.*, **109**, 667–682.

# Some critical events in the differentiation of the avian embryo

Ruth Bellairs

Department of Anatomy and Developmental Biology, University College London, Gower Street, London, WC1E 6BT, UK E-mail: r.bellairs@ucl.ac.uk

## INTRODUCTION

The term “differentiation” covers all those complex changes that occur in a developing embryo and are concerned with the conversion of a simple group of cells into a complex individual with organs and tissues. Differentiation is not the same as growth, which is usually defined as an increase in body mass, though the two are associated. If there were growth but no differentiation the embryo would never become more than a large ball of cells. There could not be differentiation without growth as there would not then be enough cells to form each organ.

Differentiation is essential for a normal embryo to develop and consists of a continuous sequence of events. Some of these are especially critical, however, and if they do not take place correctly the embryo becomes malformed or fails to develop at all. This review briefly covers the basic principles of these critical events in development.

## ESTABLISHMENT OF THE BODY AXES (POLARITY)

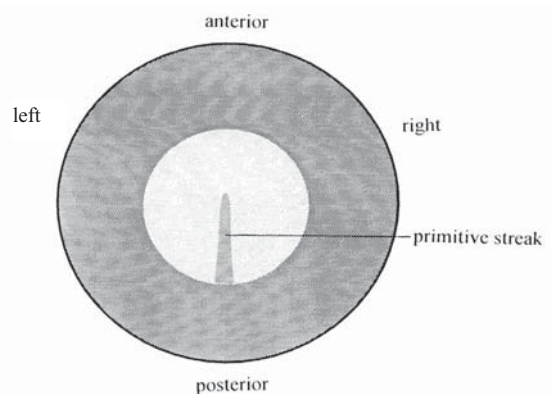
Establishment of the body axes is arguably the most important event in development because differentiation could not take place without it. First, the dorsal and ventral sides (the dorso-ventral axis) and then the head and tail ends of the body (the antero-posterior axis) become apparent. By establishing these two axes the embryo has automatically acquired a left and a right side (Figure 1).

Acquisition of the dorso-ventral axis occurs during cleavage when the egg is still in the upper part of the oviduct. In birds the embryo is a flat disc of cells, the blastoderm. Its lower surface rests on the yolk and becomes the ventral side whilst its upper surface becomes the dorsal side. It seems that the differing environments at the two sides have an important role in the establishment of the dorso-ventral polarity.

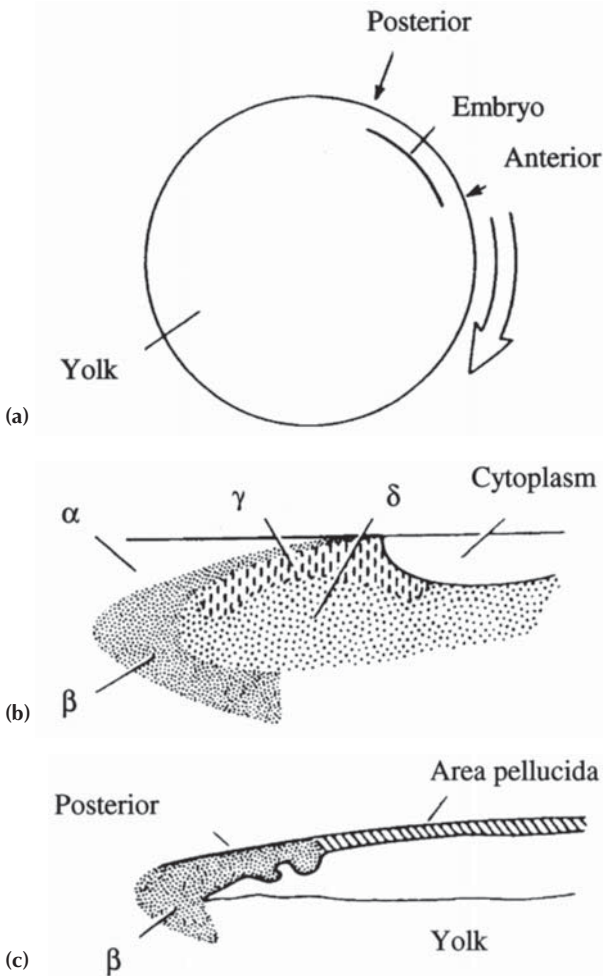
The antero-posterior axis is not obvious until after the start of incubation, although it is acquired during the period of about 20 hours spent in the shell gland. The egg slowly rotates (about 10–12 turns per hour and in the same direction) as the shell is formed. This

rotation results in the blastoderm being tipped in the direction of the rotation so that it no longer lies horizontally on the top of the yolk. The anterior (head) end forms from the side that has tipped lowest, whilst the posterior (tail) end forms from the side remaining nearer the top of the egg (Figure 2). The rotation itself is of significance for polarity only in that it brings about the tilting of the blastoderm. Experiments in which eggs have been removed from the oviduct and incubated *in vitro* have shown that the direction of the polarity depends on the position in which the egg is held, provided that one side is tipped lower than the other (Eyal-Giladi and Fabian, 1980). If the blastoderm is not tipped but is held in a horizontal position then it fails to develop properly. These experiments provide compelling evidence that gravity plays a critical role in the establishment of the antero-posterior axis. Full confirmation, however, would require that birds’ eggs be fertilised and undergo their earliest stages of development in zero gravity, *i.e.* in space (Bellairs, 1990) but these experiments have yet to be performed.

How then could gravity bring about these effects? A possible explanation was provided by the experiments of Callebaut (1983, 1987) who identified four different



**Figure 1** Diagram of the dorsal side of the embryo at the primitive streak stage. The anterior and posterior ends of the embryo are established before the egg is laid. The primitive streak has formed, dividing the blastoderm into left and right sides.



**Figure 2** Diagram to illustrate the mode of establishment of the antero-posterior axis. (a) With the rotation of the egg in the shell gland, the young embryo becomes tipped to one side of the yolk. The anterior region subsequently develops from the end that has tipped lowest. (b) Diagram to show the arrangements of the four different types of yolky cytoplasm,  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ , in a quail oocyte. (c) Diagram to show that the yolky cytoplasm comes to lie at the posterior end of the embryo and so may play a critical role in the establishment of the antero-posterior axis.

(b) and (c) after Callebaut (1987).

components of the yolky cytoplasm of the quail oocyte:  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ -ooplasm. During the rotation of the egg, these ooplasm become shifted so that the different parts of the blastoderm become underlain by different ooplasm. In particular,  $\beta$ -ooplasm becomes located in certain cells (endophyll cells) beneath the region that will become the future posterior end of the embryo (Callebaut *et al.*, 1998).

The antero-posterior axis is not, however, permanently fixed at this stage. It can be experimentally changed after laying; if the blastoderm of a new-laid egg is bisected before incubation it may develop two axes which do not necessarily possess the same orientation (Veini and Bellairs, 1983). This means

that one of them, at least, must have changed its potential orientation. Once incubation begins, this ability is rapidly lost.

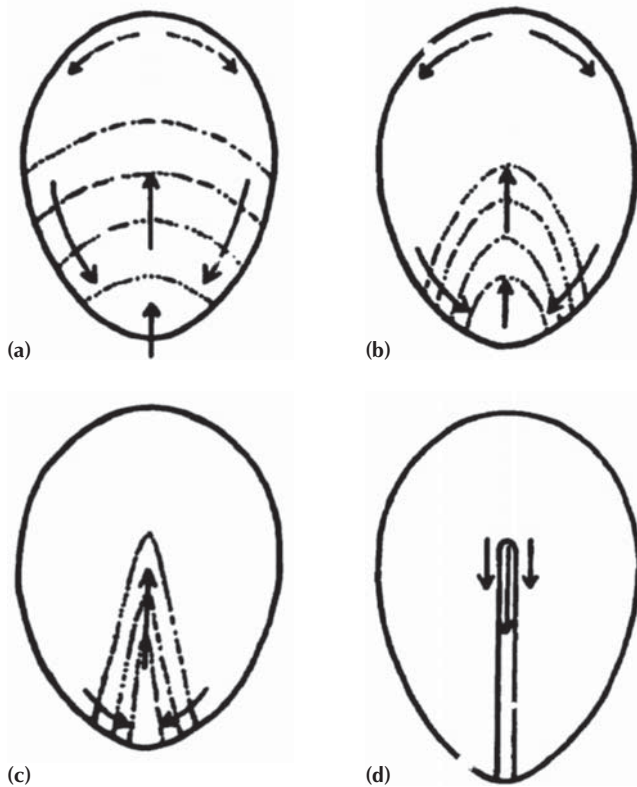
Shortly after the start of incubation a range of genes concerned with polarity start to become active (e.g. Hox genes) and apparently interpret the provisionally established antero-posterior axis. Their domains overlap one another both in the antero-posterior and the dorso-ventral axes and help to establish the differences between the different regions.

### GASTRULATION AND THE FORMATION OF THE PRIMITIVE STREAK

Gastrulation and formation of the primitive streak begins shortly after the start of incubation and is characterised by extensive migrations of cells. We know about them because many experiments have been carried out in which groups of cells have been marked in various ways, usually by applying dyes to them, and then followed through succeeding stages of development, often with time-lapse cinematography. Maps have been prepared of the patterns of migration. (e.g. Figure 3) and they consist principally of an initial forward movement in the midline. At the beginning of gastrulation the region where the embryo will form has upper and lower layers. Experiments have shown that if the lower layer (the hypoblast) is removed, the migrations in the upper layer (the epiblast) fail to take place, so the lower layer seems to be responsible for provoking these movements. By the end of gastrulation the embryo is three layered. The original lower layer (the hypoblast) has migrated away from the central region and its cells will eventually give rise to germ cells, which in turn will become sperm or ova. Two new layers, the endoderm and the mesoderm have formed from the upper layer, which is now known as the ectoderm. We, therefore, now have a three-layered blastoderm, composed of ectoderm, mesoderm and endoderm.

The endoderm and mesoderm have left the ectoderm by migrating through a region called the primitive streak (Figure 4). The primitive streak is a transitory structure formed by cells that have moved toward the midline and piled up there. Eventually it disappears as the cells leave this area and pass beneath the ectoderm, migrating out laterally.

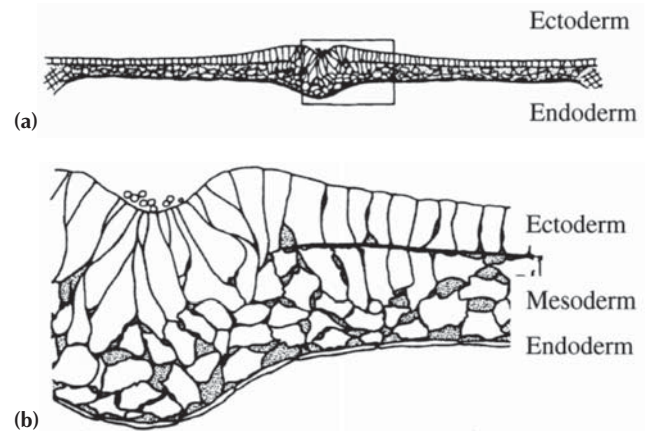
Much of the recent work on gastrulation has centred on the mechanisms by which the orderly sequence of cell migrations is controlled. There is a genetic element, but an important role is played by components of the extra-cellular materials, such as fibronectin and hyaluronic acid, which affect cell adhesion and migration.



**Figure 3** Diagram to show patterns of migrations during gastrulation in the domestic fowl embryo. In each case, the anterior end is at the top of the page, the posterior at the bottom. (a), (b) and (c) show that there is an extensive migration of cells from the posterior to the anterior end. Other movements take place around the periphery. (d) shows that once the primitive streak is fully formed, the main migration is from the anterior to the posterior end.

Once the primitive streak is fully formed the cells that will develop into the embryonic body have become located around the anterior end of the primitive streak (Figure 1). A second set of cell migrations then takes place but, whereas the first set, which formed the primitive streak, was from posterior to anterior, the second set passes from anterior to posterior. It takes place gradually as the cells leave the primitive streak to pass under the ectoderm and, as they do so, the primitive streak disappears. After about 12 hours all the material that was originally at the anterior end of the primitive streak is now relocated along the antero-posterior axis.

Let us now consider what happens if some of these migrations go wrong. If the first movement is disturbed the developing primitive streak may become split into two at its anterior end, leading to the formation of a double-headed embryo (*duplicitas anterior*). If the second movement goes wrong we may get two bodies and one head (*duplicitas posterior*). These monsters may occur naturally but are easily procured



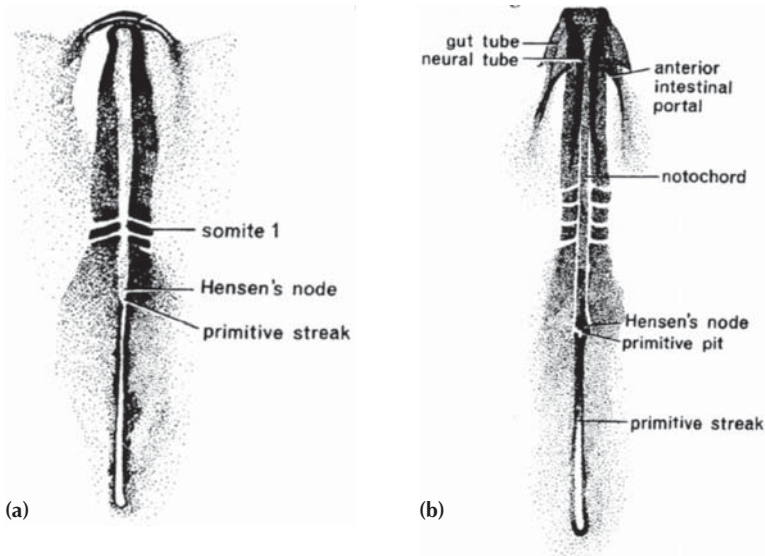
**Figure 4** (a) Diagram of the primitive streak and lateral regions of the blastoderm, seen in transverse section. (b) Enlargement of the primitive streak region. The mesoderm forms from cells that have left the ectoderm and begun to spread out laterally.

experimentally by placing obstacles in the path of the migrating cells. They demonstrate an important fact about the early embryo, that there is a great deal of flexibility in its development. Cells that would normally give rise to, say, brain tissue, may under certain circumstances form other tissues, such as epidermis (skin) or muscle. This ability to regulate their future development provides a safety valve for the young embryo, enabling it to adjust its development to abnormalities or changes in its environment and is possible because all the cells possess the same genetic makeup. Gradually this flexibility decreases as the ability of genes to express themselves becomes restricted.

## FORMATION OF THE BODY AXIS

Even before these mass migrations of cells have finished taking place at the posterior end, the basic body of the embryo begins to be visible at the anterior end. Indeed it is a characteristic of development that the differentiation of many structures starts first at their anterior end and then gradually spreads further and further towards the posterior end. This means that, developmentally, the anterior end is more differentiated than the region posterior to it.

Seen from the dorsal side the most obvious feature of the body axis is the neural plate which soon rolls up to form the neural tube, the precursor of the entire nervous system, but other important structures are the developing somites and the heart (Figure 5). These are clearly visible in transverse sections through the body (Figure 6). These structures are now described.



**Figure 5** Diagram of the embryo at (a) stage 7 (about 24 hours incubation) and (b) stage 8 (about 27 hours incubation), seen from the dorsal side. Hensen's node is the region of the primitive streak that will give rise to notochord.

**FORMATION OF THE SOMITES**

The notochord and somites (Figure 6) are structures that are present only in the early embryo because in the later stages of differentiation they become converted into other tissues. The notochord is a rod that extends down the midline of the developing body and eventually becomes part of the intervertebral discs of the vertebral column. The neural tube forms above it and eventually develops into the brain and spinal cord. The somites are derived from mesoderm and lie as paired blocks on either side of the notochord and are the first segmented structures to form in the embryo, laying the foundation for all the other segmented structures that develop, e.g. vertebrae and

ribs, cranial and spinal nerves, vertebral arteries and skeletal muscles. Each somite acquires three components: the dermatome which forms the dermis of the skin; the myotome, which gives rise to many of the muscles of the trunk and limbs; and the sclerotome, which forms the cartilage of the vertebrae and ribs.

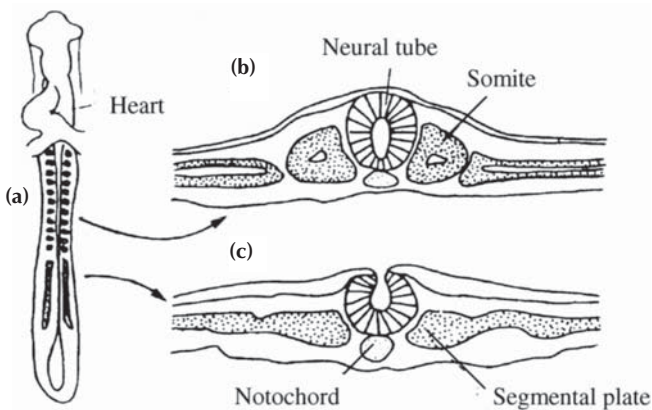
The first somites appear at about stage 7 (domestic fowl embryos are usually described according to an agreed stage of development, rather than the length of time they have been incubated [see Hamburger and Hamilton, 1951]) and the others are laid down pair by pair further and further towards the posterior. The somites arise from cells that initially lie in the ectoderm around the anterior end of the primitive streak at about stage 3 + . During the later stages of gastrulation they leave the upper surface and come to lie as two blocks of tissue, one on either side of the midline. The somites will form from these are the segmental plates (Figure 6).

There is much we do not understand about the factors responsible for the breaking up of the continuous block of tissue, the segmental plate, into individual segments. Currently, a promising clue is that there are "oscillating genes" involved, that switch on and off, causing the somites to break off at regular intervals from the continuous blocks of mesoderm (Dale and Pourquie, 1990), but other factors include the increasing adhesion of cells to one another.

**FORMATION OF THE NERVOUS SYSTEM**

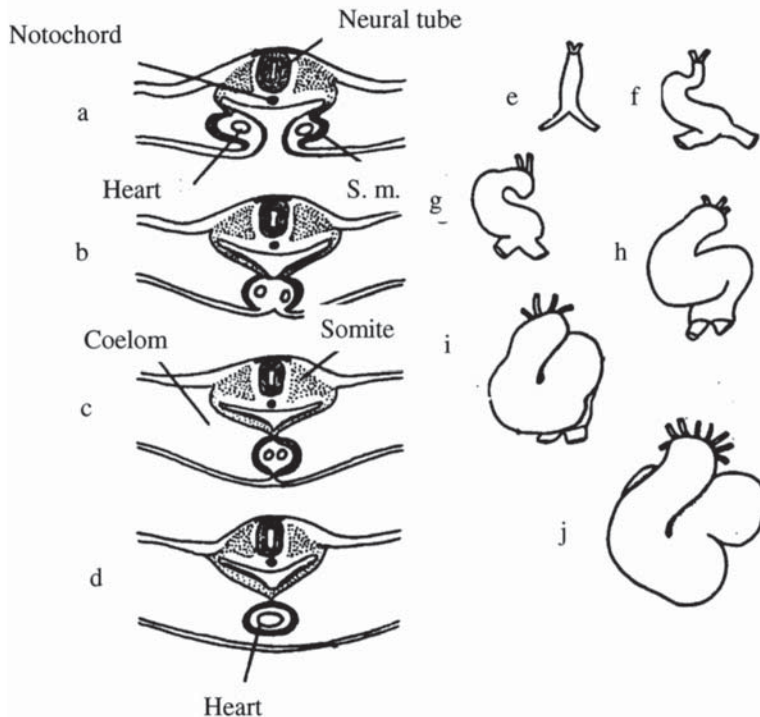
The brain and nervous system develop first as the neural plate in the ectoderm that then rolls up into the neural tube (Figure 6). Its formation has always excited interest because it develops as a result of influences from the underlying mesoderm, a process called neural induction. The anterior end of the neural tube swells into a brain, and this in turn becomes modified into its different regions. The eyes form by outgrowths from the most anterior part, the forebrain, and all the nerves grow out from the neural tube and pass to the organs and tissues that they innervate.

Many of these processes take place only after an induction from a neighbouring tissue. The mechanisms involved have resisted analysis for many years, though there are certain substances that appear to be relevant. They include fibroblast growth factor, activin and retinoic acid.



**Figure 6** Diagram of embryo at stage 11 (about 40 hours incubation). (a) viewed from the ventral side. (b) Section through the somite region. (c) Section through the segmental plate region.





**Figure 7** (a), (b), (c) and (d) transverse sections to show the folding of the splanchnic mesoderm (S. m.) to form the heart. (e), (f), (g), (h), (i) and (j) the heart at succeeding stages seen from the ventral side, to show how it changes from a straight tube (e) to an S-shape (h) and then bulges out into left and right sides (j).

### HEART AND CIRCULATION

Like the somites, the heart is formed from mesodermal cells. At the primitive streak stage the cells of the future heart lie at the anterior end of the embryo, closely associated with the lower layer (endoderm), which will form the gut. We have seen that initially the body is a flattened 3-layered structure but soon it begins to fold into a three-dimensional arrangement and this leads to the formation of the gut and a simple heart. If this folding does not take place properly the embryo remains open on its ventral side and the heart stays outside the body (exocardia). With further development, the heart becomes twisted into a loop (Figure 7) and divided into the different regions (*sinus venosus*, *atrium*, *ventricle*, and *truncus*) and then into left and right sides.

The capacity of the heart cells to beat begins as early as stage 7 and takes place even in cell culture. Even single cells will beat, each with its own rhythm, but if the cells make contact with one another, they beat in synchrony.

The red blood cells develop first in the mesoderm of the yolk sac membrane. Groups of cells clump together to form "blood islands". The outermost cells in each clump join with one another to form a

vesicle whilst the innermost become blood cells or disintegrate. Cords of cells grow out to connect one blood island to another and when these cords become canalised a continuous circulation is established (See Baggott, 2001).

Primitive blood vessels develop from mesoderm in the embryo and connect with those in the yolk sac (See Baggott, 2001). The patterns of the blood vessels in the yolk sac membrane are already laid down by the time the heart has developed sufficiently to promote blood flow, but the fine details are moulded by the passage of the circulating blood. In the differentiation of the heart itself, some cellular morphogenesis takes place even if no blood flows through it, but good development of the endocardium and myocardium depends on the flow of the blood.

### GUT AND RESPIRATORY SYSTEM

The gut is formed from the endoderm, together with a covering of mesoderm.

Initially, the walls of the gut are continuous with those of the yolk sac membrane, but the connection becomes reduced to a narrow stalk, part of the umbilical cord. The region anterior to the connection with the yolk sac is the foregut, whilst that posterior to it is the hind gut. When it is first formed the gut is a simple tube but modifications soon appear as the specialised regions become differentiated. An outgrowth from the anterior region of the foregut gives rise to the entire respiratory system, *i.e.* the bronchi, lungs and air sacs. Further modifications of the foregut include the swelling and thickening of the walls of the stomach and duodenum, and the formation of the liver and pancreas from specialised diverticula. The small intestine arises from the foregut, whilst the large intestine and the cloaca develop from the hind gut.

The intestines not only become elaborated morphologically, but also they grow in length so that at about 6 days of incubation a loop of intestines herniates outside the body into the umbilical cord and becomes twisted on itself, a process known as "rotation of the gut". It is drawn back into the body shortly before hatching, the small intestines first, so that they come to lie at the dorsal side of the body cavity, and then the large intestines, which become located more ventrally.

### FACTORS INVOLVED IN DIFFERENTIATION

Many interacting processes are involved in differentiation but in this brief resume I have touched on a few only, viz. the establishment of polarity, cell migration, cell and tissue interactions and the switching on and off of genes. The interplay of these and other factors, such as the appropriate incubation conditions, make the difference between the formation of a normal or an abnormal embryo, and frequently of the survival or death of the individual.

### REFERENCES

- Baggott, G.K. (2001) Development of extra-embryonic membranes and fluid compartments. In: *Perspectives in Fertilisation and Embryonic Development in Poultry*, pp. 23–29. Ratite Conference Books, Wallingford. Reprinted in 2008: *Avian Biol. Res.*, **1**, 21–26
- Bellairs, R. (1990) The role of gravity in bird development. *European Space Agency Publication SP-1123*. [8-10 rue Mario-Nikis, 75738, Paris, Cedex, France.]
- Callebaut, M. (1983) The constituent oocytal layers of the avian germ and the origin of the primordial germ cell yolk. *Arch. d'Anatomie Micro. Morphol. Exp.*, **72**, 199–214.
- Callebaut, M. (1987) Ooplasmic localization and segregation in quail germs: fate of the four ooplasm. *Archives de Biologie (Bruxelles)*, **98**, 441–473.
- Callebaut, M., van Nueten, E., van Nassauw, L., Bortier, H. and Harrison, F. (1998) Only the endophyll-Rauber's sickle complex and not cells derived from the caudal marginal zone induce a primitive streak in the upper layer of avian blastoderms. *Reprod. Nutr. Develop.*, **38**, 449–463.
- Dale, K.J. and Pourquie, O. (2000) A clock-work somite. *BioEssays*, **22**, 72–83.
- Eyal-Giladi, H. and Fabian, B.C. (1980) Axis determination in uterine chick blastodiscs under changing spatial positions during the sensitive period of polarity. *Develop. Biol.*, **77**, 228–232.
- Hamburger, V. and Hamilton, H.L. (1951) A series of normal stages in the development of the chick embryo. *J. Morphol.*, **88**, 49–92. Reprinted (1992) *Develop. Dynamics*, **195**, 231–272.
- Veini, M. and Bellairs, R. (1983) Experimental analysis of control mechanisms in somite segmentation in avian embryos. I Reduction of material at the blastula stage in *Coturnix coturnix japonica*. *J. Embryol. Exp. Morphol.*, **14**, 1–14.

# Development of extra-embryonic membranes and fluid compartments

Glenn K. Baggott

Department of Biology, School of Biological and Chemical Sciences, Birkbeck College, University of London, Malet Street, London, WC1E 7HX, UK E-mail: g.baggott@bbk.ac.uk

## INTRODUCTION

Seventy years ago the Cambridge embryologist Joseph Needham coined the term “cleidoic” to describe the special characteristics of the avian egg (Needham, 1931). He pointed out that the avian egg was essentially closed because nearly all the materials needed for the development of the embryo are contained within the shell. Sufficient water, nutrients and energy (in this case fats) for tissue growth and maintenance are provided. Only oxygen (and heat) is required from the environment. Whereas Needham’s perceptive analysis of the nature of the avian egg has been vindicated, the implications of this egg design for the fashioning of embryonic tissue from the fertilised ovum are still to be fully explored. One of the primary difficulties is in the supply of water to the growing mass of embryonic and extra-embryonic tissues. The latter consist of membranes and fluid compartments that develop outside of the body of the true embryo and fulfil a pivotal role in normal development. This review describes the importance of water in development of the extra-embryonic compartments of the egg in order to describe how there is a resolution of the water problem.

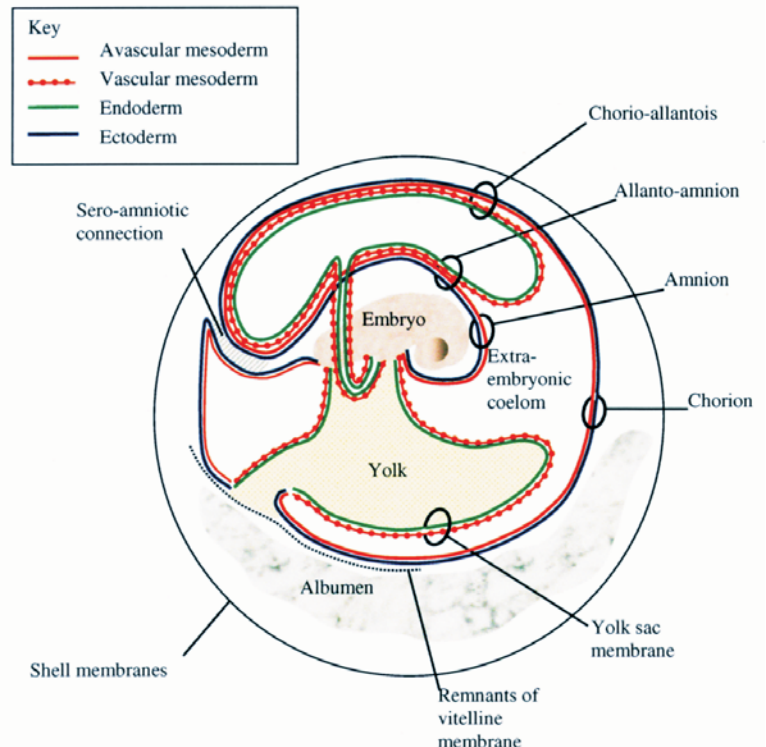
## DEVELOPMENT OF THE EXTRA-EMBRYONIC MEMBRANES

It is difficult to give an account of extra-embryonic fluid compartments without reference to the embryological development of the extra-embryonic membranes. This topic is briefly summarised below for the domestic fowl (*Gallus gallus* var. *domesticus*), to which the days of incubation indicated refer, based upon the account in Romanoff (1960) and Mossmann (1987). It is also hard to describe the development each membrane without reference to another, but as far as is possible, a self-

contained account of the development of each extra-embryonic membrane is provided. For the fowl embryo, the spatial relationships of the extra-embryonic membranes and fluid compartments at around a third of the developmental period are illustrated in Figure 1.

### Yolk sac membrane

The first of the extra-embryonic membranes to develop is the yolk sac membrane. Initially recognised as the *area vitellina*, this is the outer area of the blastoderm, consisting of three cell layers, which are a continuation of the cell layers of the embryonic disc. There is an ectodermal layer adjacent to the vitelline



**Figure 1** A diagrammatic representation of the extra-embryonic membranes and fluid compartments for the chick embryo around a third of the way through incubation. Note that the sizes of these structures, and their development relative to each other, have been modified to clarify types of cells present in each of the membranes.

**Table 1** The mass of water and solids (dry mass) of the embryo of the domestic fowl for selected days during development (from Romanoff, 1967). The maxima are indicated in bold: either the maximum absolute water or solids content, or the maximum change, increase or decrease, in water or solids content ( $\Delta$  water or  $\Delta$  solids)

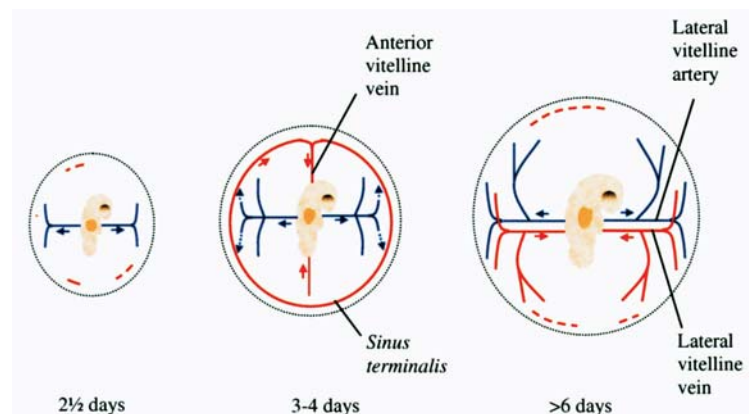
	water (g)	$\Delta$ water (g)	solids (g)	$\Delta$ solids (g)
<b>Albumen</b>				
Day 0	<b>29.9</b>		<b>4.1</b>	
Day 10	7.3	−22.6	3.6	−0.5
Day 21	1.4	−5.9	0.2	−3.4
<b>Yolk sac</b>				
Day 0	9.4		<b>9.9</b>	
Day 10	<b>9.6</b>	+0.2	8.8	−1.1
Day 21	2.2	−7.4	5.1	−3.7
<b>Embryo</b>				
Day 1	0.00014		0.00006	
Day 10	2.2	+2.2	0.2	+0.2
Day 21	<b>24.7</b>	+22.5	<b>6.5</b>	+6.3
<b>Yolk sac membrane</b>				
Day 10	1.6		0.06	
Day 15	<b>3.8</b>	+2.2	<b>0.41</b>	+0.35
Day 21	2.5	−1.3	0.28	−0.13
<b>Sub-embryonic fluid</b>				
Day 2	0.6		0.02	
Day 6	<b>12.9</b>	+12.3	0.5	+0.5
Day 10	6.3	−6.6	<b>1.1</b>	+0.6
<b>Amniotic fluid</b>				
Day 7	1.2		0.01	
Day 10	2.8	+1.6	0.03	+0.02
Day 13	<b>3.7</b>	+0.9	0.13	+0.10
Day 19	1.6	−2.1	<b>0.15</b>	+0.02
<b>Allantoic fluid</b>				
Day 7	1.5		0.01	
Day 10	4.1	+2.6	0.05	+0.04
Day 13	<b>6.1</b>	+2.0	0.09	+0.05
Day 19	1.2	−4.9	<b>0.10</b>	+0.01

membranes, an endodermal layer adjacent to the yolk and in between a layer of mesoderm cells. The mesoderm is split into two by a cavity, the extra-embryonic coelom, and only the mesoderm next to the endoderm develops blood vessels (called the vascular mesoderm). It is these two cell layers that form the definitive wall of the yolk sac. The vitelline membranes enclose the yolk sac until day 4 of incubation. Contact of embryonic tissue with the vitelline membranes alters their structure (Jensen, 1969), probably facilitating their rupture when yolk sac volume increases at this time. This causes the yolk to lose its spherical shape and the embryonic-yolk structure adopts the shape of the egg. The top of this structure is bounded by the yolk sac membrane and the lower by the vitel-

line membrane, which slips down to the pole of the yolk sac opposite to the embryo. In this way the partition of the yolk sac from the albumen (the so-called yolk sac umbilicus) is maintained and at this “vegetal” pole, the yolk sac membrane remains incomplete until day 17.

The yolk sac membrane passes the equator of the yolk sac at 5–7 days and achieves its maximum area around 10–11 days finally surrounding yolk at 14–15 days. From day 12 the yolk sac membrane changes into a flabby three-lobed mass. After day 10–11 the area of the yolk sac membrane actually decreases, although the membrane is still increasing in mass and reaches its peak weight at around 15 days (Table 1). This is due to a decrease in the size of the yolk sac as yolk is absorbed.

The primary blood system of the yolk sac membrane, the *area vasculosa*, is evident by day 2–3 of incubation when the vitelline arteries that carry blood from the heart to the periphery of the blastoderm become clearly visible (Figure 2). At the same time a vein develops at the margin of the blastoderm, the *sinus terminalis*. The blood in the peripheral capillary vessels empties into this vein, which carries blood anteriorly to the anterior vitelline vein, which then conveys blood back to the heart. A secondary blood system of new veins starts to appear whilst the primary blood system is still developing. By day 5, the lateral vitelline veins have grown parallel to the vitelline arteries, and in the peripheral capillaries, in which blood was formerly conveyed in a peripheral direction into the *sinus terminalis*, the flow is reversed. Blood now flows from periphery of the blastoderm to heart via the lateral vitelline veins, a more direct route. By day 6 the *sinus terminalis* starts to regress (Figure 2).



**Figure 2** Development of the primary and secondary blood systems of the *area vasculosa*. Blood becomes oxygenated in the peripheral areas of the area, the vitelline veins carry oxygenated blood towards the embryo, and vitelline arteries carry deoxygenated blood away from the embryonic tissue.

### Amnion

The amnion is formed from a layer of ectoderm and underlying avascular (does not develop blood vessels) mesoderm immediately adjacent to the embryo. The flat tissue rises up to form folds over both the head and tail of the developing embryo and by day 4 of incubation the folds fuse together over the embryo to enclose the amniotic sac. Consequently, the inner layer of the amnion is ectoderm, and the outer one is avascular mesoderm. Muscle cells appear within this mesoderm so that the amnion becomes contractile by day 5, but the blood vessels of the amnion arise from the subsequent fusion of this avascular mesoderm with vascular mesoderm from the allantois. On day 12 a duct develops between the albumen sac and the amniotic sac; this sero-amniotic connection (Figure 1) is the point where the head and tail folds of the amnion met on day 4 of incubation. This duct allows the movement of albumen proteins into amniotic fluid where they are swallowed ending up in the yolk sac.

### Chorion

The chorion develops from tissue continuous with the amnion and away from the embryo. It is comprised of an outside layer of ectoderm adjacent to the inner shell membrane and an inner avascular mesoderm, which lines the extra-embryonic coelom. Eventually this mesoderm fuses with vascular mesoderm of allantois, so supplying the blood vessels and forming the chorio-allantois. By day 11 the allantois has completely lined the chorion and the chorio-allantois covers 98% of the area of the eggshell membranes, thereby acting as the primary respiratory surface for the embryo during the second half of incubation.

### Allantois

At day 2 the allantois is a small bud of endodermal cells and by day 4 it is visible as a free sac growing out from the primitive hindgut of the embryo. Its inner side is endoderm and the outer surface is vascular mesoderm. It expands into, and eventually fills, the extra-embryonic coelom, so that by day 6 its vascular mesoderm is fused both with the chorion, to form chorioallantois, and with the avascular mesoderm of the amnion. Hence, the allantois supplies the blood system for both the chorio-allantois and the amnion. The allantois is a fluid-filled and is a repository for kidney excretions that first appear by day 5 and enter via the allantoic duct from the cloacal region of the hindgut.

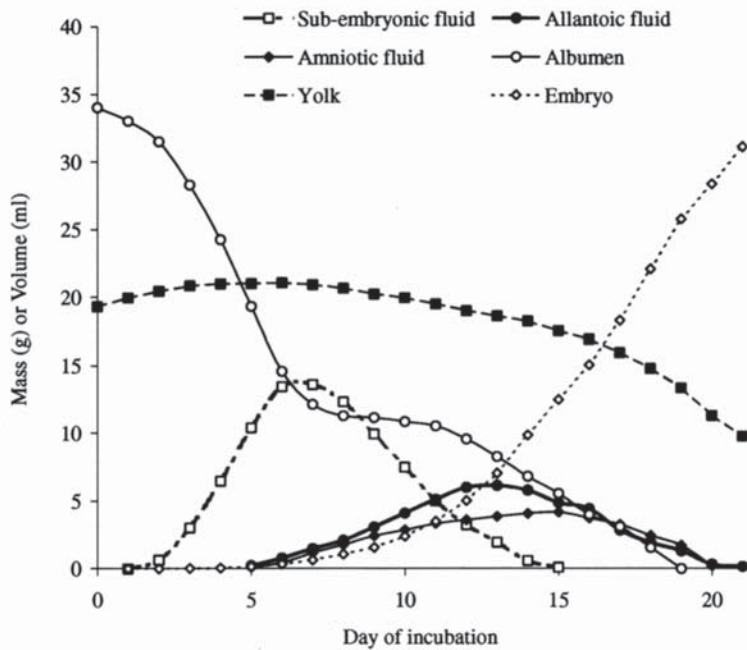
### THE WATER PROBLEM

Observations of many avian species have established that with a total water loss during incubation of about 20% of the initial egg mass, the water concentration of the hatchling (plus spare yolk) is very similar to the initial concentration in the egg at lay (Ar, 1991). This is because fat provides the energy source for embryonic growth and maintenance, and for this substrate the amount of water consumed is replaced by almost exactly the same amount of water produced by metabolism (Ar and Rahn, 1980). Thus the design of the avian egg produces "constant hydration", such that the chick has an appropriate tissue water content at hatching (Ar, 1991). So what then is the nature of the "water problem"?

At ovulation the ovum consists of a single cell containing large amounts of yolk, a store of nutrients and energy. In the upper part of the oviduct a second protein membrane is laid down over the primary vitelline membrane that contains the fertilised ovum (Bellairs, 1991). Subsequently, within the oviduct, albumen is added around the fertilised ovum, to act as a source of water, and finally the shell membranes and the shell are added around the albumen prior to oviposition (Gilbert, 1971). By the time of lay the ovum has undergone cell division to produce an embryo (Bellairs, 1991), which is physically separated from the water in the albumen by the vitelline membranes. The problem, then, is to ensure that this source of water is made accessible to the growing mass of tissue. Resolution of this difficulty is crucial for growth of the embryo.

At lay, 60% of the water in the egg is located in the albumen, which itself comprises 88% water (Romanoff, 1967). The tissues of the embryo contain about 70% water (Romanoff, 1967), and water must also be supplied to the extra-embryonic tissues. Most of the albumen water is transferred during the first half of the incubation period (Figure 3): about 76% of albumen water has disappeared by day 10 of incubation (Table 1). During this period the total amount of solids in the albumen hardly changes at all (Table 1), indicating that the water is removed preferentially. By contrast, at lay the yolk sac contains 49% water and the water content of yolk changes by only 2% over this 10 day period (Table 1).

Over the whole period of incubation 28.6 g of water disappears from albumen and 7.2 from the yolk; 24.7 g appears in embryonic tissue and 2.5 g in the yolk sac membrane (Table 1). However, up to day 10 of incubation the water content of the embryo increases by only 2.2 gms (Table 1). Thus, the largest increase in water content of the embryo is in the second half of incubation, but by 10 days 76% of



**Figure 3** The pattern of changes in the mass of the embryo, yolk and albumen, and in the volume of the fluid compartments, of the developing fowl egg. Data from Romanoff (1967).

the albumen water has been removed. So where has it gone, and how? The answers to these questions lie in the formation and depletion of three extra-embryonic fluid compartments in the egg: the sub-embryonic fluid, amniotic fluid and allantoic fluid.

#### Sub-embryonic fluid (SEF)

Apparently, therefore, water does not move directly to embryonic tissue from albumen. In fact, during embryonic development water appears and disappears from a number of separate fluid compartments. The first new compartment becomes evident around 2–3 days of incubation, when a fluid appears in the yolk sac beneath the embryo, the sub-embryonic fluid. Its water content reaches a maximum at day 6 of incubation, when it is more than 95% water, and decreases in mass thereafter (Figure 3; Table 1). There is a “critical period” for the production of SEF between days 3–7 of incubation: if eggs are not turned during this period SEF volume is decreased and the formation of other fluid compartments and embryonic growth is retarded (Deeming, 1989a, 1989b). An absence of egg turning also reduces the growth of the *area vasculosa* over the yolk sac (Deeming, 1989c), and this is most likely the cause of the reduced amount of SEF found in unturned eggs (Latter and Baggott, 2002). The endoderm cells of the *area vasculosa* that face the yolk are specialised for the transport of water and sodium ions from

albumen to yolk sac (Babiker and Baggott, 1995; Latter and Baggott, 2000), so with fewer cells less SEF will be produced. It is assumed that water from the SEF is distributed to the growing tissue, embryonic and extra-embryonic, by the blood system (derived from the vascular mesoderm), although nothing is known about this process. The transport of water into the yolk sac has an additional benefit: the yolk becomes lighter than albumen in which it is immersed and floats towards the upper surface of the egg, so placing the *area vasculosa* just beneath the shell membranes (Babiker and Baggott, 1992). As the *area vasculosa* is also a respiratory organ (Lomholt, 1984), this process improves the access of the embryo to the air. In unturned eggs the yolk sac floats less and sits deeper in the albumen (Babiker and Baggott, 1992), an outcome that may contribute to the greater embryonic mortality of unturned eggs.

