Aviagen's European Technical Seminar focuses on reproduction

Recently Aviagen hosted their European Technical Seminar in the National Museum of Scotland in Edinburgh. This well attended event focused on broiler breeders and egg management and International Hatchery Practice was there to hear some excellent presentations.

Murray Bakst from the Agricultural Research Service of USDA was the main guest speaker and he addressed the topic of 'fertility and sperm storage in the breeder hen'.

Key roles of the oviduct

He started by considering the reproductive tract and highlighted the key roles of the oviduct as:

- Egg formation.
- The immunological resistance of ingressing pathogens.
- The selection, storage and transportation of sperm.
- Hosting fertilisation and the onset of embryogenesis.

He highlighted how after semen transfer, spermatozoa ascend in the vagina to the uterovaginal junction's sperm storage tubules (SSTs) in which spermatozoa can survive for days to weeks and the ability to do this is the basis for sustained hen fertility.

Numerically an ejaculation deposits some 300 million spermatozoa in the hen and within an hour 85% have been lost and, of the remaining 15%, only about five million of these (<2%) reach the SSTs.

When semen from a pooled sample is artificially inseminated into a hen 65% of the resulting progeny come from just 25% of the males and 54% of the males account for <2% of the progeny highlighting the important fact that not all males are equal when it comes to fertilising ability.

When it came to the biological basis of sperm selection in the oviduct Murray noted that:

- The spermatozoa that swim fastest reach the SSTs and it is these which fertilise the ova.
- The surface of the selected spermatozoa that reach the SSTs possess unique proteins, lipids and carbohydrates.
- In some animals, for example insects, the female 'selects' the fertilising spermatozoa deposited in the oviduct.
- The sperm selection process occurs deep within the folds of the vagina and this process has a high fall out rate. The selected spermatozoa enter the SSTs which are tubular structures at the uterovaginal junction.

He then considered the SSTs' key role in sustained hen fertility. In particular he highlighted:

- Only fit spermatozoa that are selected in the vagina enter the SSTs and it is a misconception that SSTs serve as a 'sperm reservoir' for fit spermatozoa between successive inseminations.
- Spermatozoa are gradually released from the SSTs and transported to the infundibulum where they are fertilised. This process ensures the fertilisation of a clutch of eggs without a male presence.

The function of SSTs

In non-domesticated birds in nature the function of SSTs means that if a nest of eggs is destroyed the female is often capable of laying a new clutch of fertile eggs without further male involvement.

Also, males with poor semen quality will mate numerous times to build up the number of spermatozoa in the SSTs.

Some females will mate and then feed for 3-4 weeks before returning to the nest to lay her eggs.

The prolonged survival of spermatozoa in the SSTs is a consequence of:

- Suppressed spermatozoan motility.
- Suppressed spermatozoan metabolism.
- Secretions from the SSTs' epithelial cells which provide the spermatozoa with nutrients.
- The fact that they are immunologically privileged sites because of suppressed lymphocyte proliferation and there are no antibodies against spermatozoa.

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Fig. 1. The correlation between holes in the inner perivitelline membrane and flock fertility (Wishart, 1999).

