Embryo development and hatchery practice in poultry production

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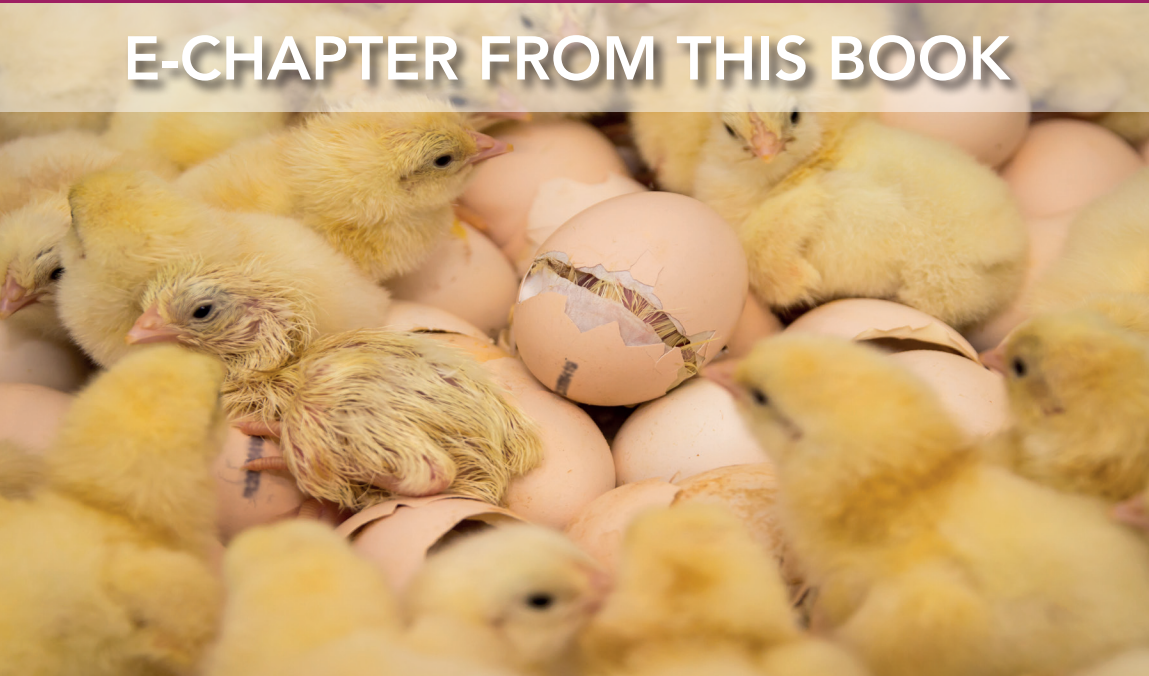
Chicken egg storage and transport

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1 Introduction

For a chicken breeding operation, one of the major production challenges is to manage the rolling stock of hatching eggs so every egg is set while still at peak quality. While hatchability holds up well for the first week or so after the egg is laid, it will start to fall in the second week, with the rate of decline increasing rapidly into the third and fourth week. A typical profile is shown in Fig. 1.

The profile shows a slight increase in hatchability over the first 4 days of storage, an uneven plateau to 10 days followed by a steady fall to 21 days. If storage conditions are inappropriate – too warm or with a fluctuating storage temperature – then hatchability will start to fall sooner, and the rate of decline will be more extreme.

A hatchery will usually be supplied with hatching eggs from multiple farms, similar in size and with flock ages managed so that in total they deliver a similar number of eggs every week. In the past, the egg utilisation versus egg age conundrum was often handled by combining eggs from
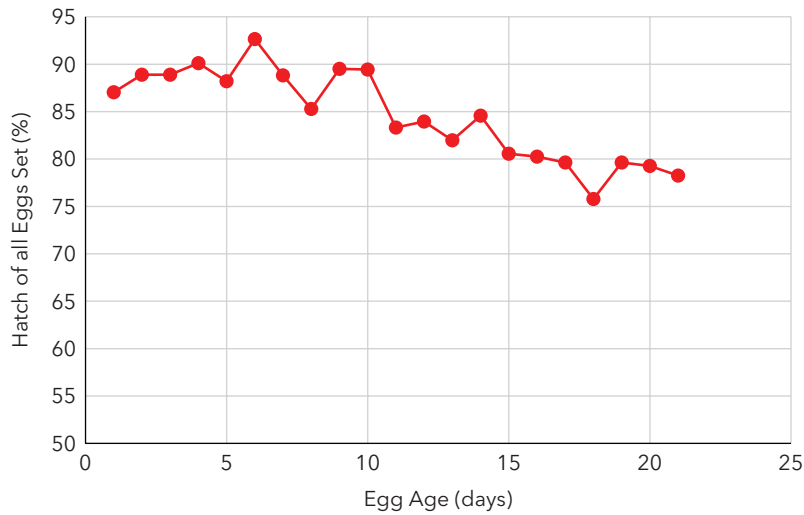
Figure 1 Profile of hatchability changes as egg age increases – Ross 308 broiler hatching eggs. Inappropriate storage conditions accelerate the rate of decline. Source: Aviagen data (2008).