#### Amniotic fluid

Amniotic fluid appears later in development than SEF and achieves peak water content at day 13 of incubation; solids accumulate slowly throughout development (Figure 3; Table 1). Amniotic fluid has a unique ionic composition (high in chloride ions), which is responsible for the inflow of water into the amnion (Faber *et al.*, 1973). Amniotic fluid volume is, apparently, unaffected by the hydration state of the egg (Faber *et al.*, 1973; Ar, 1991); it is thought that the main function of the fluid is mechanical protection of the embryo. An absence of egg turning substantially decreases the mass of amniotic fluid from days 12–18 of incubation (Deeming, 1989a). This is thought to be caused by less albumen entering the amniotic fluid *via* the sero-amniotic connection, probably due to the altered spatial relationship between albumen, amnion and allantois in unturned eggs (Deeming, 1991). Additionally, by mid-incubation the albumen volume of unturned eggs is greater than that of turned eggs, which may also impede the transfer of albumen through the sero-amniotic connection (Deeming, 1991; Babiker and Baggott, 1992).

#### Allantoic fluid

Like amniotic fluid, the maximum allantoic fluid water content is found on day 13 with largest increase in water content preceding this (Figure 3; Table 1). The source of this fluid is blood filtered by the

embryonic kidney. As for amniotic fluid, a lack of egg turning decreases the mass of this fluid compartment, but for allantoic fluid this reduction was detectable only on day 12 (Deeming, 1989a). Solids form only a small proportion of the allantoic fluid (Table 1), but can be important in determining its composition and properties. Excretory nitrogen, as ammonia, urea and uric acid, are present in allantoic fluid at day 5, but it is predominantly the uric acid content of this fluid that increases during development (Fisher and Eakin, 1957).

It has been claimed that the rate at which water is absorbed from allantoic fluid varies with the degree of embryonic desiccation, thus producing a stable water and ion content of the embryo and fluid compartments (Hoyt, 1979). However, it is well established that embryos subjected to higher water losses during incubation have a lower tissue water content and a smaller allantoic fluid volume (Vleck, 1991). Also, investigators have repeatedly emphasised the potential for alteration of allantoic fluid composition by ion and fluid transport across the allantoic membrane into the blood. However, as pointed out by Murphy (1997), changes in allantoic fluid volume can be explained adequately only if urine inflow to the allantois, and fluid re-absorption via the allantoic membrane, are both measured. Certainly, ions (and water) are transported across the membrane from allantoic fluid (Vleck, 1991), and this process, as well as a reduction in urinary output from the kidney into the allantois, is most probably enhanced by the action of hormones (Murphy, 1997). However, the relative importance of these two processes in determining the volume of allantoic fluid remains uncertain. Moreover, the ionic composition of the allantoic fluid is determined by at least three factors: the capacity of the allantoic membrane to reabsorb water and ions, the composition of urine flowing in from the kidney, and the interactions between excretory uric acid and ions in the fluid.

For example, by 15 day of incubation, calcium, sodium and sulphate ions are all reabsorbed into the blood by kidney, so less are added to the allantoic fluid (Clark *et al.*, 1993). However, uric acid in the allantoic fluid also can change the ion composition of the fluid, as solid uric acid sequesters sodium ions: under dry incubation conditions uric acid excretion is increased, allantoic fluid contains more solid uric acid and more sodium ions are removed from the fluid, so increasing the potential for water re-absorption into the blood across the allantoic membrane (Bradfield and Baggott, 1993a, 1993b). The solid uric acid (with faecal material, meconium) is discarded in the eggshell at the time of hatching (Romanoff, 1967).

## CONCLUSION

As is now clear, a number of fundamental aspects concerning the production of the fluid compartments of the avian egg remain to be explored. We are a little closer in understanding what factors, hormonal and environmental, influence the development of the *area vasculosa*, but still lack a complete account for a relatively simple environmental variable such as egg turning. We now know that both water inflow from the kidney and outflow, across allantoic membrane, determines allantoic fluid volume, but we are unsure of the relative importance of these processes, especially with regard to the hydration status of the embryo. Even more fundamentally, we have little understanding of the mechanisms whereby fluid moves between SEF, amnion, allantois and embryo. From this long list, the role of the *area vasculosa* in moving water from SEF to embryo would appear to be the process most amenable to experimental analysis.

## REFERENCES

- Ar, A. (1991) Roles of water in avian eggs. In: *Egg Incubation: Its Effects on Embryonic Development in Birds and Reptiles*, Deeming, D.C. and Ferguson, M.W.J. (eds.), pp. 229–243. Cambridge University Press, Cambridge.
- Ar, A. and Rahn, H. (1980) Water in the avian egg: overall budget of incubation. *Amer. Zool.*, **20**, 373–384.
- Babiker, E.M. and Baggott, G.K. (1992) The effect of turning upon the sub-embryonic fluid and albumen of the Japanese quail. *Brit. Poultry Sci.*, **33**, 973–991.
- Babiker, E.M. and Baggott, G.K. (1995) The role of ion transport in the formation of sub-embryonic fluid by the embryo of the Japanese quail. *Brit. Poultry Sci.*, **36**, 371–383.
- Bellairs, R. (1991) Overview of early stages of avian and reptilian development. In: *Egg Incubation: Its Effects on Embryonic Development in Birds and Reptiles*, Deeming, D.C. and Ferguson, M.W.J. (eds.), pp. 371–383. Cambridge University Press, Cambridge.
- Bradfield, P.M. and Baggott, G.K. (1993a) The effect of water loss upon the urate, urea and ammonia content of the egg of the Japanese quail *Coturnix coturnix japonica*. *Comp. Biochem. Physiol.*, **106A**, 187–193.
- Bradfield, P.M. and Baggott, G.K. (1993b) Allantoic fluid electrolytes and urate of embryonic Japanese quail subject to differential water loss. *Brit. Poultry Sci.*, **34**, 597–612.
- Clark, N.B., Feng, J.Q. and Murphy, M.J. (1993) Renal clearance measurements of electrolytes in embryonic chickens. *J. Exp. Zool.*, **265**, 107–111.
- Deeming, D.C. (1989a) Characteristics of unturned eggs: critical period, retarded embryonic growth and poor albumen utilisation. *Brit. Poultry Sci.*, **30**, 239–249.
- Deeming, D.C. (1989b) Importance of sub-embryonic fluid and albumen in the embryo's response to turning of the egg during incubation. *Brit. Poultry Sci.*, **30**, 591–606.
- Deeming, D.C. (1989c) Failure to turn eggs during incubation: development of the *area vasculosa* and embryonic growth. *J. Morph.*, **201**, 179–186.

- Deeming, D.C. (1991) Reasons for the dichotomy in egg turning in birds and reptiles. In: *Egg Incubation: Its Effects on Embryonic Development in Birds and Reptiles*, Deeming, D.C. and Ferguson, M.W.J. (eds.), pp. 307–323. Cambridge University Press, Cambridge.
- Faber, J.J., Gault, C.F., Green, T.J., Long, L.R. and Thornburg, K.L. (1973) Chloride and the generation of amniotic fluid in the early embryo. *J. Exp. Zool.*, **183**, 343–352.
- Fisher, J.R. and Eakin, R.E. (1957) Nitrogen excretion in developing chick embryos. *J. Embryol. Exp. Morph.*, **5**, 215–224.
- Gilbert, A.B. (1971) Egg albumen and its formation. In: *Physiology and Biochemistry of the Domestic Fowl, Volume 3*. Bell, D.J. and Freeman, B.M. (eds.), pp. 1291–1329. Academic Press, London.
- Hoyt, D.F. (1979) Osmoregulation by avian embryos: allantois functions like a toad's bladder. *Physiol. Zool.*, **60**, 9–17.
- Jensen, C. (1969) Ultrastructural changes in the avian vitelline membrane during embryonic development. *J. Embryol. Exp. Morphol.*, **21**, 467–484.
- Latter, G.V. and Baggott, G.K. (2000) Ion and fluid transport by the blastoderm of the Japanese quail. *Brit. Poult. Sci.*, **41**, S43–S44.
- Latter, G.V. and Baggott, G.K. (2002) Role of carbon dioxide and ion transport in the formation of sub-embryonic fluid by the blastoderm of the Japanese quail. *Brit. Poult. Sci.*, **43**, 104–116.
- Lomholt, J.P. (1984) A preliminary study of local oxygen tensions inside the bird egg and gas exchange during the early stages of embryonic development. In: *Respiration and Metabolism of Embryonic Vertebrates*, Seymour, R.S. (ed.), pp. 289–298. Dr W. Junk, Dordrecht.
- Mossmann, H.W. (1987) *Vertebrate Fetal Membranes: Comparative Ontogeny and Morphology; Evolution; Phylogenetic Significance; Basic Functions; Research Opportunities*. Rutgers University Press, New Jersey.
- Murphy, M.J. (1997) Endocrine control of hydromineral balance in bird embryos. *Amer. Zool.*, **37**, 546–552.
- Needham, J. (1931) *Chemical Embryology*. Cambridge.
- Romanoff, A.L. (1960) *The Avian Embryo*. The Macmillan Company, New York.
- Romanoff, A.L. (1967) *Biochemistry of the Avian Embryo*. John Wiley, New York.
- Vleck, D. (1991) Water economy and solute regulation of reptilian and avian embryos. In: *Egg Incubation: Its Effects on Embryonic Development in Birds and Reptiles*. Deeming, D.C. and Ferguson, M.W.J. (eds.), pp. 245–259. Cambridge University Press, Cambridge.



## Development during the first seven days post-hatching

Vern L. Christensen

Department of Poultry Science, North Carolina State University, Box 7608, Raleigh, North Carolina 27695-7608, USA E-mail: vern\_christensen@ncsu.edu

### INTRODUCTION

The neonate has basic needs that must be satisfied at the time of hatching if survivability and maximum potential are achieved. Some of these basic needs are fresh air, clean water, proper feed, and heat. The developmental state at hatching of the neonate differs among all avian species (Nice, 1962). The post-hatching period for altricial neonates is more critical than for precocial birds because they hatch in a less mature state. Differing amounts of maternal care are therefore essential for each species (Nice, 1962). Although embryonic growth among species is very similar, no two physiological systems seem to mature at the same rate (Ricklefs and Starck, 1998). In addition, maturity may be a function of the egg conductance constant (Ar and Rahn, 1978), which is determined by egg mass, eggshell conductance (or functional properties) and the length of the incubation period (see Box 1 later), all of which may constrain neonatal maturity. When viewed energetically, the difference between maturity types resides in the different water concentrations in eggs and hatchlings, in the density of chemical potential energy in the dry matter of true hatchlings, and in the different amounts of energy transferred from the egg to the spare yolk (Ar *et al.*, 1987).

On the basis of species comparisons of post-hatching growth of all birds (Lilja, 1983; Ricklefs, 1987), it has been suggested that the rate of growth after hatching is at least partially determined by the pattern of organ growth. It appears that a high rate of growth is correlated to early growth of “supply organs” (oesophagus, proventriculus, gizzard, intestines, heart and liver) at the expense of “demand organs” (breast, wings, legs and feathers). These changes begin very early in development (Lilja and Olsson, 1987), and Schmalhausen (1930) hypothesised that growth and organ function come into conflict when growth occurs too slowly or too rapidly.

Even under the most optimum conditions, a newly hatched bird is not free from stress. This is impossible because the absence of stress is death (Selye, 1951). However, hatchlings possess abilities to cope with stress via adrenal cortical hormones (Davis and

Siopes, 1989). Hatchlings have different blueprints for growth and maturation that must occur within a predetermined time frame. Many times this blueprint does not include adjustments that need to occur in maturational and growth processes in an imperfect environment. The objective of this paper is to define a “physiologically normal” hatchling and to describe the principles involved in the maturation and growth of several organ systems during the initial stages of life.

At least six physiological systems exist that require maturation during the last week of incubation or, in the case of altricial species, the initial days of life outside the shell. These systems are: (1) the circulatory system (heart and blood); (2) the kidney and body fluids system; (3) the digestive system; (4) body temperature regulation; (5) the respiratory system; and (6) the immune system. These systems are discussed here using published data to help clarify important points.

### CIRCULATORY SYSTEM

The heart and blood are among the first tissues to develop and mature in the embryo (see Baggott, 2001), and both function physiologically in the initial stages of embryonic development (Watterson and Sweeney, 1973; Tazawa and Whittow, 2000). The number of red blood cells increases with the age of the embryo until hatching (Macpherson and Deamer, 1964; Bagley, 1987). This occurs by both erythropoiesis and reticulocyte recruitment and maturation (Bagley, 1987). The processes for haemoglobin maturation and synthesis undergo similar patterns (Isaacks *et al.*, 1976). Post-hatching, erythrocyte numbers have been reported to increase initially for 3–4 days then decline to 10 days of age (Phelps *et al.*, 1987a). Sexual dimorphism affects erythrocyte numbers because in males numbers of cells declined more rapidly than in females, and erythrocyte changes were accompanied by declines in packed cell volumes, cell sizes and haemoglobin concentrations (Phelps *et al.*, 1987a).

Heart growth has been reported to be more variable than other organs during the first two weeks post-

hatching (Phelps *et al.*, 1987a). Rates of heart growth peak at about five to six days post-hatching then plateau to a rate similar to body growth at about 10 days. Spontaneous turkey cardiomyopathy (round heart disease, cardiohepatic syndrome; Magwood and Bray, 1962) and ascites in broiler chicks are manifestations of conflicts between organ growth and function. These syndromes are characterised by an enlarged heart due to extensive dilation of the ventricles (primarily the right ventricle initially), congestive heart failure and mortality. Julian *et al.* (1992) and Breeding *et al.* (1994) showed that rapid growth rates in connection with high protein diets contribute to the problem. Lilja and Olsson (1987) hypothesised that rapidly growing poultry will develop supply tissues before demand tissues.

Hearts in avian species are demand tissues because they can neither recycle lactate nor can they create glucose from gluconeogenic amino acids. Thus, heart growth, heart energy metabolism and blood cell maturation may be greatly influenced by the overall growth of the animal. These problems have been shown to occur during embryogenesis and manifest themselves throughout the life of the bird (Buyse *et al.*, 1998).

#### KIDNEY AND BODY FLUIDS SYSTEM

Embryos create urea as nitrogenous waste that is partitioned from the embryo and stored in liquid form in the allantoic fluid (Watterson and Sweeney, 1973). Approximately half way through incubation, the system begins creating insoluble uric acid, as a nitrogenous waste product. Water serving as the solvent for urea is recycled from the allantoic sac into the embryo via the chorio-allantoic circulation or the hindgut (Romanoff, 1960; Baggott, 2001). This makes it very difficult to dehydrate the embryo itself during incubation (Ar, 1991). However, egg weight losses of greater than 20% can result in physiological problems for embryos (Davis and Ackerman, 1987; Ar, 1991). The embryo relies on this system to filter nitrogenous waste until it hatches. After hatching a "new" renal system develops to maintain body fluid balances. Water is an essential nutrient for neonates while the system maintaining water balance matures (Doneen and Smith, 1982). Maturation of the renal clearance system requires a few days post-hatching whereas establishment and maintenance of acid-base balance is completed during the final stages of embryonic development (Goldstein and Skadhauge, 2000).

In birds, the renal system performs the additional functions of gluconeogenesis and maintenance of blood glucose concentrations (Fasenko, 1996). As

stress and difficulties in food acquisition are encountered, the kidney becomes essential to neonatal survival (Donaldson and Christensen, 1994). Maturation of the kidney in the gluconeogenic sense occurs whilst still in the egg but can be affected by the length of the incubation period (Fasenko, 1996). Glucose in hatchlings increases significantly from hatching until day five or six then may decrease achieving constant values by about day nine (Phelps, 1985).

#### DIGESTIVE SYSTEM

The yolk sac contains all of the enzymes and absorption mechanisms necessary for sustaining life in the shell and following yolk sac retraction into the body before hatching, it maintains these capabilities for the first few days following hatching (Romanoff, 1960; Denbow, 2000). The yolk digestive system is based primarily on lipids (Donaldson and Christensen, 1994; Donaldson *et al.*, 1994) but after hatching, the neonate must begin life on diet rich in carbohydrate (48%) and this basically requires a new metabolic process. Many intestinal mechanisms are required to be established and mature during a very short time from hatching (*circa* 24 hours) until food is consumed. The yolk sac system is drawn into the body prior to hatching to continue yolk digestion after hatching. Some estimates indicate as much as 60% of the total energy of a neonate (Fan *et al.*, 1997) is devoted to maturation and growth of intestinal tissue in the first few days after hatching.

Yolk assimilation in neonates is altered by feeding, temperature extremes and type of feed but not water (Phelps, 1985). Gender also has no effect (Moran and Reinhart, 1980). Yolk absorption is reported to be complete after five to six days post-hatching. Yolk is utilised post-hatching by two mechanisms: (1) transfer of yolk into blood in embryos and (2) anti-peristaltic movement of yolk from the distal end towards the proximal small intestine where it is absorbed by the intestine (Noy and Sklan, 1998).

Intestinal maturation post-hatching has been studied extensively (Sell *et al.*, 1989; Fan *et al.*, 1997; Uni *et al.*, 1995a, 1995b, 1999; Suvarna, 1999) because of its economic importance to commercial poultry. Intestinal disaccharidases and glucose transport mechanisms are fully functional at about 48 hours post-hatching and can adjust rapidly to different types of food even at 72 hours post-hatching (Suvarna, 1999). In most domestic species of poultry, intestinal mass increases in parallel to nutrient intake (Noy and Sklan, 1996; Uni *et al.*, 1999). Therefore, starvation or lack of intake of various food types slows maturation of the intestine

(Noy *et al.*, 1996). In genetic lines of domestic fowl (O'Sullivan *et al.*, 1992; Uni *et al.*, 1995a) and turkeys (Christensen, unpublished) selected for rapid growth, hatchlings used increased growth of the intestine, rather than improved intestinal function, to increase nutrient uptake for more rapid growth.

One of the fastest growing organs during the first 10 days of the neonatal life is the pancreas (Phelps *et al.*, 1987b). The exocrine pancreas is the source of many digestive enzymes. Little is known about the function of the pancreas at these stages although glucagon and insulin from the endocrine pancreas are readily available in the embryo and neonate (Christensen *et al.*, 2001a).

Appetite is controlled neurologically in precocial species at hatchling (Noble *et al.*, 1999). The principal neurochemicals involved are norepinephrine and epinephrine (noradrenalin and adrenalin respectively). Both neurotransmitters are functional at hatching in poultry species (Denbow and Sheppard, 1993). Manipulations of neurotransmitters by dietary tyrosine or tryptophan did not improve livability or growth of hatchlings but dietary carbohydrate did (Nicholson, 1992).

### BODY TEMPERATURE REGULATION

The zone of thermoneutrality for newly hatched avian species is very narrow. Birds vary enormously in their thermoregulatory capacities immediately after hatching, and they have been classified according to these and other capabilities (Nice, 1962). Precocial species are covered with down and are able to respond effectively to heat and to cold. By contrast, altricial birds are naked when hatched and have little ability to regulate their body temperature at air temperatures below 35°C or above 40°C. Other species are intermediate between precocial and altricial types (Dawson and Whittow, 2000). Most are not comfortable until they are provided an environment within this very narrow temperature range. During the last half of the incubation period the fowl egg maintains a temperature above that of the ambient air temperature of 37–38°C. When hatchlings are exposed to room air at 28°C, a new equilibrium state is reached in 5 hours (Tazawa and Rahn, 1987; Tazawa and Whittow, 2000). The difference between body temperature and ambient temperature increases from 6°C immediately after hatching to 10°C at five days post-hatching. Most altricial species require up to 10 days post-hatching to develop thermoregulatory abilities (Dawson and Whittow, 2000).

There are two types of thermoregulation available to the neonate. The first is thermoregulation by behavioural means (Dawson and Whittow, 2000).

Thermoregulatory behaviour involves movement of the entire bird, or part of the bird, such as a limb, in response to a change of either environmental or body temperatures, and it often requires conscious effort. This is demonstrated in cold temperatures when the neonate will reduce its surface area by "hunching" or "huddling". Behavioural thermoregulation is available to most avian species at hatching.

Both peripheral temperature receptors and temperature-sensitive neurones in the central nervous system are involved in neural thermoregulation, the second mechanism in birds. Shivering is usually the response in neurological thermoregulation. Neural thermoregulation is available to birds at differing times following hatching. Some species, such as the domestic turkey, may require as long as four weeks to become fully mature and thermoregulate without supplemental heat (Dawson and Whittow, 2000). Brooding, the process of providing supplemental heat in domestic poultry, simulates a similar response by the mother under natural conditions.

Thyroid function is associated with thermoregulation because of its involvement with feather growth and basal metabolism and has been used as a basis to discriminate between precocial and altricial neonates (McNichols and McNabb, 1988). Thyroid hormone levels in precocial species peak prior to hatching and remain elevated during the first week after hatching whereas in altricial species they peak following hatching. Weytjens *et al.* (1999) related plasma tri-iodothyronine concentrations to the onset of thermoregulation in high and low body weight lines of fowl. Greater tri-iodothyronine levels indicated earlier thermoregulatory abilities.

### RESPIRATORY SYSTEM

The final days of embryonic life for avian species are a time of respiratory transition. Respiration evolves from a system totally dependent upon diffusion of gases through the eggshell pores to one that is fully dependent upon convective forces for gas exchange in the lungs. Eggs are created with a functional property that allows respiration to occur by simple diffusion through pores created in the shell by the hen. Amazingly, these pores determine precisely the developmental time for the embryo (Ar and Rahn, 1978) as well as the time of lung inflation. The timing of the "plateau stage" in oxygen consumption determines the characteristic maturity of the respiratory system at hatching. The functional properties of the shell are thought to initiate convective mechanisms that will sustain the animal throughout the remainder of its life. The fluids must be evacuated from the lungs, which can then become functional. Fluid evacuation and

aeration are gradual process and occur over several days both within the egg and post-hatching (Vince and Tolhurst, 1975; Bagley, 1987).

At the beginning of the last third of embryonic development in the fowl, the parabronchi have reached their final number and position (Duncker, 1977). Two to three days before hatching, blood capillary connections between arterioles and venules rapidly increase and air-filled capillaries sprout from the infundibula to surround the developing blood capillaries, forming a three-dimensional network of gas-exchange tissue. At this time the lung is aerated (Powell, 2000). Thus, the lung gradually becomes used as an organ of gas exchange between the time of internal pipping and hatching (the parafoetal period). The lung therefore does not suddenly expand at the time of hatching, as is the case with mammals at birth, but gradually grows to an air-filled structure during the parafoetal period.

During post-hatching growth, the number of parabronchi in the lung does not increase; only their diameter and length increase. However, pneumatisation of the skeleton only occurs post-hatching (Duncker, 1977).

#### IMMUNE SYSTEM

The avian immune system is nearly non-existent in the embryo and is very weak at hatching (Zander, 1978). Lymphocytes are present in small numbers in the embryonic circulation during the final week of incubation and increase during the first two weeks of life (Bagley, 1987). The development of the subsystems that comprise the immune system also occur very slowly during the first two weeks post-hatching (Glick, 2000). Early studies revealed that the bursa of Fabricius grew most rapidly during the first three weeks after hatching, plateaued, and then regressed as early as eight weeks of age (Glick, 1956, 1960). The spleen and bursa also grow very slowly during the first ten days of age in turkey poults and were classified as late maturing organs (Phelps *et al.*, 1987b). Similar results were obtained for thymus maturation (McCorkle *et al.*, 1983). Detectable graft-versus-host responses were first observed at 6 days post-hatching. After 6 days post-hatching, detectable responses were noted in their xenobiologic condition but appreciable responses were noted only in the 6 to 21 day old birds (McCorkle *et al.*, 1983).

#### EFFECT OF INCUBATION CONDITIONS ON MATURATION PROCESSES POST-HATCHING

One of the discriminating features of altricial and precocial species is the existence of a plateau phase

in oxygen consumption (Ar and Rahn, 1978). Thus, it has been inferred that the existence of such a phase may play a major role in the characteristic maturity of the species at hatching. The factors determining the characteristic maturity have been summarised in what is called the conductance constant. Three variables seem to be interdependent in determining the maturity of a hatchling, and these three may be interrelated. The variables are eggshell conductance, the incubation period and the egg weight (Ar and Rahn, 1978). An example of how these measurements may be used in incubation is given in Box 1. Incubation conditions must be adjusted to account for these variables to allow optimal maturation of hatchlings (Christensen *et al.*, 2001b).

**Box 1. An example of how egg weight, eggshell conductance and the length of the incubation period may affect hatchling maturity and function (Ar & Rahn, 1978).**

The conductance constant (k) is defined as:

$$k = (G \times I) / W$$

Where k = 5.13; G = eggshell conductance in milligrams of water vapour lost per day per mmHg of pressure gradient across the shell [inside pressure (saturation) – outside pressure (incubator humidity vapour pressure)]; I = incubation period in days; and W = egg weight in grams

For example, if G = 17 and W = 90 g, which are the average values for turkey eggs, then:  $5.13 = (17 \times I) / 90$ .

Solving for I:  $I = (90 \times 5.13) / 17 = 27.2$  days for the incubation period.

Therefore, incubation conditions for these eggs need to be set to result in an incubation period of 27.2 days.

**Box 1** An example of how egg weight, eggshell conductance and the length of the incubation period may affect hatchling maturity and function (Ar and Rahn, 1978).

**Table 1** Developmental rates of different physiological systems during the first seven days post-hatching in broilers and turkeys

System	Growth	Function
Circulatory system	Rapid and early in embryogenesis	Rapid and early
Kidney and body fluids system	Rapid and early following hatching	Slow until midway through embryogenesis to hatching
Digestive system	Rapid at hatching	Rapid at hatching
Body temperature control system	Slow	Depends upon species
Respiratory system	Slow	Slow
Immune system	Slow	Slow

If the timing of the plateau phase in oxygen consumption is accelerated or decelerated by incubation conditions or physical egg properties, then the embryo makes adjustments and may delay maturation and function. Examples of incubation conditions that may affect the plateau stage in oxygen consumption are the incubation temperature or the ventilation rates of the machines. When these conditions are imposed upon embryos in late development, the results can be long term (Buys *et al.*, 1998; Weytjens *et al.*, 1999). At high altitude, eggs are produced that naturally have longer incubation periods because hens at high altitude create eggs with decreased eggshell conductance values (Rahn *et al.*, 1977). Thus, conditions for high altitude incubation must be adjusted to preserve optimal plateau phase conditions to result in a mature hatchling.

## CONCLUSIONS

The most striking feature of the initial days of life outside the shell is the rate at which different physiological systems mature (Table 1). Some systems become totally functional within a 24 hour period but others take considerably longer. As during the continued commercial development of different strains of poultry additional emphasis is placed on rapid growth, the ontogeny of some systems may be compromised (Lilja and Olsson, 1987). Management strategies need to be formulated to deal with such systems and simultaneously maintain the overall health of the neonate. Examples of tissue systems that need to mature rapidly in both size and function post-hatching are the heart, intestines and lungs.

## REFERENCES

- Ar, A. (1991) Egg water movements during incubation. In: *Avian Incubation*, Tullett, S.G. (ed.), pp. 157–173. Butterworths-Heinemann, London.
- Ar, A., Arieli, B., Belinsky, A. and Yom-Tov, Y. (1987) Energy in avian eggs and hatchlings: utilization and transfer. *J. Exper. Zool., Suppl. 1*: 151–164.
- Ar, A. and Rahn, H. (1978) Interdependence of gas conductance, incubation length and weight of the avian egg. In: *Respiratory Function in Birds, Adult and Embryonic*, Piiper, J. (ed.), pp. 227–236. Springer-Verlag, Berlin-Heidelberg.
- Baggott, G.K. (2001) Development of extra-embryonic membranes and fluid compartments. In: *Perspectives in Fertilisation and Embryonic Development in Poultry*, pp. 23–29. Ratite Conference Books, Wallingford. Reprinted in 2009: *Avian Biol. Res.*, 2, 21–26.
- Bagley, L.G. (1987) *Embryonic respiration and hatchability of turkey eggs*. Ph.D. dissertation, North Carolina State University, Raleigh.
- Breeding, S.W., McRee, W.A., Ficken, M.D. and Ferket, P.R. (1994) Effect of protein restriction during brooding on spontaneous turkey myopathy. *Avian Dis.*, **38**, 366–370.
- Buyse, N., Dewil, E., Gonzales, E. and Decuyper, E. (1998) Different CO<sub>2</sub> levels during incubation interact with hatching time and ascites susceptibility in two broiler lines selected for different growth rate. *Avian Pathol.*, **27**, 605–612.
- Christensen, V.L., McMurtry, J.P., Donaldson, W.E. and Nestor, K.E. (2001a) Incubation temperature affects plasma insulin-like growth factors in embryos from genetically selected lines of turkeys. *Poult. Sci.*, **80**, 949–954.
- Christensen, V.L., Grimes, J.L. and Wineland, M.J. (2001b) Effects of turkey breeder hen age, strain, and length of the incubation period on survival of embryos and hatchlings. *J. Appl. Poult. Res.*, **10**, 5–15.
- Davis, T.A. and Ackerman, R.A. (1987) Effects of increased water loss on growth and water content of the chick embryo. *J. Exp. Zool., Suppl. 1*: 357–364.
- Davis, G.S. and Siopes, T.D. (1989) Ontogeny of daily rhythmicity in plasma corticosterone and variation in sensitivity of the corticosterone response in turkey poults. *Poult. Sci.*, **68**, 423–427.
- Dawson, W.R. and Whittow, G.C. (2000) Regulation of body temperature. In: *Avian Physiology*, Whittow, G.C. (ed.), pp. 344–390. Academic Press, San Diego.
- Denbow, D.M. (2000) Gastrointestinal anatomy and physiology. In: *Avian Physiology*, Whittow, G.C. (ed.), pp. 299–326. Academic Press, San Diego.
- Denbow, D.M. and Sheppard, B.J. (1993) Food and water intake responses of the domestic fowl to norepinephrine infusion at circumscribed neural sites. *Brain Res. Bull.*, **332**, 121–128.
- Donaldson, W.E. and Christensen, V.L. (1994) Dietary carbohydrate effects on some plasma organic acids and aspects of glucose metabolism in turkey poults. *Comp. Biochem. Physiol.*, **109A**, 423–440.

- Donaldson, W.E., Clark, J. and Christensen, V.L. (1994) Protein, lipid and glycogen stores in newly-hatched turkey (*Melagris gallopavo*) poults as affected by post-hatch stressors and holding time. *Comp. Biochem. Physiol.*, **107A**, 559–562.
- Doneen, B.A. and Smith, T.E. (1982) Ontogeny of endocrine control of osmoregulation in chick embryo. II. Actions of prolactin, arginine vasopressin, and aldosterone. *Gen. Compar. Endocrinol.*, **48**, 310–318.
- Duncker, H.R. (1977) Development of the avian respiratory and circulatory systems. In: *Respiratory Function in Birds, Adult and Embryonic*, Piiper, J. (ed.), pp. 261–273. Springer-Verlag, Berlin-Heidelberg.
- Fan, Y.K., Croom, J., Christensen, V.L., Black, B.L., Bird, A.R., Daniel, L.R., McBride, B.W. and Eisen, E.J. (1997) Jejunal glucose uptake and oxygen consumption in turkey poults selected for rapid growth. *Poult. Sci.*, **76**, 1738–1745.
- Fasenko, G.M. (1996) *Factors influencing embryo and poult viability and growth in stored turkey eggs*. Ph.D. dissertation, North Carolina State University, Raleigh.
- Glick, B. (1956) Normal growth of the bursa of Fabricius in chickens. *Poult. Sci.*, **35**, 843–851.
- Glick, B. (1960) Growth of the bursa of Fabricius and its relationship to the adrenal gland in the White Pekin duck, White Leghorn, outbred and inbred New Hampshire. *Poult. Sci.*, **39**, 130–139.
- Glick, B. (2000), Immunophysiology. In: *Avian Physiology*, Whittow, G.C. (ed.), pp. 657–670. Academic Press, San Diego.
- Goldstein, D.L. and Skadhauge, E. (2000) Renal and extrarenal regulation of body fluid composition. In: *Avian Physiology*, Whittow, G.C. (ed.), pp. 265–297. Academic Press, San Diego.
- Isaacks, R.E., Harkness, D.R., Adler, J.L., Kim, C.Y., Goldman, P.H. and Roth, S. (1976) Studies on avian erythrocyte metabolism. 5. Relationship between the major phosphorylated metabolic intermediates and whole blood oxygen affinity in embryos and poults of the domestic turkey (*Meleagris gallopavo*). *Poult. Sci.*, **55**, 1788–1794.
- Julian, R.J., Mirsalimi, S.M., Bagley, L.G. and Squires, E.J. (1992) Effect of hypoxia on spontaneous turkey cardiomyopathy (round-heart disease). *Avian Dis.*, **36**, 1043–1047.
- Lilja, C. (1983) A comparative study of postnatal growth and organ development in some species of birds. *Growth*, **47**, 317–339.
- Lilja, C. and Olsson, U. (1987) Changes in embryonic development associated with long-term selection for high growth rate in Japanese quail. *Growth*, **51**, 301–308.
- Magwood, S.E. and Bray, D. (1962) Disease condition of turkey poults characterized by enlarged and rounded hearts. *Canad. J. Compar. Med.*, **26**, 268–272.
- MacPherson, C.R. and Deamer, J. (1964) Some observations on the normal erythrocyte development in the chick embryo. *Poult. Sci.*, **43**, 223–228.
- McCorkle, F.M., Simmons, D.G. and Luginbuhl, G.H. (1983) Graft-vs-host response in *Alcaligenes faecalis*-infected turkey poults. *Amer. J. Veter. Res.*, **44**, 1141–1142.
- McNichols, M.J. and McNabb, F.M.A. (1988) Development of thyroid function and its pituitary control in embryonic and hatchling precocial Japanese quail and altricial Ring doves. *Gen. Comp. Endocrin.*, **69**, 109–118.
- Moran, E.T. and Reinhart, B.S. (1980) Poult yolk sac amount and composition upon placement: Effect of breeder age, egg weight, sex and subsequent change with feeding on fasting. *Poult. Sci.*, **59**, 1521–1528.
- Nice, M.M. (1962) Development of behavior in precocial birds. *Trans. Linn. Soc. New York*, **8**, 1–211.
- Nicholson, L. (1992) *Nutritional aspects of early poult mortality*. MS thesis, North Carolina State University, Raleigh.
- Noble, D.O., Nestor, K.E. and Polley, C.R. (1999) Factors influencing early poult flip-overs in experimental populations of turkeys. *Poult. Sci.*, **78**, 178–181.
- Noy, Y. and Sklan, D. (1996) Uptake capacity in vitro for glucose and methionine and in situ for oleic acid in proximal small intestine of posthatch chick. *Poult. Sci.*, **75**, 998–1002.
- Noy, Y. and Sklan, D. (1998) Yolk utilisation in the newly hatched poult. *Brit. Poult. Sci.*, **39**, 446–451.
- O'Sullivan, N.P., Dunnington, E.A., Larsen, A.S. and Siegel, P.B. (1992) Correlated responses in lines of chickens divergently selected for fifty-six-day body weight. 3. Digestive enzymes. *Poult. Sci.*, **71**, 610–617.
- Phelps, P.V. (1985) *The posthatch physiology of the turkey poult*. MS thesis, North Carolina State University, Raleigh.
- Phelps, P.V., Edens, F.W. and Christensen, V.L. (1987a) The posthatch physiology of the turkey poult. II. Hematology. *Comp. Biochem. Physiol.*, **86A**, 745–750.
- Phelps, P.V., Edens, F.W. and Christensen, V.L. (1987b) The posthatch physiology of the turkey poult. 1. Growth and development. *Comp. Biochem. Physiol.*, **86A**, 739–743.
- Powell, F.L. (2000) Respiration. In: *Avian Physiology*, Whittow, G.C. (ed.), pp. 223–264. Academic Press, San Diego.
- Rahn, H., Carey, C., Balmas, K., Bhatia, B. and Paganelli, C. (1977). Reduction of pore area of the avian eggshell as an adaptation to altitude. *Proc. Nat. Acad. Sci.*, **74**, 3095–3098.
- Ricklefs, R.E. (1987) A comparative analysis of avian embryonic growth. *J. Exp. Zool.*, **51**, 309–324.
- Ricklefs, R.E. and Starck, J.M. (1998) Embryonic growth and development. In: *Avian Growth and Development, Evolution within the Altricial-Precocial Spectrum*, Starck, J.M. and Ricklefs, R.E. (eds.), pp. 31–58. Oxford University Press, New York.
- Romanoff, A.L. (1960) *The Avian Embryo*. The Macmillan Company, New York.
- Schmalhausen, L. (1930) Das wachstumgesetz als gesetz der progressiven differenzierung. *Wilhelm Rouxs Arch. Develop. Biol.*, **123**, 153–178.
- Sell, J.L., Angel, C.R., Piquer, R.L., Mallarino, E.G. and Batshan, H.A. (1991) Developmental patters of selected characteristics of the gastrointestinal tract of young turkeys. *Poult. Sci.*, **70**, 1200–1205.
- Selye, H. (1951) The general adaptative syndrome. *Ann. Rev. Med.*, **2**, 327–342.
- Suvarna, S.R. (1999) *Ontogeny of glucose transport in turkey intestines*. PhD dissertation, North Carolina State University, Raleigh.
- Tazawa, H. and Rahn, H. (1987) Temperature and metabolism of chick embryos and hatchlings after prolonged cooling. *J. Exp. Zool., Suppl.* **1**: 105–109.

- Tazawa, H. and Whittow, G. C. (2000) Incubation physiology. In: *Avian Physiology* G. C. Whittow (ed.), pp. 617–645. Academic Press, San Diego.
- Uni, Z., Noy, Y. and Sklan, D. (1995a) Posthatch changes in morphology and function of the small intestines in heavy- and light-strain chicks. *Poult. Sci.*, **74**, 1622–1629.
- Uni, Z., Noy, Y. and Sklan, D. (1995b) Development of the small intestine in heavy and light strain chicks before and after hatching. *Brit. Poult. Sci.*, **36**, 64–71.
- Uni, Z., Noy, Y. and Sklan, D. (1999) Posthatch development of small intestinal function in the poul. *Poult. Sci.*, **78**, 215–222.
- Vince, M.A. and Tolhurst, B.E. (1975) The establishment of lung ventilation in the avian embryo. The rate at which the lungs become aerated. *Comp. Biochem. Physiol.*, **52**, 331–337.
- Watterson, R.L. and Sweeney, R.M. (1973) Embryology of the chick. In: *Laboratory Studies of Chick, Pig, and Frog Embryos*, pp. 5–76. Burgess Publishing Company, Minneapolis.
- Weytjens, S., Meijerhof, R., Buyse, J. and Decuypere, E. (1999) Thermoregulation in chicks originating from breeder flocks of two different ages. *J. Appl. Poult. Res.*, **8**, 139–145.
- Zander, D.V. (1978) Principles of disease prevention: diagnosis and control. In: *Diseases of Poultry*, Hofstad, M.S., Calnek, B.W., Helmboldt, C.F., Reid, W.M. and Yoder, H.W. (eds.), pp. 3–48. The Iowa State University Press, Ames.

# Developmental plasticity during embryonic and post-hatching growth of birds

J. Matthias Starck

*Institute of Zoology and Evolutionary Biology, Friedrich-Schiller-Universitaet Jena, Erbertstrasse 1, D-07743 Jena, Germany*

*Present address: Department of Biology II, University of Munich (LMU), Großhaderner Straße 2, D-82152 Planegg-Martinsried, Germany E-mail: starck@uni-muenchen.de*

## INTRODUCTION

Developmental plasticity is the ability of a developing organism to respond to fluctuating environmental conditions by invoking different developmental programs. Therefore, variable conditions during development may result in different phenotypes of the adult. Temperature dependent sex determination in reptiles (see review by Deeming and Ferguson, 1991) is among the more spectacular cases of such developmental plasticity (or “developmental conversion”) *i.e.* different genetic programs being read. By contrast, the more or less constant incubation conditions for bird eggs have meant that embryogenesis is quite unaffected by fluctuations of the environment (except for maternal effects, such as yolk contents of eggs, or concentrations of hormones and immunoglobulins deposited in the yolk). However, during post-hatching growth birds may experience considerable fluctuations of their natural environment.

Within the theoretical framework of developmental plasticity it would be expected that growing birds adjust their developmental patterns in response to changes in environmental conditions. This may occur by invoking new genetic programs (developmental conversion) or by changing quantitative expression of existing genetic programs (developmental modulation). Availability of food, nutrient composition, and changes in ambient temperature are the major sources of environmental variation for a young bird.

Developmental plasticity and compensatory growth of birds have been widely investigated (*e.g.* Schew and Ricklefs, 1998). In particular, laboratory studies have investigated the effects of fluctuating food supply on growth and intestinal function in the altricial nestlings of the song thrush (*Turdus philomelos*) and the precocial hatchlings of Japanese quail (*Coturnix japonica*). Song-thrush nestlings were characterised by a limited degree of plasticity of their developmental program which prohibited overfed nestlings from significantly up-regulating their gut function to

accommodate increased food intake. This suggests that they were already growing at a rate close to their physiological maximum. By contrast, young of Japanese quail had a flexible developmental program and were able to interrupt growth for extended periods of post-hatching development.