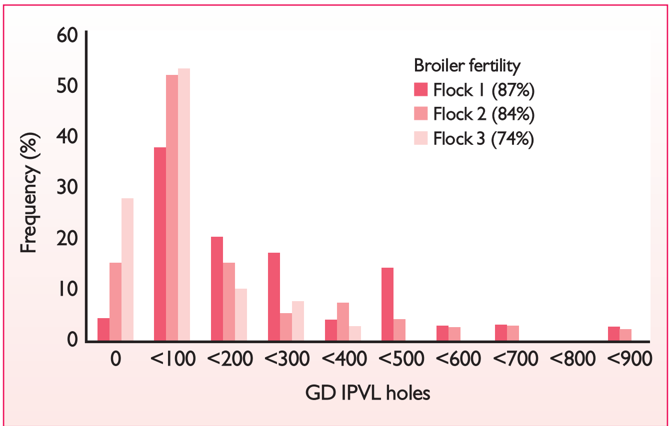
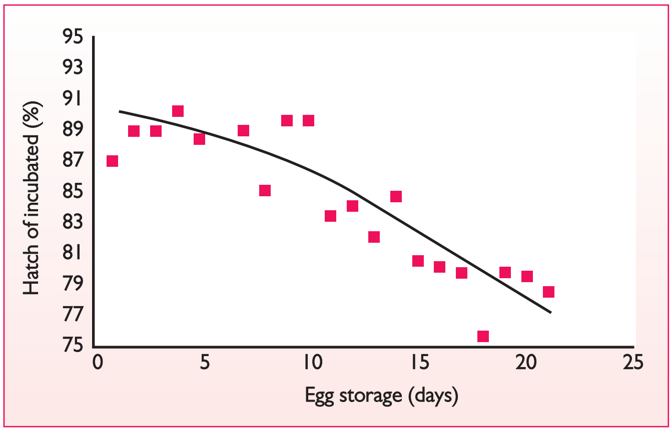


Fig. 2. The effect of egg storage on hatchability (if possible, set eggs when they are 3-7 days old).



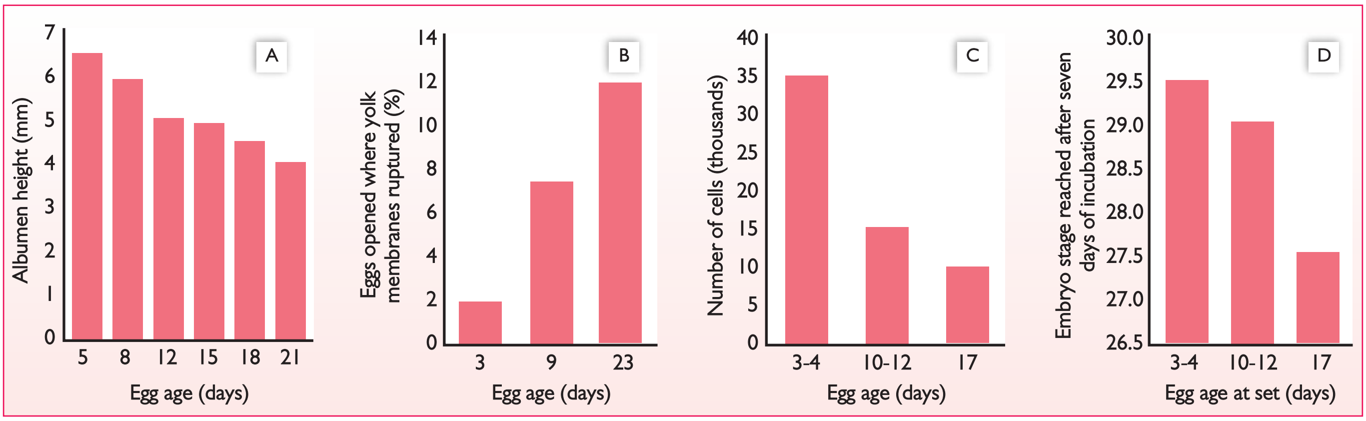


Fig. 3. Effects of egg age. A. Thick albumen thins and gets more runny. B. Yolk membranes become more fragile. C. Cells within the germinal disc die. D. Once incubation starts, the embryos have to recover the lost cells, so development is delayed.

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The SSTs are a key to sustained fertility because:

- After mating or artificial insemination, a fit population of spermatozoa enter the SSTs.
- The SSTs act as 'sperm reservoirs' for these fit spermatozoa.
- Fertility percentage is directly correlated to the number of spermatozoa released from the SSTs. If there is no spermatozoa in the SSTs there is zero fertility; with low numbers there is some fertility; and with high numbers fertility is maximised.

The process of fertilisation requires one or more spermatozoa to attach to the inner perivitelline membrane. These attached spermatozoa then digest holes through this membrane over the germinal disc and, once inside the disc, the chromosomes from one spermatozoon fuse with those of the female (syngamy).