different breeder flocks (and even breeds at the broiler generation) so that eggs could be set together to meet the needs of a day's hatch with minimal egg age. However, modern production has become steadily more demanding of uniformity in broiler flocks. In addition, food retailers often expect reliable traceability of meat deliveries back to the breeder source farm. These constraints, added to those of variable demand through the year and destination flock sizes getting steadily larger, make it more and more likely that eggs from one source flock will be collected over several days in order to fill a single broiler or breeder house. As a consequence, egg age at set in commercial hatcheries can sometimes be significantly higher than is optimal for good hatchability.

The effects of egg storage on hatchability, chick quality and progeny performance and the techniques that might improve storability have been of interest to researchers for many years, with reviews published by Mayes and Takeballi (1984), Butler (1991), Meijerhof (1992), Brake et al. (1997), Fasenko (2007), Reijerink et al. (2008) and most recently by Nasri et al. (2020). Rather than attempt to improve on an already well-covered topic, this chapter will focus on the techniques that are in practical use in commercial poultry operations, followed by a section describing the experiments and field trials implemented by specialists employed by Aviagen to develop a technique using short periods of incubation during egg storage (SPIDES) to improve the hatchability of stored eggs.

The most important factors that make achieving good hatch and chick quality from stored eggs so difficult are caused by

- changes to the blastoderm during egg storage causing an increase in the percentage of abnormal and dead embryos identified by Mather and Laughlin (1977), Fasenko et al. (1992b), Bakst and Akuffo (1999) and Bakst et al. (2012);
- deterioration in internal egg quality, especially albumen and the vitelline membrane – Walsh et al. (1995), Lapao et al. (1999) and Fasenko et al. (2009); and
- bacterial growth in eggs contaminated immediately after lay, leading to an increase in rots and exploding eggs during incubation – Gesche and Schuler (1979) and Humphry and Whitehead (1993).

2 Changes to the blastoderm during egg storage

Eyal-Giladi and Kochav (1976) published a system for staging chicken embryos in the very early stages of development – from fertilisation to the period immediately after the eggs are laid. They described the 14 stages of development that happen before Hamburger and Hamilton (1951) stage 2, which is usually achieved after 6–7 h of incubation.

At oviposition, a fertile hatching egg will hold a blastoderm on the yolk surface which contains around 60 000 cells and will have developed to on average EGK stage 10 (Eyal-Giladi and Kochav, 1976). Various factors will affect the mean and distribution of the embryo stage at and immediately after oviposition. Fasenko et al. (1991), using eggs from a naturally mated layer breed, showed that eggs left in the nest for 6 h after oviposition had blastoderms which were more advanced than those in eggs collected and cooled within an hour of lay (12.33 vs 11.11, P = 0.0001). She did not report hatchability but did suggest that there might be an optimal stage of development to permit the best hatchability. She also reported (Fasenko et al., 1992a), this time using artificially inseminated broiler breeders, that the first egg in a sequence tends to have a slightly more advanced embryo at oviposition than all the other eggs (10.36 vs 10.05, P = 0.0001). In this trial, hatchability was reported and shown to be significantly higher in eggs from within the sequence, i.e. those with a lower embryo stage.

If cooling is impeded in parts of the egg pack, the eggs which remain at or close to incubation temperature longer will continue to develop past EKG stage 10. Figure 2 shows data collected on behalf of Aviagen (Bakst, 2012, personal communication).