It is interesting to place the developmental plasticity observed in birds in relationship to that reported in other sauropsids (*i.e.* reptiles and dinosaurs). Studies have concentrated on investigating the bone microstructure of Japanese quail growing under different experimental conditions. These observations were compared with that of extant and extinct birds, and dinosaurs. The rate of bone formation is significantly affected by environmental/experimental conditions, skeletal element, and age. In the quail, the experimental conditions did not result in formation of lines of arrested growth. In contrast to extant birds, variable incidence of lines of arrested growth can be observed among several dinosaur species, including fossil birds (from the Cretaceous), extant sauropsids (reptiles) as well as non-mammalian synapsids, and some extant mammals. This suggests that developmental plasticity was much common in the ancestral condition and that the response of bone to environmental conditions was more variable than what is observed in living birds. It is suggested that during the evolution of the ornithurine birds (which died out at the end of the Cretaceous Period), such high degree of developmental plasticity was reduced in modern birds in association with the shortened developmental time.

## REFERENCES

- Deeming, D.C. and Ferguson, M.W.J. (1991) *Egg Incubation: Its Effects on Embryonic Development in Birds and Reptiles*. Cambridge University Press, Cambridge.
- Schew, W.A. and Ricklefs, R.E. (1998) Developmental plasticity. In: *Avian Growth and Development. Evolution within the Altricial-Precocial Spectrum*. Starck, J.M. and Ricklefs, R.E. (eds.), pp. 288–304. Oxford University Press, New York.



# Assessment and significance of fertility in commercial poultry production

Graham J. Wishart

*Avian Reproduction Group, University of Abertay Dundee, Bell Street, Dundee, DD1 1HG, UK*

## INTRODUCTION

Breeding efficiency in poultry is usually assessed in terms of "percentage fertile eggs" laid following artificial insemination (AI) or natural mating. Fertility of eggs can be assessed "non-destructively" by examining eggs that have failed to hatch or by candling during incubation, or "destructively" by opening a sample of eggs to look for the presence of an embryo – either before or during incubation.

All of these systems have particular disadvantages. In the first two methods, any fertility or insemination "problems" will be two or more weeks old by the time that fertility data is obtained. In the second two methods, there are the statistical problems associated with sampling as well as the loss of eggs. Additionally, assessing fertility by macroscopic analysis of unincubated eggs can be unreliable.

However, all of these methods have another common disadvantage that is inherent in the measurement of fertility itself. It is that assessment of fertility only tells us what happened to one sperm, the one that fertilised the egg, whilst we know that hundreds of millions of spermatozoa are transferred in an AI schedule or by natural mating. So, if we accept that breeding efficiency is simply the transfer of sperm from the male to the female reproductive tracts (with or without AI), then the proportion of fertile eggs is not a particularly good measure of this.

There are more direct ways of measuring successful sperm transfer. Evidence of sperm that associate with the peri-vitelline layers that surround the ovum at or around the time of fertilisation in the upper oviduct can be found in laid eggs. In fact, evidence of up to a thousand sperm per egg can be readily obtained to provide an extended scale of breeding efficiency compared to the binary "fertility", *i.e.* the egg is fertile or not. This chapter described the principles, use and application of some of the methods to detect 'sperm-in-eggs'.

## SPERM IN THE FEMALE REPRODUCTIVE TRACT—HOW SPERM REACH THE OVUM

Inseminated sperm are deposited in the distal vagina (see Brillard, 2002), from where they are mostly

ejected into the cloaca. Around 1% of the inseminated dose of sperm (*e.g.* 1 million out of 100 million) enters the "sperm storage tubules" at the utero-vaginal junction. These tubules provide a reservoir from which sperm are released at a rate of around 30% per day in chickens and 10% per day in turkeys. Released spermatozoa migrate up the hen's oviduct to fertilise the newly ovulated ovum at the upper end of the oviduct. However, if the oviducal sperm reservoir is not replenished by further inseminations, then there will eventually be insufficient sperm to fertilise the ova and so the "fertile period" will come to an end. This occurs after about 2 weeks in chickens and 4–6 weeks, or longer, in turkeys.

## FERTILISATION—WHEN SPERM ENCOUNTER THE OVUM

Fertilisation takes place in the infundibulum, the funnel-like opening of the oviduct adjacent to the ovary (see Brillard, 2002). To enter the ovum, sperm must penetrate the inner peri-vitelline layer (IPVL) that surrounds the ovum and they do this by enzymatic hydrolysis of the layer's fibres to create small holes in the IPVL. These "IPVL-holes" are most frequent in the IPVL over the germinal disc where over 1,000 can be found, although eggs are likely to be fertile if only 6 IPVL-holes are present. If the numbers are lower than 6, fertility is less likely and is zero if there are no IPVL-holes.

Fertilisation has to take place in the period (~15 minutes) between ovulation and deposition of the outer peri-vitelline layer (OPVL), which is the first oviducal secretion to surround the yolk. After fertilisation, sperm that have not penetrated the ovum are trapped in the OPVL. In the final accounting of IPVL-holes and OPVL-sperm, up to 50,000 sperm can be shown to interact with the peri-vitelline layers.

The numbers of sperm interacting with the egg are proportional to the numbers in the sperm storage tubules and so they are a measure of sperm transfer. Most importantly, they can be demonstrated in the laid egg, which therefore represents a useful "reporter" of oviducal sperm numbers and so of successful transfer of sperm into the hen's reproductive system.



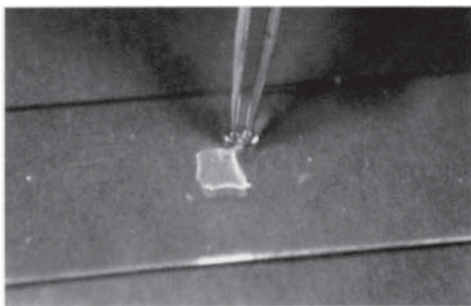
**Figure 1** Cutting a small 4 cm<sup>2</sup> piece of peri-vitelline layer from around the germinal disc.

#### METHODS FOR MEASURING OPVL-SPERM AND IPVH-HOLES

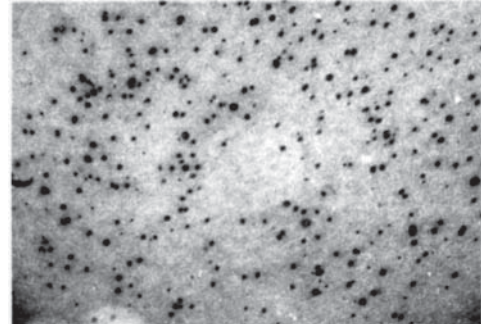
Whichever measurement is to be made or technique to be followed, the first steps involve removing the whole yolk from an unincubated egg, washing it in a simple salt solution, and then cutting a 2 cm-squared piece of whole peri-vitelline layer from over the germinal disc (Figure 1). This piece of layer is then washed free of yolk and spread on a microscope slide (Figure 2). For viewing OPVL-sperm the membrane should be stained with a DNA-specific fluorochrome. OPVL-sperm nuclei can be seen using a fluorescent microscope at around  $\times 400$  magnification. IPVH-holes can be seen in glycoprotein-stained samples by light microscopy at  $\times 40$  magnification or, for unstained samples, at the same magnification using dark-ground optics.

OPVL-sperm require staining with potentially toxic dyes and they must be viewed at high magnification, which takes a long time. So IPVH-holes measurements are preferable and the author's method of choice, on the basis of simplicity, is to examine IPVH-holes in unstained samples. However, in all the following work, OPVL-sperm measurement can be applied in exactly the same way as that for IPVH-holes.

IPVH-holes are most frequent in a broad ring that corresponds to the region over the germinal disc



**Figure 2** Spreading the washed peri-vitelline layer on a microscope slide.

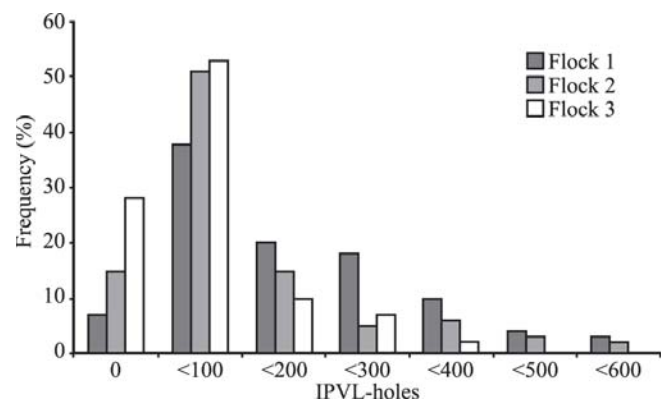


**Figure 3** The holes in the IPVH from over the germinal disc (the image is  $\sim 2.5$  mm across).

(Figure 3). We usually report the whole numbers of holes in this region, although if the numbers are high, we only count the holes in a region of the area and calculate the total number by proportion.

#### Application of measuring sperm-in-eggs in commercial broiler-breeder flocks

We have measured the IPVH-holes in samples of 60 eggs from broiler breeder flocks and have found distributions similar to those shown in Figure 4. The distributions are skewed, more so in flocks with lower fertility, where there are more eggs with either low numbers, or no, IPVH-holes. What is remarkable about these distributions is the range of sperm-in-eggs, which can range from zero to many hundreds. This highly differential distribution of sperm among the females represents the most significant contribution to flock infertility. It may be affected by female reproductive physiology, but it is more likely to reflect mate choice. In fact, in all broiler breeder flocks studied so far, infertile eggs can be shown by IPVH-hole analysis, to be laid by a separate population of hens that do not appear to be involved in mating.



**Figure 4** Frequency distribution of the IPVH-holes from over the germinal disc in samples of 60 eggs from three flocks of broiler breeders. The fertility of all birds in the flocks was 87%, 84% and 74% for flocks 1, 2 and 3, respectively.

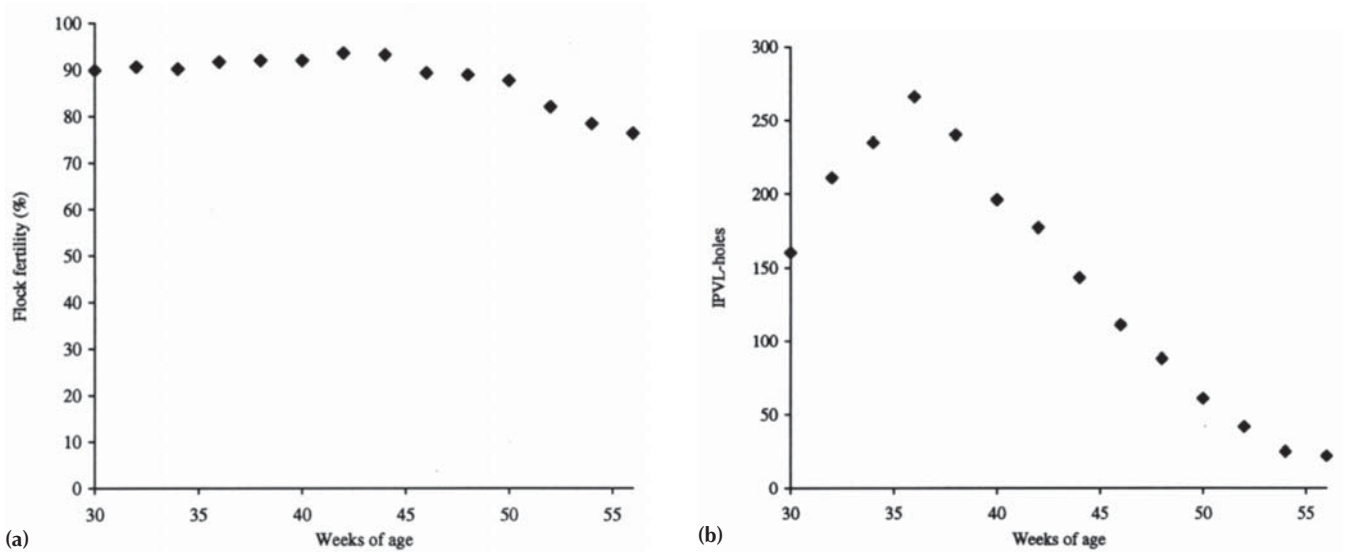


Figure 5 Weekly flock fertility (a) and mean IPVL-holes of a sample of 60 eggs (b) during the life of a broiler breeder flock.

Despite the large variation in IPVL-holes among eggs (and thus hens), the median IPVL-holes in samples of broiler eggs is highly correlated with overall flock fertility and so can be used to monitor flock fertility and identify sub-fertile flocks.

It is commonly found that the fertility of broiler breeder flocks becomes reduced after 50 weeks of age. It is often assumed that mating may decrease after that time due to lowered mating frequency or success, usually blamed on male "leg problems". Figure 5 illustrates this by showing a typical pattern, with fertility dropping by around 10% between weeks 50 and 55. However, the pattern of the median IPVL-holes of samples of eggs shows that real mating efficiency, in terms of the numbers of sperm passed

into eggs, follows a quite different pattern. In fact, oviducal sperm numbers (IPVL-holes) increase in eggs from hens of up to 38 weeks of age, after which there is a steady decrease from around 250 IPVL-holes to only 20 IPVL-holes at 55 weeks of age (Figure 5). Thus not only does the greater quantitative range of the IPVL-holes assay show that the magnitude of the problem is that of a 90%, rather than 10% reduction in mating success, but that also the reduction in mating efficiency actually occurs more than 10 weeks before a significant drop in fertility is seen. This might suggest that the fertility loss in broiler breeders is more likely to be the result of social changes in mating systems, rather than of infirmity in ageing birds.

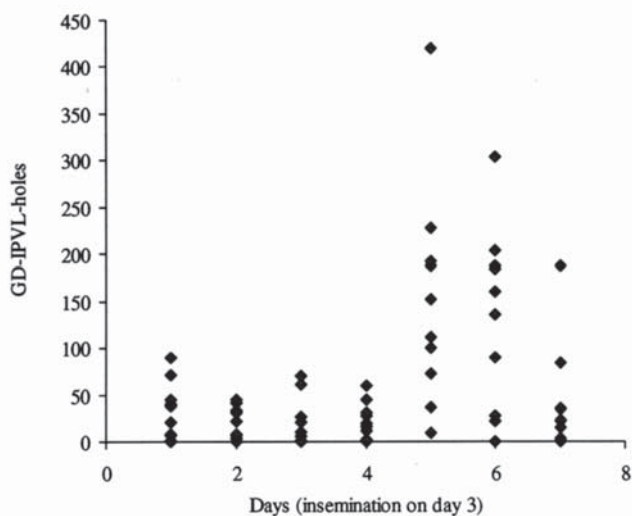


Figure 6 Number of IPVL-holes over the germinal disc (GD) from samples of 10 turkey eggs taken daily before and after artificial insemination.

#### Application of measuring sperm-in-eggs in commercial turkey breeder flocks

The distribution of OPVL-holes in commercial turkey flocks is similar to that found in broiler breeders, although the range is less since artificial insemination negates the added variable of mate choice. However, in the same way as for broiler breeders, the median IPVL-holes of samples of 60 eggs is related to flock fertility. When comparing IPVL-holes in eggs from turkey flocks, it is important to take samples on the same number of days after artificial insemination, since this makes a significant difference in the numbers of IPVL-holes (Figure 6). Examining the IPVL-holes in eggs take before and after insemination can be used to assess the efficiency of AI.

### Other applications of measuring sperm in eggs to assess breeding efficiency

In many research applications, measuring sperm-in-eggs has proved to be a much more sensitive measure of breeding efficiency than the proportion of fertile eggs. Thus, measuring sperm-in-eggs, often under conditions where there was no apparent difference in fertility, has highlighted differences in sperm quality, nutritional regimes, environmental conditions and male to female ratios. Thus these techniques have superseded the traditional 'fertility trial'.

### CONCLUSIONS

Measuring sperm-in-eggs provides a useful alternative to measuring the proportion of fertile eggs laid by chickens and turkeys. It has found application in analysis of various breeding systems and on both a research and a commercial scale. Its value lies in its greater quantitative range and sensitivity and more direct relevance to breeding efficiency, in terms of numbers of sperm transferred from males to females. It also provides a more immediate analysis, avoiding the wait for incubated eggs, and can even identify a reduction in breeding efficiency before any infertile eggs are seen. In research, it has replaced the 'fertility trial' as the best means of experimentally highlighting the differences in fertilising ability between samples of semen or in breeding efficiency under different environmental or nutritional regimens. On a commercial scale, it can be used for monitoring flock per-

formance and provides the only means by which mating and AI efficiency can be properly analysed.

### FURTHER READING

Reviews by Bakst *et al.* (1994), Wishart (1995), Wishart and Staines (1999), Wishart *et al.* (2001) and Brillard (2002) should be consulted for further information and details of original articles on measuring sperm-in-eggs by Bramwell, Brillard, Donoghue, Wishart and their colleagues.

### REFERENCES

- Bakst, M.R., Brillard, J.-P. and Wishart, G.J. (1994) Sperm selection, transport and storage in the avian oviduct. *Poult. Sci. Rev.*, **5**, 143–177.
- Brillard, J.-P. (2002) Practical aspects of fertility in poultry. In: *Practical Aspects of Commercial Incubation in Poultry*, Deeming, D.C. (ed.), pp. 7–10. Ratite Conference Books, Lincoln. Reprinted in 2009. *Avian Biol. Res.*, **2**, 41–46.
- Wishart, G.J. (1995) New approaches to evaluating male and female fertility. In: *Proceedings of the 1st International Symposium of AI in Poultry*, Bakst, M.R. and Wishart, G.J. (eds.), pp. 207–223. Poultry Science Association, Savoy, Illinois.
- Wishart, G.J. and Staines, H.J. (1999). Measuring sperm:egg interaction to assess breeding efficiency in chickens and turkeys. *Poult. Sci.*, **87**, 428–436.
- Wishart, G.J., Staines, H.J. and Hazary, R.C. (2001) Evaluation of Fertility: Biological Basis and Practical Application. *World's Poult. Sci. J.*, **57**, 309–314.

# Practical aspects of fertility in poultry

Jean-Pierre Brillard

INRA, Station de Recherches Avicoles, 37380-Nouzilly, France E-mail: [brillard@tours.inra.fr](mailto:brillard@tours.inra.fr).

## INTRODUCTION

Birds, reptiles and insects (e.g. Hymenoptera) share fundamental similarities in their reproductive strategies due to the presence, in the female tract, of specialised sites in which spermatozoa may reside for prolonged periods upon a single mating. In avian species, two distinct storage sites are present, one located in the utero-vaginal junction and the other in the lower portion of the infundibulum. At both sites, spermatozoa are stored in sperm storage tubules (SSTs) which are discrete, generally non-branched invaginations of the luminal epithelium (see review by Bakst *et al.*, 1994). The SSTs located in the utero-vaginal junction are considered as the main site of residence of spermatozoa upon their deposition in the lower portion of the vagina. The fertilising potential of females along the reproductive season is at first dependent on their ability to store and maintain adequate populations of spermatozoa in their SSTs in order to repeatedly provide the site of fertilisation with sufficient numbers of “fit” spermatozoa after each ovulation. As a consequence in poultry species, eggs from females with prolonged sperm storage potential have also a natural tendency to maintain optimal fertility rates. This situation may be highly desirable to sustain high fertility in the case of partial failure of the males during the season.

This chapter is an attempt to address the main intrinsic and extrinsic factors capable of favouring or altering fertility in poultry species. Unfortunately, most of the information available is obtained from two species, namely the domestic fowl and turkey, which, in certain circumstances, may reveal insufficient to pinpoint the specificity of fertility in other poultry species.

## CONTROL OF FERTILITY BY INTRINSIC FACTORS

Fertility is, basically, the ability to reproduce. This definition should however be more accurately targeted in poultry as it is *per se* applicable to both an animal: (e.g. hen fertility) and to its eggs each of which seen as an entity (egg fertility). Indeed, a common practice in poultry species is to restrict the definition of “fertility” to the level of fertilisation of

laid eggs (fertile eggs/incubated eggs  $\times$  100). This criterion can be estimated from eggs at one or the other stages of their development (*i.e.* before or during incubation). Egg fertility intrinsically depends on hen fertility in a manner that, following mating or insemination, a hen will or will not store spermatozoa in its oviduct. Therefore, she will or will not provide sufficient numbers of spermatozoa to ensure the fertilisation of oöcytes within minutes after ovulation. From a female standpoint, the genetic origin and the age in the reproductive season are the two major intrinsic factors which, ultimately, interfere with the degree of sperm storage/release at the oviducal level.

## Genetic origin

The duration of the fertile period can be defined as the number of days during which a female lays fertile eggs following a single mating or insemination. From an applied standpoint, the above definition can be expressed either as the number of days with 100% fertile eggs (efficient duration or *De*) or as the number of days up to the last fertile egg (maximum duration or *Dm*; Brillard *et al.*, 1989). Previous observations of domestic fowl have established that a selection of females on the basis of their duration of fertility is feasible (Pingel and Planert, 1978; Brillard *et al.*, 1998). Using a divergent approach, the latter authors also demonstrated that the duration of the fertile period is, at least in part, dependent on the population of sperm storage tubules located in the utero-vaginal junction. Previous observations have confirmed that the population of sperm storage tubules can be modified by selection (Brillard *et al.*, 1998; also see Table 1). Moreover, a selection based on overall reproductive performances (e.g. number of hatched chicks per female in a season) modifies the number of eggs capable of developing viable embryos, a factor that may ultimately increase or affect the overall reproductive performances in breeder flocks. Currently, as far I am aware, a selection of female lines based on their duration of fertile period and/or degree of embryo survival in fertile eggs has still not been of practical use in commercial parent stocks.

**Table 1** Some reproductive traits in females from domestic fowl selected during five generations on the basis of their high (H) or low (L) potential to produce hatched chicks<sup>1</sup>

Line	Maximum (SD) duration of fertility (days)		F/I% (2–8 days post-AI)	H/F%	F/I% (9–15 days post-AI)	H/F%	SSTs × 10 <sup>3</sup> /hen (SD)	
H-line	15.1	(0.5)	94.8	89.2	89.4	82.2	2957	(140)
L-line	10.7	(0.6)	70.2	69.9	36.4	49.1	2241	(121)

<sup>1</sup>Differences were significant between lines for all parameters tested ( $P < 0.05$ ). F = fertile; I = incubated; SST = sperm storage tubules.

### Seasonal variations of fertility

It has long been established that poultry species undertake more or less marked variations of their reproductive performances over the reproductive season, a consequence of their physiological age and of photoperiod. For example, the physiological status of the hen at the onset of the reproductive season can play a major role on subsequent performances of fertility. Brillard and Bakst (1990) demonstrated that turkey hens inseminated just prior to the onset of the laying season store higher populations of sperm compared to hens inseminated just (equates to one day) after the onset of lay. The rationale for such a higher sperm storage efficiency in pre-laying hens has still not been established. Hypothetically, the absence of oviducal contractions in pre-laying hens may facilitate the migration of spermatozoa up to the storage sites. Notwithstanding the origin of such differences, it appears that high fertility rates obtained early in season will generally sustain persistent high fertility performances over the entire season. By contrast, low fertility performances at the onset of the season generally result in persistently poor fertility performances over the season. Whether or not such differences can be attributed to a more functional release of spermatozoa in hens having sperm storage tubules primarily filled with large populations of "fit" spermatozoa also remains to be established.

A general feature of fertility in fowl and turkey hens is its progressive increase over the 3–5 first weeks of the reproductive period (up to peak of lay) followed by a plateau maintained up to 2/3–3/4 of this period. Following this plateau, fertility progressively declines with age, a consequence of a faster release of spermatozoa from the SSTs rather than impaired storage of spermatozoa in the oviduct (Brillard, 1993). In order to persistently obtain pre-cited profiles of fertility rather than profiles with successive, temporary drops revealing the existence of impaired sperm storage, an adequate management of the hens with regards to filling of the SSTs is necessary. Over the past years, research to assess the population of sperm present at the fertilisation sites has provided a series of tools which, at many instances, may reveal useful to point

out the existence of defective sperm deposition (Wishart and Staines, 1995) or low sperm quality (Donoghue *et al.*, 1995). These tools are either based on the measuring the population of spermatozoa present in the peri-vitelline membrane (Wishart, 1987; Brillard and Antoine, 1990) or on the number of holes hydrolysed by spermatozoa in this membrane (Robertson *et al.*, 1997). Each of these techniques may be of practical interest in order to early detect the causes of impaired fertility over the reproductive season in breeder flocks or in parental lines (see Wishart, 2002).

### CONTROL OF FERTILITY BY EXTRINSIC FACTORS

Any factor impairing the initial sperm storage in the SSTs will also affect the population of "fit" spermatozoa at the infundibular level as these two populations are closely linked (Brillard and Antoine, 1990). From a practical standpoint, a number of factors may impair sperm fertility such as defective mating behaviour (in the fowl), or, in artificially inseminated flocks, any factors capable of influencing the quantity and quality of sperm migration (e.g. sperm dose, *in vitro* storage of semen, timing of insemination with oviposition).

#### Alterations of sperm migration

Several early reports described a "late, non reversible decline of fertility" occurring in some but not all breeder of turkey hens (Harper and Arscott, 1969; van Krey and Leighton, 1970; Sexton, 1977). Such a situation is with a very few exceptions, no longer observable at the present times, an indication that the reproductive potential of turkey breeder hens or their management, or both, have been significantly modified over the past decades. Whether or not the causes of a late seasonal decline of fertility have an intrinsic or extrinsic origin is questionable. Meanwhile, some evidence exists that both local infections (reversible by antibiotic treatment) and/or inadequate use of artificial insemination (AI) technology may have been responsible for the presence of local inflamma-

tions in the vaginal wall. Such inflammations may durably impair the migration of spermatozoa to their storage sites, which results in variable reductions in fertility. The fact that in most circumstances, turkey hens used for a second laying period had recovered normal fertility levels supports the hypothesis of an inflammatory problem disappearing after the suppression of its causal factor, *i.e.* repeated inseminations inadequately performed.

### Sexual behaviour

Despite the extensive use of natural mating conditions to produce day-old chicks in the fowl, the number of studies dedicated to the behavioural aspects of this method of reproduction have been relatively few. It should be noted that fertility traits are negatively correlated with growth rate, which may result in increased problems of fertility in breeder facilities with low quality management.

In my experience, in a majority of situations, leg problems (including foot pad and articulation infections) are often associated with an insufficient control of body growth in males or females or both, should be considered as responsible for the persistence of low fertility during the second half of the laying season. By contrast, delayed sexual maturity of the males may affect sexual behaviour in young flocks. Such a situation may have several nutritional causes, including excessive feed restriction during the growing period, and/or insufficient balance in minerals/vitamins (*e.g.* biotin, B-complex vitamins).

Another problem often affecting sexual behaviour in young and ageing flocks is the incidence of inappropriate light stimulation of the males. Indeed, the reproductive season in avian (including poultry) species is highly dependent on their photoperiodic environment. Thus, males subjected to "long" or increasing photoperiods (>10–12 hours) during the first 12–14 weeks of life will develop precocious but non-persistent testicular growth. This will result in the need for an early (<40–45 weeks) and risky replacement of most of these males before the end of the reproductive season.

### Artificial insemination: sperm quantity and quality

In contrast to sexual behaviour, the rationale for successful use of artificial insemination procedures has been extensively studied in fowl and turkey breeders. (see review by Bakst and Brillard, 1995). Among factors known to influence the subsequent fertility performances, the initial quantity and quality of spermatozoa at the time of insemination exert a major role. This is because they directly influence

the degree of selection exerted by the hen on subsequent sperm populations stored in the oviduct. A series of experiments conducted over the past years have revealed that: (1) following a single insemination, a maximum of approximately 1% of the initial population of spermatozoa deposited intravaginally reaches the storage sites both in the fowl and turkey hen (Brillard and Bakst, 1990; Brillard, 1993). (2) The quantity of spermatozoa present in the infundibulum at the time of fertilisation, *i.e.* during the 8–9 minutes following ovulation (Wishart and Fairweather, 1998) can be estimated in freshly laid eggs. This is either directly from their population trapped in the peri-vitelline membrane (Wishart, 1987) or indirectly from the number of holes created by the hydrolysis of the inner peri-vitelline layer (Wishart and Wood, 1994). The number of peri-vitelline sperm is itself highly correlated with the actual population of spermatozoa stored in the utero-vaginal junction (Brillard, 1993).

From a practical standpoint, both techniques are appropriate to rapidly detect whether or not a fertility problem can originate from a lack of sperm storage efficiency. If such a problem is confirmed, several quality tests are now available to pinpoint if whether or not the problem is qualitative or quantitative (see Bakst and Cecil, 1997).

### Timing of insemination with oviposition

A series of experiments conducted both in the chicken and in the turkey have demonstrated that most spermatozoa deposited within 1–3 hours prior to or just after oviposition are eliminated by the vaginal contractions involved in the process of oviposition (Brillard *et al.*, 1987; Brillard and Bakst, 1990). For example in the fowl hen, the relative efficiency of sperm storage around the time of oviposition can be reduced as much as 30–40 times that observed in hens inseminated well apart (>4 hours) from oviposition.

From a practical standpoint, the period of insemination in a given breeder house should be carefully scheduled in order to minimise the risks of performing a majority of inseminations at or around the time of lay. Chicken breeder hens at peak lay most of their eggs between the 5th and the 8th hour following the onset of the photoperiod (model: 16L:8D) while turkey breeder hens at peak would lay mainly from the 7th to the 9th hour of lay (model: 14L:10D). Both species would have their laying period delayed about 2 hours in ageing flocks.

### AI technique/AI crew

Fertility problems in breeder flocks should be considered as originating, in a majority of cases, from "human" rather than from technical problems. Among factors often encountered are: (1) Lack of training of AI crews (e.g. replacement of employees during vacations). (2) Inappropriate number of people in the crew. For example, large flocks of hens should be inseminated with appropriate numbers of technicians to reduce the risks of AI performed during the laying period (see above). (3) Inadequate use of a diluent.

Sperm diluents are common practice to facilitate the transportation and short term storage of semen prior to insemination. However, their optimal use is subjected to some simple rules which, if not adequately performed, may severely impair sperm survival. Among mistakes most often observed are: (1) The absence of diluent at the time of semen collection. (2) The absence of agitation of the collection tube between two semen collections (very common). In this case, the technician is convinced that semen storage conditions are optimal which is truly the case only for one portion of the first ejaculate. (3) Inadequate temperature of semen storage. A general rule should be: The longer the sperm to be stored, the cooler the semen should be with temperatures ranging between 10–4°C although lower temperatures (particularly 0°C) should be avoided.

### CONCLUSIONS

Unfortunately this short review of the main factors involved in the practical aspects of fertility in poultry species cannot be exhaustive. Thus, some aspects of mating behaviour in relation to heat stress have not been treated here. Indeed, the focus has been on practical rather than generic questions of fertility, but a number of problems remain unanswered. In a large majority of cases, questions relevant to AI technology and, to a lesser extent, to interactions between nutrition and behaviour, can be solved if sufficient information on the history of the flock has been recorded and is rapidly available to decision makers. However, in other occasions, an appropriate answer to a fertility problem may not be available immediately.

Such a situation is quite frequently observed when a lack of recovery of fertility is observable upon partial replacement of the males at the onset of the second half of the reproductive season (*i.e.* around 43–45 weeks of age in the fowl or 45–50 weeks of age in

turkey breeder flocks). At this time, the partial replacement of males is performed to boost the fertility of a flock over the remaining weeks of its season. On many occasions, the causes for such a declining fertilising potential in males are either an inadequate light stimulation or a too severe or, even, too liberal feed restriction (see above). In this latter case, an acceptable solution is unlikely as the flock is under natural mating (e.g. chickens) and the introduction of new males has created a major problem of social behaviour within the flock. Alternatively, the hens are artificially inseminated and the recovery of fertility has been unsuccessful because the problem was not directly related to a question of sperm availability but, rather, to a question of AI management. In both cases, the only acceptable solution becomes the early elimination of the flock.

### REFERENCES

- Bakst, M.R., Brillard, J.-P. and Wishart, G.J. (1994) Sperm selection, transport and storage in the avian oviduct. *Poult. Sci. Rev.*, **5**, 143–177.
- Bakst, M.R. and Cecil, H.C. (1997) *Techniques for Semen Evaluation, Semen Storage, and Fertility Determination*. Poultry Science Association Publishers, Savoy, Illinois.
- Brillard, J.-P. (1993) Sperm storage and transport following natural mating and artificial insemination. *Poult. Sci.*, **72**, 923–928.
- Brillard, J.-P. and Antoine, H. (1990) Storage of sperm in the utero-vaginal junction and its incidence on the numbers of spermatozoa present in the perivitelline layer of hen's eggs. *Brit. Poult. Sci.*, **31**, 701–710.
- Brillard, J.-P. and Bakst, M.R. (1990) Quantification of spermatozoa in the sperm storage tubules of turkey hens and the relation to sperm numbers in the perivitelline layer of eggs. *Biol. Reprod.*, **43**, 271–275.
- Brillard, J.-P., Beaumont, C. and Scheller, M.F. (1998) Physiological responses of hens divergently selected on the number of chicks obtained from a single insemination. *J. Reprod. Fert.*, **114**, 111–117.
- Brillard, J.-P., Galut, O. and Nys, Y. (1987). Possible causes of subfertility in hens following insemination near the time of oviposition. *Brit. Poult. Sci.*, **28**, 307–318.
- Brillard, J.-P., McDaniel, G.R., De Reviers, M. and Drane, J.W. (1989) Expression of several traits of fertility in young and old dwarf broiler breeder hens inseminated with duplicate doses of semen. *Poult. Sci.*, **68**, 558–563.
- Donoghue, A.M., Bakst, M.R., Holsberger, D.R. and Donoghue, D.J. (1995) Effect of semen storage on the number of spermatozoa in the perivitelline layer of laid turkey eggs. *J. Reprod. Fert.*, **105**, 221–225.
- Harper, J.A. and Arscott, G.H. (1969) Seasonal decline in fertility of turkey eggs. *Poult. Sci.*, **48**, 2109–2113.
- Pingel, H. and Planert, C. (1978) Untersuchung über die möglichkeiten der verlängerung der befruchtungspersistenz. *Proc. 16th World Poult. Congr., Rio de Janeiro*, **2**, 116–125.



- Robertson, L., Brown, H.L., Staines, H.J. and Wishart, G.J. (1997) Characterisation and application of an avian *in vitro* spermatozoa-egg interaction assay using the inner perivitelline layer from laid chicken eggs. *J. Reprod. Fert.*, **110**, 205–211.
- Sexton, T.J. (1977) Relationship between number of sperms inseminated and fertility of turkey hens at various stages of production. *Poult. Sci.*, **56**, 1054–1056.
- van Krey, H.P. and Leighton, A.T. (1970) Sperm gland populations, oviduct homogenates and late season declines in fertility. *Poult. Sci.*, **49**, 1447.
- Wishart, G.J. (1987) Regulation of the length of the fertile period in the domestic fowl by numbers of oviductal spermatozoa as those trapped in laid eggs. *J. Reprod. Fert.*, **80**, 493–498.
- Wishart, G.J. (2002) Assessment and significance of fertility in commercial poultry production. In: *Practical Aspects of Commercial Incubation in Poultry*, Deeming, D.C. (ed.), pp. 1–6. Ratite Conference Books, Lincoln. Reprinted in 2009: *Avian Biol. Res.*, **2**, 37–40.
- Wishart, G.J. and Fairweather, J. (1998) The chicken “fertilization window”. *Avian Poult. Biol. Rev.*, **9**, 124.
- Wishart, G.J. and Staines, H.J. (1995) Assessing the breeding efficiency of broiler breeder flocks by measuring sperm transfer into laid eggs. *Brit. Poult. Sci.*, **36**, 317–323.
- Wishart, G.J. and Wood, L.K. (1994) Evaluation of tetrazolium reduction assays for assessing poultry sperm metabolism and predicting fertilizing ability. *Proc. European Poult. Conf.*, pp. 310–311.

## Shell formation and function and its role in incubation

Nick H.C. Sparks

Avian Science Research Centre, Scottish Agricultural College, Auchincive, Ayr, KA6 5HW, UK  
E-mail: Nick.Sparks@sac.ac.uk..

### INTRODUCTION

Poultry embryos develop within an egg that will both protect them during the incubation process and, with the exception of oxygen and heat energy, provide all that the embryo requires to allowing develop into a viable hatchling. The importance of the contribution made by the shell to these needs is difficult to over-estimate because it provides physical protection and is a source of calcium for the embryo. An understanding of how the shell carries out its various functions should be underpinned by a basic knowledge of how the shell is formed. Flawed or incomplete shell formation is often the cause of the shell failing to perform the functions required of it during incubation.

### SHELL FORMATION

Shell formation takes place in the oviduct (Figure 1). This organ and its associated (left) ovary, forms the reproductive structure of the domestic fowl. The oviduct may be divided into a number of functional regions viz: the infundibulum, magnum, isthmus, tubular shell gland, shell gland pouch and the vagina. While shell mineralisation begins in the tubular shell gland, factors that affect shell formation are influenced during albumen deposition in the magnum, and during shell membrane formation in the isthmus.

Once the two fibrous shell membranes (Figure 2) have formed around the albumen (from which they are separated by the so-called limiting membrane) the egg moves into the tubular shell gland where shell mineralisation begins at discrete sites on the outer surface of the outer shell membrane. These sites, sometimes known as mammillary cores, are chemically and structurally modified causing the calcium salts that are released into the lumen of the tubular shell gland to mineralise around the organic, shell membrane-derived, core (Figure 3). This process firmly anchors the shell to the shell membranes. The egg then moves to the shell gland pouch where the bulk of the mineralisation process takes place, accompanied during the early stages by a process known as plumping.

Plumping is the process by which primarily water moves into the albumen and causes the flaccid shell membranes to become turgid. The mammillary cores, spatially separated as result of plumping, continue to be mineralised and become encased in a sphere of radiating calcium carbonate (Figure 4). Mineralisation continues until contact is made with neighbouring spheres. At this point the cone or mammillary layer is complete. The flow of calcium carbonate to the hemisphere that faces the shell membranes is now disrupted and shell formation moves into a new phase—the formation of the palisade layer.

The palisade layer is frequently represented in schematic diagrams as a series of columns, each

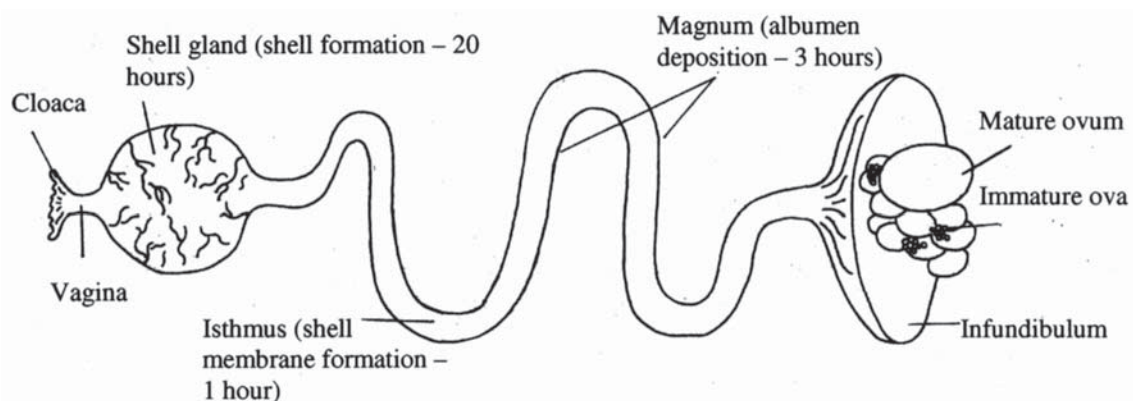
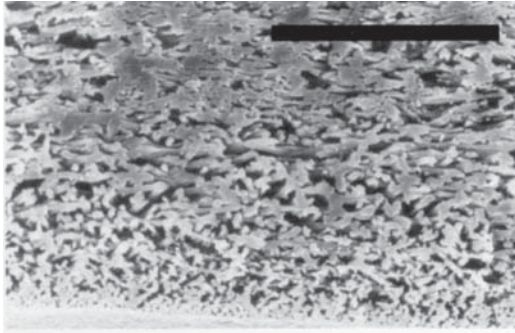


Figure 1 Diagram showing the functional regions of the hen's oviduct.

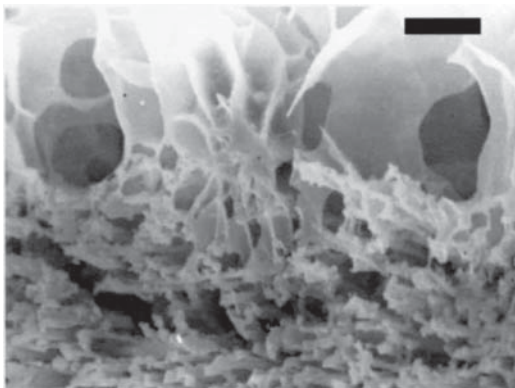


**Figure 2** Cross section through the shell membranes of a hens egg showing the fine inner, and the thicker outer, membrane fibres. Bar marker 100  $\mu\text{m}$ .

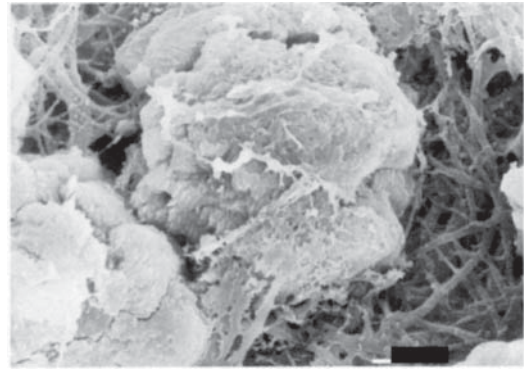
emanating from a mammary core. The outer region of the palisade layer tends to be less dense than the inner region, giving this part of the shell its alternative name, the spongy layer. A thin surface crystal layer caps the palisade layer. The crystals in the surface crystal layer are distinct from those in the palisade layer, almost certainly reflecting the changing environment within the lumen of the shell gland as shell formation draws to a close.

The palisade layer, which makes up the bulk of the shell, consists of 95% calcium carbonate and 5% organic material. While constituting a proportionately minor component, this organic matrix plays an important role in conferring structural integrity upon the shell. Shell membranes are composed primarily of type X collagen, which inhibits mineralisation, while the nucleation sites contain mammillan (a mammary keratin sulphate proteoglycan) and the palisade layer contains dermatan sulphate proteoglycan (Arias and Fernandez, 2001).