However, it should be noted that too many spermatozoa kill the embryo after semen transfer (pathological polyspermia) and the outer perivitelline layer blocks excessive spermatozoal penetration.

Fertility can be assessed by enumerating these holes in the inner perivitelline membrane. The correlation between these holes and flock fertility is shown in Fig. 1.

Known as the sperm hole assay it can be used to determine true fertility immediately after lay and for the prediction of duration of fertility. It can also be used to assess the impact of changes such as spiking, artificial insemination, flock age and farm.

Murray then went on to consider the staging of embryonic development which is reflected in the hatch window but is also evident at time of lay and throughout incubation. If this is to be described it must be on the basis of the stage of embryonic development.

He concluded with the following remarks:

- Fertility is a complex interaction of many biological factors.
- Hens must be capable of storing sufficient numbers of spermatozoa in SSTs to assure fertility, be it by more SSTs, more fit sperm or a slower release of spermatozoa.
- How you manage the laid egg during storage and incubation will influence the future development of the hatched chick.

Egg storage

Aviagen's global manager for hatchery development and support, Dinah Nicholson, then shared her latest thoughts on egg storage. She started by considering why egg storage conditions are important:

- Suitable egg storage conditions keep the embryo and egg contents in the best possible condition for good hatchability and egg quality.
- Effective collection, disinfection, cooling and storage of eggs will impact on egg quality and embryo growth.
- Each process must be carried out so that embryonic survival and development are not compromised.

Dinah highlighted the following rules for egg storage:

- Cool eggs as soon after lay as possible to below physiological zero (around 24°C – the temperature at which the embryo starts/stops development).
- Disinfect the egg shell shortly after collection and again before the eggs are set.
- Do not let the temperature fluctuate around physiological zero.
- Set eggs that are between three and seven days old.
- Use humidity to prevent excessive weight loss during storage.

She then went on to consider the effects of egg storage. The effect on hatchability is shown in Fig. 2. As eggs age there are physical changes to the egg contents, and to the blastoderm.

When they are incubated, stored eggs produce more early deads (clears) and any bacterial contamination will be worse and the chicks that do hatch will take longer to emerge from their eggs. This rate of

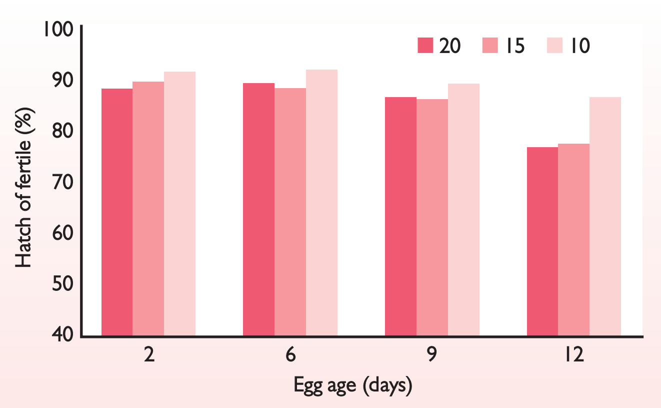

Fig. 4. Effect of storage temperature and egg age on hatch of fertile (Meijerhof et al 1994).

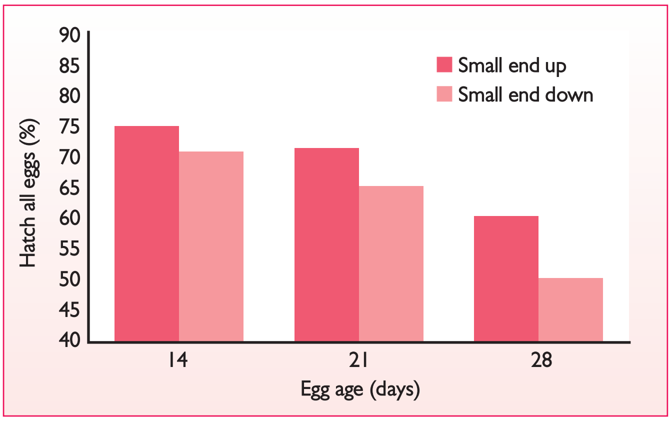

Fig. 5. The benefit of upside down egg storage. Store eggs upside down (small end up) (Proudfoot 1969).

deterioration can be slowed by using appropriate storage treatments.