The embryos were staged on the day after the eggs were laid (using eggs from a flock aged 36 weeks). Rather than a tight distribution around stage EGK
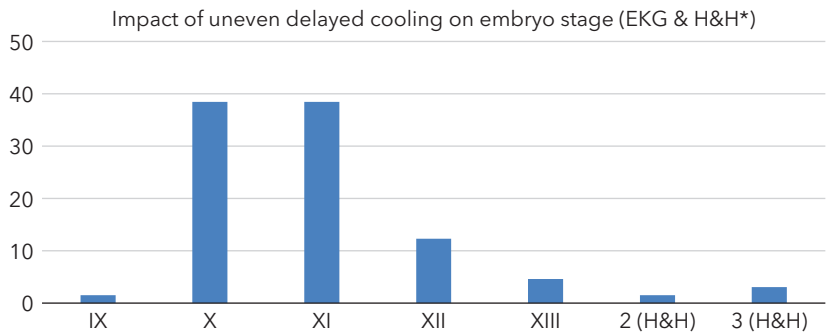
Figure 2 The effect of uneven cooling of hatching eggs packed in close-stacked plastic setter trays on the stage of embryo development 24 h after egg collection on farm. *EGK = Eyal-Giladi and Kochav (1976), H&H = Hamburger and Hamilton (1951).
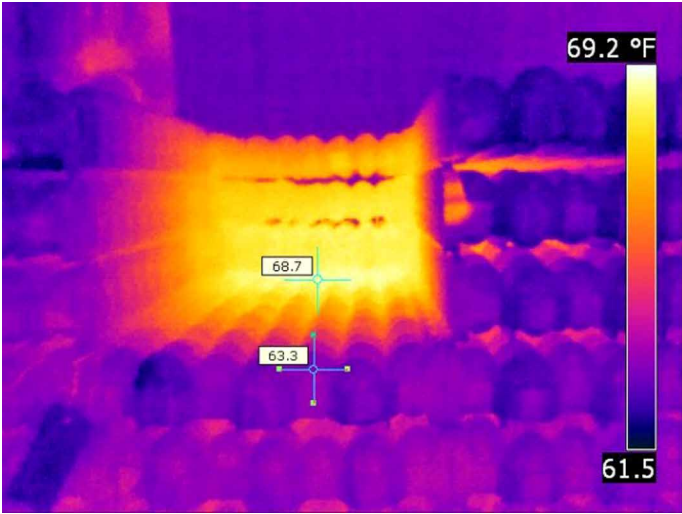

Figure 3 Thermal image of eggs the morning after collection when packed close and unable to cool evenly. Eggs in the centre of the stack were 5°F warmer than those at the periphery.

X, as reported by Selliers et al. (2006), there was a skewed distribution (Fig. 2), with a small number of eggs developing as far as Hamburger and Hamilton stages 2 and 3.

Rather than the more usual system for collecting hatching eggs onto setter trays, which are then placed on well-spaced racks in buggies, this operation collects eggs onto close-stacked plastic trays which delay cooling if eggs are packed when still warm. Thermal images of the system, taken the day after eggs were laid, showed that eggs in the centre of the pack were 5°F warmer than those at the edges (Fig. 3).

The hatchery involved was not reporting any worries about hatchability using this system, but the likelihood that the wide range of embryo stages in the egg pack would also widen the hatch window and the possibility of raised embryo mortality in the embryos which had reached the two Hamburger and Hamilton (H&H) stages remain of concern.

When fertile hatching eggs are stored, cells in the blastoderm start to die and the percentage of viable cells drops from 27.3% at 3–4 days of age to 17.4% and 10.5% at 10–12 and 17 days, respectively (Bakst et al., 2012). Mather and Laughlin (1976), looking at delays in early development, showed that eggs incubated for 42 h were delayed by 5.3 h after 7 days storage and 12.2 h after 14 days. Similarly, Fasenko (2009) also showed that embryos stored for 14 days can be slower to start growing; after 96 h at incubation temperature, they were some 10 h behind embryos from eggs stored only 4 days. Bakst (personal communication) showed that eggs stored for 17 days and then incubated for 7 days had an embryo stage of H&H 27.6, two stages, behind eggs stored for 3–4 days before set. It is standard practice in hatcheries where egg age is variable to advance the set time of stored eggs to take account of the delay and allow the chicks to emerge at roughly the same time after incubation.

By contrast, the EGK stage does not necessarily change much when eggs are stored for up to 21 days at a low enough temperature; stored at 14°C, the EGK stage remained the same but the number of dead blastoderms was significantly ($P < 0.00001$) increased after 14 days and 21 days of storage (Fasenko et al., 1992b). This ties in with observations by Mather and Laughlin (1976) and Bakst and Akuffo (1999) that long storage leads to a proportion of the blastoderms developing abnormally. In practical hatchery terms, these will be the embryos which never start to grow again after storage or which start to grow and then very soon die in the early embryonic development stage.

Brady et al. (2022) analysed the transcriptome activity of blastoderms exposed to prolonged egg storage. Eggs were stored for 21 days at 17°C, and their gene expression was compared with blastoderms from eggs stored for 4 days. They concluded that prolonged storage was associated with the enrichment of pathways associated with cell stress and death.

3 Changes to egg quality during storage

For hatching eggs, the most significant components of the egg in terms of egg storage are the shell cuticle, the vitelline membrane and the albumen. All show signs of change with egg storage, with changes generally being detrimental to hatchability.