It is proposed that the matrix controls crystal morphology, orientation and growth (Nys *et al.*, 1999, 2001; Lavelin *et al.*, 2000; Gautron *et al.*, 2001). Given this it is predictable and has been



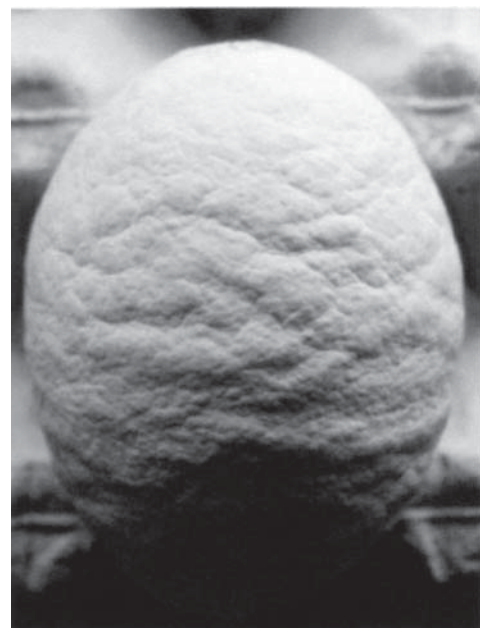
**Figure 3** Cross-section of a de-mineralised shell showing the close-knit membrane fibres, organic core (towards centre of micrograph) and organic shell matrix. Bar marker 10  $\mu\text{m}$ .



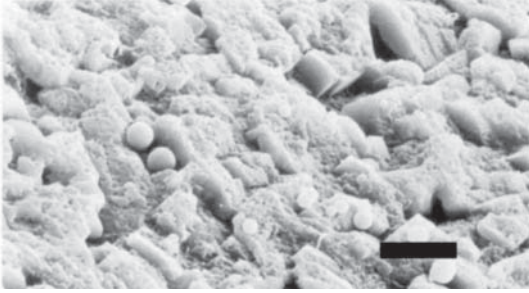
**Figure 4** Mineralised mammary core. Bar marker 10  $\mu\text{m}$ .

demonstrated, that disruption of the organic phase in the albumen, shell membrane or shell may result in abnormal shell development. Board and Sparks (1991) noted that disrupting the cross-links within the membrane fibres (e.g. copper deficient diets or exposure to lathrogen-like compounds in the feed) will result in abnormal, typically wrinkled in appearance, shell formation. Similarly, disease conditions that affect plumping or albumen viscosity, and hence the tensioning of the shell membranes, will result in shells that have a wrinkled or corrugated appearance (Figure 5). This highlights the importance of the organic component of the bird's nutrition in respect of shell formation, something that is sometimes overlooked when considering shell quality problems.

It is also now increasingly recognised that exposing the laying bird to environmental stress (e.g. unusual levels or types of noise, unusual visual stimuli, altered housing) can affect the mineralisation process and



**Figure 5** Corrugated shell resulting from watery albumen.

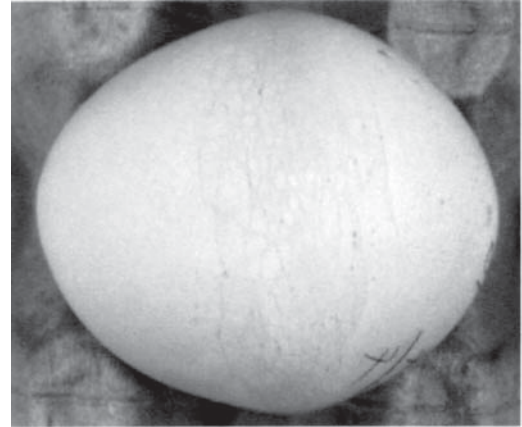


**Figure 6** Spherical crystals of vaterite, induced by stress, overlying calcite crystals within the palisade layer of a ducks egg. Bar marker 10  $\mu\text{m}$ .

hence shell quality. These effects, which have been reviewed by Solomon (1991) and Board and Sparks (1991), include the formation of calcium carbonate in the vaterite form (*cf* the normal calcite) and the deposition of calcium phosphate on the outer surface of the shell giving it a white or purple bloom. The effect of these changes on hatchability varies. Layers of vaterite (Figure 6), which is spherical (*cf* calcite that has flat sides) within a shell can affect shell strength whereas a layer of calcium phosphate over the cuticle can decrease a shell's conductance to gases (see below). The severity of the effect depending on the thickness of the overlying layer.

Stress, if experienced by the bird when an egg is in the early stages of palisade mineralisation may also cause the oviduct to contract around the egg, causing the shell to break around the egg's equator. Subsequent mineralisation, which relies upon epitaxial growth (at the risk of over simplifying this means to copy what went before) is disrupted, the crystals forming an irregular layer. The finished egg appears to have a thickened band around the equator (Figure 7) but in reality the band represents an area of weakness and an area in which the pore structure has been lost. These so-called 'body-checked' eggs suffer from weakened shells and reduced shell conductance, neither of which are conducive to producing a high quality hatchling.

The mechanics of shell mineralisation are, from a bio-mineralisation standpoint, relatively easy to understand. However, one aspect of shell formation that is more difficult than almost any other to explain is the mechanism that allows pores to be formed and maintained in the shell structure. Pore structure ranges from the very simple, *e.g.* the trumpet-shaped hen's egg pore, to the complex, *e.g.* the multi-branched structure observed in ostrich eggshells (Board *et al.*, 1977). While the number, size, shape and position of the pores is, to a greater or lesser extent, under genetic control the environment can also influence the mechanics of pore formation. A good example of this is the change in pore number that occurs in



**Figure 7** Shell showing so-called "equatorial bulge" following the shell cracking in the early stages of mineralisation due to a stress reaction.

birds that are moved from low to high altitudes (Rahn, 1977; Rahn *et al.*, 1977).

Immediately prior to oviposition, the shell of hen, duck and turkey egg (and many other species of bird including most game birds) is coated with a thin organic layer or cuticle. In other species (*e.g.* the megapodes) the layer is inorganic and in some ratites (*e.g.* ostrich) the layer is omitted.

#### SHELL INTEGRITY

The bird embryo is naturally incubated under an adult bird (see Deeming, 2002a), or in the case of megapodes, under composting organic material, or soil or sand (Booth and Jones, 2002). The egg may rest in a lined nest cup or on an unforgiving rocky ledge. The egg may be incubated in isolation or with a large number of other eggs. Environmental pressures such as these has resulted in bird eggs evolving structural variations, such as tough flexible or hard brittle shells or surface layers of shock absorbing crystal spheres (Sparks, 1994).

Eggshell strength has also been the focus of specific selection pressures by poultry breeders. While the objective is straightforward, to select for an eggshell that does not crack during the incubation process but does not unnecessarily hinder the emergence of the hatchling, achieving it can be problematical. In part this reflects difficulties in measuring shell strength in a meaningful way. Methods available for the direct measurement of shell strength include quasi-static compression and impact tests while indirect tests include specific gravity, shell thickness and non-destructive deformation testing. The difficulty, and it is one that is still to be resolved, is to derive a test that reproducibly models the stresses applied to an egg's shell in the field.

While the effective thickness of the shell (*i.e.* shell thickness excluding the cuticular and mammillary layers) is important in minimising crack propagation, the density of calcite columns and the arrangement of the columns are also important. Shell strength is improved when the column density is high and the number of aligned columns is low (Solomon, 1991).

If the shell breaks to the extent that the contents leak out, then the embryo's development will almost certainly be arrested due to disruption of the extra-embryonic membranes. Should the shell be cracked but the underlying membranes remain intact the development of the embryo may be affected either due to infection of the egg contents by micro-organisms or excessive water loss from the egg causing dehydration of the embryo.

#### MOVEMENT OF GASES ACROSS THE SHELL

Birds' eggshells are required to fulfil two conflicting functions. A shell needs to prevent microbial contaminants penetrating the shell and reaching the egg contents while being sufficiently porous for respiratory gases to diffuse through the shell without subjecting the embryo to hypoxia or similar.

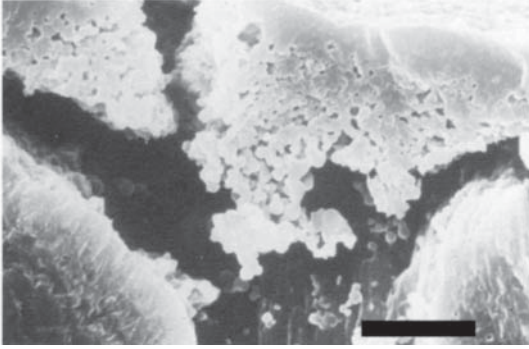
Gases move across the shell through the pores by diffusion, the rate of diffusion being determined by the effective pore area, the length of the diffusion pathway (*e.g.* shell thickness or length of the pore canal and associated overlying shell cover) and the concentration difference of the gas across the shell. It is notable that there is no correlation between the rate at which gases may move from one side of the shell to the other and the rate that liquids or contaminants may travel across the shell (Sparks and Board, 1984; Berrang *et al.*, 1998). For the fowl's egg this is a reflection of the role of the cuticle which acts as a barrier to the movement of liquids and solids across the shell while having a small or negligible impact on the movement of gases (Deeming, 1987).

The shell needs to allow the passage of oxygen, carbon dioxide and water in concentrations that vary during the incubation process. Thus, as the embryo grows, the amounts of oxygen and carbon dioxide that need to be exchanged across the shell increase during incubation. By contrast, with the exception of the first 24 h or so, the amount of water that needs to diffuse across the shell can be relatively constant throughout the incubation period (The reasons for this are discussed by Ar and Deeming, 2002). However, given that shell conductance is fixed at oviposition, the requirement to have a shell that can meet the embryo's peak requirement for oxygen,

while not being so porous that it allows the embryo to dehydrate during the incubation process, requires shell conductance to be under strict control. The mechanism that controls pore formation also needs to be flexible in so far as the requirements will change during a normal production cycle. Thus as egg weight increases the volume to surface area ratio of the egg changes requiring shell conductance to change. However, as was illustrated when French and Tullett (1991) reviewed this aspect of shell conductance, the mechanisms for achieving this are not identical either between or within a species. Responses in shell conductance have also been noted in response to moving birds from low to high altitude sites. The conductance of the eggs changing, although not always in the same direction, in response to the reduced gaseous concentrations at the higher altitudes (Wangensteen *et al.*, 1974; Rahn *et al.*, 1977; Monge *et al.*, 2000).

There are occasions, however, more often linked to the influence of breeding programmes than aberrations occurring in wild population, when the eggshell does not meet the requirements of the embryo. Thus breeding programmes can result in similar lines having significantly different shell conductances (French and Tullett, 1991; Sparks, unpublished observations). Reducing the age at which the parent bird starts to produce eggs can also cause result in eggs being laid which have a sub-optimal shell conductance value (French and Tullett, 1991). Probably the most common example of this occurs in ducks where the removal of the cuticle from Pekin-type (*cf* Muscovy) duck eggs does, unlike the equivocal evidence for chickens (Deeming, 1987; Shafey, 2002), increase shell conductance and, as a consequence, improves hatchability. While removing the cuticle from duck (Deeming, 1987) and turkey eggs (Christensen and Bagley, 1984) significantly increases shell conductivity, as noted above this does not apply to all species and in these circumstances other strategies have been used. For example it has been reported (Meir and Ar, 1996) that drilling holes through the shell in the latter half of the incubation period has improved the hatchability of early laid goose eggs.

While species and breeding may influence shell conductance, in commercial production the impact of abnormal shells arguably has the biggest day-to day impact on production. For example, the rate of water loss from either thin-shelled eggs (*i.e.* eggs laid before mineralisation is complete), or eggs with cracked shells, will lose water at a faster rate than predicted causing the embryo to be at best dehydrated when it hatches and at worst to die *in ovo*.



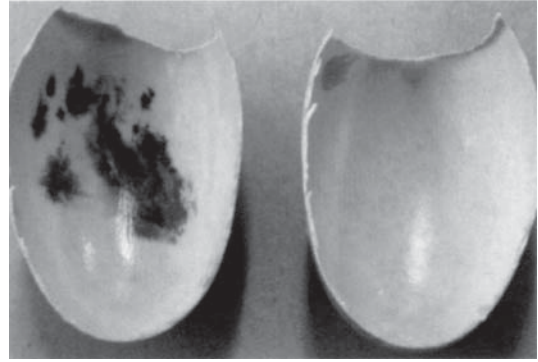
**Figure 8** Cross-section of cuticle forming a loose plug in the neck of a pore canal in a hen's eggshell. Bar marker 10  $\mu\text{m}$ .

### MOVEMENT OF LIQUID AND SOLID CONTAMINANTS ACROSS THE SHELL

Eggs have evolved to be incubated in a range of environments. Those like poultry that may be laid in muddy, damp conditions have developed a surface layer or cuticle (Figure 8) that restricts the passage of water or mud into the mouth of the pore canal while allowing gaseous diffusion to continue relatively unimpeded (Sparks, 1994). Other species, such as the ostrich, which incubates its eggs in dry environments, produce eggs that have uncapped pores.

The dual role of the cuticle in preventing the pores from being blocked by, for example, mud or faecal material and in restricting the movement of liquid water through the shell is critical to the survival of the embryo. If the mouth of the pore were to become blocked then the conductance of the shell would be reduced in proportion to the surface area of the shell affected. As mentioned above and elsewhere in this book, this can adversely affect the embryo in a number of ways. Restricting the movement of liquid water is important not only because the efficacy of a water logged pore is significantly reduced but because, with the exception of fungi, micro-organisms tend not to penetrate the shell in the absence of water (Sparks, 1994; see also Berrang *et al.*, 1999). It is notable that bacterial contamination of hatching eggs poses a significant problem for producers who breed ostriches in the UK (Deeming, 1997). This is a problem due in significant part to the absence of a cuticle or cover on an egg that has evolved to be laid in a dry environment.

The structure of the cuticle deposited on eggs will change as the bird ages and, on a particular egg, as the storage time increases. However the cuticle is at its most vulnerable to challenge immediately following oviposition (Figure 9) when it has still to dry (Sparks and Board, 1985). In this respect, it is difficult to overemphasise the importance of nest-box hygiene in preventing internal contamination of the hatching egg.



**Figure 9** Bacterial growth underlying section of shell contaminated (with faecal material) within minutes of oviposition compared with no growth in section underlying shell challenged several minutes post-oviposition.

While, in some respects, the cuticle on poultry eggs faces fewer challenges in the commercial production environment with its clean nest boxes, fumigation and artificial incubation those challenges that it does face can be more severe than those that would be found in the wild. For example, the movement of cooled eggs out of storage can cause condensation (sweating) to form on the shell creating good conditions for bacteria to move through the shell. Another challenge faced by the cuticle is egg washing or sanitising. If carried out correctly, egg washing should pose little or no challenge to the egg but if carried out incorrectly wash-water can be readily moved through the shell carrying contaminants with it. This problem can be compounded by the likelihood of washing degrading the cuticle reducing its efficacy significantly (Sparks, 1994). In theory, the removal of the cuticle should not increase the incidence of bacterial contamination of hatching eggs that are handled with due regard to the risks. This is the case, for example, with duck eggs where the cuticle may be removed deliberately to improve shell conductance—but it does remove one of the most effective barriers to bacteria if the egg should be challenged subsequently.

This section has focussed on the role of the cuticle but this is predicated on the understanding that, should the shell be malformed (e.g. thinner than normal) or cracked, then the influence of the cuticle is negligible, contaminants being relatively free to move from the outer to the inner surface of a cracked shell.

### ION RESERVOIR

The developing embryo requires calcium for a range of biochemical processes however, proportionately, it is skeletal development that is the most demanding, requiring some 125 mg of calcium. While some calcium is contained within the yolk,

the majority, approximately 100 mg, is reabsorbed from the shell (Simkiss, 1967). The embryo accesses the calcium in the shell via its opposes the limiting membrane (lining the inner shell membrane). The calcium is reabsorbed from the shell at the point where the shell membrane fibres attach to the shell (*i.e.* the cone tip). Carbonic acid releases the calcium ions which are then bound by a calcium binding protein which in turn is absorbed into the chorio-allantoic membrane, the calcium being transported to the developing embryo (Tuan *et al.*, 1991). As the calcium is reabsorbed from the cone tip, moving back along the membrane fibres to the chorio-allantoic membrane, so the bond between the shell membranes and shell weakens. This explains in part why membranes that can be readily separated from the shell post-hatching are difficult to remove prior to incubation. There is no evidence to date that skeletal abnormalities are a function of poor or abnormal shell mineralisation.

#### EMBRYO MORTALITY RELATED TO SHELL FUNCTION

As far as commercial poultry incubation, and more specifically shell function, is concerned embryos tend to die as a result of: (1) High conductance shells causing excessive water loss leading to dehydration; and (2) Low conductance shells causing hypoxia, hypercapnia or oedema, due to inadequate water loss which hinders the embryo when trying to rotate within the egg at hatching. Arguably, with the exception of the very low conductance shells, shells with an abnormal conductance do not increase embryo mortality *per se*. Rather, it is the need to incubate eggs in groups of tens or more commonly thousands, and the inability to tailor conditions to meet the requirements of embryos whose shells have conductances that vary significantly from the mean that is the problem.

Although shell conductance is normally fixed at oviposition (but see Deeming [2002b] for discussion of those species that exhibit an increase in conductance during incubation), the effects of shells with high or low conductance values often do not become apparent until the latter half of incubation. This is a result of the demand for oxygen remaining relatively low during the first half of incubation but then increasing dramatically to a peak at hatching. It is increasing appreciated that poor incubation conditions can also affect post hatch performance. A good example of this is evidence of low shell conductance predisposing fast growing lines of chicken to the metabolic disorder, ascites.

#### REFERENCES

- Ar, A. and Deeming, D.C. (2002) Roles of water and gas exchange in determining hatchability success. In: *Practical Aspects of Commercial Incubation in Poultry*, Deeming, D.C. (ed.), pp. 21–25. Ratite Conference Books, Lincoln. Reprinted in 2009: *Avian Biol. Res.*, **2**, 61–66.
- Arias, J.L. and Fernandez, M.S. (2001) Role of extracellular matrix molecules in shell formation and structure. *World's Poult. Sci. J.*, **57**, 349–357.
- Berrang, M.E., Cox, N.A., Frank, J.F. and Buhr, R.J. (1999) Bacterial penetration of the eggshell and shell membranes of the chicken hatching egg: A review. *J. Appl. Poult. Res.*, **8**, 499–504.
- Board, R.G. and Sparks, N.H.C. (1991) Shell structure and formation in avian eggs. In: *Egg Incubation: Its Effects on Embryonic Development in Birds and Reptiles*, Deeming, D.C. and Ferguson, M.J.W. (eds.), pp. 71–86. Cambridge University Press, Cambridge.
- Board, R.G., Tullett, S.G. and Perrott, H.R. (1977) An arbitrary classification of the pore system in avian eggs. *J. Zool., Lond.*, **182**, 251–265.
- Booth, D.T. and Jones, D.N. (2002) Underground nesting in the megapodes. In: *Avian Incubation: Behaviour, Environment and Evolution*, Deeming, D.C. (ed.), pp. 192–206. Oxford University Press, Oxford.
- Christensen, V.L. and Bagley, R.A. (1984) Vital gas exchange and hatchability of turkey eggs at high altitude. *Poult. Sci.*, **63**, 1350–1356.
- Deeming, D.C. (1987) Effect of cuticle removal on the water vapour conductance of eggshells of several species of domestic bird. *Brit. Poult. Sci.*, **28**, 231–237.
- Deeming, D.C. (1997). *Ratite Egg Incubation*. Ratite Conference Books, Buckinghamshire.
- Deeming, D.C. (2002a). *Avian Incubation: Behaviour, Environment and Evolution*. Oxford University Press, Oxford.
- Deeming, D.C. (2002b). In: Functional characteristics of eggs. *Avian Incubation: Behaviour, Environment and Evolution*, Deeming, D.C. (ed.), pp. 8–27. Oxford University Press, Oxford.
- French, N.A. and Tullett, S.G. (1991) Variations in the eggs of poultry species. In: *Avian Incubation*, Tullett, S.G. (ed.), pp. 59–77. Butterworth-Heinemann, London.
- Gautron, J., Hincke, M.T., Panheleux, M., Garcia-Ruiz, J.M., Boldicke, T. and Nys, Y. (2001) Ovotransferrin is a matrix protein of the hen eggshell membranes and basal calcified layer. *Connective Tiss. Res.*, **42**, 255–267.
- Lavelin, I., Meiri, N. and Pines, M. (2000) New insight in eggshell formation. *Poult. Sci.*, **79**, 1014–1017.
- Meir, M. and Ar, A. (1996) Artificial increase of eggshell conductance improves hatchability of early laid goose eggs. *Brit. Poult. Sci.*, **37**, 937–951.
- Monge, C.C., Ostojic, H., Aguilar, R. and Cifuentes, V. (2000) Reduced oxygen diffusion across the shell of gray gull (*Larus modestus*) eggs. *Biol. Res.*, **33**, 209–214.
- Nys, Y., Hinche, M.T., Arias, J.L., Garcia-Ruiz, J.M. and Solomon, S.E. (1999) Avian eggshell mineralization. *Poult. Avian Biol. Rev.*, **10**, 143–166.
- Nys, Y., Gautron, J., McKee, M.D., Garcia-Ruiz, J.M. and Hincke, T. (2001) Biochemical and functional characterisation of eggshell matrix proteins in hens. *World's Poult. Sci. J.*, **57**, 401–413.

- Rahn, H. (1977) Adaptation of the avian embryo to altitude. In: *Respiratory Adaptations, Capillary Exchange and Reflex Mechanisms*, Paintal, A.S. and Gill-Kumar, P. (eds.), pp. 94–105. University of Dehli, Dehli.
- Rahn, H., Carey, C., Balmas, K., Bhatia, B. and Paganelli, C.V. (1977) Reduction of pore area of the avian eggshell as an adaptation to altitude. *Proc. Nat. Acad. Sci.*, **74**, 3095–3098.
- Shafey, T.M. (2002) Eggshell conductance, embryonic growth, hatchability and embryonic mortality of broiler breeder eggs dipped into ascorbic acid solution. *Brit. Poult. Sci.*, **43**, 135–140.
- Simkiss, K. (1967). *Calcium in Reproductive Physiology*. Publishing Corporation, New York.
- Solomon, S.E. (1991) *Egg and Eggshell Quality*. Wolfe Publishing Ltd, London.
- Sparks, N.H.C. (1994) Shell accessory materials: structure and function. In: *Microbiology of the Avian Egg*, Board, R.G. and Fuller, R. (eds.), pp. 25–42. Chapman and Hall, London.
- Sparks, N.H.C. and Board, R.G. (1984) Cuticle, shell porosity and water uptake through hens' egg shells. *Brit. Poult. Sci.*, **25**, 267–276.
- Sparks, N.H.C. and Board, R.G. (1985) Bacterial penetration of the recently oviposited shell of hens' eggs. *Aust. Vet. J.*, **62**, 169–170.
- Tuan, R.S., Non, O., Akins, R.E. and Koide, M. (1991) Experimental studies on cultured, shell-less fowl embryos: calcium transport, skeletal development and cardio-vascular functions. In: *Egg Incubation: Its Effects on Embryonic Development in Birds and Reptiles*, Deeming, D.C. and Ferguson, M.J.W. (eds.), pp. 419–433. Cambridge University Press, Cambridge.
- Wangenstein, O.D., Rahn, H., Burton, R.R. and Smith, A.H. (1974) Respiratory gas exchange of high altitude adapted chick embryos. *Resp. Physiol.*, **21**, 61–70.



# The critical importance of incubation temperature

Nick A. French

*Aviagen Turkeys Ltd, Chowley Five, Chowley Oak Business Park, Tattenhall, Cheshire, CH3 9GA, UK.  
E-mail: nick.french@aviagen.com*

## INTRODUCTION

Incubation is defined as the process of applying heat to eggs and control of this process is critical for successful hatching. Most birds apply heat to their eggs by sitting on them, although there are some notable exceptions such as the megapodes, which use heat from rotting vegetation or volcanic activity. However, it is the purpose of this chapter to discuss the third method of incubation as used by man for poultry and other avian species, artificial incubation.

Before proceeding incubation temperature must first be defined. For most hatchery managers it is the temperature that they set their machines to operate at and is shown on the outside of the incubator. However, for the developing embryo it is the temperature it experiences inside the egg that is the incubation temperature and as this is what determines the development of the embryo this must be considered the real incubation temperature. Machine operating temperature and embryo temperature are by no means necessarily the same thing (French, 1997).

## RELATIONSHIP BETWEEN INCUBATOR AND EGG TEMPERATURE

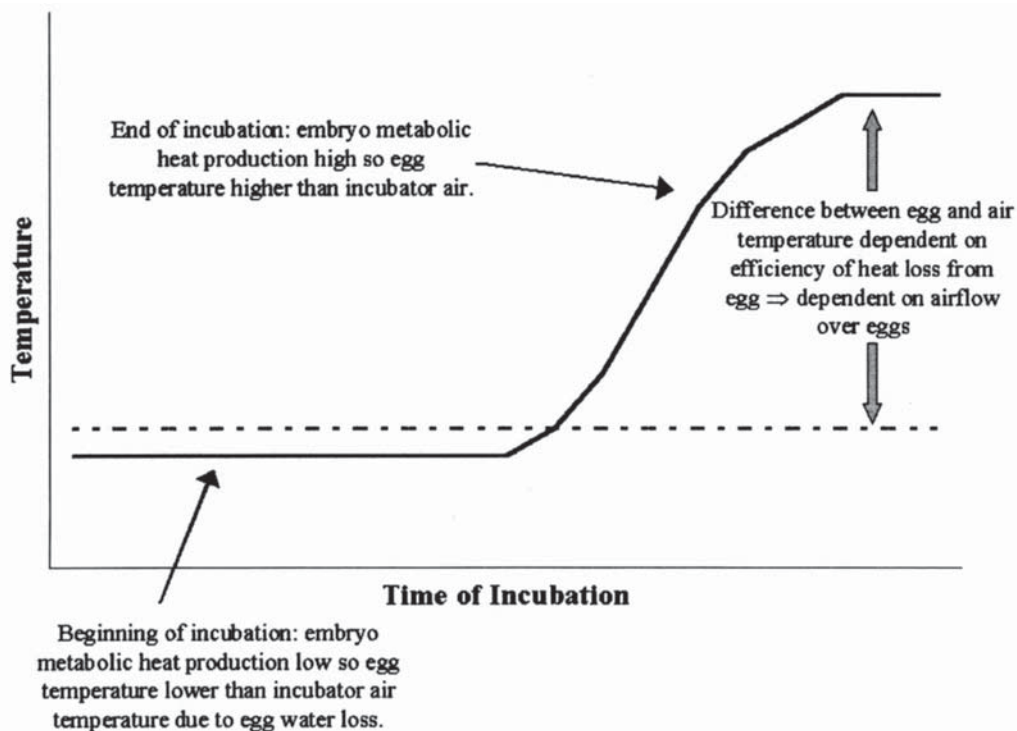
Measurements of air temperature around the eggs within incubators have shown that, depending on the design of the machine, temperatures can differ between 0.4 and 3.0°C from the set temperature (Kaltofen, 1969; Mauldin and Buhr, 1995; French, 1997). Similarly, studies have shown that as incubation progresses the internal egg temperature changes from being slightly cooler than the surrounding air in the first half of incubation to being hotter than the surrounding air in the second half of incubation (Tazawa and Nakagawa, 1985; French, 1997). Understanding why these temperature differences occur between egg and incubator requires an understanding of how heat is transferred within the system.

The temperature experienced by an embryo during incubation depends on: (1) the metabolic heat production of the embryo itself which in turn is dependent on the size of the embryo, (2) the slight cooling effect of water lost from the egg during

incubation, (3) the temperature of the incubator and (4) the ability of the heat to transfer from embryo to incubator air. Several authors have produced thermal energetic models describing how these four factors interact to determine the temperature within the egg during artificial incubation (Kashkin, 1961; Sotherland *et al.*, 1987; Meijerhof and van Beek, 1993; French, 1997).

While incubators are designed with heating and cooling systems to control the temperature within the machine, the eggs within the machine also have an important effect on temperature. Embryos at the start of incubation are very small and so generate very little metabolic heat and so incubation heat needs to be supplied to the embryo by the incubator. Indeed, the internal egg temperature at this stage is slightly cooler than the incubator air as there is a slight evaporative cooling effect due to water being lost from the egg (Figure 1). At the end of incubation, the embryo is large and generates a significant amount of metabolic heat, approximately 130 mW in a chicken egg (Romijin and Lokhorst, 1960), that must be removed from the egg. In a study of temperatures within a turkey tunnel incubator, French (2001) showed that there was a strong correlation between the estimated total metabolic heat production of the eggs within the machine and the air temperature around the eggs. The total metabolic heat production of the eggs was dependent of the size of the egg and their fertility so that as either egg mass or fertility increased so did the temperature within the machine.

How effectively heat is transferred from the egg to the surrounding incubator air and control system is mainly determined by the rate of airflow over the eggs (Sotherland *et al.*, 1987; Owen, 1991). The greater the airflow over the egg the more efficiently heat is transferred either to or from the egg. Variations in rates of airflow are a major reason for temperature variations within incubators. Monitoring temperatures within commercial incubators have found variations of up to 1.2°C within machines (French, 2002) and reducing this temperature variation requires a more uniform airflow over the eggs in all locations within the incubator.



**Figure 1** The relationship between egg temperature (—) and incubator air temperature (---) in an artificial incubator.

Other factors that can influence the uniformity of temperature within a machine are the heater and cooler units, evaporative cooling from humidifiers and cool air entering the machine through the ventilation system. A well-designed incubator will ensure that the localised air heating or cooling caused by these elements will not effect the eggs by ensuring good mixing of the air before it reaches the eggs.

Where multi-stage incubation is used, *i.e.* incubating eggs at different stages of incubation together within the same machine, there are some additional considerations to ensure uniform temperatures. Multi-stage incubators rely on the metabolism of the embryos at the end of incubation to heat the eggs at the start of incubation. For this to work successfully it is important to ensure that there are equal numbers of embryos at each stage of incubation and different stages of incubation are equally spaced apart. Major hatch problems can occur if there are too many eggs at either the end or the start of incubation within the machine. Multi-stage incubators also rely on good airflow amongst the eggs to ensure efficient heat transfer between the eggs.

#### INCUBATION TEMPERATURE TOLERANCE OF POULTRY EMBRYOS

Many studies have investigated the optimum incubation temperature for poultry species but most have

reported the effects of incubator operating temperature rather than embryo temperature on hatching success. As the operating and embryo temperatures are not necessarily the same and the difference will depend on incubator design, it is very difficult to summarise results between different studies. Optimum operating temperatures for poultry species appear to be between 37–38°C and deviations from this optimum can have a major impact on hatching success (Wilson, 1991). For example, French (1994) showed that increasing the incubation temperature of turkey eggs from 37.5 to 38.5°C resulted in a 30–60% decline in hatchability.

Recent studies have also shown that incubation temperature will not only affect hatching success but also post hatching performance (*e.g.* Lourens and Middelkoop, 2000; Gladys *et al.*, 2000; Hulet *et al.*, 2000). It is therefore important to consider all the effects when determining the correct incubation temperature for poultry species (Decuypere and Michels, 1992).

Rather than using a single optimum incubation temperature, it would be better to define a temperature range over which incubation will be successful. Ideally this would be the temperature at the embryo level, although it may be more practical to use the temperature of the air immediately surrounding the egg. The degree of temperature tolerance will depend on whether the temperature is high or low, the length of time applied and the stage of embryo development.

So what happens as incubation temperature changes away from the optimal temperature range for successful development? The first effect of high or low incubation temperature is to alter the rate of embryo development resulting in an altered time of hatch (French, 1997). High incubation temperatures will advance the hatch whereas low incubation temperatures will delay hatch time and this can be a useful early indicator for the hatchery manager that temperature is sub-optimal. However, it should be noted that if incubation temperature continues to increase further it can also slow down development (Romanoff, 1935).

If incubation temperature further increases above the optimum, then the next effect would be to alter the embryo's development so that post-hatch performance is affected. A further increase in temperature would result in a delayed mortality: French (2000) showed that incubating turkey eggs at 38.0°C in the second week of incubation resulted in an increase in mortality in the fourth week of incubation. Immediate embryo death occurs when the internal egg temperature reaches 46.5°C (Ono *et al.*, 1994).

The effects of decreasing incubation temperature are less dramatic. Embryos are able to tolerate quite long periods of low incubation temperature without adverse effects. Lancaster and Jones (1988) showed that broiler embryos were able to tolerate cooling to 21°C for 24 hours after day 13 of incubation without any adverse effect on hatchability. Indeed embryos were able to tolerate 22°C for up to 48 hours on day 16 of incubation without any adverse effect on hatch, although exposure for more than 30 hours increased the incidence of hatched chicks with down abnormalities. These experiments were carried out at temperatures below 27°C, the temperature at which embryonic development stops (Wilson, 1991). Prolonged incubation at temperatures between 27–35°C are more of a problem and will cause abnormal development of the embryo (Wilson, 1991). Immediate embryonic death at low temperatures will only occur if internal egg temperature drops below freezing and allows ice crystals to form inside the egg (Lundy, 1969).

The effects of temperature on embryo development are summarised in Table 1. The temperature ranges shown are not exact and may vary between poultry species, eggs of different sizes (French, 1997) and stage of embryo development. Studies in both chickens (Romanoff *et al.*, 1938; Morgan and Tucker, 1967; Moreng and Shaffner, 1951; Ande and Wilson, 1981) and turkeys (French, 2000) have found that the tolerance of embryos to high incubation temperature varies with the stage of incubation.

**Table 1** Typical effects of temperature on development of the avian embryo—temperature ranges shown are estimates based on available evidence

Incubation Temperature (°C)	Effect on Embryo
47	Immediate mortality
39–47	Mortality dependent on length and timing of exposure
38–40	Altered rate of development and post hatch performance
37–38	Normal embryo development
35–37	Altered rate of development and post hatch performance
27–35	Mortality dependent on length and timing of exposure
14–27	Embryo development stops, no adverse effects of holding for 24 hours in embryos in last third of incubation
0–14	Mortality dependent on length and timing of exposure
<0	Immediate mortality

#### IDENTIFICATION OF INCUBATION TEMPERATURE PROBLEMS

In commercial hatcheries the rapid identification and correction of incubation temperature problems is essential for maximising hatchability. There are two techniques that are routinely used in the hatchery to ensure that the correct incubation temperature is being used: (1) monitoring hatch time and (2) measuring incubation temperature within incubator.

As noted above, an early indication that incubation temperature may be sub-optimal is an alteration in hatch time. Hatching early can indicate high incubation temperatures whereas late hatching can indicate low or very high incubation temperatures. It should be noted that other factors can also influence the length of the incubation period, such as length of pre-incubation egg storage and age of breeder flock, and so a change of incubation time does not necessarily indicate a change of incubation temperature.

Monitoring hatch time can be done in several ways, assessing chick quality (thin chicks—hatched early, fat chicks—hatched late), counting how many chicks have hatched 18 hours before take-off and by weighing chicks. The simplest method to check that the chicks are hatching at the right time is to weigh the chicks and express this weight as a percentage of the fresh egg weight (chick yield). If hatch time is correct then chicks yield should be between 66–68% when they are removed from the hatcher. If the weight is too low, it is an indicator that the chicks have been hatched a long time before removal from the hatcher. If the weight is too high it indicates that the hatch is late. Chick yield will also be affected by how

much water is lost from the egg during incubation although the effect is relatively small in comparison to the effect of changing hatch time.

Recently it has been suggested that measuring chick length at hatch is an indicator of normal or abnormal embryo growth and could be a useful technique for identifying high incubation temperature problems (Hill, 2001). Further studies are required to determine how chick length is affected by incubation temperature but if a relationship is demonstrated then this could be a very useful tool for the hatchery manager.

Measuring temperatures within incubators can also be very useful for solving hatch problems. This can be done with thermistors attached to data-loggers so that temperatures can be recorded at several locations within an incubator throughout the incubation cycle. There is a wide range of data-logging equipment available on the market but whatever system is chosen it is important that the thermistor should be able to read to 0.1°C as a minimum and to be as accurate as possible.

Measuring internal egg temperatures would be the ideal situation but practically difficult to achieve within commercial incubators. Measuring eggshell surface temperature is less problematic as it does not require placing a thermistor within the egg and has been shown to be similar to measuring internal temperature (Sotherland *et al.*, 1987). Measuring air temperature in amongst the eggs within the incubator will not be as close to internal egg temperature as measuring shell surface temperature but can still be a useful method of checking temperature within the machine.

## THE FUTURE

Whilst the importance of temperature for the successful incubation of poultry eggs has been long understood it is only in recent years that the clear distinction between egg temperature and incubator temperature been made. An improved understanding of how egg temperatures with incubators are controlled has resulted in improvements in incubator design resulting in less within machine temperature variation. What are now required are guidelines as to what are the maximum and minimum egg temperatures that the poultry embryo can tolerate without adverse effect on hatch or post-hatch performance. The temperature tolerance of embryos may not only vary between poultry species but also between breeds or types within species (Decuypere and Michels, 1992). Determining embryo tolerance to temperature will be essential if hatchery managers are to maximise hatchability.

## REFERENCES

- Ande, T.B. and Wilson, H.R. (1981) Hatchability of chicken embryos exposed to acute high temperature stress at various ages. *Poult. Sci.*, **60**, 1561–1566.
- Decuypere, E. and Michels, H. (1992) Incubation temperature as a management tool: A review. *World's Poult. Sci. J.*, **48**, 28–38.
- French, N.A. (1994) Effect of incubation temperature on the gross pathology of turkey embryos. *Brit. Poult. Sci.*, **35**, 363–371.
- French, N.A. (1997) Modelling incubation temperature: the effect of incubator design, embryonic development and egg size. *Poult. Sci.*, **76**, 124–133.
- French, N.A. (2000) Effect of short periods of high incubation temperature on hatchability and incidence of embryo pathology of turkey eggs. *Brit. Poult. Sci.*, **41**, 377–382.
- French, N.A. (2001) Temperatures in a tunnel incubator—a long term study. *Avian Poult. Biol. Rev.*, **12**, 184–186.
- French, N.A. (2002). Managing the Incubation environment in commercial hatcheries to meet the requirements of the embryo. *Avian Poult. Biol. Rev.*, **13**, 179–185.
- Gladys, G.E., Hill, D., Meijerhof, R., Saleh, T.M. and Hulet, R.M. (2000) Effect of embryo temperature and age of breeder flock on broiler post-hatch performance. *Poult. Sci.*, **79** (Supplement 1), S179.
- Hill, D. (2001) Chick length uniformity profiles as a field measurement of chick quality? *Avian Poult. Biol. Rev.*, **12**, 188.
- Hulet, R.M., Gladys, G., Hill, D. and Meijerhof, R. (2000) Embryonic temperature effects on post-hatch performance in broilers. *Avian Poult. Biol. Rev.*, **11**, 298–299.
- Kaltofen, K.S. (1969) The effect of air movements on the hatchability and weight loss of chicken eggs during artificial incubation. In: *The Fertility and Hatchability of the Hen's Egg*, Carter, T.C. and Freeman, B.M. (eds.), pp. 177–190. Oliver and Boyd, Edinburgh.
- Kashkin, V.V. (1961) Heat exchange of bird eggs during incubation. *Biophysica*, **6**, 57–63.
- Lancaster, F.M. and Jones, D.R. (1988) Cooling of broiler hatching eggs during incubation. *Brit. Poult. Sci.*, **29**, 597–604.
- Lourens, S., and Middelkoop, J. H. (2000) Embryo temperature affects hatchability and grow-out performance of broilers. *Avian Poult. Biol. Rev.*, **11**, 299–301.
- Lundy, H. (1969) A review of the effects of temperature, humidity, turning and gaseous environment in the incubator on the hatchability of the hen's egg. In: *The Fertility and Hatchability of the Hen's Egg*, Carter, T.C. and Freeman, B.M. (eds.), pp 143–176. Oliver and Boyd, Edinburgh.
- Mauldin, J.M. and Buhr, R.J. (1994) What is really happening in your incubator? *Int. Hatch. Pract.*, **9**(5), 19–22.
- Meijerhof, R. and van Beek, G. (1993) Mathematical modelling of temperature and moisture loss of hatching eggs. *J. Theor. Biol.*, **165**, 27–41.
- Moreng, R.E. and Shaffner, C.S. (1951) Lethal internal temperatures for the chicken, from fertile egg to mature bird. *Poult. Sci.*, **30**, 255–256.
- Morgan, W. and Tucker, W.L. (1967) Influence of thermal incubation stresses. *Poult. Sci.*, **46**, 1172–1176.

- Ono, H., Hou, P.-C.L. and Tazawa, H. (1994) Responses of developing chicken embryos to acute changes in ambient temperature: Noninvasive study of heart rate. *Israel J. Zool.*, **40**, 467–480.
- Owen, J. (1991). Principles and problems of incubator design. In: *Avian Incubation*, Tullett, S.G. (ed.), pp. 205–224. Butterworth-Heinemann, London.
- Romanoff, A.L. (1935) Influence of incubation temperature on the hatchability of eggs, post-natal growth and survival of turkeys. *J. Agric. Sci.*, **25**, 318–325.
- Romanoff, A.L., Smith, L.L. and Sullivan, R.A. (1938) Biochemistry and biophysics of the developing hen's egg. III. Influence of temperature. *Cornell Agric. Exp. Stat. Bull.*, **216**, 1–42.
- Romijn, C. and Lokhorst, W. (1960) Foetal heat production in the fowl. *J. Physiol.*, **150**, 239–249.
- Sotherland, P.R., Spotila, J.R. and Paganelli, C.V. (1987) Avian eggs: Barriers to the exchange of heat and mass. *J. Exp. Zool.*, **Supplement 1**, 81–86.
- Tazawa, H. and Nakagawa, S. (1985) Response of egg temperature, heart rate and blood pressure in the chick embryo to hypothermal stress. *J. Comp. Physiol.*, **B155**, 195–200.
- Wilson, H.R. (1991) Physiological requirements of the developing embryo: temperature and turning. In: *Avian Incubation*, Tullett, S.G. (ed.), pp. 145–156. Butterworth-Heinemann, London.

# Roles of water and gas exchange in determining hatchability success

Amos Ar<sup>a\*</sup> and D. Charles Deeming<sup>b,c</sup>

<sup>a</sup>Department of Zoology, Tel-Aviv University, Ramat-Aviv 69978, Israel

\*E-mail: AmosA@tauex.tau.ac.il.