Fig. 3 summarises the effects of egg age on albumen quality (height), yolk membranes, cells in the germinal disc and embryonic development.

Fig. 4 shows the effect of storage temperature and egg age on hatch of fertile.

When it comes to egg storage the following action points to improve the hatch from stored eggs were noted:

- If eggs are still warm when they are collected they need the chance to cool down evenly.
- Cooling does not have to be fast and 4-5 hours is acceptable but consistency across eggs is important.
- It is best to pack eggs directly on to setter trays and to place these in spaced racks or buggies as this allows air to flow across the eggs as they cool.
- Load buggies from the bottom so that the warm eggs are always placed above cooler ones so rewarming can not occur from rising warmed air.
- If eggs are to be boxed, cool the trays of eggs on racks before they are cooled.
- Setter trays stacked without spaces are slow to cool down in their centres, so stack them with spaces.
- Eggs do not need a sequence of different storage temperatures – if any eggs are to be stored for 14 days, embryo survival will be improved if they are held at 12°C for the whole storage period.
- Adjust egg store temperatures at the hatchery – leave the farm stores at 18-20°C to prevent condensation.
- Eggs set fresh will not be damaged by low storage temperatures – adjust the temperature to suit the oldest eggs.

The benefits of storing eggs upside down (small end up) are shown in Fig. 5. Benefits have also been seen from turning eggs during storage, although some researchers have seen little benefit in eggs stored for less than 14 days.

In nature each time an egg is laid all the eggs are warmed and turned.

The SPIDES concept

In these scenarios hatchability and hatch window are often better than one would normally expect given the age of the first egg. Due to this observation a whole new concept, SPIDES (Short Periods of Incubation During Egg Storage), came into being. Aviagen started trials on this in 2010, which have been covered in previous issues.

Dinah concluded that:

- SPIDES can be made to work.
- The amount of lost hatch recovered will be affected by the amount of time over 32°C with the best results coming from 5-15 hours and >24 hours giving no benefits.
- Absolute rates of recovery of lost hatchability varied between Aviagen's trials but typically was 50-60% and the best recovery achieved was 78%.
- It does not matter whether it takes four or eight hours to reach temperature.
- Age of eggs at time of treatment appears not to matter.

She believes that SPIDES works because the paused embryo develops to a later stage which survives long storage better and warming >32°C gives embryonic cells and cell membranes a chance for self-repair. In addition, there may also be some repair of egg membranes and systems.

In the field Aviagen have been investigating the effectiveness of SPIDES since 2011 in hatcheries around the world and many trials have been completed across a range of GGP and GP lines and crosses using a variety of incubators.

Fig. 6 shows the benefits achieved using SPIDES.

When it comes to SPIDES the following are found to have worked well:

- The use of Tiny Tags to monitor egg shell temperatures made it easier to interpret and improve results.
- Eggs can be cooled evenly by using the pre-warm setting on a single stage incubator.
- For long storage of over 12 days treatments every 6-7 days work best, but they must be short.
- The first treatment needs to be before the seventh day.

Dinah's overall conclusions were:

- Re-evaluate your egg store temperature – any eggs kept for longer than three days will probably benefit from a lower holding temperature.
- Control egg storage temperatures to avoid inadvertent fluctuation.
- Set eggs within seven days if possible.
- Hatchability after longer storage will be better if egg store temperatures are reduced, eggs are turned during storage and SPIDES is implemented. ■

Fig. 6. Aviagen's experience with SPIDES. Mean improvement at each egg age.