3.1 The cuticle

The cuticle, the final layer of the egg shell to be formed before oviposition, forms the main barrier to bacterial penetration of the egg. Sparks (1985) showed that the cuticle will only prevent bacterial penetration of the egg shell once it has cured – this broadly corresponds to the newly laid egg losing the appearance of wetness which is associated with the uncured cuticle, typically within 15 min after oviposition. It follows that any contaminated material that the egg encounters immediately after it is laid, particularly if that material is wet, may allow bacteria to enter the egg shell.

Egg storage does not seem to have a commercially significant effect on cuticle quality. Ball et al. (1975) used a green stain (Edicol Supra Pea Green) to show the cuticle cover of eggs. The dye is protein specific, and the intensity of the stain reflects the cuticle quality of the egg. Eggs stored at 24°C showed a worsening of cuticle cover after 18 days and again after 49 days. For eggs stored at 5°C, there was no change up to 36 days. Given that eggs are unlikely to be stored as long, or as warm, as these were, storage *per se* is unlikely to be an issue.

3.2 Albumen

Albumen is deposited around the egg yolk in the magnum section of the oviduct. The absolute weight increases with egg weight as flocks age, but in general albumen makes up 57–65% of the total weight of the hen's egg (Nys and Guyot, 2011), with the higher values generally seen in eggs laid by commercial layer lines and by relatively immature hens (Peebles et al., 2000). The albumen can be divided into four parts: an inner liquid layer in contact with the vitelline membrane; a layer of thick albumen; the chalazae, which help to hold the yolk in position; and an outer liquid layer. The thick albumen makes up 57% of the total albumen by weight (Nys and Guyot, 2011). With increasing egg age, the thick albumen deteriorates. Walsh et al. (1995), working with broiler lines, showed that long storage (14 days vs 7 days) and high storage temperature (23.9°C vs 12.8°C) were detrimental to albumen quality (as albumen height, mm). The observed effects of both factors were significantly different ($P < 0.001$), and there was also a significant interaction ($P < 0.001$) between storage time and storage temperature where the impact on early mortality + infertility due to storage temperature was much greater in eggs stored for 14 days. Plotting mean albumen height against the percentage of infertile and early dead embryos recorded in the trial showed a steady increase in embryo mortality as albumen height deteriorated (Fig. 4).

Lapao et al. (1999), storing eggs at 16°C, showed that albumen height was reduced as eggs were stored and was able to show a similar
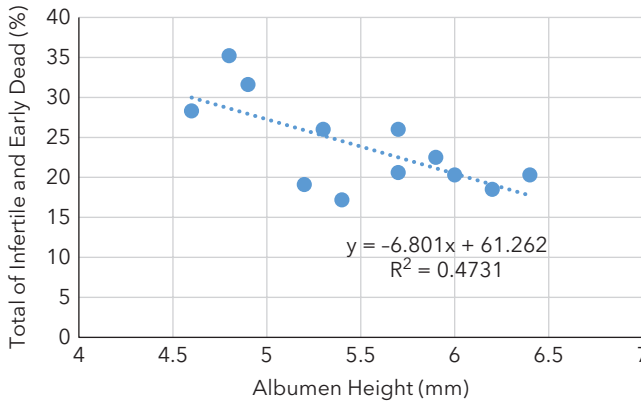
Figure 4 The relationship between albumen height and losses due to early embryo mortality in broiler hatching eggs. Source: data from Walsh et al. (1995).

deterioration due to hen age. Differences due to egg and flock age were both significant ($P < 0.0001$) but there was no interaction between egg age and flock age.

3.3 The vitelline membrane and mottling

The vitelline membrane is formed in the infundibulum, immediately after ovulation, and is complete within 20 min of ovulation (Nys and Guyot, 2011). It is composed of an inner and outer fibrous layer, sandwiching an amorphous middle layer. The membrane tends to deteriorate with egg age; at the most basic level, any attempt to isolate the blastoderm for staging becomes increasingly difficult in stored eggs (Fasenko et al., 2009). Bakst (personal communication), isolating blastoderms for staging from broiler hatching eggs, showed an increase in the number of broken yolks from 2% after 3 days storage up to 12% after 23 days. When the vitelline membrane is not functioning properly, water moves from the albumen into the yolk, causing a phenomenon called mottling, where the egg surface is marked with darker areas, as shown in the photograph in Fig. 5. It is known that mottling gets worse with egg age (Lyon et al., 1972).

Fasenko et al. (2009) suggested that in long-stored eggs, the embryo does not cling to the inner peri-vitelline layer as closely as it does in fresh eggs and that any alteration to the membrane may influence early embryo development, reducing survival rates. But the fragility of the membrane under stress also means that in some eggs the yolk will rupture early in incubation, and in others it will rupture as the residual yolk is drawn into the body cavity just before hatch (personal observation).

Figure 5 Egg yolk with extensive mottling after 5 days of storage.