<sup>b</sup>Hatchery Consulting and Research, 9 Eagle Drive, Welton, Lincoln, LN2 3LP, UK

<sup>c</sup>Present address: Department of Biological Sciences, University of Lincoln, Riseholme Park, Lincoln, LN2 2LG, UK

## INTRODUCTION

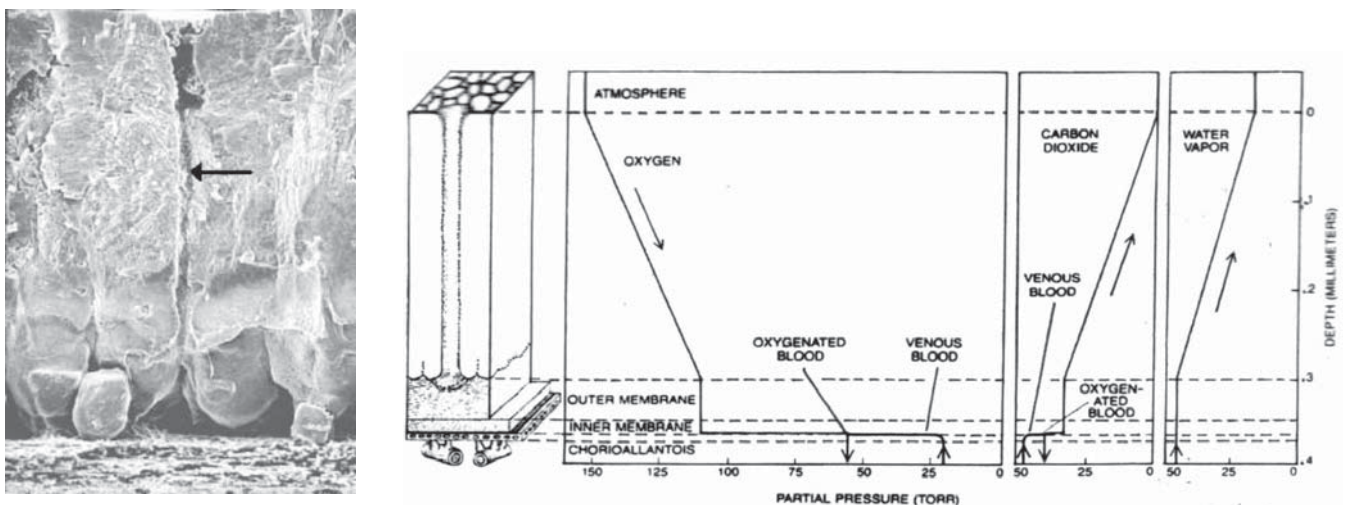
Among egg-laying terrestrial vertebrates, birds are unique in that they lay only one egg at a time (related to the demands of flight), and that the eggs are almost fully exposed to the atmosphere. Bird embryos require relatively high temperatures for their relatively fast development and thus for their short incubation. These can only be provided by contact incubation with birds and good thermal insulation. As a result, avian eggs generally develop in low humidity conditions and their embryos face the danger of desiccation. The embryo is partially protected from desiccation by the eggshell and also by the bird maintaining a microenvironment in the nest. The latter has not only a temperature but also a humidity higher than the ambient conditions (There are, however, cases where ambient temperature is higher than optimal nest temperature. In these cases the bird cools its eggs). If normal development is to occur the eggshell must be permeable to oxygen and

carbon dioxide and water is still lost through the shell in the form of vapour from the moment the egg is laid. Avian embryos have evolved to cope with this set of conflicting conditions and successful development is due to the interaction between the bird, its nest and the eggs (Deeming, 2002).

Artificial incubation has either to mimic this interaction or to generate equivalent solutions by providing an appropriate humidity to ensure an optimal rate of water loss from the eggs, the supply of oxygen and the elimination of carbon dioxide. The principles on which these are based may be the same as in nature but the mechanisms by which they are controlled can differ greatly.

## GAS DIFFUSION THROUGH THE EGGSHELL

Movement of water vapour, oxygen and carbon dioxide across the eggshell is achieved by diffusion through pores within the calcitic eggshell (Figure 1).



**Figure 1** Scanning electron micrograph of an avian eggshell (left) with the arrow indicating a pore (courtesy of Glenn Baggott). An illustration (right) showing the partial pressure differences across the eggshell (from Rahn *et al.*, 1979).

Diffusion through the long narrow pores makes gas exchange independent of airflow around the egg and dependent only on the partial pressure difference across the shell. However, laterally, under the shell, within the shell membranes from and to the inner pore orifice, the exchange of gases is limited (Visschedijk *et al.*, 1988) and therefore a certain density of pores in the shell must be provided to supply the blood in the chorio-allantoic membrane with sufficient oxygen. This dictates a link between diffusive water loss from eggshell pores and respiratory gas exchange.

Gas exchange across the eggshell is dictated by Fick's law:

$$M_x = G_x \times (P_{e_x} - P_{i_x})$$

where  $x$  is the gas involved ( $H_2O$ ,  $O_2$  or  $CO_2$ ),  $M$  is the rate of gas exchange,  $G$  is the conductance of the shell to the gas,  $P_e$  is the partial pressure of the gas outside the egg, and  $P_i$  is the partial pressure of the gas under the shell. For water vapour,  $P_{eH_2O}$  is 48 Torr at 37.5°C (the air under the shell is considered to be saturated with water vapour, *i.e.* 100% relative humidity at egg temperature which, in turn, is considered to be equal to the incubator temperature although small deviations from this scheme do exist.) and the rate of water vapour loss ( $M_{H_2O}$ ) from an egg is controlled by the combination of water vapour conductance ( $G_{H_2O}$ ) and the humidity of the air outside of the shell ( $P_{iH_2O}$ ).

In reality, for many bird species,  $G_{H_2O}$  is fixed at laying and so  $M_{H_2O}$  can only be adjusted by changing the humidity in the air around the egg if incubation temperature (and thus  $P_{iH_2O}$ ) is constant. In their natural habitat birds choose to build their nests in specific locations and with specific construction characteristics and at a given season. The presence of the bird on eggs in a nest is sufficient to modify humidity in relation to the ambient and nest humidity, water vapour conductance and fractional mass loss as water vapour are intimately linked.

Fractional mass loss values for bird eggs from a variety of bird species averages at 0.15. Actually, this 15% of the initial egg mass is a calculated loss for the entire incubation period based on average daily mass loss (Rahn and Ar, 1974). Additional water (*circa* 4–5%) is lost between external pipping and hatching. The range of average values observed in birds generally lay between 10–22% and in nests individual eggs can lose any value in this range without apparent ill effect. This makes sense because a requirement for a narrow range of mass loss would prove to be far from advantageous. Probability of hatching drops for low rates of loss (<10%) due to problems with exchange of respiratory gases in particular at hatch (see below)

and losses higher than 22% cause problems with dehydration of the egg contents.

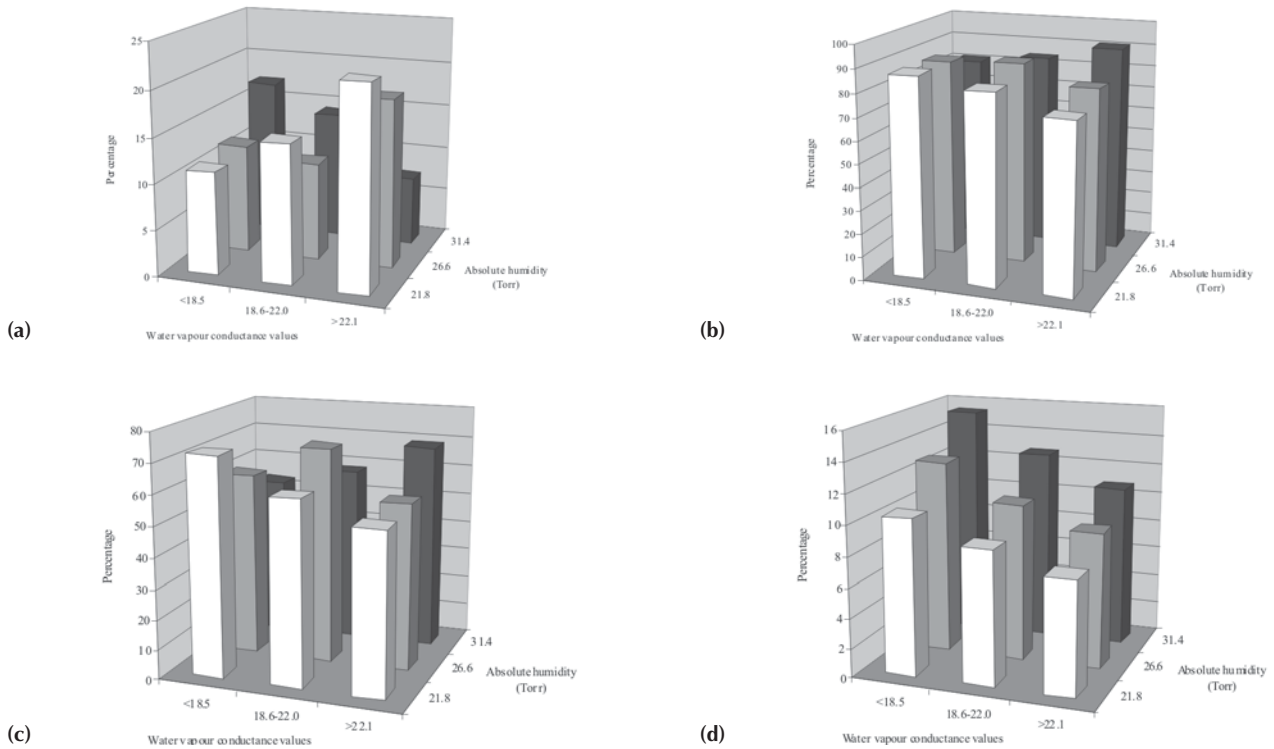
It is interesting to note at this point that water vapour and oxygen, which exchange through the same pores, are also linked by the fact that the end products of aerobic metabolism are metabolic water production and carbon dioxide (and energy). Metabolism utilises solids stored in the egg, which, together with the water produced, would have increased water concentration in the egg to an intolerable level if no water loss would have occurred. Under normal conditions the water evaporation/water production/solid elimination ratios are such that the water concentration of the hatchling is almost identical to that of the fresh egg.

A batch of eggs under artificial incubation would be normally composed of eggs from a large number of individual hens and so there is variation in the values for  $G_{H_2O}$ , which are typically distributed around a mean value. When dealing with thousands of eggs within a setter, commercial incubation needs to set the humidity to match the eggshell characteristics of the average egg of a particular batch so that it attains the appropriate mass loss (*e.g.* 15% to external pipping). In this way the majority of the eggs will lie within the 10–22% mass loss range and stand a good chance of hatching normally. Significant departures from this average mass loss using inappropriate incubator humidity will cause a significant increase in embryonic mortality (Meir and Ar, 1987).

## REGULATION OF WATER LOSS AND HATCHABILITY

In commercial poultry production egg laying within a flock persists for many weeks and is associated with changes in egg composition as well as increases in egg size and eggshell conductance (French and Tullett, 1991). Changes in size and water vapour conductance over a 10-week laying season are also reported for commercial geese and pheasant eggs (Meir and Ar, 1996; Deeming, 2000). Astute artificial incubation procedures should take into account all these interacting variables, in order to maximise hatchability, by monitoring and controlling incubator temperature, humidity and respiratory gas levels, in accordance to the stage of embryonic development. Several studies have shown that monitoring mass loss of eggs and subsequent adjustment of the humidity regimen can have beneficial effects.

Tullett (1981) demonstrated that the water vapour conductance of turkey eggs changed as flock age increased. As a result the humidity required to ensure a standard amount of mass loss has to change as the flock gets older. Several other studies on turkeys (Meir *et al.*, 1984; Meir and Ar, 1987),



**Figure 2** Effects of matching incubator humidity to mass specific eggshell conductance (mgH<sub>2</sub>O per 100-g per Torr per day) on (a) late embryonic mortality, (b) hatchability of fertile turkey eggs, (c) Grade A turkey poults, and (d) percentage weight loss of turkey eggs to 25 days of incubation. \* Indicates the results where each range in mass specific conductance was matched with the appropriate humidity. Data from Meir and Ar (1987).

chickens (Bamelis *et al.*, 2001), geese (Meir and Ar, 1996) and ostriches (Ar *et al.*, 1996) have shown that measuring mass specific conductance of eggshells can be used to adjust incubator humidity, which has beneficial effects on both hatchability and chick quality. For instance, Meir and Ar (1987) showed that the highest hatchability of turkey poults was achieved by matching eggshell conductance with the appropriate humidity and that the improvement in hatchability was associated with decreases in late mortality (Figure 2). The highest poult quality was also achieved under these conditions, which produced a percentage mass loss to 25 days of 10.4–10.8%. Lower or higher values were associated with decreases in hatchability and chick quality (Figure 2).

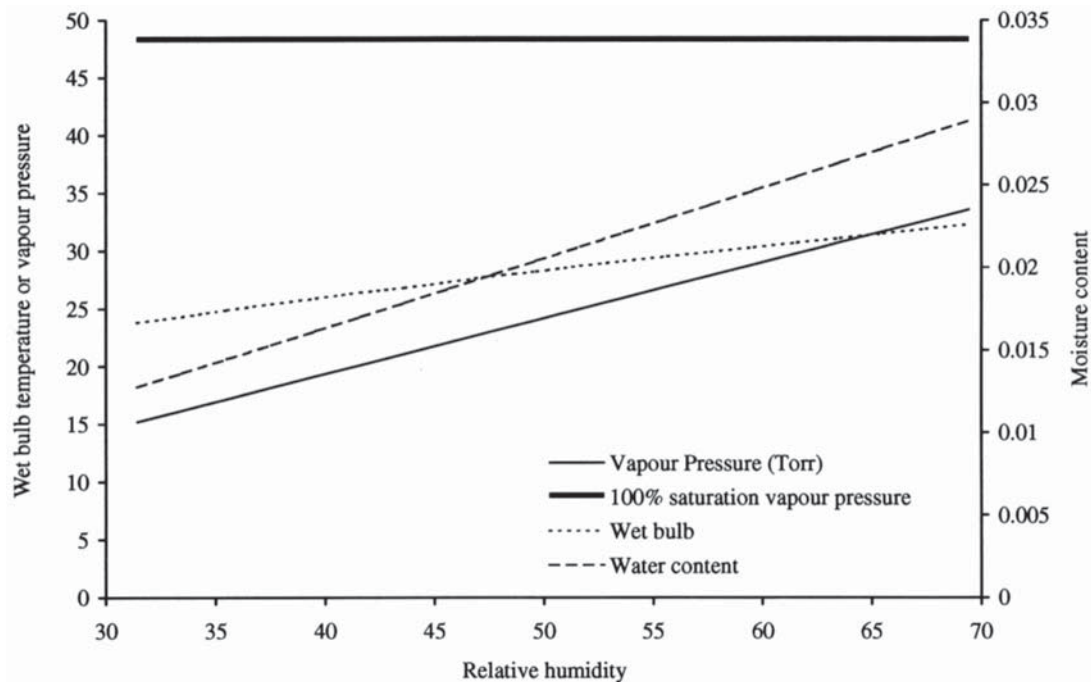
An important conclusion from these studies is the fact that by monitoring mass loss in the incubator, early during incubation, humidity can be changed accordingly to prevent eventual late embryonic mortality and to increase chick quality. In single stage incubators this can be easily applied but also in multi-stage incubators humidity can be dynamically controlled, from week to week and even in various parts of the incubator, according to the special demands of the eggs in question and their age.

Unfortunately, commercial practice usually fails to take advantage of these benefits. If water loss is

monitored it is usually recorded at transfer, a time when nothing can be done to improve the actual mass loss of the eggs being incubated. It would be more effective to measure mass loss, at least for representative samples of the batches, after 3–7 days and then adjustments can be made to the humidity setting to better match the conductance of the eggshells involved. Whilst it could be argued that it would be impossible at a practical level to monitor mass loss of every batch of eggs, there is still considerable scope for improvement of humidity management during incubation. For instance, setting eggs in incubators on the basis of flock age would allow for regular monitoring of mass loss and adoption of humidities that better match the needs of particular eggs at different stages of the laying cycle. Therefore, the larger eggs from older flocks have a higher water vapour conductance and should need a higher humidity in order to maintain an appropriate level of water loss. This would be applicable to eggs under both single stage and multi-stage incubation regimens.

Finally, humidity is typically measured by a wet bulb thermometer, which provides a wet bulb temperature that is used to determine the relative humidity at the dry bulb temperature. Other systems employ a dry humidity sensor that gives a value for





**Figure 3** Relationships between relative humidity (%), wet bulb temperature ( $^{\circ}\text{C}$ ), vapour pressure (Torr) and absolute moisture content (kg per kg dry air) at a dry bulb temperature of  $37.5^{\circ}\text{C}$ . The vapour pressure under the eggshell (100%RH) is also shown.

percentage Relative Humidity (%RH) directly. However, in terms of monitoring mass loss and adjusting humidity it is better to use measures of vapour pressure for water (Absolute Humidity) vapour measured in Torr ( $1\text{ Torr} = 1\text{ mm Hg} = 7.5\text{ kPa}$ ). Relationships between %RH, absolute water content and vapour pressure are shown in Figure 3.

#### EMBRONIC WATER BALANCE

The fractional water content of the egg contents is equal to the fractional water content of the hatchling ( $\sim 73\%$  for poultry species). The water balance of the egg during incubation is a function of the initial water fraction of the contents, the rate of water lost through diffusion across the eggshell and the amount of water produced by metabolism. Normal respiration produces water as a waste product and as a result the total water content of the egg remains constant during the second half of development. This is despite of some increase in the rate of water vapour loss due to increasing egg temperature as its metabolic activity increases. Metabolic water production accounts for 10% of the total water content of the hatchling and is critical in the overall water balance and in maintaining the osmolarity of the egg contents (Ar, 1991).

Another link between embryonic respiration and egg water content emerges towards hatching: incubation water loss creates the air space under the rigid eggshell. Sufficient water must be lost to create an air

space volume adequate for complete inflation of the respiratory air sacs of the hatchling during the period between internal and external pipping (Ar, 1991).

Furthermore, the water content of the egg compartments provides the embryo with heat inertia, thus preventing fast changes in embryonic metabolism due to temperature changes. As the embryo develops, water is shifted among compartments within and around it, each of which has an optimal volume and ionic composition at a given stage of development. This requires the maintenance of the appropriate water content and ionic composition at each stage (Ar, 1991; Baggott, 2001).

Metabolic rate and egg water vapour pressure comply in the same way to changes in egg temperature. The eggshell, carbon dioxide and water interact to supply dissolved calcium to the developing embryo. Many studies have shown that imbalance in any of the above-discussed parameters will reduce hatchability success, and optimising them will increase it. It becomes clear that appropriate incubation atmosphere has to comply not only with temperature and humidity demands of the developing embryo but at the same time with appropriate exchange of its respiratory gases.

#### RESPIRATORY GAS EXCHANGE

The eggshell acts a mediating barrier for the diffusion of oxygen into, and carbon dioxide out, of the egg via the pores. Shell conductance values are normally

matched to the nesting environment of the bird and so eggs will usually incubate successfully within only a narrow range of humidities. The conductance of the eggshell is also matched to the respiratory exchange of the embryo but problems arise with low conductance eggshells. In average and above-average values for conductance, the embryo is free to exchange oxygen and carbon dioxide without restriction, while water vapour loss can be controlled by setting the appropriate incubator humidity. This is because the eggshell conductance does not act to limit the potential rate of oxygen consumption or carbon dioxide production. However, in eggshells with lower than average conductance the eggshell limits the maximum gas exchange value and forces the embryo to conform to the maximum rate of diffusion that it will physically allow. It should be mentioned here that while the shell comprises the only resistance to diffusion for water vapour and most of this resistance for carbon dioxide, about one third to one half of the resistance for oxygen diffusion lies in the chorio-allantoic membrane (Rahn *et al.*, 1979). For eggs at the lower end of the range of eggshell conductance, this can mean a reduction in oxygen consumption as early as day 15 in the fowl (Tullett and Deeming, 1982), and retention of carbon dioxide. This can affect rates of embryonic growth (Burton and Tullett, 1983), presumably causing longer incubation periods.

Shell-induced hypoxia and hypercapnia are reasons why embryos in low porosity eggshells can fail to hatch even if the incubator humidity is lowered to facilitate the appropriate rate of mass loss. Increasing mass loss across a low conductance eggshell will not improve rates of oxygen diffusion. In practice, shell induced hypoxia may not kill many embryos *in ovo* but it can be a contributing factor in increasing the spread of hatch events such as internal and external pipping (Visschedijk, 1968) and lowering the quality of some chicks.

Provision of oxygen to developing embryos means that ventilation of the setters and in particular hatchers, where all the embryos are of the same age, at their maximal development and active in hatching hence, consuming oxygen at a high rate, should be sufficient and should be supplied by a body of air that is itself being continuously replenished. The importance of hatchery ventilation is described elsewhere (Mauldin and MacKinnon, 2002).

## IN CONCLUSION

From the point of view of improving hatchability and chick quality there is much that can be done in monitoring and modifying humidity to improve hatch-

ability and chick quality even without any further research. So far, 6000 years of domestication (and selection) of the hen has not yielded a uniform egg with fixed shell porosity and fixed composition. A certain variability in these properties seems to be a built in repertoire of the egg for species survival in nature. It looks like that even intense selection in this direction (of which we are not aware) would not yield good results because of the multiple genetic and environmental interacting factors involved.

Theoretically, a possibility exists of "helping nature" and treating eggs artificially pre-incubation such as to make them all uniform, of the same shell conductance and thus all could be incubated under the same conditions. It all depends on the so-called "cost benefit ratio". This goes also to the other possibility, to the amount of technology incubators and hatchers producers would be willing to invest in their products. Incubating and hatching machines can be made to monitor and deal with changing properties of egg batches and non-conservative variable incubation conditions. Depending on the future market demands there a hope to see advancement in one or more of these avenues.

## REFERENCES

- Ar, A. (1991) Egg water movements during incubation. In: *Avian Incubation*, Tullett, S.G. (ed.), pp. 157–173. Butterworth-Heinemann, London.
- Ar, A., Meir, M., Aisik, N. and Campi, D. (1996) Standard values and ranges of ostrich egg parameters as a basis for proper artificial incubation. In: *Improving our Understanding of Ratites in a Farming Environment*, Deeming, D.C. (ed.), pp. 144–146. Ratite Conference Books, Oxfordshire.
- Baggott, G.K. (2001) Development of extra-embryonic membranes and fluid compartments. In: *Perspectives in Fertilisation and Embryonic Development in Poultry*, Baggott, G.K., Bakst, M.R., Bellairs, R., Christensen, V.L., Fassenko, G.M. and Starck, J.M. (eds.), pp. 23–30. Ratite Conference Books, Lincoln. Reprinted in 2009: *Avian Biol. Res.*, **2**, 21–26.
- Bamelis, F., Tona, K., De Baerdemaeker, J. and Decuyper, E. (2001) Increasing chicken eggs hatchability by matching incubator humidity to eggshell conductance. *Avian Poult. Biol. Rev.*, **12**, 169.
- Burton, F.G. and Tullett, S.G. (1983) A comparison of the effects of eggshell porosity on the respiration and growth of domestic fowl, duck and turkey embryos. *Comp. Biochem. Physiol.*, **75A**, 167–174.
- Deeming, D.C. (2000). *Principles of Artificial Incubation for Game Birds*. Ratite Conference Books, Oxfordshire.
- Deeming, D.C. (2002). Functional characteristics of eggs. In: *Avian Incubation: Behaviour, Environment and Evolution*, Deeming, D.C. (ed.), pp. 28–42. Oxford University Press, Oxford.

- French, N.A. and Tullett, S.G. (1991) Variations in the eggs of poultry species. In: *Avian Incubation*, Tullett, S.G. (ed.), pp. 59–77. Butterworth-Heinemann, London.
- Mauldin, J.M. and MacKinnon, I.R. (2002) Hatchery ventilation and environmental control. In: *Practical Aspects of Commercial Incubation in Poultry*, Deeming, D.C. (ed.), pp. 47–52. Ratite Conference Books, Lincoln. Reprinted in 2009: *Avian Biol. Res.*, **2**, 87–91.
- Meir, M. and Ar, A. (1987) Improving turkey poult quality by correcting incubator humidity to match eggshell conductance. *Brit. Poult. Sci.*, **28**, 337–342.
- Meir, M. and Ar, A. (1996) Artificial increase of eggshell conductance improves hatchability of early laid goose eggs. *Brit. Poult. Sci.*, **37**, 937–951.
- Meir, M., Nir, A. and Ar, A. (1984) Increasing hatchability of turkey eggs by matching incubator humidity to shell conductance of individual eggs. *Poult. Sci.*, **63**, 1469–1496.
- Rahn, H. and Ar, A. (1974) The avian egg: incubation time and water loss. *Condor*, **76**, 147–152.
- Rahn, H., Paganelli, C.V. and Ar, A. (1979) How bird eggs breathe. *Sci. Amer.*, **240**, 46–55.
- Tullett, S.G. (1981) Theoretical and practical aspects of egg shell porosity. *Turkeys*, **29**, 24–28.
- Tullett, S.G. and Deeming, D.C. (1982) The relationship between eggshell porosity and oxygen consumption in the domestic fowl. *Comp. Biochem. Physiol.*, **72A**, 529–533.
- Visschedijk, A.H.J. (1968). The air space and embryonic respiration 2. The times of pipping and hatching as influenced by an artificially changed permeability of the shell over the air space. *Brit. Poult. Sci.*, **9**, 185–196.
- Visschedijk, A.H.J., Girard, H. and Ar, A. (1988) Gas diffusion in the shell membranes of the hen's egg: lateral diffusion *in situ*. *J. Comp. Physiol.*, **B158**, 567–574.

# The role of egg turning during incubation

D. Charles Deeming

Hatchery Consulting and Research, 9 Eagle Drive, Welton, Lincoln, LN2 3LP, UK

Present address: Department of Biological Sciences, University of Lincoln, Riseholme Park, Lincoln, LN2 2LG, UK E-mail: cdeeming@lincoln.ac.uk

## INTRODUCTION

Egg turning is a crucial aspect of normal incubation under a bird or in a machine. With some exceptions all birds turn their eggs and commercial incubators employ systems which move whole trays of eggs typically through 90° once every hour in the setter (usually 45° either side of the horizontal). After transfer into the hatcher the eggs do not need to be turned. It has long been understood that failure to turn eggs during incubation lowers hatchability and chick quality. This chapter investigates the reason why egg turning is so important during incubation and provides information on the kinds of problems encountered when there is a problem with turning during artificial incubation.

## EXPLANATIONS FOR EGG TURNING

Observations of incubating birds led the Ancient Egyptians to realise that turning of eggs was necessary during incubation. In the oven incubators they used the eggs were turned by hand 2–3 times per day during incubation. The Egyptians did not, however, appear to understand why eggs needed to be turned.

Over the years reasons for egg turning have included the need for re-distribution of heat to break down temperature gradients within a clutch of eggs (de Réaumur, 1751). This has been discounted largely because in artificial incubators temperature gradients are abolished yet turning is necessary.

The commonest explanation for egg turning is that it prevents adhesion of the embryo to the inner shell membrane early in development. Whilst this problem does occur in poultry eggs, most of the depression in hatchability is associated with late mortality, with dead embryos being characterised by small size, poor growth of the chorio-allantois and residual albumen in the bottom of the egg (Tullett and Deeming, 1987). Research over the past 20 years has shown that many of these problems are associated with physiological effects of the lack of egg turning (see reviews by Deeming, 1991, 2002; and Baggott *et al.*, 2002).

## PHYSIOLOGICAL EFFECTS OF EGG TURNING

Studies into the effects of turning on embryonic development have largely been based on not turning eggs during incubation. Most of the work has been carried out in the domestic fowl (*Gallus gallus*) and the Japanese quail (*Coturnix coturnix japonica*). This work has revealed that lack of turning affects a variety of aspects of embryonic development including membrane growth, fluid transport, embryonic growth and hatchability.

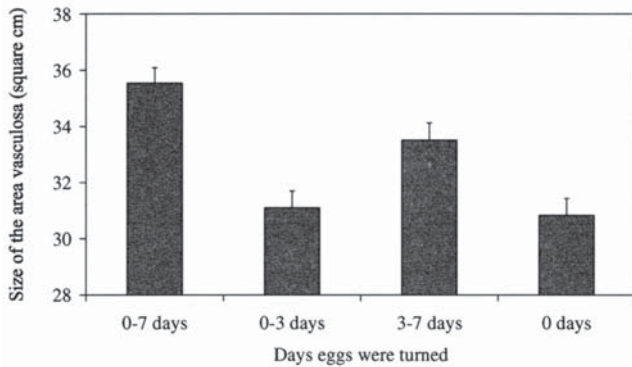
An important aspect is the need to turn eggs during a critical period in the first third of incubation. Turning during days 3–7 in the fowl embryo (New, 1957) is quite sufficient for normal hatchability and growth even if there is no other turning during incubation (Table 1; Deeming, 1989d). Similarly, turning at all times other than this critical period of 3–7 days significantly prevents normal development and depresses hatchability (Table 1). The effects of turning during the critical period are further illustrated by the depression in growth of the *area vasculosa* (Figure 1).

During this time there is massive transfer of water from the albumen into the yolk sac to form the sub-embryonic fluid (SEF) and there is rapid growth of the *area vasculosa* of the yolk sac membrane (Baggott

**Table 1** Effects of turning during the critical period (3–7 days) for the fowl embryo on hatchability and embryonic growth at 16 days of incubation (Deeming, 1989d)

Days turned	Hatchability (%)	Embryo mass at 16 days (g)
0–21	78.6	18.5 <sup>1</sup>
0–7	86.7	17.5
3–7	76.9	17.0
3–21	73.3	16.9
Mean	78.9	17.5
7–21	64.3	16.5
0–3	64.3	15.6
0–3 and 7–21	42.9	15.2
0	50.0	14.5
Mean	55.4	15.5

<sup>1</sup>Eggs were turned to 16 days.



**Figure 1** Effects of turning during the critical period (3–7 days) on the growth of the *area vasculosa* at 7 days in the fowl embryo. Values are means plus SE. Data from Deeming (1989b).

*et al.*, 2002). Failure to turn eggs causes a reduction in the mass and solid content of SEF and there is a slower rate of growth of the *area vasculosa*.

Later in development the spatial arrangements between the embryo, yolk and albumen are different in unturned eggs. The embryo tends to sink down into the yolk with the albumen lying below in the bottom of the egg. This prevents the normal transfer of albumen proteins into the amniotic fluid via the sero-amniotic connection (SAC; Figure 2). This process involves the ectoderm tissue of the SAC (where the head and tail folds of the amnion meet during the first days of development) breaking down exposing a link between the dehydrated albumen proteins and the amniotic fluid. The proteins move through into the fluid and are then swallowed with the amniotic fluid, entering the alimentary tract and moving through into the yolk sac via the yolk sac stalk.

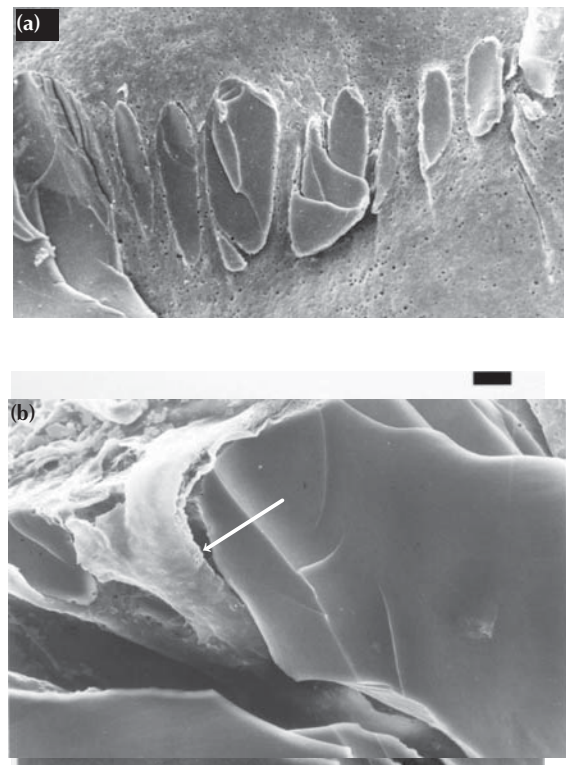
Volumes of allantoic and amniotic fluids are reduced by lack of turning (Deeming, 1989a) because of the reduced amount of SEF. Furthermore, the amount of protein in amniotic fluid is significantly lower in unturned eggs (Deeming, 1991). The rate of embryonic growth is slower in unturned eggs from day 12 of incubation onwards, which adversely affects their oxygen consumption (Tazawa, 1980). However, Deeming (1991) showed that incubation in an atmosphere of 40% oxygen does not alleviate the symptoms of failure to turn the eggs.

Deeming (1989c) investigated whether SEF or albumen was more critical in affecting the physiology of unturned embryos. Experimental removal of SEF in turned eggs led to a reduced volume of allantoic fluid but did not affect either the amniotic fluid or the rate of embryonic growth. By contrast, removal of albumen at 3 days of incubation in turned eggs produced similar effects observed for unturned eggs in the volume of allantoic and amniotic fluids. Lack of

albumen proved to be more important than a reduction in SEF in determining rates of embryonic growth. This evidence suggested that utilisation of albumen was ultimately the primary embryonic process affected by turning. However, SEF is formed from albumen and the effects of turning on its quantity and composition have proved to be a very useful tool in the study of SEF formation.

## TURNING AND SEF FORMATION

Deeming *et al.* (1987) first demonstrated the effects of lack of turning on SEF formation and suggested that "unstirred layers" were generated in the albumen. These supposedly prevented normal transfer of sodium ions from the albumen across the *area vasculosa* and into the yolk. Water and chloride moved down the electro-osmotic gradient to form SEF. During the 1990s Baggott, Latter and Babiker took up the challenge of investigating this concept further and showed that "unstirred layers" did exist although not in the form envisaged by Deeming *et al.* (1987).



**Figure 2** Scanning electron micrographs of the sero-amniotic connection (SAC) in a fowl egg. (a) A cast (membranes have been removed) of the SAC in solidified albumen proteins. Note the crescent shape and irregular oval holes. (b) Cast of the solidified albumen proteins protruding through the SAC. Arrow shows the membrane lying below the albumen. In both pictures scale bar = 100  $\mu\text{m}$ .

From Deeming (1989d).

Careful analysis has revealed that the concentration of sodium ions was higher in the albumen towards the shell than it was towards the yolk and that this difference was seen in unincubated eggs. In incubated eggs this sodium gradient persisted but was smaller in turned eggs. Therefore, the embryo does not generate the unstirred layers but rather turning during incubation, and perhaps during storage, breaks down diffusion gradients for sodium ions that already exist in the albumen (Latter and Baggott, 1996; Baggott *et al.*, 2002).

Work with Japanese quail eggs has revealed the process by which SEF is formed from albumen. Space prevents a full description of this process but see Latter and Baggott (2001) and Baggott *et al.* (2002) for further information.

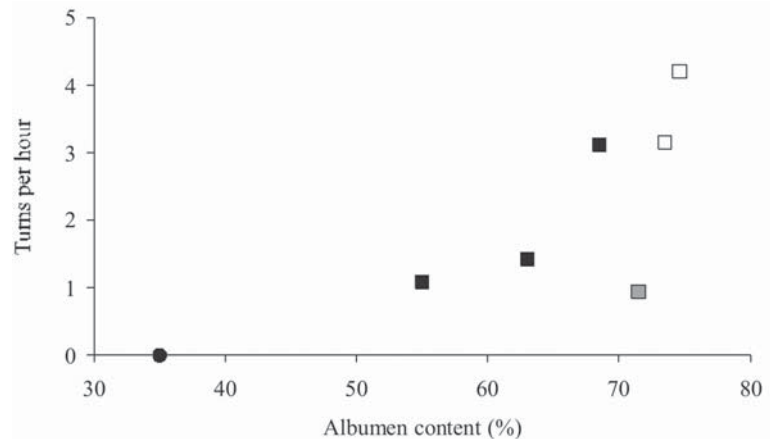
#### A HYPOTHESIS TO EXPLAIN EGG TURNING

Deeming (1991) used the results of previous studies to suggest that egg turning had a crucial role in the utilisation of albumen by the embryo. Short incubation periods require that the water reservoir in the albumen is moved efficiently into the yolk. Turning destroys unstirred layers within the albumen thereby preventing diffusion limitations to SEF formation. The high protein content is a valuable resource for the embryo and so turning ensures that there is complete transfer into the amniotic fluid. In the alligator, where the albumen is thin and incubation periods are long, turning the normally unturned egg has no physiological effect (Deeming and Ferguson, 1991).

Deeming (1991) concluded that complete utilisation of albumen (fluids and proteins) was dependent upon egg turning. This hypothesis led to the suggestion that the albumen content of eggs will be correlated with the turning frequency during incubation. Therefore, eggs from altricial species, which have a high albumen content, should be turned more frequently than eggs from precocial species, which have a relatively larger yolk and less albumen.

#### EGG TURNING BY BIRDS IN NESTS

Although anecdotal observations lent some support, this hypothesis has recently been tested by examining the literature for reports of the rate of turning (turns per hour) during natural incubation (Deeming, 2002). The results of this review has provided 82 reports from 61 species from 14 orders of birds and showed that there



**Figure 3** Correlation between average percentage albumen content of the egg and turns per hour for different groups of birds based on hatching maturity. The black circle represents kiwis and megapodes, black squares are precocial species, the grey square represents semi-precocial gulls and the white squares indicate semi-altricial and altricial species.

was considerable variation in the turning rates between different groups of bird.

Kiwis, megapodes and palm swifts do not turn their eggs during incubation. In precocial and semi-precocial species rates of egg turning were between 0.75–1.50 turns per hour compared with rates of 2–12 turns per hour in semi-altricial and altricial birds. Turning frequency showed a significant ( $p < 0.01$ ) positive correlation with relative albumen content of eggs (Figure 3). There were also significant ( $p < 0.01$ ) negative correlations for turning frequency with both initial egg mass and incubation period. The hypothesis that egg turning is needed to ensure full utilisation of water and proteins in albumen is strongly supported by this data.

It is likely that the archosaurian ancestors of birds did not turn their eggs. During the initial stages of the evolution of contact incubation any accidental movement of eggs may have proved to have a selective advantage. An increased rate of transfer of water from the albumen to the yolk may have allowed a shortening of the incubation period, which in turn would have reduced the risk of predation on the incubating birds. Further selection for more albumen rich eggs, which produce altricial young, meant that the rate at which eggs were turned had to increase in order to ensure maximal formation of sub-embryonic fluid from the water in the albumen. Again as egg mass decreased then incubation periods could be reduced minimising the time spent by the birds sitting on eggs.

Another interesting possibility for the evolution of egg turning lies in the results that show the density of blood vessels in the chorio-allantoic membrane is affected by exposure to a brood patch (Ar and Sidis, 2002). Application of an artificial brood patch to the shell of fowl eggs from 24 hours reduced the density

of blood vessels compared with uncovered areas of eggshell. This effect was enhanced by 48 hours under the brood patch.

### TURNING AND COMMERCIAL INCUBATION

Turning during commercial incubation of poultry eggs usually continues up to the day of transfer—18 days in the domestic fowl. Recently there have been studies into the effects of stopping turning earlier than at transfer.

Wilson and Wilmering (1988) showed that cessation of turning after day 10 of incubation decreases hatchability but stopping turning after 13 days had variable results. Stopping turning after 16 days had no significant effect on hatchability. Similarly, Lourens and Deeming (1999) showed that stopping turning at 15 days of incubation had no adverse effect on hatchability or pattern of embryonic mortality. However, even though there was a lowering in egg temperature, due to better air flow over the stationary horizontal egg trays, there was no beneficial effect on hatchability.

By contrast, Tona *et al.* (2001) showed that stopping turning at days 15, 16 or 17 (*i.e.* eggs were transferred to stationary hatcher baskets) depressed hatchability relative to stopping turning at transfer at 18 days. There was also a pronounced strain effect and young and old flocks were also adversely affected by stopping turning at days earlier than 18 days.

However, if turning is associated with the utilisation of albumen then stopping turning should have little effect on embryonic development. This is because most of the albumen proteins have moved into the amniotic fluid by day 15 of incubation in the domestic fowl (Romanoff, 1967; Deeming, 1989a). By contrast, stopping turning at 12 days may have a significant effect on albumen utilisation because this is the time that the sero-amniotic connection opens (Hirota, 1891; Deeming, 1991). It is unclear why Tona *et al.* (2001) demonstrated the opposite effect but perhaps it related to other environmental conditions the eggs experienced in the hatcher baskets rather than lack of turning *per se*.

At present there is no evidence to suggest that stopping turning after 15 days of incubation in the domestic fowl has any deleterious effects on development or hatchability.

Egg turning has also been recently studied in the large eggs of the ostrich (van Schalkwyk *et al.*, 2000). In terms of the effect of angle of turn this species, resembles poultry but orientation of eggs is also critical. Eggs set in a horizontal position for 2–3 weeks before being positioned vertically with the air space upwards have a much higher hatchability than

eggs set vertically from the start of incubation or those that remain horizontal during incubation. This time period for turning in the horizontal position approximates to the critical period of fowl eggs.

To date many designs of small-scale incubators have utilised rates of turning based on poultry species (one turn per hour). In light of the results from the study on turning in nests rates of turning may need to be changed to accommodate the higher rates of turning required by eggs from semi-altricial and altricial species. This could assist in improving the success rate of artificial incubation in conservation programmes.

### REFERENCES

- Ar, A. and Sidis, Y. (2002) Nest micro-climate during incubation. In: *Avian Incubation: Behaviour, Environment and Evolution*, Deeming, D.C. (ed.), pp. 143–160. Oxford University Press, Oxford.
- Baggott, G.K., Deeming, D.C. and Latter, G.V. (2002) Electrolyte and water balance of the early avian embryo: effects of egg turning. *Avian Poult. Biol. Rev.*, **13**, 105–119.
- Deeming, D.C. (1989a) Characteristics of unturned eggs: A critical period, retarded embryonic growth and poor albumen utilisation. *Brit. Poult. Sci.*, **30**, 239–249.
- Deeming, D.C. (1989b) Failure to turn eggs during incubation: development of the *area vasculosa* and embryo growth. *J. Morph.*, **201**, 179–185.
- Deeming, D.C. (1989c) Importance of sub-embryonic fluid and albumen in the embryo's response to turning of the egg during incubation. *Brit. Poult. Sci.*, **30**, 591–606.
- Deeming, D.C. (1989d) *An Investigation into the Physiological Basis for Egg Turning during Incubation in Birds*. Unpublished PhD Thesis, University of Reading.
- Deeming, D.C. (1991) Reasons for the dichotomy in the need for egg turning during incubation in birds and reptiles. In: *Egg Incubation: Its Effects on Embryonic Development in Birds and Reptiles*, Deeming, D.C. and Ferguson, M.W.J. (eds.), pp. 307–323. Cambridge University Press, Cambridge.
- Deeming, D.C. (2002) Patterns and significance of egg turning. In *Avian Incubation: Behaviour, Environment and Evolution*, Deeming, D.C. (ed.), pp. 161–178. Oxford University Press, Oxford.
- Deeming, D.C. and Ferguson, M.W.J. (1991) Egg turning has no effect on growth and development of embryos of *Alligator mississippiensis*. *Acta Zool.*, **72**, 125–128.
- Deeming, D.C., Rowlett, K. and Simkiss, K. (1987) Physical influences on embryo development. *J. Exp. Zool.*, **Suppl. 1**, 341–345.
- Hirota, S. (1894) On the sero-amniotic connection and the foetal membranes in the chick. *J. College Sci., Univ. Tokyo*, **6**, 337–397.
- Latter, G.V. and Baggott, G.K. (1996) Effect of egg turning and fertility upon the sodium concentration of albumen of the Japanese quail. *Brit. Poult. Sci.*, **37**, 301–308.

- Lourens, S. and Deeming, D.C. (1999) Effect van niet meer keren na twee weken broeden. *Praktijkonderzoek*, **99/1**, 20–24. (In Dutch).
- New, D.A.T. (1957) A critical period for the turning of hens' eggs. *J. Embryol. Exp. Morphol.*, **5**, 293–299.
- de Reamur, R.A.F. (1751). *Konst om Tamme-vogelen van Allerhandesoort in alle Jaartyden Uittebroeijen en Opiebrengen zp door 't Middle van Mest als van't Gewone Vuur*, Vol. **1**, pp. 1–384. Pieter de Hondt, s'Gravenhage.
- Romanoff, A.L. (1967) *Biochemistry of the Avian Embryo*. John Wiley and Sons, New York.
- Tazawa, H. (1980) Adverse effect of failure to turn the avian egg on the embryo oxygen exchange. *Resp. Physiol.*, **41**, 137–142.
- Tona, K., Decuypere, E. and Coucke, W. (2001). Effects of strain, hen age and transferring eggs from turning to stationary trays after 15 to 18 days of incubation. *Brit. Poult. Sci.*, **42**, 663–667.
- Tullett, S.G. and Deeming, D.C. (1987) Failure to turn eggs during incubation, The effects on embryo weight, development of the chorioallantois and absorption of albumen. *Brit. Poult. Sci.*, **28**, 239–249.
- van Schalkwyk, S.J., Cloete, S.W.P., Brown, C.R. and Brand, Z. (2000) Hatching success of ostrich eggs in relation to setting, turning and angle of rotation. *Brit. Poult. Sci.*, **41**, 46–52.
- Wilson, H.R. and Wilmering, R.F. (1988) Hatchability as affected by egg turning in high density plastic egg flats during the last half of incubation. *Poult. Sci.*, **67**, 685–688.



# Effects of long term storage on the egg, embryo and chick

Gaylene M. Fasenko<sup>1</sup>, Frank E. Robinson<sup>2</sup> and Vern L. Christensen<sup>2</sup>

<sup>1</sup>Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta T6G 2P5, Canada E-mail: Gaylene.Fasenko@ualberta.ca

<sup>2</sup>Poultry Science Department, North Carolina State University, Raleigh, North Carolina 27695-7608, USA

## INTRODUCTION

When most people think of a chicken egg, they think of something nutritious to eat. From an avian embryo's point of view the egg provides the same function. At the time the egg is laid, if the oöcyte has been successfully fertilised, almost all of the nutrients required for the embryo to grow and develop are enclosed within the egg. There are two main elements missing; one is oxygen and the other is heat. The importance of both of these factors in embryonic growth and development are addressed elsewhere in this book (see French, 2002; Ar and Deeming, 2002).

In most commercial poultry breeding operations, hatching eggs are produced by flocks of breeders housed in barns. Male and female broiler breeders are housed together so that natural mating can occur, while turkey breeder hens are housed separately from the toms and therefore must be artificially inseminated. Most broiler breeder eggs are laid in nests and collected by mechanical belts while turkey breeder eggs are still hand-collected. Irrespective of the method of insemination or egg collection, once the eggs are collected they are stored in on-farm cool stores, usually for a few days, until they can be transported to the hatchery. This is done as daily egg transport from the breeder farms to the hatchery would be inefficient. Once transported to the hatchery, the eggs are again stored in large egg stores. Egg storage at the hatchery usually occurs for two reasons. First, hatching eggs are stored until enough eggs are available to fill large incubator racks. Second, stock-piling of eggs occurs in anticipation of fluctuations in egg production or demand for broiler chicks during the production year.

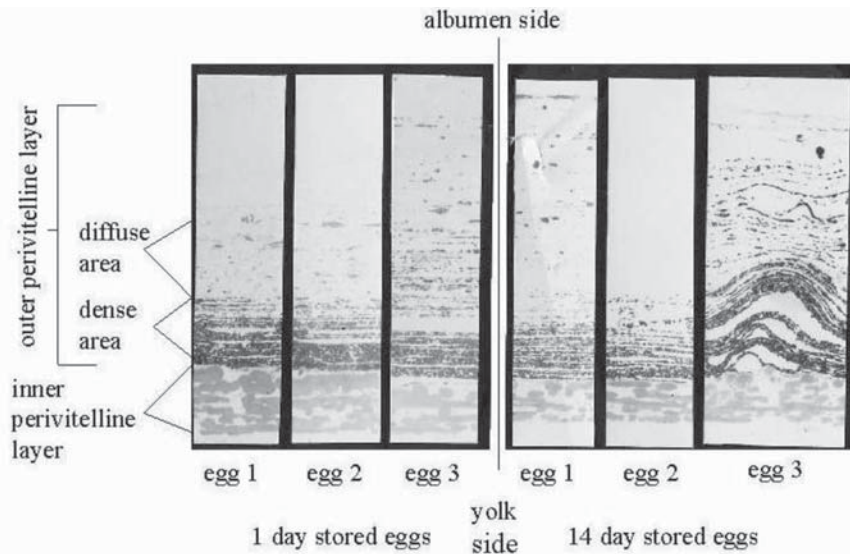
Storage of fertile eggs both on farm and at the hatchery occurs at temperatures below 21°C. This can prevent the growth of bacteria, but the main purpose is to stop development of the very young embryo. After oviposition, temperature is the primary catalyst which influences embryonic development (see French, 2002). As assessed by microscopic embryonic staging methods (Eyal-Giladi and

Kochav, 1976), storage of fertile Single Comb White Leghorn eggs for various lengths of time at 14°C stopped all observable embryonic development (Table 1; Fasenko *et al.*, 1992). Using a similar microscopic staging technique developed for turkey embryos (Gupta and Bakst, 1993), it was shown that turkey embryonic development during storage for 3, 7 or 14 days at 15°C continued from Stage 7 (the stage most common at the time of lay) to a Stage of 8, and then ceased (Bakst and Gupta, 1997). The minimum temperature above which embryonic development occurs (physiological zero) has been reported at two different levels. Edwards (1902) reported the minimum temperature for embryonic development to be 21°C, while Funk and Biellier (1944) found this minimum temperature to be 28°C. Previous researchers have hypothesised that the minimum temperature for embryonic development is not the same for all developing tissues of the early growing embryo (Kaufman, 1948). Therefore the objective of storing eggs at temperatures well below physiological zero is to prevent abnormal growth of the embryo which could occur if eggs were held at temperatures between physiological zero and normal incubation temperatures of 37.5°C. The generally accepted optimal range for hatching egg storage in the industry is between 14 and 18°C. Different storage temperatures and conditions are recommended depending on the length of storage (See reviews by Proudfoot and Hamilton, 1990; and Meijerhof, 1992).

**Table 1** Mean stage of chicken embryonic development after various storage periods at 14°C

Duration of egg storage (days)	Mean stage of embryonic development <sup>1</sup>
0	9.90 ± 0.04
4	9.97 ± 0.04
7	9.93 ± 0.04
14	9.93 ± 0.05
21	9.89 ± 0.05

<sup>1</sup>Least square means and SEM. Embryonic development (Eyal-Giladi and Kochav, 1976) not affected by length of egg storage ( $P = 0.5815$ ).



**Figure 1** Comparison of photomicrographs of six peri-vitelline complexes from eggs stored for 1 or 14 days. (Print magnification 3000X; scale: 30  $\mu\text{m}$  = 10  $\mu\text{m}$ .)

Hatching eggs can be successfully stored for up to seven days with little or no effects on hatchability. However, when fertile eggs are stored for more than one week, embryonic abnormalities and mortality increases, which causes a decline in hatchability. Mayes and Takballi (1984) and Meijerhof (1992) have reviewed the relevant literature on this topic. In addition to this, the quality of chicks hatching from long-term stored eggs is reduced (Becker, 1960; Merrit, 1964; Whitehead *et al.*, 1985). Many research projects have been devoted to the subject of long-term storage of fertile eggs by the primary author and the results are described in more detail below.

## EFFECTS OF LONG-TERM EGG STORAGE

### Egg quality

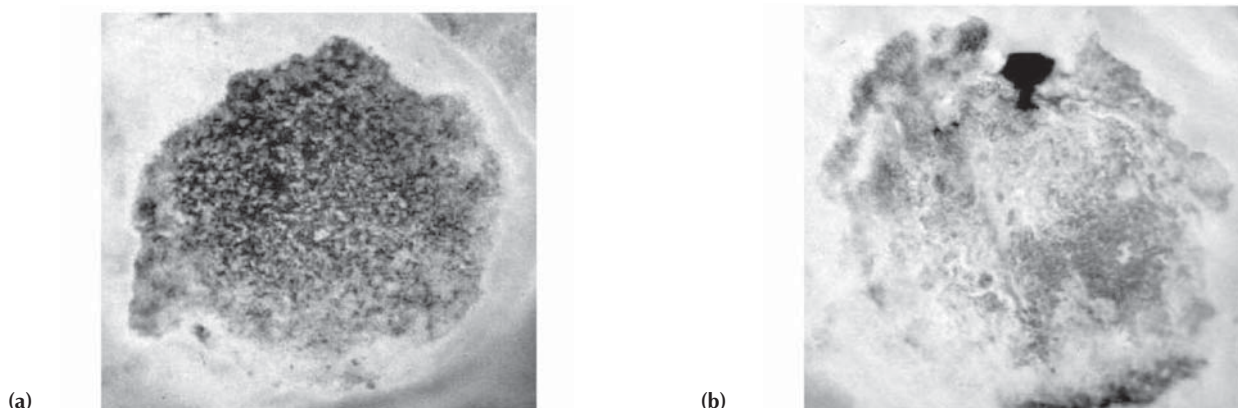
It was observed that the yolk membranes (peri-vitelline complex) of eggs stored for 14 days were extremely weak and ruptured very easily (Fasenko,

unpublished data). This observation had been noted by previous investigators (Fromm, 1964; Britton, 1973). The incidence of yolk mottling is higher in long-term stored eggs (Fasenko, personal observations; Britton, 1973). Yolk mottling describes a change in the coloration of the yolk, giving it a patchy appearance. The mechanisms involved in yolk mottling are unknown, but water movement from the albumen into the yolk is associated with the mottling. From these observations a hypothesis was formed that the thickness of the peri-vitelline layers in 14 day versus 1 day stored eggs would be reduced due to water movement into the yolk and a subsequent distension and weakening of the peri-vitelline complex. To test this hypothesis, fertile turkey eggs were stored for 1 or 14 days, the peri-vitelline complexes were sampled at the circumference area of the yolk (vertically between the animal and vegetal poles, and horizontally between the two chalazae), and prepared for transmission electron microscopy (Fasenko, 1996). The thicknesses of the

**Table 2** Average thickness of the peri-vitelline complex and the layers of which it is composed for eggs stored for 1 or 14 days

Duration of egg storage	Egg no.	Thickness ( $\mu\text{m}$ )					
		PC +	PC -	IPL	OPL	OPLden.	OPLdif.
1 day	1	22.0	11.2	5.2	16.6	6.0	10.5
	2	21.8	11	5.0	16.5	6.0	10.3
	3	51.0 +	8	4.0	46.0 +	4.0	41.9
14 days	1	33.3	8.6	3.4	32.8	5.2	24.4
	2	9.3	7.6	3.2	6.1	4.4	1.7
	3	24.9	11.5	3.9	20.9	7.6	11.6

PC + = inner peri-vitelline layer (IPL) + outer peri-vitelline layer (OPL); PC - = inner peri-vitelline layer (IPL) + outer peri-vitelline dense area (OPLden.); OPL = outer peri-vitelline diffuse area (OPLdif.) + outer peri-vitelline dense area (OPLden.).



**Figure 2** Photomicrographs of an Eyal-Giladi and Kochav (1976) Stage X domestic fowl blastoderm (embryo) (a) and an abnormal/dead blastoderm (b). Both embryos were sampled prior to incubation. Print magnification is 16X.

inner peri-vitelline layer, and of the dense area of the outer peri-vitelline layer (Figure 1) were reduced due to storage (Table 2). In addition the density of the inner and outer peri-vitelline dense layers decreased in eggs stored for 14 versus 1 day (Figure 1; Fasenko, 1996).

It is known that the peri-vitelline complex plays an integral role in normal early avian embryonic development. New (1959) found that contact between the inner surface of the inner peri-vitelline layer of the peri-vitelline complex and the outer edges of the blastoderm is necessary for the blastoderm to expand properly. In long-term stored eggs, the "adhesiveness" of the embryo to the inner peri-vitelline layer is often observed to be greatly reduced in eggs stored for long periods (Fasenko, personal observations). There appears to be a special affinity between the cells of the early embryo and the peri-vitelline complex. Hence, because of this intimate relationship, it is logical to hypothesise that any alteration in the structure of the peri-vitelline complex may influence early embryonic development. The fact that the peri-vitelline complex structure is modified during long-term storage may provide a partial answer as to why embryos of stored eggs are more likely to die in early embryogenesis.

#### Embryonic viability

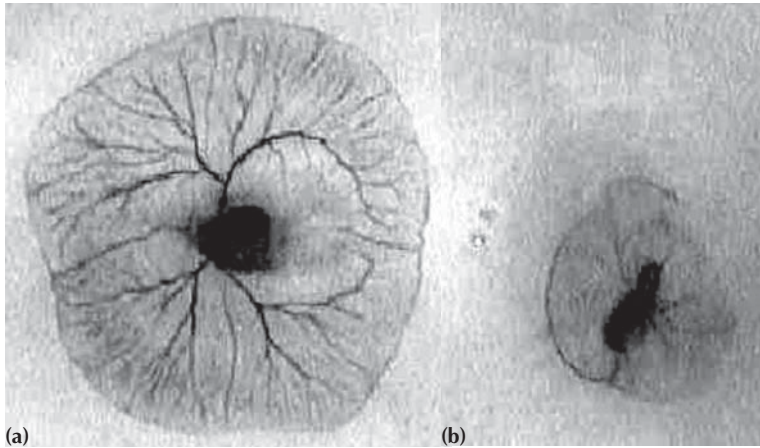
Previous researchers studying embryonic mortality in turkeys (Arora and Kosin, 1966) and chickens (Mather and Laughlin, 1976, 1977, 1979; Reinhart and Hurnik, 1976; Fasenko *et al.*, 1992) have shown that the incidence of abnormal and dead embryos (Figure 2) significantly increased as storage was lengthened. Arora and Kosin (1968) established that the number of embryonic cells with necrotic nuclei increased as the length of the storage period

increased. This reduction in viable embryonic cells may be the reason behind the observations of blastoderm shrinkage when eggs are stored for 7 or 14 days (Mather and Laughlin, 1979).

A study by Bloom *et al.* (1998) showed that egg storage for 14 days at 12°C increases the number of apoptotic cells (cells programmed to die). It is suggested that long-term exposure of avian embryos to storage below physiological zero may increase the number of necrotic (dead) cells or may induce unscheduled apoptosis, thus increasing the ratio of non-viable to viable embryonic cells. This may explain why there is a higher percentage of abnormal or dead embryos occurring in long-term stored eggs as there may be an optimum number of viable embryonic cells required for initiation of normal growth and development. Research currently underway by the principle author and colleagues is aiming to clarify the relationship between long term storage and the incidence of necrotic and apoptotic cells.

#### Embryonic development

It is well known that incubation time required for a chick to hatch is extended when hatching eggs are stored for long periods (Kosin and Konishi, 1973; Mather and Laughlin, 1976, 1977). Researchers have suggested two possible reasons for the increased incubation period needed. The first reason may be that embryonic development in stored eggs does not begin immediately after normal incubation temperatures are provided (Arora and Kosin, 1966). The second is that embryonic development in embryos from stored eggs proceeds at a slower rate throughout the first periods of incubation. Mather and Laughlin (1977) showed that embryonic growth in embryos



**Figure 3** An embryo from an egg stored for 4 days (a) versus and embryo from an egg stored for 14 days (b). Both eggs were incubated for 96 hours post-storage. The biological age of the embryo from the 14 day stored egg lags behind the chronological age by approximately 10 hours.

from eggs stored for 14 days was about 12.2 hours behind that of embryos from non-stored eggs.

It has been verified that embryos of eggs stored for 14 days were at a less developed biological age than eggs stored for 4 days even though the chronological age (amount of incubation time) in both groups was equal (Fasenko, unpublished data; Figure 3). In this study, at three hours of incubation there was no significant differences in development between the embryos from 14 versus 4 day stored eggs, but as early as six hours of incubation the development of the embryos from 14 day stored eggs began to lag behind (Table 3). Further examination of the embryonic development during the first 12 hours of incubation showed that not all embryos react in the same manner to long term storage. Even after 12 hours of incubation some embryos of 14 day stored eggs still had not initiated development and were at Stage X, which is the most common stage of embryonic development at the time of lay (Table 3). Other embryos began

development, but at a slower rate and only reached Stages XIII or XIV, while the development of other embryos appeared unaffected by the 14 day storage period, and these embryos managed to reach stages of development that embryos from their 4 day stored counterparts attained (Table 3).

### Embryonic metabolism

In another study it was shown that the metabolism of embryos from 15 day stored eggs, as measured indirectly by embryonic CO<sub>2</sub> output, proceeds at a slower rate than embryos from 4 day stored eggs (Fasenko, unpublished data; Figure 4). This indicates that not only do the embryos from long-term stored eggs lag behind in development, but

their metabolism is somehow altered as well. Evidence for this comes from recent studies in turkeys (Fasenko, 1996) and in broiler breeders (Christensen *et al.*, 2001). In turkeys, embryos from 14 day stored eggs relied more upon gluconeogenesis during pipping and hatching than did embryos from 4 day stored eggs (Fasenko, 1996). Long-term egg storage may change the biological quality of an embryo such that its metabolism is not as efficient as an embryo from a short-term stored egg. Christensen *et al.* (2001) showed that the carbohydrate metabolism of a line of broiler breeders that was resistant to the deleterious effects of storage was compared to a broiler breeder line susceptible to embryonic mortality due to storage. Embryos from the line resistant to storage maintained larger glycogen reserves in the muscle and heart tissues than did embryos from the line susceptible to storage. This fact may provide an advantage in that adequate carbohydrate reserves in the storage resis-

**Table 3** Comparison of the numbers of embryos at each stage of embryonic development for each egg storage length at 3 ( $P=0.3450$ ), 6 ( $P=0.0043$ ), and 12 hours ( $P=0.0031$ ) of incubation

Incubation (hours)	Storage length (days)	n <sup>1</sup>	Stages of embryonic development (as you go from left to right embryonic development is more advanced)								
			EKG <sup>2</sup> IX	EKG X	EKG XI	EKG XII	EKG XIII	EKG XIV	HH <sup>3</sup> 2	HH 3	HH 3 +
3	4	12	0	6	4	2	0	0	0	0	0
3	14	12	0	9	3	0	0	0	0	0	0
6	4	18	0	<b>1</b>	12	<b>5</b>	0	0	0	0	0
6	14	16	1	<b>7</b>	8	<b>0</b>	0	0	0	0	0
12	4	19	0	<b>0</b>	0	0	<b>1</b>	8	6	2	2
12	14	19	0	<b>5</b>	0	0	<b>7</b>	5	2	0	0

<sup>1</sup>n = total number of embryos staged for development. <sup>2</sup>Eyal-Giladi and Kochav (1976) stages of development. <sup>3</sup>Hamburger and Hamilton (1951) stages of development.

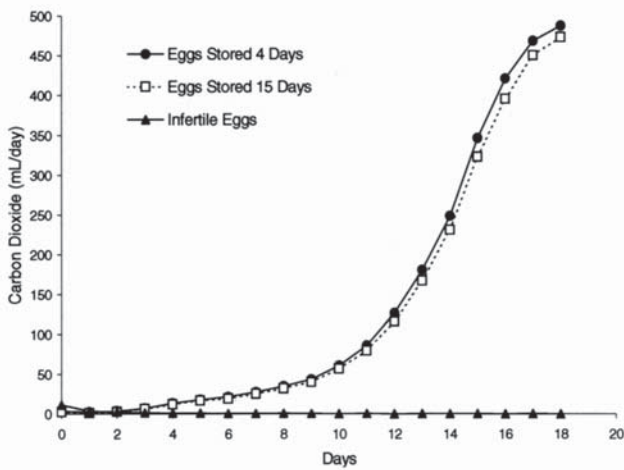


Figure 4 Carbon dioxide production of incubating eggs stored for 4 and 15 days before Incubation.

tant embryos are maintained and available for growth and function of demand tissues.

**Hatchability and chick quality**

There are negative effects of long-term egg storage on embryonic metabolism and viability and so it is not surprising that egg storage has been determined to reduce hatchability (See reviews by Mayes and Takeballi, 1984; and Meijerhof, 1992). In a recent study on broiler breeders it was hypothesised that each two days of egg storage would produce a concomitant reduction in hatchability (Fasenko, unpublished data). The results, however, showed that egg storage less than 8 days does not significantly

affect hatchability, but hatchability does significantly decline after 8, 12, and 16 days of storage (Figure 5).

Previous researchers have observed negative effects of storage on broiler body weights (Becker, 1960), mortality (Merritt, 1964) and chick quality as measured by red blood cell counts and navel condition (Whitehead *et al.*, 1985). A recent study showed that the percentage of culled chicks due to unhealed navels, deformities, and general signs of weakness was significantly higher in chicks from eggs stored for 14 days versus eggs stored for 4 days (Fasenko, unpublished data). In this study the body weight of chicks at placement was also 1.4 g lighter in the 14 versus the 4 day stored eggs, however, by 3 weeks of age this difference was erased. Irrespective of this, chicks with greater placement body weights may be better able to withstand environmental or disease challenges placed upon them.

**MANAGEMENT METHODS TO REDUCE THE NEGATIVE EFFECTS OF LONG-TERM EGG STORAGE**

In both turkeys and chickens research on incubation of eggs prior to long-term storage has proven fruitful with respect to improving hatchability. Fasenko *et al.* (2001a) found that hatchability of turkey breeder eggs was reduced from 70.9% in 4 day stored eggs to 64.4% in 14 day stored eggs. When eggs destined to be stored for 14 days were incubated for 12 hours prior to storage, the hatchability significantly improved back to the same level (70.9%) as the eggs stored for 4 days. After 12 hours of incubation, the development of the turkey embryo at the time of storage had completed hypoblast formation.

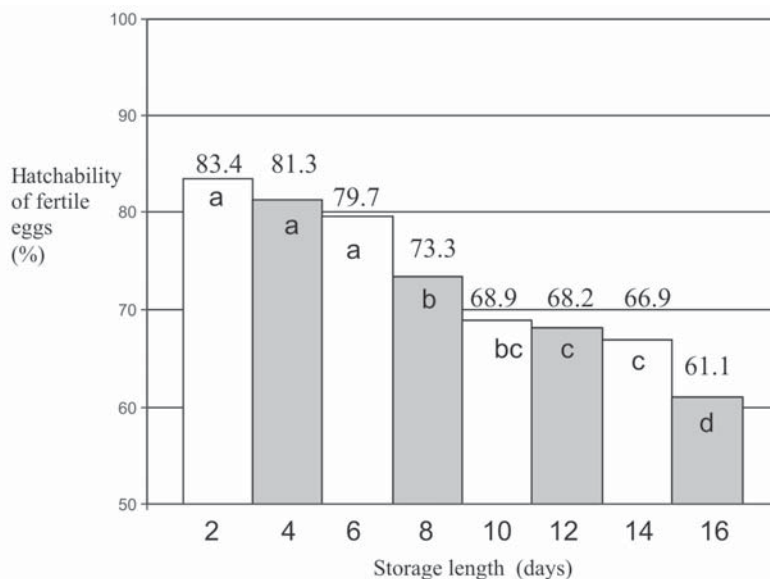


Figure 5 Hatchability of broiler breeder eggs after storage for 0, 2, 4, 6, 8, 10, 12, 14, or 16 days.

Similar research on broiler breeders has supported the results found in turkeys. The hatchability of broiler breeder eggs stored for 4 days (89.7%) significantly declined when eggs from the same flock were stored for 14 days (72.2%), but hatchability significantly improved (78.1%) when the eggs destined for 14 days of storage were incubated for 6 hours prior to storage (Fasenko *et al.*, 2001b). As with the turkey embryos, 6 hours of pre-storage incubation brought the embryonic development up to the point where hypoblast formation was complete. Without full observation of the data, it could be argued that pre-storage incubation simply provides the extra time needed for embryos of stored eggs to complete hatching. However, pre-storage incubation for 18 hours prior to storage of eggs for 14 days reduced the hatchability to a mere 11.5% (Fasenko *et al.*, 2001b). It was hypothesised that turkey and chicken embryos destined to be stored long-term do have optimal stages of embryonic development at which they should be stored. Embryos reaching hypoblast formation are at a relatively quiescent developmental period and may withstand developmental arrest better than embryos that are undergoing active periods of cellular division, migration and/or differentiation.

There may be problems with the logistics in incubating eggs before any storage and so a second study was undertaken to determine if incubation for 4, 6, or 8 hours after 3 days of on-farm storage, but before 11 days of hatchery storage would provide the same improvements in hatchability (Fasenko, unpublished data). If the results of this study showed improvements in hatchability of 14 day stored eggs, it would be easier to incubate the eggs at the hatchery rather than at the breeder farm. Additionally, holding of breeder eggs at the farm at high room temperatures (26.6°C) for 28 hours was examined prior to any storage as another method for incubating the eggs to be stored long-term. Hatchability of 14 day stored eggs declined by 19.4% compared to eggs stored for 4 days. The treatment of holding eggs at room temperature advanced embryonic development to the same developmental stage as eggs incubated for six hours. In spite of this the hatchability of eggs incubated for 6 hours and stored for 14 days total was not different from eggs not incubated prior to long-term storage. This was in contrast to the previous study that clearly demonstrated that 6 hours of incubation prior to storage for 14 days improved hatchability (Fasenko *et al.*, 2001b). The three days of storage, simulating on-farm storage, prior to the incubation treatments may have somehow reduced the beneficial effects of the incubation treatments prior to the long-term storage. Furthermore, the hatchability of the eggs held at room temperature actually declined compared

with the control of no post-farm, pre-hatchery storage incubation. This may be attributed to the fact that different embryos and embryonic cells may respond differently to the lower than normal incubation temperatures which are above or near physiological zero (the temperature below which embryonic development ceases).

## IN CONCLUSION

Researchers in the previous four decades have helped to shed light on the detrimental effects of long-term egg storage on hatching eggs and chick quality. Recent research has tried to elucidate the physiological reasons behind the reduced embryonic development and viability in embryos from long-term stored eggs. Future research on this topic should be aimed at examining how some embryos withstand the negative effects of storage and how we can use this information to improve the hatchability in all long-term stored eggs.

## REFERENCES

- Ar, A. and Deeming, D.C. (2002) Roles of water and gas exchange in determining hatchability success. In: *Practical Aspects of Commercial Incubation in Poultry*, Deeming, D.C. (ed.), pp. 21–25. Ratite Conference Books, Lincoln. Reprinted in 2009: *Avian Biol. Res.*, **2**, 61–66.
- Arora, K.L. and Kosin, I.L. (1966) Developmental responses of early turkey and chicken embryos to preincubation holding of eggs: Inter- and intra-species differences. *Poult. Sci.*, **45**, 958–970.
- Arora, K.L. and Kosin, I.L. (1968) The response of the early chicken embryo to pre-incubation temperature as evidenced from its gross morphology and mitotic pattern. *Physiol. Zool.*, **41**, 104–112.
- Becker, W.A. (1960) The storage of hatching eggs and the post-hatching body weights of chickens. *Poult. Sci.*, **39**, 588–590.
- Bloom, S.E., Muscarella, D.E., Lee, M.Y. and Rachlinski, M. (1998) Cell death in the avian blastoderm: resistance to stress induced apoptosis and expression of anti-apoptotic genes. *Cell Death Differ.*, **5**, 529–538.
- Britton, W.M. (1973) Vitelline membrane chemical composition in natural and induced yolk mottling. *Poult. Sci.*, **52**, 459–464.
- Christensen, V.L., Wineland, M.J., Fasenko, G.M. and Donaldson, W.E. (2001) Egg storage effects on plasma glucose and supply and demand tissue glycogen concentrations of broiler embryos. *Poult. Sci.*, **80**, 1729–1735.
- Edwards, C.L. (1902) The physiological zero and the index of development from the egg of the domestic fowl. *Amer. J. Physiol.*, **6**, 351–397.
- Eyal-Giladi, H. and Kochav, S. (1976). From cleavage to primitive streak formation: A complementary normal table and a new look at first stages of the development of the chick. I. General Morphology. *Develop. Biol.*, **49**, 321–337.

- Fasenko, G.M. (1996). *Factors influencing embryo and poul viability and growth during long term storage of turkey eggs*. Ph.D. Thesis. North Carolina State University, Raleigh, North Carolina.
- Fasenko, G.M., Christensen, V.L., Wineland, M.J. and Petite, J.N. (2001a) Examining the effects of prestorage incubation of turkey breeder eggs on embryonic development and hatchability of eggs stored for four or fourteen days. *Poult. Sci.*, **80**, 132–138.
- Fasenko, G.M., Robinson, F.E., Hardin, R.T. and Wilson, J.L. (1992) Variability in preincubation embryonic development in domestic fowl. 2. Effects of duration of egg storage period. *Poult. Sci.*, **71**, 2129–2132.
- Fasenko, G.M., Robinson, F.E., Whelan, A.I., Kremeniuk, K.M. and Walker, J.A. (2001b) Prestorage incubation of long-term stored broiler breeder eggs: 1. Effects on hatchability. *Poult. Sci.*, **80**, 1406–1411.
- French, N.A. (2002) The critical importance of incubation temperature. In: *Practical Aspects of Commercial Incubation in Poultry*, Deeming, D.C. (ed.), pp. 17–20. Ratite Conference Books, Lincoln. Reprinted in 2009: *Avian Biol. Res.*, **2**, 55–59.
- Fromm, D. (1964) Strength, distribution, weight, and some histological aspects of the vitelline membrane of the hen's egg yolk. *Poult. Sci.*, **43**, 1240–1246.
- Funk, E.M. and Biellier, H.V. (1944). The minimum temperature for embryonic development in the domestic fowl (*Gallus domesticus*). *Poult. Sci.*, **23**, 538–540.
- Gupta, S.K. and Bakst M.R. (1993) Turkey embryo staging from cleavage through hypoblast formation. *J. Morph.*, **217**, 313–325.
- Hamburger, V. and Hamilton, H.L. (1951) A series of normal stages in the development of the chick embryo. *J. Morph.*, **88**, 49–92.
- Kaufman, L. (1948) The effect of certain thermic factors on the morphogenesis of fowl embryos. In: *Official Report of the Eighth Worlds' Poultry Congress*. *World's Poultry Science Association*, Baelum, J. and Nielsen, A. (eds.), pp. 351–356. Nielsen and Lydich, Copenhagen.
- Kosin, I.L. and Konishi, T. (1973) Pre-incubation storage conditions and their effect on the subsequent livability of chicken embryos: exogenous CO<sub>2</sub>, plastic bags and extended holding periods as factors. *Poult. Sci.*, **52**, 296–302.
- Mather, C.M. and Laughlin, K.F. (1976) Storage of hatching eggs: The effect on total incubation period. *Brit. Poult. Sci.*, **17**, 471–479.
- Mather, C.M. and Laughlin, K.F. (1977) Storage of hatching eggs: The effect on early embryonic development. *Brit. Poult. Sci.*, **18**, 597–603.
- Mather, C.M. and Laughlin, K.F. (1979) Storage of hatching eggs: the interaction between parental age and early embryonic development. *Brit. Poult. Sci.*, **20**, 595–604.
- Mayes, F.J. and Takeballi, M.A. (1984) Storage of the eggs of the fowl (*Gallus domesticus*) before incubation: a review. *World's Poultry Sci. J.*, **40**, 131–140.
- Meijerhof, R. (1992) Pre-incubation holding of hatching eggs. *World's Poultry Sci. J.*, **48**, 57–68.
- Merritt, E.S. (1964) Pre-incubation storage effects on subsequent performance of chickens. *Brit. Poult. Sci.*, **5**, 67–73.
- New, D.A.T. (1959) The adhesive properties and expansion of the chick blastoderm. *J. Embryol. Exp. Morphol.*, **7**, 146–164.
- Proudfoot, F.G. and Hamilton, R.M.G. (1990) *Care of Hatching Eggs Before Incubation*. Publication 1573/E, Communications Branch, Agriculture Canada, Ottawa, Ontario, Canada.
- Reinhart, B.S. and Hurnik, J.F. (1976) The effect of temperature and storage time during the pre-incubation period. 1. The influence of storage changes on hatchability and first ten days chick performance. *Poult. Sci.*, **55**, 1632–1640.
- Whitehead, C.C., Maxwell, M.H., Pearson, R.A. and Herron, K.M. (1985) Influence of egg storage on hatchability, embryonic development, and vitamin status in hatching broiler chicks. *Brit. Poult. Sci.*, **26**, 221–228.

# Principles and practice of incubator design

Ron Meijerhof

*Hybro bv, P.O. Box 30, 5830 AA Boxmeer, The Netherlands*

## INTRODUCTION

To be able to incubate with success, commercial incubators have to provide an optimal climate for the developing embryo. To understand what are the crucial factors in this optimal climate, we have to look into the process of embryo development. These factors have to be facilitated in the incubation process. In this respect, we can learn from natural incubation (see Deeming, 2002a). After all, the bird is the most experienced in how to incubate, and if they find certain parts of the process of particular importance, they probably have a good reason for that.

From a biological point of view incubation, the process of turning an egg into a living healthy day old bird, is of a complexity far beyond imagination, and nothing less than a miracle. However, to be able to understand the concepts of incubator design, we can simplify the model of the process enormously.

## WHAT HAPPENS DURING INCUBATION

During incubation, the embryo builds up its body from the contents of the egg using energy obtained by burning yolk fat with oxygen. This process also produces waste products: carbon dioxide, water and heat energy. To incubate successfully in a machine, environmental conditions have to be created to facilitate this process. Heat has to be provided and the heat produced by the eggs has to be removed to keep the embryo at the correct temperature. We have to ventilate to provide the eggs with the required oxygen and remove the carbon dioxide produced. Any excess water lost from the egg that increases relative humidity above that required by the eggs has to be removed, which is also done by ventilation. Turning the egg is crucial for the developing embryo, especially during the first part of incubation (see Deeming, 2002d). Turning also influences the air velocity over the egg, and with that heat transfer.

Temperature is the most crucial climatic condition during incubation. Besides turning, egg temperature is the only process that the bird in nature controls accurately. Slight differences in temperature can have dramatic effects on embryo development and incubation results.

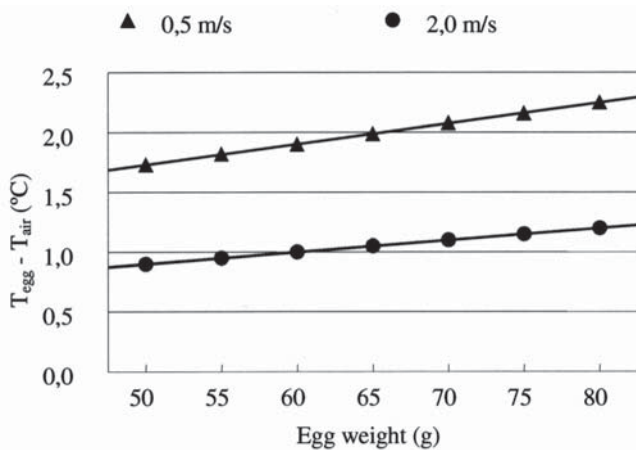
Normal embryonic development is highly dependent on temperature. This is why all commercial incubators put so much effort in controlling and maintaining a constant temperature throughout the machine. However, it can be questioned if creating a constant air temperature by itself is enough for creating optimal temperature conditions. After all, the embryo itself only experiences the temperature inside the shell, the so-called embryo temperature. Controlling the air temperature by itself assumes that embryo temperature is equal or at least highly correlated with air temperature. Although this seems logical at first sight, this assumption is in fact not correct. The embryo temperature is a result of the heat production inside the egg (metabolic heat production) and the transport of heat through the egg content and to and from the eggshell. This transport of heat is a function of several mechanisms, of which air temperature is just one.

During the development of the embryo, metabolic heat is produced, resulting in an increased temperature in the shell. About 40 years ago, this metabolic heat was determined at approx 0.14 watt per 60 g egg at 18 days of incubation (Romijn and Lokhorst, 1961). This figure is still used in the design of incubators. However, it has to be questioned whether this figure is still correct (Deeming, 2002b). After all, genetic selection has resulted in an enormous increase in growth in the broilers and it is likely that this difference in growth rate will have already had an influence on rates of embryonic growth. Recent experiments (Hulet and Meijerhof, 2001a) have shown that heat production of eggs from modern broilers is substantially higher than reported in 1960. If the heat transfer of the eggs is left unchanged this increased heat production will result in a higher embryo temperature. Interestingly, my field experience indicates that the metabolism of the embryo goes down if the embryo temperature is too high. This results in a less developed chick (Romanoff *et al.*, 1938), but also in a reduction of the heat production during incubation.

## HEAT TRANSFER

At the start of the incubation process, to create the correct temperature inside the shell for the embryo to





**Figure 1** Theoretical relation between egg weight, air velocity and the difference between embryo and air temperatures calculated for a modern broiler egg at 18 days of incubation (metabolic heat production 4,000 W per  $\text{m}^3$ ).

develop heat energy has to be transported from the air to the egg. Later in incubation the embryo starts to produce noticeable amounts of heat, which has to be removed by the air to avoid overheating of the egg. A simple model of heat exchange was given by Owen (1991) and Meijerhof and van Beek (1993) developed a more complex theoretical model to determine heat transfer of eggs. In principle there are three factors, temperature, heat capacity and velocity of the air, that influence the transfer of heat between the eggs and the air. Evaporation also influences the loss of heat from eggs. The temperature difference between the egg and surrounding air forces a flow of heat from hot to cold. The larger the temperature difference, then the more heat transfer will be obtained.

The amount of heat that a volume of air can hold, *i.e.* its heat capacity, depends on air temperature and humidity. Heat capacity also affects the thermal conductivity of air, *i.e.* the speed of the transmission of heat through a layer of heat. In general, at any given temperature difference, a high humidity in the air improves heat transfer. The influence of the thermal conductivity of air will be more noticeable for higher temperature gradients. Therefore, a large heat capacity of the air will equalise embryo temperature differences between eggs, as relatively warm eggs will lose more heat.

Air velocity has a major influence on heat transfer. High air velocity will induce a high heat transfer, where low air velocity will limit heat transfer (Figure 1). Air velocity is also an equalising factor for embryo temperature, as warm eggs will lose more heat than cold eggs with increasing air velocity.

Evaporation of water utilises a lot of energy. Droplets of water in air will cause a reduction in temperature, simply because the energy for the

evaporation will be taken from the environment. The temperature of the water, or the size of the droplets, has no influence on the energy required for this evaporation, only on the particular places in a machine where the water will be evaporated and the energy for that will be taken. Theoretically, the evaporation of water from the shell (*i.e.* moisture loss) also has a cooling effect on eggs. However, the amount of water evaporated (0.020–0.025 g per egg per hour) is not enough to make a major contribution to the temperature of the egg.

#### IMPORTANT ASPECTS OF ENVIRONMENTAL CONTROL DURING THE FIRST DAYS OF INCUBATION

During the first stages of development, the embryo is very sensitive to environmental conditions. Too high or too low temperature, no turning, and chemicals such as formaldehyde, can have enormous negative influences during the first days of embryo development.

Below a certain "physiological zero" temperature the embryo is unable to develop. Although several temperatures are reported for this physiological zero, it is generally accepted that the critical temperature for the start of development is approximately 25–27°C (Meijerhof, 1992). Little is published about the influence of the time that it takes to heat eggs from the physiological zero to incubation temperature. Our own experiments (Meijerhof and Meter, unpublished results) show that if eggs are quickly heated to incubation temperature then this has a positive effect on hatchability, especially after prolonged storage.

The temperature at which the eggs are held during storage not only has an influence on the loss of hatchability, but also on the time which is needed to heat up the egg to incubation temperature. In a practical situation we often set huge numbers of eggs in one machine. If we set 100,000 eggs of 60 grams at one time, this means that we have to heat up an egg mass equal (by volume and thermal properties) to 6  $\text{m}^3$  of water. When checking temperatures at yolk level, it shows that in some occasions it will take up to 36 hours before the last eggs are completely at incubation temperature. This will not only increase the number of early dead embryos, but also the spread in development and hatch time. For optimal incubation results, it is important that all eggs are warmed as quickly as possible to incubation temperature. This means that incubators must allow a high heat transfer during the start of incubation. Warming the eggs prior to incubation will increase the speed of temperature increase. Furthermore, after setting machines the ventilation holes in the machine should be closed

and the humidity turned off to prevent energy being used to evaporate water during this time.