4 Improving the survival of embryos in stored eggs

4.1 Storage temperature and humidity

Breeding companies and integrators tend to define acceptable storage temperatures depending on their usual egg age at set. Eggs stored for a short time can cope with a higher temperature than eggs stored for longer (Meijerhof, 1994). The ideal will be a compromise between the temperature needed to maintain egg contents in good condition, protecting the embryo and avoiding condensation at any point in the egg collection – storage–transport–storage–setting chain. For a commercial operation, there will also be some pressure to keep the costs of building the eggs store down and to minimise the running costs by not overcooling.

Edwards (1902) suggested a value of 20.5°C for physiological zero – the temperature below which all embryonic development stops. By contrast, Funk and Biellier (1944) who simply measured the diameter of the blastoderm, showed no change in the diameter at storage temperatures between –1°C and 24.4°C (30°F and 76°F). Possibly, the most satisfactory measure is that of Fasenko et al. (1992b), who used the EGK staging system to show that at a storage temperature of 14°C, the embryo shows no sign of further development during egg storage of 21 days (although the number of dead embryos still rose after 14 days and 21 days). More recently, Özlü et al. (2022), using EGK staging over a range of egg storage temperatures, were able to show significant increases in embryo stage over storage periods of 3, 7 and

14 days at temperatures of 18°C, 21°C and 24°C but no development in eggs stored at 15°C. The possibility of embryo development, particularly over a long storage period, explains why fluctuating temperatures, even if below physiological zero, can still be detrimental to hatchability. Özlü et al. (2018) compared the EGK score and hatchability of broiler embryos and hatching eggs from a young and an old flock which were either kept at a constant 17.9°C or allowed to rise from that baseline to 21°C 3 times a day. Set at 7 days, the eggs on the fluctuating treatment had a significantly ($P < 0.05$) raised EGK score. However, it was only in the eggs laid by older hens that the fluctuating temperature affected hatchability – the temperature fluctuation was associated with a hatch drop of 3.3% points, due to an increase in early dead embryos. The young flock, despite an increase in the EGK score, did not lose any hatchability.

Meijerhof et al. (1994) stored hatching eggs laid by hens of 37 weeks or 59 weeks of age at 10°C, 15°C or 20°C for 2, 6, 9 or 12 days. The eggs laid by the older birds were much less able to cope with adverse treatment. High storage temperatures and storage over 9 days both reduced hatchability. The 10°C treatment gave numerically better hatch than the 15°C treatment for all egg storage periods, although the difference was only statistically significant ($P < 0.05$) at 12 days storage. For the younger birds, the 10°C treatment still performed well, but only the 20°C temperature held for 12 days significantly reduced hatchability.

Relative humidity during storage is not particularly important (Brake et al., 1997), provided it is not too low so as to cause eggs to dehydrate (Proudfoot and Hulan, 1976) or too high so as to permit mould growth on walls and ceiling of the egg store (personal observation). In most egg stores, humidity will build naturally due to evaporation from the stored eggs and after the routine washing of floors and walls. Provided the weight loss during storage is included in any assessment of incubation weight loss to 18 days, this is probably the best way to manage egg store humidity.

The temperature response data cited above tend to suggest that keeping hatching eggs consistently below 15°C will improve the overall hatch results. This is probably true, but only if the operation can manage the risk of condensation when the eggs are moved from the cold store to a warmer, more humid environment. Surface moisture seems to be necessary for bacterial penetration of the egg shell pores, which is why it is so important to avoid conditions likely to cause condensation on the shell surface (Bruce and Drysdale, 1994). For this reason, a dew point chart is a useful tool, helping to identify which moves are safe, and which might allow condensation and thus cause problems. Aviagen (2018) publish such a table, focused on changes often observed in hatching egg storage and incubation. If eggs are held at 15°C, and moved to a warmer room, then condensation will occur at 26°C if the humidity

is held at 50% relative humidity (RH). If the storage temperature is dropped to 12°C, then the dew point will be at 23°C at the same humidity level. When the eggs are stored at 15°C, they can safely be moved straight to the setter room corridor (around 23°C and 50% RH) to make up the set. Storing the eggs at 12°C can only be done safely if the humidity is kept below 50%. Otherwise, an additional store held at an intermediate temperature will be needed. The risk of condensation on the egg shell is the main reason that many integrations are reluctant to reduce egg store temperatures to a more appropriate level when egg age is high.

5 Turning eggs during storage

Landauer (1961) referred to four publications in the first half of the twentieth century, three of which showed no benefit to turning during storage and the fourth (Funk and Forward, 1951) showed a benefit only if eggs were stored for more than 7 days.

Proudfoot (1966) stored White Leghorn eggs from a 60-week-old flock for between 14 and 28 days. Half the eggs were held at 50° from the vertical and turned through 180° once a day. The other half were held with the air cell facing upwards. Storage periods of up to 14 days showed no benefit to turning eggs during storage, but by 21 days a 10% point gap had opened up which persisted to the 28th day of storage. A later, more complex series of experiments (Proudfoot, 1969), this time using meat type lines of unspecified age, looked at the effect of storing eggs with the air cell downwards (small end up, SEU), as well as that of the same turning treatment as in the earlier experiments. He showed that for a storage of 7 days, hatch was better if eggs were stored with the air cell upwards (small end down, SED), compared with eggs stored SEU. Hatchability was 78.3% for the SED and 70.4% for the SEU treatments, $P < 0.05$. Longer storage periods, of 2, 3 or 4 weeks, showed numeric advantage to storage SEU, although the difference was statistically significant ($P < 0.05$) in only one of the three trials. There was no significant advantage to turning nor was there any treatment interaction. Again, the treatment effects were only apparent in eggs of 21 and 28 days, with an average gap of 10.5% points at 21 days widening to 16.55% points at 28 days. Although not statistically significant, turning the SEU and SED eggs daily narrowed the gap between them from 10–16% points down to 2–3% points.

Becker et al. (1969) looked at storage periods of up to 5 weeks, turning boxed eggs either through 180° (as did Proudfoot) or through 90° (equivalent to turning in a commercial setter), twice a day. They showed that the 90° turn gave better results than either no turning or a 180° turn.

Bowman (1969) and Insko and Martin (1933) both showed that lines with different selection histories may respond to in-storage turning differently. Given

the considerable amount of time that has elapsed since both publications, it is unlikely that either paper is of any predictive value, but they do raise the possibility that lines with different selection histories may differ in their response to storage treatment.

Elibol et al. (2002) reported trials investigating the interaction of broiler breeder flock age, egg age and turning frequency during storage. They compared eggs from young (30–31 weeks) and old (52–53 weeks) flocks over two experiments. The experiment used more eggs per replicate and more replicates per treatments than most earlier reports. Eggs were stored for 3, 7 or 14 days and either not turned or turned through 90°, 4 or 24 times a day. Trays were distributed throughout both setter and hatcher to avoid any systematic interaction between treatment and local conditions in the incubators. The two experiments showed a significant benefit to turning four times a day, which was especially apparent in eggs from the older flocks. Turning every hour showed a small numeric improvement in experiment 1 and a small decrease in performance in experiment 2. Again, the older flock was more sensitive. However, this 2002 trial showed turning to have an effect at 14 days storage, while Proudfoot (1966, 1969) only showed improvements from 21 days storage.

Elibol and Brake (2008) looked at eggs from 60- to 62-week-old breeders, stored for 3 days or 14 days at 18°C. The eggs were either stored SED (controls) or SEU. The rate of fall of hatchability with increasing egg age was quite high in this trial (82.9% vs 53.6% hatch of fertile at 3 days and 14 days respectively,
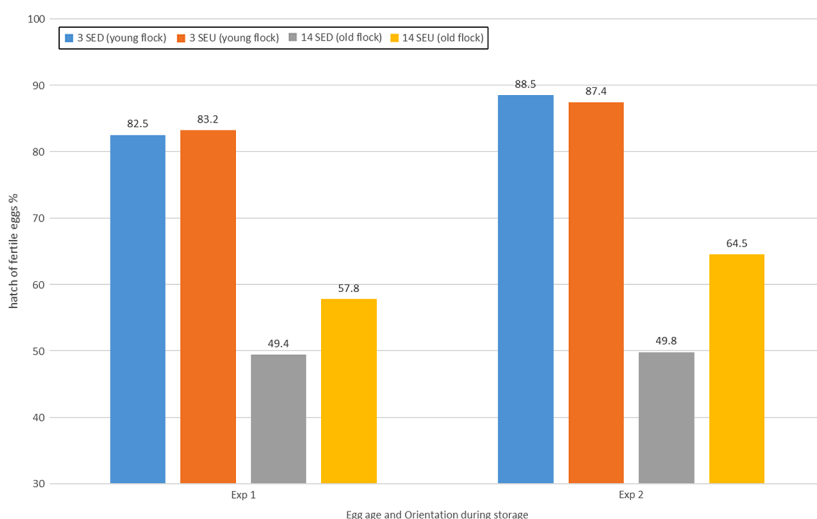

Figure 6 Elibol and Brake (2008) hatchability of eggs laid by young or old flocks, stored for 3 days or 14 days small end down or up.

$P < 0.05$); this was probably because of the relatively high storage temperature. Storing eggs SEU improved hatchability from 49.4% to 57.8% in eggs stored for 14 days. Fresh eggs were not significantly improved by the SEU orientation during storage but were no worse (Fig. 6).