## IMPORTANT ASPECTS DURING THE LAST DAYS OF INCUBATION

### Temperature

During the latter stages of incubation, metabolic heat production causes embryo temperature to rise significantly above air temperature. Due to differences in heat transfer conditions and additional effects of evaporation of water, huge differences in embryo temperature between eggs are often found at different spots in the machine. A spread of 1.5°C is quite common, and differences up to 2.5°C can also be found, even though the air temperature is at the set point. These differences are not due to differences in controlled air temperature in different parts of the cabinet, but are due to variations in air velocity and rates of evaporation of water.

Embryo temperature at the end of the incubation process has a large influence on hatch results and chick quality. We know that relatively small changes in embryo temperature can have a large effect on embryo development. In my opinion, a range of 0.2°C in the spread of embryo temperatures within a cabinet should be the goal for incubator manufacturers to achieve in their machines.

### Moisture loss

It is well known that moisture loss during incubation influences hatchability and chick quality, and that a total moisture loss of 12–14% of the initial egg weight should be achieved at external pipping. (Meijerhof, 1992). Ar and Rahn (1980) found that for a wide range of species an average moisture loss of 15% at external pipping. This value was in line with maintaining water balance within the egg. In general, losing not enough moisture seems to be more detrimental than losing too much moisture. Romanoff (1930) suggested that a limited respiratory gas exchange will contribute to this fact and low shell porosities have been shown to limit oxygen consumption in poultry species (Tullett and Deeming, 1982; Burton and Tullett, 1983). Tullett (1981) observed in commercial hatcheries that turkey eggs had good hatchability values despite a relatively wide range in moisture loss.

I believe that the fact that in nature birds are not really able to control moisture losses confirms this observation (but see Ar and Deeming, 2002). Hence if hatchability is very sensitive to rates moisture loss, birds or eggs would have a mechanism to control moisture loss. I think moisture loss becomes important

only once embryo temperature pattern is correct. It makes no sense to focus on moisture loss as a major factor if the embryo temperature is not well controlled.

One of the practical problems with moisture loss is that it coincides with heat loss. To increase moisture loss from an egg, relative humidity of the air has to be reduced. This will mean a reduction of the heat capacity and heat conductivity of the air, and therefore, will limit heat transfer and lead to an increased embryo temperature. If moisture is not needed for heat transfer, the optimal incubation settings for relative humidity might be lower than currently used allowing more moisture loss. This would be in line with the observation of Ar and Rahn (1980).

### Carbon dioxide

During the development of the embryo, oxygen and carbon dioxide have to pass continuously through the shell and membranes. Although Taylor and Kreutziger (1965, 1966, 1969) and Taylor *et al.* (1956, 1971) have shown that embryos are rather insensitive to high CO<sub>2</sub> levels at different stages of incubation, it is common practice to limit carbon dioxide levels in setters and hatchers to a certain set point to avoid damage to the developing embryo. For setters a maximum set point of 0.6–0.7% is commonly used, for hatchers this is often limited to 0.4–0.5% CO<sub>2</sub>. At the end of the incubation process, carbon dioxide levels in the air cell are 6–10 times higher than in the outside air, due to metabolic activity in the shell and limited permeability through the shell (Visschedijk, 1987). The same author reported that a certain level of carbon dioxide in the air cell is a stimulus to the embryo to hatch.

Based on this, it is often assumed that a certain minimum level of carbon dioxide has to be obtained in setter and especially in the hatcher. Although the influence of carbon dioxide in the air cell was demonstrated in experiments, it can be questioned whether this influence is that great in a commercial situation. Besides the fact that a bird doesn't seem to feel any need to control carbon dioxide, also the common use of *in ovo* injection techniques seem to indicate that this influence is limited. In large parts of the world the majority of the broiler eggs are injected at 18 days of incubation to administer Marek's vaccine, and the presence of a relatively large hole in the air cell, preventing the build up of carbon dioxide, doesn't seem to have a negative influence on hatchability.

Also carbon dioxide levels in setters and hatchers can be related to maintenance of embryo temperature. To obtain low levels of carbon dioxide, the

amount of ventilation in the machine has to be high. This means that the machines take in large amounts of relative cold and dry air that has to be heated and moisturised, which will increase the spread in embryo temperature.

### Oxygen and high altitude incubation

Under normal conditions at sea level, oxygen is not a limiting factor for incubation, especially in multi-stage machines. However, at high altitude, incubation becomes more difficult due to the limited amount of oxygen molecules per unit volume of air (Visschedijk and Rahn, 1981). Whilst this is well known there is also an effect on embryo temperature. At altitude the oxygen content of the air is reduced due to the lower pressure. This does not only hold for oxygen content but also for all the other gases, including water vapour. This means that at high altitude the moisture content of the air is reduced and with that the heat transfer of the eggs. This explains why incubators at high altitude show much more variability in embryo temperature than at sea level, although machine settings are identical.

### WHAT WILL INCUBATORS LOOK LIKE IN THE FUTURE?

Although the design of incubators has not basically changed over the last 40 years (Deeming, 2002c), I think that we have to reconsider the basic principles of the design. Incubators have worked very well over the last decades, but a continuous selection on broiler growth and meat yield puts more pressure on the incubation process.

#### Size

Over the years, setting capacity of machines has been increased to reduce the cost per egg set. It has been stated (Deeming, 2002c) that this tendency has a negative influence on hatchability, and that these problems could be reduced by bringing down the number of eggs set in a machine in order to improve the flow of air over the eggs. This by itself is true, but is not necessary the only or even best solution. In the field we are often able to improve results by setting limited number of eggs to improve air flow. I am convinced that improving the design of machines, paying more respect to heat transfer than to air temperature, will improve the results, even with increased setting capacity. In fact, I think we can increase the density of eggs in future machines even more if we take the laws of physics into account in the design.

#### Design

The design of incubators must focus primarily on controlling embryo temperature. The machines have to provide a uniform embryo temperature for all eggs, and have the capacity to control this embryo temperature. Other systems, like turning, ventilating, and humidifying, should not interfere with embryo temperature, at least not in an uncontrollable way. A maximum spread in embryo temperature of 0.3°C (0.5–0.6°F) between individual eggs in the machine must be a target.

Given the importance of air velocity on heat transfer, a big variation in air velocity is not acceptable as it will give a big variation in embryo temperature. This means that incubators must be designed to provide a forced and controlled air velocity. This can best be achieved when the basic principles of a laminar air flow are used (Owen, 1991; Meijerhof and van Beek, 1993).

A laminar air flow is heated up or cooled down when it passes over the eggs, depending on heat transfer conditions. This limits the number of eggs that air can pass before it has to be reconditioned again, to meet the goals on spread in embryo temperature. This number of eggs can be estimated by using a rather simple model (Owen, 1991) or a more detailed approach (Meijerhof and van Beek, 1993) and depends on heat production, air velocity and heat capacity of air. However, when the calculations are made, the number of eggs that can be passed before reconditioning is necessary is surprisingly low (less than 30 eggs, depending on climatic characteristics of the air used). When air velocity drops below a certain point, the air flow will change from laminar to turbulent. As turbulent air flows are more difficult to control and tend to show high variation in velocity, a minimum air velocity is required to maintain the laminar flow.

In any design, the humidifier should not interfere with embryo temperature. Energy for evaporation should not be provided by the eggs, but the water must be evaporated completely before the air reaches the eggs. The typical design with the humidifier within the cabinet and close to the eggs is unlikely to achieve this, unless steam humidification is used (which is unlikely nowadays). With any other type of humidification the air has to be conditioned to the correct water content outside the egg cabinet. If the heat transfer by air velocity and temperature difference is optimal, I expect it is possible to incubate at lower relative humidity than commonly used nowadays, resulting in a reduced negative effect of humidifiers inside the machine.

At the start of incubation, it is important that there is fast heat transfer to achieve the correct embryo temperature as quickly as possible. This means that the heating capacity of the machines must be high, which can be assisted by closing ventilation holes, to prevent loss of energy and moisture and to increase heat transfer.

With modern broiler breeds, field data shows that we have to start reduce the air temperature in single stage incubation early in the incubation process, often after only one week rather than from 12–13 days typically used. When we have optimal development of the embryo, it produces large amounts of heat in the final stages of incubation. Machines that are originally designed for water-cooling are often too limited in cooling capacity and have to rely partly on air-cooling. As this extra air has to be humidified, this often results in an increase in spread of embryo temperature.

#### Multi-stage versus single-stage

Besides beneficial effects in hygiene, it is obvious that single-stage machines have benefits over multi-stage machines in controlling embryo temperature at the desired level. The range in heat production between start (no embryonic heat) and end (maximum heat) of incubation is higher for modern high-yielding breeds as it was with the more classical breeds. This suggests that increasing difficulties can be expected with multi-stage incubation in the future (Hulet and Meijerhof, 2001b). However, the fact that single-stage machines lack the levelling effect on heat transfer of a multi-stage system will make it more important for single stage machines to have a design that pays attention to the physical laws of heat transfer.

#### Control systems

Traditionally, the control mechanisms in incubators focus on conditioning the air in terms of air temperature and relative humidity. Recently, more interactive control mechanisms have been developed that control directly or indirectly the responses of the eggs. Controlling ventilation by carbon dioxide levels can be seen as a very simple form of interactive system. A better example is recent development of the control of moisture loss by an automatic weighing system. Recent research has showed that tools can be developed that control embryo development during incubation. A relative simple solution would be to attach temperature sensors on eggs to measure continuously embryonic temperature. Hulet and Meijerhof (2001a) have shown that the incubation process can be controlled by measuring the changes

in carbon dioxide output as an indicator for development. In this way, controllers can be build that adjust machine settings according to responses in embryo development. This will really take incubation into the 21st century.

#### IN CONCLUSION

The most important climatic condition during incubation process is temperature. However, the difference in temperature of the air and the temperature that the embryo experiences can be substantial, due to metabolic heat production and factors influencing heat transfer. As modern, high yielding broilers have an increased heat production compared to the more traditional lines, the differences in embryo temperature can be substantial in modern machines, with consequently an effect on embryo development.

To control this embryo temperature and to have it uniform at any place in the machine, design of incubators must deal with the physical laws of heat transfer. Besides air temperature, the most important factors for heat transfer in incubators are air velocity, relative humidity and evaporation of water. Especially a uniform laminar air flow is essential in maintaining a uniform embryo temperature.

In my opinion, the controls systems on incubators have to change from controlling air temperature, relative humidity and ventilation (carbon dioxide levels) towards controlling embryo development and metabolic activity. Embryo temperature but also changes in carbon dioxide output can provide valuable information for the process of incubation.

#### REFERENCES

- Ar, A. and Deeming, D.C. (2002) Roles of water and gas exchange in determining hatchability success. In: *Practical Aspects of Commercial Incubation in Poultry*, Deeming, D.C. (ed.), pp. 21–25. Ratite Conference Books, Lincoln. Reprinted in 2009: *Avian Biol. Res.*, **2**, 61–66.
- Ar, A. and Rahn, H. (1980) Water in the avian egg: overall budget of incubation. *Am. Zool.*, **20**, 373–384.
- Burton, F.G. and Tullett, S.G. (1983) A comparison of the effects of eggshell porosity on the respiration and growth of domestic fowl, duck and turkey embryos. *Comp. Biochem. Physiol.*, **75A**, 167–174.
- Deeming, D.C. (2002) *Avian Incubation: Behaviour, Environment and Evolution*. Oxford University Press, Oxford.
- Deeming, D.C. (2002b) Incubation of eggs from our modern strains of chicken. *Elsevier World Poult.*, **18(3)**, 22–23.
- Deeming, D.C. (2002c) Taking hatchery management into the 21st century. *Poultry International*, March 2002, 8–15.
- Deeming, D.C. (2002d) Patterns and significance of egg turning. In: *Avian Incubation: Behaviour, Environment and Evolution*, Deeming, D.C. (ed.), pp. 161–178. Oxford University Press, Oxford.

- Hulet, R.M. and Meijerhof, R. (2001a) Real time incubation temperature control and heat production of broiler eggs. *Poult. Sci.*, **80** (Suppl. 1), 528.
- Hulet, R.M. and Meijerhof, R. (2001b) Multi- or single-stage incubation for high-yielding broilers? *Poult. Sci.*, **80** (Suppl. 1), S156.
- Meijerhof, R. (1992) Pre-incubation holding of hatching eggs. *World's Poult. Sci. J.*, **48**, 57–68.
- Meijerhof, R. and van Beek, G. (1993) Mathematical modelling of temperature and moisture loss of hatching eggs. *J. Theoret. Biol.*, **165**, 27–41.
- Owen, J. (1991). Principles and problems of incubator design. In: *Avian Incubation*, Tullett, S.G. (ed.), pp. 205–224. Butterworth-Heinemann, London.
- Romanoff, A.L. (1930) Biochemistry and biophysics of the developing hen's egg. I. Influence of humidity. *Mem. Cornell Agr. Exp. Stat.*, **132**, 1–27.
- Romanoff, A.L., Smith, L.L. and Sullivan, R.A. (1938) Biochemistry and biophysics of the developing hen's egg. III. Influence of temperature. *Cornell Agric. Exp. Stat. Bull.*, **216**, 1–42.
- Romijn, C. and Lokhurst, W. (1961) Some aspects of energy metabolism in birds. In: *2nd Symposium on Energy Metabolism*, Brouer, E. and van Es, A.J.H. (eds.), pp. 49–58. European Association of Animal Production publication No. 10. Wageningen.
- Taylor, L.W. and Kreutziger, G.O. (1965) The gaseous environment of the chick embryo in relation to its development and hatchability. 2. Effect of carbon dioxide and oxygen levels during the period of the fifth through the eighth days of incubation. *Poult. Sci.*, **44**, 98–106.
- Taylor, L.W. and Kreutziger, G.O. (1966) The gaseous environment of the chick embryo in relation to its development and hatchability. 3. Effect of carbon dioxide and oxygen levels during the period of the ninth through the twelfth days of incubation. *Poult. Sci.*, **45**, 867–884.
- Taylor, L.W. and Kreutziger, G.O. (1969) The gaseous environment of the chick embryo in relation to its development and hatchability. 4. Effect of carbon dioxide and oxygen levels during the period of the thirteenth through the sixteenth days of incubation. *Poult. Sci.*, **48**, 871–877.
- Taylor, L.W., Kreutziger, G.O. and Abercrombie, G.L. (1971) The gaseous environment of the chick embryo in relation to its development and hatchability. 5. Effect of carbon dioxide and oxygen levels during the terminal days of incubation. *Poult. Sci.*, **50**, 66–78.
- Taylor, L.W., Sjodin, R.A. and Gunns, C.A. (1956) The gaseous environment of the chick embryo in relation to its development and hatchability. 1. Effect of carbon dioxide and oxygen levels during the first four days of incubation upon hatchability. *Poult. Sci.*, **35**, 1206–1215.
- Tullett, S.G. (1981) Theoretical and practical aspects of egg shell porosity. *Turkeys*, **29**, 24–28.
- Tullett, S.G. and Deeming, D.C. (1982) The relationship between eggshell porosity and oxygen consumption in the domestic fowl. *Comp. Biochem. Physiol.*, **72A**, 529–533.
- Visschedijk, A.H.J. (1987) Air space: the embryonic medium. *J. Exp. Zool.*, **Suppl. 1**, 193–201.
- Visschedijk, A.H.J. and Rahn, H. (1981). Incubation of chicken eggs at altitude: theoretical consideration of optimal gas composition. *Brit. Poult. Sci.*, **22**, 451–460.

# Hatchery ventilation and environmental control

Joseph M. Mauldin<sup>1</sup> and Ian R. MacKinnon<sup>2</sup>

<sup>1</sup>*Poultry Science Department, The University of Georgia, Athens, Georgia, USA*

*E-mail: jmauldin@arches.uga.edu*

<sup>2</sup>*Jamesway Incubator co. Inc., 30 High Ridge Court, Cambridge, Ontario, N1R 7L3, Canada*

## INTRODUCTION

Ventilation and the maintenance of proper environmental control within the hatchery have always been critical to good hatchability and chick quality. However, in the last 10 years proper ventilation and environmental control have become more important than ever before. The poultry industry has entered an era where egg size and egg density are restricting the normal airflow patterns within incubators (Deeming, 1996). The resulting failure to remove heat, moisture, and carbon dioxide away from the developing embryos is indeed a challenge. Incubation specialists have found it necessary to initiate a different set of parameters to reduce the effects of this problem. This chapter intends to outline the principles of ventilation and environmental control and to describe the current success achieved in some commercial hatcheries. This will be done from an incubation specialist's point of view.

## SOME GENERAL PRINCIPLES

The first goal of ventilation is to bring fresh air into the hatchery to replace stale and possibly contaminated air. An operation that hatches a million chicks per week will have four or five million embryos in one building that must receive oxygen and eliminate carbon dioxide on a continuous basis. Secondly, the hatchery and ventilation system must be designed so that the fresh air does not accumulate contamination on its way to the developing embryos. The environment of each room within the hatchery must be properly conditioned with temperature and humidity to achieve optimum results.

There are three important parameters to providing good hatchery ventilation: temperature, humidity, and air volume. Heating and cooling control air temperature; humidifiers and air conditioning control humidity; and air volume is controlled by fans and dampers. There are also other factors that influence control of the hatchery environment and these are outlined below.

## Climate

It is important to consider climate in designing and operating a ventilation system. Climatic conditions dictate the type of ventilation equipment needed, the type of hatchery construction, and the amount of insulation required. Climates with distinct seasons require a full range of ventilation equipment to control temperature, humidity, and airflow. In every climate, ventilation equipment should be designed to handle all anticipated conditions. In the southeastern USA, summers are very warm and humid. Although a large percentage of hatcheries in this area use evaporative coolers they would be better served by air conditioning, especially in the setter and hatcher rooms. Evaporative coolers add moisture to the air and thereby reduce the temperature. This cooling "effect" is due to the conversion of heat energy from one form to another, namely converting sensible heat to latent heat. The water absorbs the heat of vaporisation from the surrounding air, which in turn reduces the temperature of this air. This process works very well in dry climates, as there is a need to reduce temperature and increase humidity at the same time in these climates. In warm and humid climates, temperature and humidity both need to be reduced and air conditioning is the best method to achieve this result.

## Humidification methods

Most locations will have some period of the year when the air is too cold. During these times heating and humidification will be necessary. There are several common methods to humidify air in hatcheries and these can be grouped into two main categories; methods that add heat with the moisture and methods that do not. The methods that do not add heat with the moisture are the water spray nozzle and centrifugal discharge type atomisers. Those that add heat with the moisture are steam systems. New modular type steam generation systems have created renewed interest in introducing humidity using steam. There are several advantages and disadvantages when comparing humidification that the user should be fully

**Table 1** Comparison of air conditioning equipment needed with and without insulation for a building holding 10 tunnel-type incubators (ambient air 40°C dry bulb, 24.5°C wet bulb)

Material	U value	British thermal units (Btu) per h	Tons	Estimated cost	Savings
No insulation	0.25	438,488	37	US\$73,081 (£47,149)	
5 cm insulation	0.10	369,188	31	US\$61,531 (£39,697)	US\$11,550 (£7,450)
10 cm insulation	0.05	346,088	29	US\$57,681 (£37,214)	US\$15,400 (£9,935)

U value = overall coefficient of heat transmission, Btu per hour per ft<sup>2</sup> per °F; Tons = ton of refrigeration effect where 1 ton = 12,000 Btu per hour.

aware of before investing. The most common method in the industry is to use a spray, mist or centrifugal type device. This creates fine droplets by mechanical separation and heat energy from the surrounding air is absorbed to turn the droplets into vapour form. It is essential to have sufficient heating capacity in the room heating system to supply this heat of vaporisation during the cold weather season as well as heating the incoming fresh air.

#### Heat recovery

Heat Recovery systems to capture waste heat from incubators have been attempted in various locations around the world with, at best, mixed results. While it may be argued that this exhaust is a waste of energy, it has proven more difficult to implement a simple and effective system to recover this energy. In most installations observed by the authors, the efficiency of the heat recovery was more than negated by the energy used to operate the system, therefore creating a net loss in energy.

#### Insulation

The use of insulation is vital to cost saving in a number of ways. The size and cost of the heating, ventilation, and air conditioning units are smaller when insulation is used. MacKinnon (2002) provided an example of cost savings in the size of air conditioning equipment needed in a 23 cm thick brick wall hatchery housing 10 tunnel-ventilated incubators. Comparisons of estimated equipment cost were made between no insulation, 5 cm, and 10 cm of insulation (Table 1). In addition to equipment cost savings, the added insulation insured significant savings in energy expenditure.

#### Biosecurity

One of the most basic principles of ventilation that hatcheries employ is to bring the fresh air into the hatchery from the roof, filter it, and to exhaust air from the sidewalls. In many hatcheries it is not possible to

follow this principle in every room environment due to older design, construction, and building additions. Air that is exhausted through the roof has the potential of re-entering the hatchery through the roof ventilation equipment and is a biosecurity hazard.

One must view hatchery ventilation as an essential part of the whole biosecurity program. The hatchery should be constructed away from sources of contamination or dust. For example, there are many hatcheries that are located in the vicinity of the company feed mill. As a result, the hatchery must clean and sanitise filters many times a week to reduce the potential for contamination. Other dust producing enterprises that should be avoided include processing plants, lumber yards, and live animal farms.

As another biosecurity precaution the "clean" and "dirty" areas within the hatchery may be isolated from each other by controlling the pressure within each hatchery room. The vaccine preparation room and setter rooms are examples of clean areas and must have positive pressure to avoid the entrance of contamination. Negative pressure in the dirty rooms, such as the chick take-off room, will also help achieve biosecurity. A frequent mistake is that setter and hatcher room doors are left open for several hours each day for ease of flow of workers and equipment. This results in loss of air pressure control and subsequently, hatchability performance.

#### Hatchery room environments

Environmental control within each of the various hatchery room environments may have significant influence on hatchability and chick quality. Components of a properly conditioned room environment include temperature, humidity, and pressure. Table 2 provides acceptable ranges of these conditions for the most important hatchery rooms.

The egg storage room shows a wide range in temperature recommendations. This range accounts for variable lengths of egg storage. For hatcheries of integrated poultry companies, the egg storage time is generally less than a week. For this situation, it is best to store eggs at the upper limit of the temperature

**Table 2** Recommended environmental conditions for selected hatchery rooms

Room	Temperature range (°C)	Optimal temperature (°C)	Humidity (%RH)	Pressure (kPa)
Egg storage	13–19	Depends on length of storage	75–80	Neutral
Setter and hatcher	24–29	26.6	75–80	1.2–3.7
Clean	22–26	24	Non added	6.2–8.7
Chick take-off and wash	24–27	25	Non added	– 1.2 – – 3.7
Chick holding	24–27	25	55	Neutral to outside (slightly negative)

range. Storing at the cooler end may result in condensation (sweating) on the eggs when they are brought out of storage, making them susceptible to microbial penetration. Primary breeder hatcheries typically use longer storage times, frequently 10 days or longer so that sufficient quantities of eggs from breeding stock are incubated at the same time. In this situation, egg quality can be maintained by reducing the temperature of storage to the lower range of recommended temperature. For extremely long storage (more than two weeks), other management practices should be considered to preserve egg quality. Placing trays of eggs in plastic bags and infusing with 95% nitrogen and 5% oxygen will help to maintain egg quality under long storage conditions. Storing the eggs with the small end up will reduce the egg moisture loss during storage. Some hatcheries that store eggs for a long period turn the eggs similar to when turning in the setter to prevent adhesions to the shell membranes. However, Deeming (2000) suggests that turning renews the albumen over the early embryo helping to maintain its viability. To the knowledge of the authors, there has been no research to support this practice, however, no apparent damage to the eggs occurs because of turning during storage.

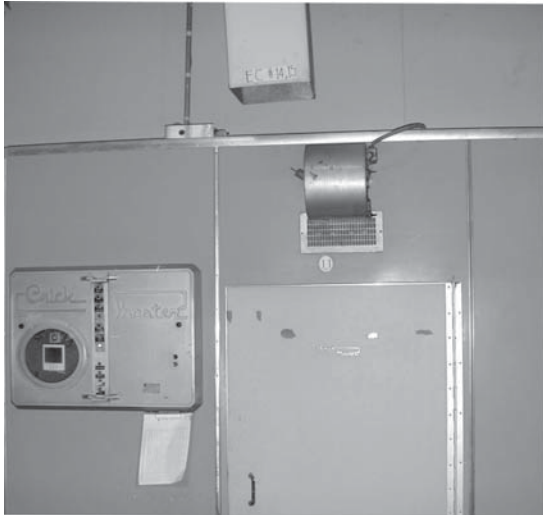
#### SETTER AND HATCHER ROOM ENVIRONMENTAL CONTROL

Proper environmental control is critical in the setter and hatcher rooms. An incubator will successfully incubate eggs even when it is placed outdoors. However, in this situation, it will neither operate efficiently nor economically, and the performance in chick quality and hatchability may be lower. For optimum performance, the incubators need to be enclosed in a room where there is plenty of fresh air that has been preconditioned with temperature and humidity, with a slightly positive air pressure compared with adjacent rooms or hallways. The content of the air with regard to temperature, humidity, oxygen, and carbon dioxide is important

for the incubators to work at their best in these rooms (see Table 2). Temperature, humidity, and pressure control insure that the machines perform at their optimum. For example, when a setter room is too cool, the incubator will provide additional heat to achieve the correct incubation temperature. However, heating the air with electric heating coils in the setter will cost about three times as much as when the room air is properly heated with a gas furnace before it enters the incubator. Furthermore, incubator dampers will close, reducing air exchange, and may result in elevated carbon dioxide levels. Similar non-optimum results will occur when the room air is too dry. Incubators respond to dry air by adding mist to elevate humidity. The mist results in evaporative cooling, closing of dampers, and elevation of CO<sub>2</sub>.

The location of the heater and cooler vents are important to provide patterns of airflow that will facilitate machine performance. Air outlets should be located near the ceiling and have their louvres positioned so that the air is thrown into the room horizontally. When the horizontally moving air from one outlet meets the air from another outlet coming in the opposite direction, the airstreams will be forced downward and eventually into a cyclic pattern of flow. These patterns insure proper mixing of the air so that uniform temperature and humidity conditions are maintained throughout the room. The incorrect placement of air vents, pointed downward or at a slant, will not result in good flow patterns or optimum mixing. More serious mistakes are made when the vents throw air directly over the air intakes of the incubators. An example of this type of mistake is illustrated in Figure 1. Here the fresh air from the vent is directed exactly into the intake of an incubator. During the winter the vents deliver heated air with very little humidity. Humidity is provided with humidifiers and is uniform in the air only after proper mixing. The incubator in this photo will have to work inefficiently and with reduced effectiveness.





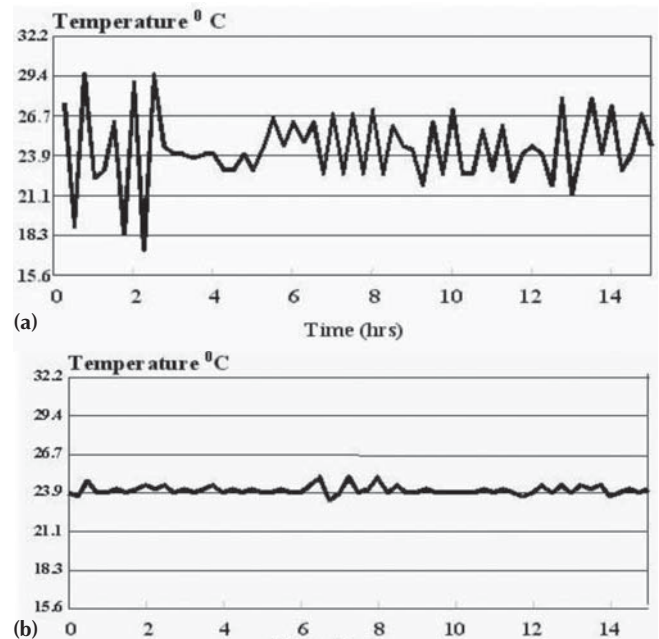
**Figure 1** An example of an incorrect location of air vent.

### Thermostat and humidistat location

The placement of thermostats and humidistats is critical to provide the correct ambient conditions in the setter and hatcher rooms. These environmental control devices work much better when they are placed in the airflow of the room and not in a “dead” spot. A common, but incorrect, location for thermostats and humidistats has been the setter or hatcher room end wall. When they are flush mounted on the end wall, it is unlikely that they will correctly “read” the room conditions and the corrections they make will be arbitrary. Mauldin (2001) provided a dramatic example of incorrect thermostat placement in a hatcher room. Figure 2A shows the temperature for a hatcher room during a 14-hour period. The thermostat was located in a “dead” air spot and made arbitrary corrections and as a result, wild fluctuations in temperature were noted. Figure 2B provides temperature recordings in the same room after the thermostat was correctly placed in the air stream. Note the dramatic difference in temperature control after correct thermostat placement. In these examples, moving the thermostat further into the room significantly improved room conditions, and subsequently machine performance.

### MICROENVIRONMENTS IN INCUBATORS

Even assuming general conditions are correct in the setter and hatcher rooms, micro-environmental conditions may occur within the incubators that lower performance. These may be due to poor machine maintenance or increasing egg size. Air, like water, flows in the path of least resistance. It is easier for air to pass through poor door seals or warped, open baffle doors than for it to flow through the mass of eggs in an

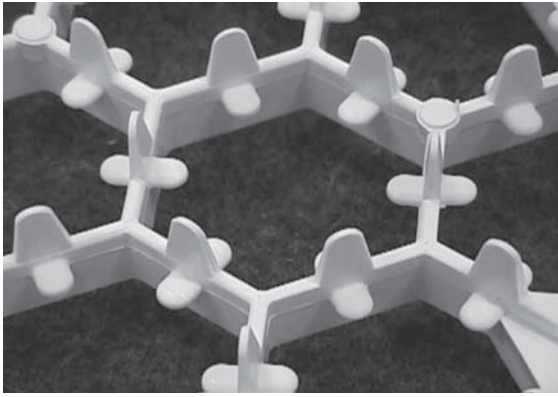


**Figure 2** The temperature of a hatcher room before (a) and after (b) the correct placement of the thermostat.

incubator. Mauldin and Buhr (1994) demonstrated the effects on temperature within masses of eggs due to a minor machine maintenance problem. Data-logging thermometers were placed in several locations within two setters. One setter had a curtain with two broken snaps and the other incubator had no apparent maintenance problem.

High temperature spikes were observed in the incubator with the broken curtain snaps while the other machine showed much more uniform temperature around the eggs. Small tasks in incubator maintenance, such as removing lime-scale formations from fan blades, make the incubators work far more efficiently.

One of the most significant advances in modern incubation has been the development of the plastic egg tray (or flat). Plastic trays were responsible for an increase in hatchability for several reasons including fewer cracks, increased egg density within the machine, and improved airflow around the eggs. However, a similar restriction of airflow due to large egg size as was seen in times of metal trays is now being observed. Some poultry personnel are convinced that the observed increase in embryonic mortality is due to recent intensified genetic selection for high meat yielding birds. Their belief is that the current embryo is in some ways different than the embryo of one decade past and has different incubation requirements. Deeming (2002) and Meijerhof (2002) have supported this view. It is commonly seen in commercial broiler hatchers that late stage embryo temperatures exceed 38.5°C. Embryo over-



**Figure 3** Plastic egg tray cells designed for increasing air space around the incubating egg.

heating is responsible for high embryo mortality, malformations, and poor chick quality (French, 1994). While it is true that embryo overheating and mortality are more common now than in the past, it may be due to restricted airflow within incubators due to the large egg size and egg density within the modern incubator. If the industry agrees to reduce the density of eggs set on each tray, as was suggested by Deeming (1998), a significantly improved hatchability would probably result. The results would probably be similar to what was observed three decades past when the industry started using plastic trays instead of metal trays. Plastic trays (Figure 3), designed with more space around each egg would significantly increase the airflow around the eggs and more efficiently remove the heat, moisture, and carbon dioxide surrounding the eggs.

Since embryo heat has recently become a significant concern, it is important for hatcheries to frequently include this parameter as a quality control check. A popular method to measure embryo temperature is the use of an inexpensive infant infrared ear thermometer, which can readily be purchased from pharmacies and department stores. Place the thermometer against the side of the egg (halfway from top) and trigger the infrared pulse for an instant digital reading of embryo temperature. When there are many embryos with temperatures exceeding 38.5°C adjustments must be made in incubator operation such as increasing airflow, reducing set tempera-

ture and humidity, and transferring eggs earlier from setter to hatcher. There is some discussion in the industry that adding oxygen to the incubator would help. In recent years single stage incubation is receiving renewed interest as a means to control embryo heat, moisture loss, and carbon dioxide.

### SUMMARY

Hatchery ventilation and environmental control play a major role in the success of commercial hatcheries. Manipulating hatchery, room and incubator environmental parameters is necessary to keep pace with the rapidly changing breeding stock and increasing automation in the industry. Fine tuning environmental control may postpone significant changes in the type of ventilation needed and the need for expensive changes such as adding oxygen to the incubator environment.

### REFERENCES

- Deeming, D.C. (1996) Large eggs - an incubation challenge. *Poult. Int.*, **35**(14), 50–54.
- Deeming, D.C. (1998) Hatchery design into the 21st century: an embryologist's perspective. *Elsevier World Poult.*, **14**(8), 25–27.
- Deeming, D.C. (2000) Storage of hatching eggs. *Poult. Int.*, **39**(13), 44–50.
- Deeming, D.C. (2002) Incubation of eggs from our modern strains of chicken. *Elsevier World Poult.*, **18**(3), 22–23.
- French, N.A. (1994) Effect of incubation temperature on the gross pathology of turkey embryos. *Brit. Poult. Sci.*, **35**, 363–371.
- MacKinnon, I.R. (2002) Principles of hatchery ventilation. *Proc. Univ., Georgia Hatchery Breeder School*, Athens, Georgia.
- Mauldin, J.M. (2001) Factors affecting hatchability. In: *Commercial Chicken Meat and Egg Production*, Bell, D.D. and Weaver, W.D. (eds.), pp. 727–774. Kluwer Academic Publishers, The Netherlands.
- Mauldin, J.M. and Buhr, R.J. (1994) What is really happening in your incubator? *Int. Hatch. Pract.*, **9**(5), 19–22.
- Meijerhof, R. (2002) Design and operation of commercial incubators. In: *Practical Aspects of Commercial Incubation in Poultry*, Deeming, D.C. (ed.), pp. 41–46. Ratite Conference Books, Lincoln. Reprinted in 2009. *Avian Biol. Res.*, **2**, 81–86.

# Hygiene and microbiological control in hatcheries

**Paul F. McMullin**

*Poultry Health Services, Poultry Health Centre, Main Site Lane, Dalton, Thirsk,  
North Yorkshire, YO7 3JA, UK E-mail: PaulMcMullin@poultry-health.com*

## INTRODUCTION

The development of artificial incubation is probably the technological advance that has had the greatest impact on the structure of the poultry industry. Since its inception it has been recognised that the hatchery provides some benefits in hygiene by forming a physical separation between the generations but it also introduces risks that may be summarised thus:

Firstly, the hatchery is a node of connection between multiple breeding farms, receiving eggs and dispatching trays, trolleys *etc.*, to the farms and can, therefore, facilitate movement of infections between different breeding farms.

Secondly and similarly the hatchery can be node of connection between different customer farms and facilitate movement of infections between them.

Thirdly, either breeding or customer farms can introduce infections into the hatchery through the movement of vehicles, people and equipment. Some such infections can persist and multiply in the hatchery environment.

Fourthly, the close proximity of large numbers of eggs and chicks from different parent sources facilitates transfer of infections among them.

Finally, hatchery staff can introduce further infections in handling eggs and chicks and also become infected with infections of zoonotic significance.

The major tools used in controlling these risks are disinfectants and sanitisers. The principles and practices governing their use is covered in detail elsewhere (Lister, 2002). However, given the range of risks involved, this subject is much broader than disinfectants.

For instance, full consideration needs to be given to the key role of hatching egg hygiene prior to delivery at the hatchery. This is influenced by the health of the parent flock, both with respect to its ability to produce eggs of normal internal and shell quality, and to its status with respect to infections which may be transmitted vertically through the egg (Mauldin, 1983). Numbers of floor eggs, nest box hygiene, method of cleaning and on-farm storage and sanitation can all

significantly alter the microbial burden coming into the hatchery. Exposure of eggs to moisture by whatever means (*e.g.* fogging, condensation, *etc.*) can considerably increase microbial risks by facilitating bacterial movement through the egg shell. Washing of hatching eggs, if practised, needs to be very carefully controlled with respect to water quality, temperature and sanitiser strength. If these aspects are neglected then egg washing is capable of doing more harm than good.

It must also be recognised that chick quality is an amalgam of physical quality and microbiological quality. Problems of chick development relating to disease or malnutrition of the parents, or poor or variable physical parameters in incubation will tend to increase susceptibility to the effects of microbial contamination, for instance by allowing tissue invasion from poorly healed navels.

## MICROBIAL TARGETS FOR HYGIENE

A broad range of micro-organisms may be transmitted through the hatchery and many can reside and multiply therein. Most are bacteria commonly associated with the farm environment (*Pseudomonas sp.*, *Proteus sp.*, *Escherichia coli*, Staphylococci, Enterococci). Currently the fowl-specific *Salmonella* species (*S. pullorum* and *S. gallinarum*) and the 2 major serotypes of human health significance (*S. enteritidis* and *S. typhimurium*) are well controlled in Europe and some other countries. A range of other Salmonellae are occasionally transmitted on the surfaces and on shell membranes of hatching eggs (and more rarely in the egg contents). Some of these (*e.g.* *Salmonella senftenberg*) are particularly prone to become resident in the hatchery environment.

The role of the hatchery in transmitting anaerobic organisms such as *Clostridium perfringens* is unknown but clostridial enteritis can certainly be seen in chicks as young as 4–5 days of age. *Mycoplasma synoviae* and *M. gallisepticum* cannot reliably be controlled by egg and hatchery hygiene as the organisms survive inside the egg and embryo. The main role of hygiene with these infections is to control cross-contamination when a hatchery is hatching both positive and

negative chicks. Of the fungi, *Aspergillus fumigatus* is by far the most important.

Brief mention should also be made of viruses. Hatcheries were identified to be of significance in the Newcastle Disease (ND) outbreaks in Northern Ireland in 1998 and in Italy in 2001. Since this virus is lethal for embryos it is suggested that the virus persisted on shell membranes or the shell and infected the next generation after pipping. Many viruses are more resistant than ND virus so it seems likely that poor eggshell surface sanitation could be a factor in the transmission of other viral infections. However, unlike bacteria they do not multiply until they actually induce an infection in an embryo or newly-hatched chick.

#### PLANNING FOR HYGIENE IN HATCHERY DESIGN AND OPERATION

It is well recognised by the industry that progeny flocks derived from a single parent flock have improved health status and productivity as compared to those derived from multiple parent flocks. The exact mechanism of this effect is not known but it may well be related to the mixing of micro-organisms from different flocks. The pressure in some countries to reduce use of antibiotics in agriculture, both as "growth promoters" and as therapeutic agents, may well exacerbate this difference.

Planning egg movements and sets to facilitate the composition of sets to the required progeny flock composition may be regarded as the basis for good hatchery hygiene. The microbiological risks highlighted above will be further magnified if there are regular movements of eggs between different hatcheries, so this should be minimised. Eggs are usually sanitised by fumigation on arrival at the hatchery even if they have been previously sanitised on farm. Good planning facilitates sanitation of the eggs and of transport vehicles. Planning of hatchery layout and work practices also had a major impact on hygiene. Wherever possible, people, eggs, chicks and equipment should move through the hatchery in a consistent fashion (from egg reception to chick dispatch). Where necessary, the risks associated with "counter-flow" events may be controlled by appropriate attention to equipment hygiene, protective clothing, footwear and hands. Education of hatchery staff as to the reasons for plans and procedures should improve compliance and also result in a better outcome when staff have to respond to contingencies not envisaged in the planning process.

#### SANITATION AND DISINFECTION PROCEDURES

Hatchery hygiene programmes may be divided into the following general areas: (1) Sanitation of eggs and buggies arriving from farms (usually by fumigation). (2) Cleaning and disinfection of single-stage setters and all hatchers between batches. (3) Periodic cleaning and disinfection of multi-stage setters and all areas not directly associated with the hatch. (4) Rigorous cleaning and disinfection of areas and equipment associated with the hatch. (5) Washing and disinfection of setter trays, egg buggies, hatcher trays, chick boxes.

In each application the key aspects are effective removal of organic material usually with the aid of detergents, followed by application of disinfectant at the required concentration and under physical conditions to optimise efficacy. Consideration needs to be given to possible adverse interactions among different chemicals. For most purposes it is preferable that the product used has some residual activity. The exception relates to vaccination equipment (see below). In all such applications it is important to work in a consistent fashion and take care not to re-contaminate disinfected surfaces. Particular attention should be given to surfaces which come in contact with large numbers of eggs (e.g. automated transfer systems). Drains need to be regularly cleared of debris, washed clean and disinfected.

Equipment used for the application of vaccines, especially those administered by injection, require special care. Vents on Marek's Disease vaccine bottles are best fitted with filters to avoid contamination during vaccination. All vaccination equipment needs to be effectively sanitised or replaced between different hatches. For injection equipment this is usually achieved by heat treatment or use of disposable components. For spray vaccination systems it is usual to use chlorine-based sanitisers which do not have residual properties. Even so they must be thoroughly rinsed to avoid contaminating the next batch of vaccine with the sanitiser.

Hygiene is also a major consideration where different chicks must be vaccinated with differing live vaccines by spray. Care should be taken to separate the batches both in the hatchery and transit as contamination of chicks with low doses of live vaccines can result in adverse effects through "rolling vaccinal reaction". This occurs when some chicks are contaminated with a dose of vaccine which is insufficient to induce adequate immunity. Such chicks replicate up the virus and it successively infects other in-contact chicks over a prolonged (e.g. 2–3 week) period.