Turning during storage can make a useful contribution to improved hatchability, especially if eggs are being stored for longer than a week. For most hatcheries, the most practical method for turning the eggs will be to use setter trollies which can be connected up to the appropriate turning mechanism installed in a part of the egg store. This will turn the eggs through 90° and can be programmed to turn as often as desired – although it is probably better to stick to frequencies on or below 4 times per day. When planning which eggs to turn, eggs from older (>50 weeks) flocks which will be stored for more than 7 days should be prioritised.

Holding eggs SEU is also very effective in improving hatchability of stored eggs, but in the majority of commercial hatcheries, it is not particularly practical to implement this on a large scale. It is likely to be most useful in production systems which store cooled eggs in boxes, which can then be inverted as required. Turning the eggs as well as inverting them may give additional benefits provided the boxes can be turned daily without damaging the contents. It is probably safer to turn eggs through 180° than 90° if they are packed in boxes, to make the most of the structural integrity of the fibre trays.

6 Transport

Strategies for handling hatching egg holding temperatures from farm to hatchery egg store tend to vary depending on local climate. In temperate regions, with good roads, eggs will usually be packed straight on to setter trays and allowed to cool to the agreed storage temperature on farm. They will be collected from the farm one or more times per week, in a temperature-controlled truck with air suspension and taken to the hatchery store where they will be stored until they are set. The main vulnerabilities in this scenario are the transfers from farm store to truck in periods of hot, humid weather when cold eggs are briefly taken into hot, humid air and condensation occurs, bringing with it the risk of bacterial contamination. In tropical climates, the condensation risk of moving cooled eggs outside becomes constant, and as a consequence many operations do not cool the eggs on the farm at all but move them daily to the hatchery to be cooled. Both systems work well in the appropriate climate.

Poor road conditions can cause problems, especially if the eggs are transported on plastic rather than fibre trays. Jarring due to uneven road surfaces or potholes will increase the number of cracked eggs but may also cause the air cell membrane to detach from the shell membrane (Knox and

Olsen, 1936). The affected eggs had a hatchability some 20% points worse than eggs where the air cells appeared normal. A subsidiary study reported in the same paper showed that jarring only caused the membranes to detach if the eggs were packed SEU. Torma and Kovácsné (2012), comparing outcomes of hatching eggs produced in Hungary and either hatched locally or in Russia, observed that the transported eggs hatched less well, with a distinctive pattern of malformed embryos showing duplications of body parts. Torma and Kovácsné (2019) reported that vibration of between 10 Hz and 30Hz lasting for 10 min was enough to depress hatchability and that the effect was worse in eggs packed on a plastic tray than a fibre one. (From a control value of 91.06%, hatch of fertile was reduced by 4.16% in fibre trays and 8.24% in plastic trays.) No solution other than avoidance of transporting eggs over poor road conditions was identified, although the results do suggest that regular maintenance of farm tracks and driveways under a production company's control could be worthwhile in cases where road conditions are otherwise good.

Finally, Landauer (1961) cited Dareste writing between 1883 and 1891, who reported that eggs allowed to rest for 2 days after transport hatched normally, while if set immediately the same batch of eggs produced largely abnormal embryos. More than a century later, most hatchery managers still aim for at least 24 h rest between the eggs' arrival at the hatchery and starting incubation.

7 Egg shell contamination and disinfection

The hatching egg is well protected from bacterial contamination under most conditions (as discussed by Bain elsewhere in this book). This section covers some of the main reasons why eggs become contaminated under commercial conditions and some methods to limit contamination.

The first line of defence against bacterial contamination is the cuticle, which once cured covers the openings of the egg shell pores and limits the ability of bacteria to penetrate the shell. Haines and Moran (1940) showed that eggs immersed in a suspension of pseudomonas while still warm all went rotten. Similar exposure once the egg was fully cooled substantially reduced the incidence of rots (from 100% to just under 20%). This observation is behind still-current advice to only wash eggs (if unavoidable) in water warmer than the egg (Sparks, 1994). When eggs were placed on straw which had recently been sprayed with pseudomonas, none of the eggs rotted; the authors concluded that moisture is also important. Sparks (1985) showed how in a freshly laid egg, with the cuticle still wet, pushing the egg shell against a fresh dropping allowed bacteria to penetrate the shell. Once the cuticle was dry, similar exposure did not allow any bacteria into the egg. In practical terms, this means that nest

boxes should be checked daily, and any droppings or other source of bacteria removed. Floor eggs should be discarded.

It is important to avoid any cleaning or disinfection treatment that might damage the cuticle. Scraping or sanding the egg surface to remove patches of dirt come into this category, as do disinfectants such as peracetic acid which can strip the protein coat from the egg surface (Burgess, 1994) or spray application methods which are excessively vigorous (Sparks and Burgess, 1993).