**Table 1** Scoring system where each swab is applied to a third of a 90 mm petri dish. Total viable count and coliforms are read after 24 hours incubation, yeast and mould after 48 hours incubation, all at 37°C

Score	Colonies	Status
0 or -	0	Very clean
1 or +	1-10	Slightly contaminated
2 or ++	11-100	Moderately contaminated
3 or +++	More than 100	Heavily contaminated
4 or ++++	Confluent	Very heavily contaminated

Where *in ovo* vaccination is practised in a hatchery the equipment manufacturer’s recommendations must be followed to the letter. General hatchery hygiene must also be of a high standard given that eggs are punctured and left unsealed at transfer. *Aspergillus* is a special hazard in this circumstance.

**MONITORING HYGIENE IN HATCHERIES**

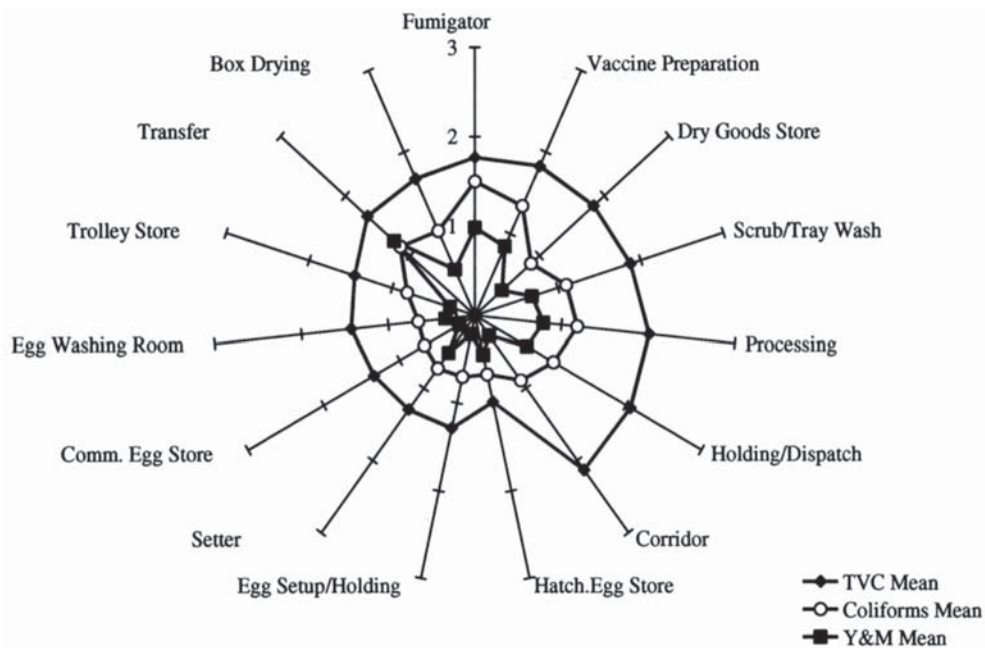
The *Assured Chicken Production* scheme in the United Kingdom requires that participating hatcheries maintain a “Health Plan” in relation to any medicines or vaccines used in chicks produced in the hatchery. It is helpful if the health plan also describes the sanitation programme and system for monitoring its efficacy and these have a major impact on the health status of chicks produced. Such plans are especially useful for demonstrating compliance with the requirements of customers, regulators, retailers, and assurance schemes.

The backbone of the monitoring programme should be simple checks of physical cleanliness (e.g. by visual inspection and use of check lists), stock control and usage of disinfectants, concentration of active ingredients, temperatures, etc.. This should be supplemented with appropriate microbiological testing carried out in a consistent fashion to facilitate the recognition of trends. It is recommended to carry out full veterinary audits associated with microbiological sampling at 2-4 month intervals according to the requirements of the hatchery and to arrange intercalated testing by hatchery staff. Some authorities recommend that this testing be done on non-hatch days but this would prevent the identification of significant incidents of recontamination which occur during normal operation.

**Hygiene testing**

Microbiological sampling should be conducted in the normal flow of eggs, typically testing 30-40 surfaces (each of an area of approximately 16 cm<sup>2</sup>) with use of the single moistened swab to provide a score for Total Viable Count (TVC), coliforms, and yeast and moulds. Surfaces sampled should include eggs, surfaces which come in contact with eggs and/or chicks, and cleaning equipment. A scoring system is shown in Table 1.

Most sanitised surfaces should achieve a score of 0 to 2 (most of the 2’s will be at the lower end of the range). Figure 1 shows the average scores obtained from various areas of hatcheries over a 6



**Figure 1** Mean scores, based on the scheme showed in Table 1, from each area tested and representing all hatchery hygiene swabs received over a 5 year period.

**Table 2** Mean scores of the same results shown in Figure 1, but summarised according to year of submission

Year	Total Viable Count	Coliforms	Yeast and moulds
1997	1.71	1.07	0.62
1998	1.55	1.18	0.79
1999	1.30	0.89	0.66
2000	1.09	0.61	0.35
2001	0.75	0.46	0.41

year period (1996–2001), representing almost 10,000 individual swabs (McMullin, unpublished observations). These sanitation results include samples taken during visits to hatcheries and samples submitted by hatcheries for testing. Note that scores tend to increase from the egg end of the hatchery towards the chick end. This is in part related to the fact that surfaces at the chick end are usually more recently disinfected when tested and full efficacy has not yet developed. Alternatively it may be due to recontamination and lack of residual activity. One area which may seem to have an unexpectedly high score is the fumigator. This is because some hatcheries test eggs prior to and after fumigation—the scores from eggs pre-fumigation bias the mean value upwards.

Recording of the results in a computerised recording system (McMullin, 1997) facilitates extraction and summarisation of data in various ways. Table 2, for instance, shows the progressive reduction in average scores from the tested hatcheries between 1997 and 2001. This is partly a result of improved facilities and partly from improved procedures and responses to monitoring results.

**Air sampling**

Helpful as a guide to airborne flows of contamination through the hatchery, air sampling can be carried out by using calibrated air samplers or, somewhat less accurately, by the use of “settle plates”. TVC results are usually under 500 per m<sup>3</sup> in a well-ventilated hatchery in areas away from post-pip hatcher and chicks, where it can rise to 10,000 per m<sup>3</sup> or more. Nutrient agar plates, if subjected to incubation for an extra 24 hours, allow the identification of *Aspergillus fumigatus* colonies derived from airborne spores. Table 3 shows a report format incorporating a semi-graphic output and actual results from a hatchery in which chicks were still present in the chick holding area. On this occasion there were high air counts samples were taken nearer the chick areas. The transfer area was used for take-off also and was directly connected to the scrub area.

**Salmonella**

In the United Kingdom, current legislation (Breeding Flocks and Hatcheries Order 1993, associated with

**Table 3** A sample report format for air sample reports

A series of 13 air samples was collected from the hatchery environment for microbiological examination. The results were as follows

Ref	Area sampled	Litres sampled	Counts per m <sup>3</sup>		Log <sub>10</sub> Total bacteria					
			Total bacteria	<i>Aspergillus</i>	1	2	3	4	5	
1	Egg store	060	0	0						
2	Trolley store	060	0	0						
3	Setter room 2	060	50	0	####					
4	Setter S9	060	33	0	####					
5	Setter room 1	060	33	0	####					
6	Setter S5	060	50	0	####					
7	Transfer	030	2483	0	#####					
8	Box drying	060	33	16	####					
9	Hatcher room 2	060	223	0	#####					
10	Hatcher H9	060	332	0	#####					
11	Hatcher room 1	060	153	0	#####					
12	Scrub/tray wash	010	9826	0	#####					
13	Holding/dispatch	005	26066	0	#####					
	Overall mean counts		3021	1	#####					

the Zoonosis directive 92-117) places the onus on the hatchery to monitor the *Salmonella* status of breeding flocks. For broilers this requires the submission of groups of dead-in-shell and/or cull chicks from each parent flock every 2 weeks. Samples are tested by prescribed methods by enrichment culture. This is reasonably sensitive but not very specific. Hence, a positive isolate from progeny can sometimes be actually due to transmission from another parent flock, because of cross-contamination of progeny or sample in the hatchery. When a positive is obtained all parent flocks hatching on the same day should be considered suspect until proven to the contrary. The Zoonosis Directive is currently being re-drafted. It appears that no major changes in actual hatchery monitoring are envisaged however the introduction of differential actions for “*Salmonellas* of human health significance” and targets for *Salmonella* control may well affect the way we do things.

The hatchery environment should also be routinely checked for *Salmonella*. Whilst it may be possible to identify *Salmonella* on hygiene swabs it is more usual to submit each group of such swabs for enrichment testing. Suspect areas should be tested by the use of a large pad swab. Hatcher floors are a good sample source for this purpose. Perhaps the most sensitive approach to the assessment of breeder and hatchery *Salmonella* status is to test a composite of 5 chick papers from boxes collected when the chicks are tipped. This is because it is possible to use more sensitive laboratory techniques for such non-statutory testing and also the extra time delay before sample collection and large numbers of chicks represented allows ready detection of infection. Positive results should only be considered indicative because it is possible for such papers to be contaminated on the broiler farm. Identification of the bacterial serotype will usually help confirm the likely significance of any such positive isolates.

### **Aspergillus**

Settle plates of Sabouraud agar are commonly used to check for mould contamination. *Aspergillus* is sometimes associated with accumulated fluff in ventilation ducts, but can also be associated with contamination of hatching eggs entering the hatchery. The fungus grows profusely in the air space of such eggs. This may be checked by opening the blunt end of unhatched eggs in the hatch debris. It is important to take precautions to avoid the dissemination of spores from such eggs and their inhalation by the operator.

### **SUMMARY**

This paper briefly reviews the key issues relating to the practice and monitoring of hatchery hygiene. The activities described are best incorporated in a hatchery health plan and specific operating procedures. Much useful information is available from equipment manufacturers and suppliers of disinfectants. Each hatchery should have access to a poultry veterinarian to help them apply these principles in a way tailored to their own business and to take into account the health status of supply and progeny flocks.

### **REFERENCES**

- Lister, S.A. (2002) Hygiene and disinfection. in poultry management. In: *Poultry Diseases 5th Edition*, Jordan, F., Pattison, M., Alexander, D. and Faragher, T. (eds.), pp. 52–54. W.B. Saunders, New York.
- Mauldin, J.M. (1983) A Hatchery and Breeder Flock Sanitation Guide *University of Georgia Bulletin 888*. See also <http://www.ces.uga.edu/pubcd/b888-w.html>.
- McMullin, P.F. (1997) Poultry Health Monitoring—Benefits of Computerised Systems. *Proceedings of WPSA Symposium “Transfer of Knowledge in Poultry Production”*, 17–19 November Utrecht. See also <http://www.poultry-health.com/library/vetco-mputing/wpsa97co.htm>.

# Chick transport and welfare

**Malcolm A. Mitchell**

*Roslin Institute, Roslin, Midlothian, EH25 9PS, UK*

*Present address: Roslin Welfare Consultants, 12 Charlton Grove, Roslin,*

*Midlothian, EH25 9NX, UK E-mail: malcolm.mitchell5@btinternet.com*

## INTRODUCTION

"Welfare is top of the agenda for, politicians, food retailers, consumers and farmers alike. Its importance is such that all sectors of the poultry industry are subject to a plethora of welfare regulation embroiled in Legislation, Codes of Recommendation and Industry Codes of Practice." (Cruickshank, 2000).

Despite regional economic pressures world-wide poultry production continues to expand. In the year 2000 the number of broiler chick placings in the European Union was approximately 4.3 billion, for layer chicks 290 million and for turkey poults 245 million. The corresponding production figures for the UK were 809 million broiler chicks, 32.5 million layer chicks and 27 million poults (Poultry World, 2001). The placement of broiler breeder chicks in the EU during the same period was approximately 30 million with the UK contributing about 7 million to this total (Poultry Bulletin, 2002). All these birds are transferred from hatcheries to their sites of production or rearing within 1–3 days of their hatching. Chicks are transported mainly by road or in the case of breeder birds by air and road to these destinations. It is widely recognised that the husbandry of the birds during this period and the conditions under which they are maintained immediately prior to and after placement are vital in determining subsequent performance and health status (e.g. Decuyper *et al.*, 2001; Langhout, 2001).

Transport of all livestock, including poultry, is regarded as a major source of stress and reduced welfare and a major cause of these problems is the thermal micro-environment in transit (Mitchell and Kettlewell, 1998; Cockram and Mitchell, 1999; Mitchell *et al.*, 2000, 2001; Hunter *et al.*, 2001; Kettlewell and Mitchell, 2001a, 2001b; Nilipour, 2002). Many other factors may also contribute to transport stress such as handling, feed and water withdrawal, vibration, space restrictions upon behaviour, noise and pollutants (Mitchell and Kettlewell 1998; Mitchell *et al.*, 2000). A number of previous studies have addressed some aspects of the transportation environments of day-old chicks but with a

primary focus of minimising in transit losses and maximising subsequent performance. Transport conditions for day-old chicks have been reported as influencing subsequent incidence of ascites and "sudden death syndrome" (Maxwell and Robertson, 1998). It may clearly be argued that assessing the well being of chicks during a journey or appropriate experimental simulations by analysis of environmental variables and the concomitant physiological and metabolic responses, will facilitate identification and definition of optimal transport conditions and practices. These will be consistent with the imposition of minimal physiological stress and as such will ensure optimum welfare and productivity in transit and during the immediate post-transport period and later development and growth phases. This approach may be termed "physiological stress modelling" (Mitchell and Kettlewell, 1998; Mitchell *et al.*, 2000, 2001). It is the purpose of this chapter to examine the existing knowledge and scientific advances relating to the micro-environments and conditions encountered during the transportation of day-old chicks and to consider relevant associated topics and issues including welfare assessment and legislation.

## WELFARE ASSESSMENT

In order to assess the welfare of an animal under a particular set of conditions or during a specific procedure two obvious questions must be addressed. What do we mean by "welfare" and what are the appropriate and objective scientific methods that can be most usefully applied to the assessment? These apparently simple questions have challenged the minds of scientists from multifarious disciplines, moral and ethical philosophers, farmers, veterinary surgeons and all those concerned with animal well being for many years. The topic has been discussed widely (e.g. Broom, 1991) and it is beyond the scope of the present review to detail all the views and arguments offered other than to present some selected points that are directly relevant to the topic of chick transport. Two working definitions of welfare are "the state of an individual in relation to its environment"



**Table 1** Animal welfare: The five freedoms and provisions as proposed by the UK Farm Animal Welfare Council (1993)

<i>Freedom from thirst, hunger and malnutrition</i>	By ready access to fresh water and a diet to maintain full health and vigour
<i>Freedom from discomfort</i>	By providing a suitable environment including shelter and a comfortable resting area
<i>Freedom from pain, injury and disease</i>	By prevention or rapid diagnosis and treatment
<i>Freedom to express normal behaviour</i>	By providing sufficient space, proper facilities and company of the animal's own kind
<i>Freedom from fear and distress</i>	By ensuring conditions which avoid mental suffering

(Broom, 1991). This definition is employed in conjunction with measures of the individual's ability to cope with environmental challenge where difficulty in coping or failure to cope are regarded as indicators of poor welfare. Some proposed indicators of poor welfare are reduced life expectancy, impaired growth, impaired reproduction, body or tissue damage, disease, immuno-suppression, increased adrenocortical activity, behavioural anomalies and self-narcotisation (Broom, 1991). In particular, it is proposed that a reduction in an individual's control over its interactions with its environment can lead to reduced welfare.

A complementary definition of welfare (or the welfare state of a sentient animal) is "the capacity to sustain physical fitness and avoid mental suffering" (Webster, 1998). Numerous methodologies have been proposed for the measurement of these conditions but frequently the experimental design and objectives have been flawed (Webster, 1998) and have focused upon the demonstration of stress with the purpose of "proving" a preconceived prejudice. The analysis of stress responses can, however, contribute to the assessment of welfare status if employed objectively.

Thus whilst it is recognised that no stress does not necessarily equate to good welfare it may be proposed that elevated levels of stress associated with disruption of physiological homeostasis and high demands upon homeostatic effort are consistent with reduced welfare. Physiological stress modelling may thus allow definition of acceptable ranges and limits for physiological stressors (Mitchell and Kettlewell 1998; Mitchell *et al.*, 2000, 2001). In turn this facilitates identification of those environmental challenges that are outwith this envelope of "comfort" and which therefore may impose conditions likely to induce poor welfare. The objective should be the minimising of detrimental stress and the prevention or avoidance of suffering. Such an approach should be supported by other studies including behavioural analyses aimed at asking animals "what matters to them" and "how much it matters" (Dawkins, 1993; Webster, 2001). It must be recognised that assessment

of welfare is necessarily extremely complex and always requires a multi-disciplinary approach (Webster, 1998). The integration of available scientific knowledge and understanding of good husbandry practices has resulted in the "Five Freedoms" (Table 1) proposed by the UK Farm Animal Welfare Council (1993).

These freedoms "identify the elements that determine the animals' perception of their welfare state and define the provisions necessary to promote that state" (Webster, 2001). When considering the welfare status of chicks in transit it is imperative to examine how the process will influence these freedoms and to identify where the major risks to the animals' welfare may lie. Careful quantification of the environmental and procedural challenges imposed upon the animals will then allow the characterisation of the "best practice" to reduce unnecessary or excessive stress and optimisation of welfare.

It may be proposed that the approaches to definition and assessment of animal welfare presented herein are still limited in relation to the implementation of higher welfare standards and that the topic should be viewed in a wider context. For example whilst an objective scientific measure of a physiological stress response and its interpretation should be free from moral judgements clearly it is impossible to divorce moral and ethical standards from animal welfare in practice. New challenges, which impinge upon all animal production, including poultry, are arising in concert with scientific developments.

An example is the increasing understanding of fear in animals (Jones, 1996), an issue which is of central importance in procedures such as transportation. Fear can be reduced, and thus welfare improved, by a range of strategies such as environmental modification, improved human-animal interactions and nutrition (Jones, 1996). In addition selective breeding or genetic modification might be employed to alter animal behaviour or fear thus effectively reducing the welfare risk. Such approaches raise ethical issues (Grandin and Deesing, 1998; Jones and Hocking, 1999), however, often crystallised in questions such

**Table 2** Specific regulations relating to the transport of poultry chicks as presented in the Welfare of Animals (Transport) Order 1997

Space allowance	21–25 cm <sup>2</sup> per chick for day-old chicks in transport containers
Feeding, watering, travelling times and rest periods	During a journey suitable food and liquid should be available, in suitable quantities and at suitable intervals save in the case of a journey lasting less than 24 hours for chicks of all species, provided that it is completed within 72 hours after hatching
Ventilation	Means of transport and receptacles shall be constructed, maintained, operated and positioned so as to provide appropriate ventilation and sufficient air space above the animals to allow air to circulate properly
Segregation of birds	Chicks shall be segregated from other poultry except their mothers or other chicks. Birds shall not be transported in proximity to any animal which is likely to be hostile to them or the presence of which is likely to cause them unnecessary suffering

as "is it appropriate to modify the nature of an animals physiology and behaviour in order to accommodate imposed stress and abuse?" This debate will undoubtedly continue but a more balanced and holistic approach to the problems is recommended consistent with the proposals of Webster (2001) where three distinct but interdependent lines of reasoning have been integrated into a strategy for action for improved welfare: (1) A scientific understanding of the factors contributing to the welfare state of farm animals. (2) An ethical understanding of how and why we *should* respect the intrinsic value of these animals. (3) An economic understanding of the factors that determine the extrinsic value we actually *do* give to these animals. Adoption of such an approach should provide a sound platform for improvements in the welfare of livestock during production and handling, transport and slaughter procedures.

#### WELFARE LEGISLATION AND CHICK TRANSPORT

In the UK the legislation relating to the transport of chicks is encompassed by the Welfare of Animals (Transport) Order 1997–Statutory Instrument 1480 based upon Directive 95/29EC. The general provisions and requirements covering the transport of chicks are set down in Schedules I and III. All the general provisions relating to protection of animals in transit (prevention and avoidance of suffering), transport containers, fitness of animals to travel, sick animals, duties of the transporter, framework of competencies, special provisions for the transport of animals by air and animal transport certificates apply to the transportation of chicks. Other key specific issues are shown in Table 2. Noticeably absent from these regulations are specific prescriptions of thermal microenvironments. The general provisions state that the means of transport (e.g. vehicles) and receptacles shall be constructed, maintained and operated so as to protect animals against inclement weather, adverse

sea conditions, marked fluctuations in air pressure, excessive humidity, heat or cold. These provisions are supported by the designated general ventilation requirements. For other species (e.g. cattle, sheep and pigs) acceptable temperature ranges in transit have been defined (411/98 EC) and ventilation regimes and performance recommended (The Report of the Scientific Committee on Animal Health and Animal Welfare–Standards for the physical environment of animal transport road vehicles EC 1999). More recently the Report of the Scientific Committee on Animal Health and Animal Welfare "The welfare of animals during transport" (European Commission, 2002) has reiterated the importance of the in transit thermal environment and ventilation regimes and the prevention of unacceptable heat and cold stress. Continuous monitoring and recording (mandatory) of the thermal environment in transport containers has also recommended. It may be confidently proposed that similar legislation and codes of practice applying to poultry transport including day-old chicks will be adopted in the future.

#### IN-TRANSIT THERMAL MICRO-ENVIRONMENT AND WELFARE

The commercial chick transport environment may potentially compromise at least four of the "five freedoms" but that the risk of thermal stress may be major source of welfare risk. Whilst commercial breeders and producers have long recognised the necessity to maintain an appropriate thermal environment for chicks in transit (Tamlyn and Starr, 1987; Freij, 1988; Laughlin, 1989; van der Hel and Henken, 1990; Qureshi, 1991; van der Hel *et al.*, 1991) the conditions employed have been largely defined by empirical means and have been based upon minimisation of mortality rates during and following transport, and efficient productivity during the subsequent rapid growth phase. In current practice the recom-

mended temperature for chick transport is 24–26°C (Ross Breeders, 1996; Meijerhof, 1997; Weeks and Nichol, 2000) although only the breeder company's advice includes a recommendation of controlled humidity (75%RH at 24°C).

In many transported animals the physiological challenges presented by the thermal conditions are compounded by extended periods without access to food or water. It has long been thought that the day-old chick may be partially protected from such stresses by the presence of energy and water reserves in the yolk sac. Older studies proposed that yolk stores in the newly hatched chick constitute 18% of total body weight and contain approximately 2 g of lipid and 2.5 ml of water, which in the absence of excessive thermoregulatory demands represent energy (75–80 kJ) and water supplies sufficient for 3 days without further provision of food and water (MacLeod, 1980; Freeman, 1984). More recent studies have indicated that in modern day-old chicks high metabolic rate and rapid utilisation of resources in the first 24 hours post-hatch coupled to delays in transit and placement result in poorer performance and health status throughout flock life (Tanaka and Xin, 1997a; Xin and Lee, 1997; Hackl and Kaleta, 1997; Viera and Moran, 1999; Bigot *et al.*, 2001).

Major causes of in-transit and post-transport mortality and morbidity are dehydration and under-nutrition (Xin and Lee, 1997). A suggested strategy to reduce metabolic depletion during extended transport is the exploitation of the reduction in metabolic rate in crated chicks in the dark (Tanaka and Xin, 1997a). The quantity and rate of use of metabolic reserves by basal metabolism, however, is clearly not the only factor that will influence chick survival during transportation. The prevailing microenvironment may impose thermoregulatory and metabolic demands upon the chicks that will require the rapid mobilisation and utilisation of lipid or the evaporation of water. Neonatal chicks do not possess fully developed effective homeothermic mechanisms (Lamoreux and Hutt, 1939; Freeman, 1964; Dunnington and Siegel, 1984) and consequently are vulnerable to the detrimental effects of thermal loads and fatigue and dehydration. In the immediate post-hatch chick both body temperature and metabolic rate increase (Freeman, 1964), however body temperature remains labile during exposure to sub-optimal thermal environments (van der Hel *et al.*, 1991). Thus, if transportation environments are unduly hot or cold then immaturity of thermoregulatory homeostasis, including inadequacy of lipid mobilisation or efficient evaporative heat loss during thermal polypnea may result in stressful or life threatening hypothermia or hyperthermia. In addition the accelerated rates of

utilisation of energy and water reserves may result in premature depletion. Freeman (1984) estimated that reserves may be completely exhausted in as little as 8–10 hours at a temperature of 40°C.

It may therefore be proposed that in order to simultaneously optimise survival, productivity and welfare of the newly hatched chick in transit an effective strategy would be to match the thermal characteristics of the microenvironment to the biological requirements of the birds. Some previous studies have attempted to define thermoneutral or optimal environments for neonatal chicks on the basis of metabolic heat production and body temperature responses (Misson, 1976; Henken *et al.*, 1989; Gates *et al.*, 1989; van der Hel *et al.*, 1991). Generally these studies did not measure other indicators of homeostatic effort that might better define the physiological impact of the thermal microenvironment. More recently Xin and Harmon (1996) examined the effects of a range of temperatures and humidities (20–35°C and 40–17%) upon day-old chicks by measuring metabolic rate and mortality. They concluded that optimum or thermoneutral conditions occurred between 30–32°C. Xin (1997) also reported that chicks held at a constant 29°C do not exhibit a different mortality or body weight loss compared to birds exposed to as much as a 16°C cycling temperature around the same mean temperature.

Studies in my laboratory, the subject of preliminary reports (Mitchell *et al.*, 1996a, 1996b) have employed physiological stress modelling, measurement of metabolic rate and the concept of Apparent Equivalent Temperature or (AET) derived from the dry bulb temperature, water vapour density and the psychrometric constant (Mitchell and Kettlewell, 1998; Mitchell *et al.*, 2000, 2001) to determine optimum transport thermal environments for day-old chicks. All measurements were performed on chicks in commercial transport containers in calorimeter chambers housed in controlled climate rooms. Temperatures of 20–35°C accompanied by relative humidities of 50–65% and duration of exposure from 3–12 hours were employed. Metabolic heat productions ranged from  $7.8 \pm 0.3$  to  $8.7 \pm 0.9$  Wkg<sup>-1</sup> in close agreement with previously published values (van der Hel *et al.*, 1991; Tanaka and Xin, 1997a). On the basis of minimal change in body temperature and minimal alterations in basal metabolic rate, hydration state, electrolyte balance, body weight loss and plasma metabolite concentrations an optimal temperature-humidity range of 24.5–25.0°C and 63–60%RH for the transport of chicks at commercial stocking density was identified. It was emphasised that these physiologically ideal conditions are very similar to those currently employed by commercial breeders and

producers. The studies also provided evidence that if the thermal microenvironment is appropriately controlled then journey durations of at least 12 hours are wholly acceptable.

It is concluded that both productivity and welfare of day-old chicks in transit can be maintained by careful regulation of the temperature and water vapour density to these prescribed limits inside the transport containers. Further work on behavioural identification of the preferred thermal conditions for chicks in commercial transport simulations will provide further refinement and support to these strategies.

#### VEHICLE AND TRANSPORT CONTAINER VENTILATION

The ultimate determinants of the localised on-board vehicle (chick transporter) microenvironment are the prevailing climatic conditions, the addition of heat and water vapour to the load space from all sources including the bio-load (chicks), and the ventilation rate and distribution. All these issues have been extensively addressed in relation to the transport of broiler chickens at slaughter age (e.g. Hoxey *et al.*, 1996; Kettlewell *et al.*, 2000) but the corresponding characteristics of chick transporters have received less detailed study.

Up to date measurements of heat and moisture production of chicks are available for calculation of vehicle ventilation requirements (Tanaka and Xin 1997a; Mitchell *et al.*, unpublished) but only the work of Quinn and Baker (1997) appears to have examined in detail the ventilation characteristics of commercial chick transporters. That work employed full-scale experimental determination of ventilation patterns and prediction of the same by Computational Fluid Dynamics. The important findings included the observation that the presence of the load of stacked chick boxes had a channelling effect upon the air flow through the load space with significant amounts of air by-passing the chick boxes and being re-circulated. The implications of this ventilation regimen for air flow in the chick containers was seen in the temperature distributions with peak temperatures occurring in the front central boxes and cooler air by-passing the load. In addition cooler air entered from beneath the vehicle in the fully loaded configuration and reduced flow through the load as well as potentially introducing exhaust fumes in to the load space.

It must be concluded that considerably more research must address these issues and that those approaches so successful in optimising mechanical ventilation regimens for vehicles carrying other poultry and red meat species (Kettlewell and Mitchell, 2001a, 2001b; Kettlewell *et al.*, 2001a,

2001b) should be applied in future to chick transporters.

#### AIR TRANSPORT OF CHICKS

Ever increasing numbers of chicks are transported by air over long distances and may be subject to delays and periods of holding in less than optimal conditions constituting significant welfare concerns. Schlenker and Muller (1997) claim high mortalities occur in air transit due to long periods of inanition and dehydration and that these problems will be significant if the birds are still in transit 48 hours after hatching and no food or fluids are provided. Transport conditions also appear to be closely correlated with air transport when thermal conditions in the aircraft hold are poorly controlled on long haul flights (Xin and Rieger, 1994). Chick container temperatures fell rapidly by 7°C upon departure and increased by up to 10°C upon touchdown. Stressful elevated temperatures were observed during holding on the aircraft prior to take off and again upon landing.

A further issue associated with aircraft hold conditions is the reduced barometric pressure and the reduction in water vapour density observed in the recycled air. Recent unpublished studies (Mitchell and Kettlewell, 2002) have monitored temperatures and humidities throughout the load space of both Boeing 747 and MD 11 freighter aircraft carrying animals on long haul flights. Accompanying varying and heterogeneously distributed temperature profiles water vapour densities as low as  $2\text{ gm}^{-3}$  were observed. These conditions would encourage high rates of evaporation from both skin and respiratory tract and would result in potential dehydration and hypothermia in chicks under certain thermal loads.

The design of chick containers and their configuration in stacking has also been questioned (Tanaka and Xin, 1997b). In adequate passive ventilation flow through the boxes when chicks are held in warehouses prior to flights or during other holding periods in aircraft holds before take off and after landing quickly resulted in potential heat stress conditions even when external temperatures were only moderately warm. For all long distance transport of chicks it has been recommended that water and feed should be available in transit to reduce mortality and maintain welfare and productivity (Xin and Lee, 1996; Xin, 1997; Tanaka and Xin, 1997a). Water may be provided in the form of commercial hydration gels, e.g. Aqua-Jel® or Pacific Oasis®, which are simply cut in to slices and placed in each container or box.

As with road chick transporters further research is required to characterise the prevailing on-board environments, the nature of the ventilation regimes and the

consequences for the birds. Only in this way can strategies for improved conditions and welfare be developed.

## SUMMARY

The welfare of chicks of all poultry species during transportation by road or air is a matter for concern. Thermal conditions (high thermal loads or chilling) may represent one of the main sources of physiological stress and reduced welfare as well as mortalities, morbidity and production losses. Physiological modelling combined with appropriate behavioural analysis can identify the optimum transport thermal environments for chicks. When these have been established it is possible to re-examine limitations on journey duration and feeding and watering strategies. The solutions to many of these problems lie in an improved understanding of ventilation flows and regimes, the design of transport containers and vehicles and the implementation of practical engineering remedies. This applies to transport by road and air. Only by the application of sound basic science to the welfare problems of chicks in transit can practical improvements in transporters, transport procedures, codes of practice, education of personnel and supporting relevant legislation be formulated.

## REFERENCES

- Bigot, K., Tesseraud, S., Taouis, M. and Picard, M. (2001) Posthatch feeding and early development in broiler chicks. *Prod. Animales*, **14**, 219–230.
- Broom, D.M. (1991) Animal welfare—concepts and measurement. *J. Anim. Sci.*, **69**, 4167–4175.
- Cockram, M.S. and Mitchell, M.A. (1999) Role of research in the formulation of rules to protect the welfare of farm animals during road transportation. In: *Farm Animal Welfare—Who writes the rules?* Russel, A.J.F., Morgan, C.A., Savory, C.J., Appleby, M.C. and Lawrence, T.L.J. (eds.), pp 43–64. British Society of Animal Science Occasional Publication No 23, Edinburgh.
- Cruikshank, G. (2000) *Personal communication*: paper presented to Poultry World Industry Conference—Welfare Guideline Sponsors 2000—copies available from original author upon request.
- Dawkins, M. (1993). *Through our Eyes Only? The Search For Animal Consciousness*. Freeman, Oxford.
- Decuyper, E., Tona, K., Bruggenan, V. and Bamelis, F. (2001) The day-old chick: a crucial hinge between breeders and broilers. *World's Poultr. Sci. J.*, **57**, 127–138.
- Dunnington, E.A. and Siegel, P.B. (1984) Thermoregulation in newly hatched chicks. *Poult. Sci.*, **63**, 1303–1313.
- Freeman, B.M. (1964) The relationship between oxygen consumption, body temperature and surface area in the hatching and young chick. *Brit. Poultr. Sci.*, **6**, 67–72.
- Freeman, B.M. (1984) Transportation of poultry. *World's Poultr. Sci. J.*, **40**, 19–30.
- Freiji, M. (1988) Chicks need cool transport. *Poult. Int. (Misset)*, **4(3)**, 18–19.
- Gates, R.S., Turner, L.W., Turner, G.M. and Tuttle, J.W. (1989) Measured heat production in chicks. *Proc. Am. Soc. Agric. Engineers*, Paper no. 894513.
- Grandin, T. and Deesing, M.J. (1998) Genetics and animal welfare. In: *Genetic and Behaviour of Domestic Animals*, Grandin, T. (ed.), pp. 319–341. Academic Press, San Diego California.
- Hackl, G. and Kaleta, E.F. (1997) Methods for the estimation of the age of broilers and effects of water and feed deprivation within the first days of life. *Arch. Geflugel.*, **61**, 280–286.
- Henken, A.M., van der Hel, W., Hanssen, E.J.M., Scheele, C.W. and van Kampen, N.M. (1989). The effect of dry and wet bulb temperature on heat production of newly hatched chicks. In: *Energy Metabolism of Farm Animals*, Verstegan, M.W.A. and Henken, A.M. (eds.), pp. 291–294. Martinus Nijhoff, Netherlands.
- Hoxey, R.P., Kettlewell, P.J., Meehan, A.M., Baker, C.J. and Yang, X. (1996). An investigation of the aerodynamic and ventilation characteristics of poultry transport vehicles: Part 1 Full scale measurements. *J. Agric. Eng. Res.*, **65**, 77–83.
- Hunter, R.R., Mitchell, M.A. and Matheu, C. (2001) Mortality of broiler chickens in transit—correlation with the thermal micro-environment. In: *Proceedings of the 6th International Livestock Environment Symposium*, Stowell, R.R., Bucklin, R. and Bottcher, R.W. (eds.), pp. 542–549. Louisville, Kentucky, U.S.A., 21st–23rd May 2001.
- Jones, R.B. (1996) Fear and adaptability in poultry: Insights, implications and imperatives. *World's Poultr. Sci. J.*, **52**, 131–174.
- Jones, R.B. and Hocking, P.M. (1999) Genetic selection for poultry behaviour: Big bad wolf or friend in need? *Anim. Welf.*, **8**, 343–359.
- Kettlewell, P.J., Hampson, C.J., Green, N.R., Teer, N.J., Veale, B.M. and Mitchell, M.A. (2001a) Heat and moisture generation of livestock during transportation. In: *Proceedings of the 6th International Livestock Environment Symposium*, Stowell, R.R., Bucklin, R. and Bottcher, R.W. (eds.), pp. 519–526. Louisville, Kentucky, U.S.A., 21st–23rd May, 2001.
- Kettlewell, P.J., Hoxey, R.P., Hampson, C.J., Green, N.R., Veale, B.M. and Mitchell, M.A. (2001b) Design and operation of a prototype mechanical ventilation system for livestock transport vehicles. *J. Agric. Eng. Res.*, **79**, 429–439.
- Kettlewell, P.J., Hoxey, R.P. and Mitchell, M.A. (2000) Heat produced by broiler chickens in a commercial transport vehicle. *J. Agric. Eng. Res.*, **75**, 315–326.
- Kettlewell, P.J. and Mitchell, M.A. (2001a). Comfortable ride: Concept 2000 provides climate control during poultry transport. *Res. Eng. Tech. Sust. World*, **8**, 13–14.
- Kettlewell, P.J. and Mitchell, M.A. (2001b) Mechanical ventilation: Improving the welfare of broiler chickens in transit. *J. Roy. Agric. Soc.*, **162**, 175–184.
- Lamoreux, W.F. and Hutt, F.B. (1939) Variability in body temperature in the normal chick. *Poult. Sci.*, **18**, 70–75.
- Langhout, P.J. (2001) Feeding during the first week of life. *Int. Poultr. Prod.*, **9(1)**, 7–10.
- Laughlin, K.F. (1989) Computerised customer care. *Int. Hatch. Pract.*, **4**, 43–45.

- Maxwell, M.H. and Robertson, G.W. (1998) UK survey of broiler ascites and sudden death syndrome in 1993. *Brit. Poult. Sci.*, **39**, 203–215.
- MacLeod, M.G. (1980) The biology of survival: The effects of day old travel in chicks. In: *Airfreighting of Hatching Eggs and Poultry*, pp. 3–5. West of Scotland College of Agriculture Ayr.
- Meijerhof, R. (1997) The importance of egg and chick transportation. *World Poult.*, **13**(11), 17–18.
- Misson, B.H. (1976) The effects of temperature and relative humidity on the thermoregulatory responses of grouped and isolated neonate chicks. *J. Agric. Sci. (Cambridge)*, **86**, 35–43.
- Mitchell, M.A., Kettlewell, P.J., Carlisle, A.J., Hunter, R.R. and Manning, T. (1996a) Defining the optimum thermal environment for the transportation of one-day old chicks: physiological responses during transport simulations. *Brit. Poult. Sci.*, **37**, S89–S91.
- Mitchell, M.A., Kettlewell, P.J., Carlisle, A.J., Hunter, R.R. and Manning, T. (1996b) Optimo transporte de pollitos de 1 dia. *Avicultura Prof.*, **14**(3), 47.
- Mitchell, M.A. and Kettlewell, P.J. (1998) Physiological stress and welfare of broiler chickens in transit: Solutions not problems! *Poult. Sci.*, **77**, 1803–1814.
- Mitchell, M.A., Carlisle, A.J., Hunter, R.R. and Kettlewell, P.J. (2000). The responses of birds to transportation. In: *Proceedings of the World Poultry Congress*, pp. 1–14. Montreal, Canada, 22nd–24th August 2000.
- Mitchell, M.A., Kettlewell, P.J., Hunter, R.R. and Carlisle, A.J. (2001) Physiological stress response modelling—application to the broiler transport thermal environment. In: *Proceedings of the 6th International Livestock Environment Symposium*, Stowell, R.R., Bucklin, R. and Bottcher, R.W. (eds.), pp. 550–555. Louisville, Kentucky, U.S.A., 21st–23rd May 2001.
- Nilipour, A.H. (2002) Poultry in transit are a cause for concern. *World Poult.*, **18**(2), 30–33.
- Poultry Bulletin (2002) *Chicken market quarterly*, Taylor, D. (ed.), p. 15. National Farmers Union, Newmarket, Suffolk, January 2002.
- Poultry World (2001) EU facts and forecasts. *Poult. World*, September, 14–15.
- Qureshi, A.A. (1991) Losses due to dehydrated broiler chicks. *World Poult. (Misset)*, **7**(4), 75–79.
- Quinn, A.D. and Baker, C.J. (1997) An investigation of the ventilation of a day-old chick transport vehicle. *J. Wind Eng. Ind. Aerodyn.*, **67** and **68**, 305–311.
- Ross Breeders (1996). *Producing Quality Broiler Meat*. Ross Broiler Management Manual.
- Schlenker, G.R. and Muller, W. (1997) The transportation of day-old chicks by aircraft with regard to animal welfare. *Berl. Munch. Tier. Wochen.*, **110**, 315–319.
- Tamlyn, J. and Starr, J.R. (1986) Monitoring the environment during transportation of day olds. *Int. Hatch. Pract.*, **6**(1), 11–15.
- Tanaka, A. and Xin, H. (1997a) Energetics, mortality, and body mass change of breeder chicks subjected to different post-hatch feed dosages. *Trans. Am. Soc. Agric. Eng.*, **40**, 1457–1461.
- Tanaka, A. and Xin, H. (1997b) Effects of structural and stacking configuration of containers for transporting chicks in their microenvironment. *Trans. Am. Soc. Agric. Eng.*, **40**, 777–782.
- van der Hel, W. and Henken, A.M. (1990) Give newly hatched chicks a safe flight. *Poult. Int. (Misset)*, **6**(1), 8–9.
- van der Hel, W., Verstegen, M.W.A., Henken, A.M. and Brandsma, H.A. (1991) The upper critical temperature in neonatal chicks. *Poult. Sci.*, **70**, 1882–1887.
- Vieira, S.L. and Moran, E.T. (1999) Effects of delayed placement and used litter on broiler yields. *J. Appl. Poult. Res.*, **8**, 75–81.
- Webster, A.J.F. (1998) What use is science to animal welfare? *Naturwissen.*, **85**, 262–269.
- Webster, A.J.F. (2001) Farm animal welfare: the five freedoms and the free market. *Vet. J.*, **159**, 1–9.
- Weeks, C. and Nicol, C. (2000) Poultry handling and transport. In: *Livestock Handling and Transport*. 2nd Edition, Grandin, T. (ed.), pp. 363–384. CAB International, Wallingford.
- Welfare Of Animals (Transport) Order (1997) Statutory Instrument 1997 No 1480, Ministry of Agriculture, Fisheries and Food, London.
- Xin, H. (1997) Mortality and body weight of breeder chicks as influenced by air temperature fluctuations. *J. Appl. Poult. Res.*, **6**, 199–204.
- Xin, H. and Harmon, J.D. (1996) Responses of group-housed neonatal chicks to post-hatch holding environment. *Trans. Am. Soc. Agric. Eng.*, **39**, 2249–2254.
- Xin, H. and Lee, K. (1996) Use of aqua-Jel® and feed for nutrient supply during long journey air transport of baby chicks. *Trans. Am. Soc. Agric. Eng.*, **39**, 1123–1126.
- Xin, H. and Lee, K. (1997) Physiological evaluation of chick morbidity during extended post-hatch holding. *J. Appl. Poult. Sci.*, **6**, 417–421.
- Xin, H. and Rieger, S.R. (1995). Environment and mortality associated with remote air-transport of day-old chicks. *Proceedings of the American Society of Agricultural Engineers*, 1994 Winter Meeting Paper No. 944557.