The risk of condensation during egg storage and transport has already been discussed and is an extremely frequent cause of contamination problems.

The first possibility of bacterial contamination, before the cuticle has cured, occurs in nest boxes before the majority of eggs are collected. The only way to prevent contamination at this point is by keeping next boxes and belts free of droppings and other sources of bacteria.

It is still advisable (and often a legal requirement) to disinfect the egg shell surface as soon as possible after the eggs have been collected. The disinfectant used should be checked to make sure it does not damage the cuticle (either due to formulation or application method), is effective on the main organisms of concern (bacteria and fungi), does not wet the eggs in the process of application and does not damage the embryo. Safety of the people applying the chemical is an important consideration.

Formaldehyde gas remains the most effective disinfectant for hatching eggs, effective on bacteria, viruses and fungal spores, with a good residual effect (Cardirci, 2009). Unfortunately, formaldehyde is also a carcinogen and as such has been removed from use in some countries. Ultraviolet (UV) light (Scott, 1993; Burgess 1994; Coufal et al., 2003) can be a good alternative for on-farm use – it is not effective on as many organisms as formaldehyde and has no residual activity, but it is effective on bacteria and although capable of causing damage to the operator can be effectively shielded so that workers are safe. Disinfection with UV light on farm should usually be followed by formaldehyde fumigation on arrival at the hatchery to control bacterial and fungal spores.

8 Short periods of incubation during egg storage

When a hen lays a series of eggs in a nest, the eggs laid early in the sequence will be partially re-warmed each time she returns to the nest to lay the next egg. Trials exploring whether the partial rewarming makes any difference to the hatchability of the early eggs in the clutch have been reported since Jackson (1912), who warmed the eggs by placing them under a broody hen and found that stored eggs hatched better as a result. Kosin (1956), Kan et al. (1962), Meir and Ar (1998) and Ar and Meir (2002) all reported trials where short periods

of heating repeated once or twice a day for 1–5 h gave some hatch benefit. However, Reijrink et al. (2009) who heated eggs for half an hour every other day in a 37.8°C water bath did not report any difference in hatchability or hatch of fertile after 15 days storage.

Despite the positive reports in the literature, heat treatment of eggs during storage was not being widely used – occasional parent stock and commercial layer hatcheries were using some sort of heat treatment during storage, but they were very much in the minority. It seemed likely that some aspect of how treatments were implemented in a research setting was not transferring adequately to commercial practice. Given the perennial problems seen in keeping egg age at set within reasonable limits, hatchery specialists employed by Aviagen started a programme of trials designed to develop robust advice for operations needing to preserve the hatchability of stored eggs.

8.1 Replicated experiments

The first three experiments were carried out at the Aviagen Product Development Centre in Albertville, AL, USA. The trials hatchery is equipped with fixed-rack multistage Chickmaster setters.

For the first trial, carried out in 2010, the heat treatment followed that of Meir and Ar (1998) and was defined in terms of time spent in the incubator. The treated eggs were placed in the setter corridor for 4 h on 8 days and 15 days of
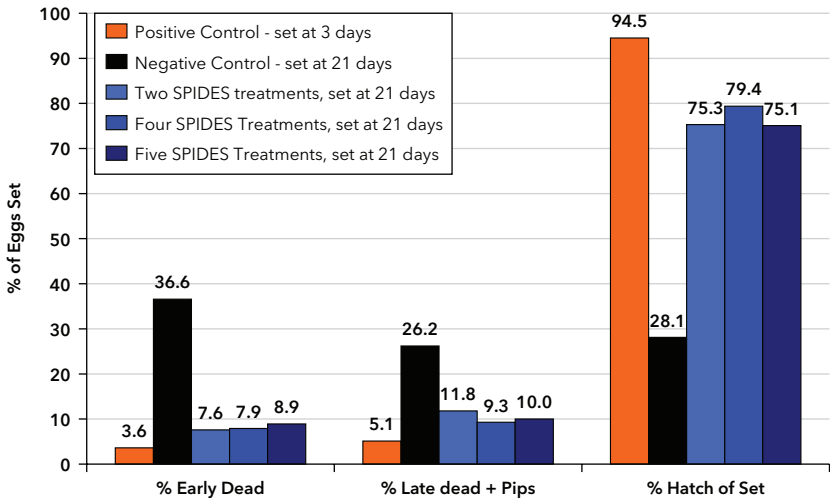

Figure 7 Experiment 1 – hatchability and late and early embryo mortality after 3, 4 or 5 heat treatments.

storage (treatment 1), on 5, 10, 15 and 18 days of storage (treatment 2) or for 4 h on days 3, 8, 12, 15 and 19 of storage (treatment 4). They were then returned to the cooled egg store. There was a positive control where the eggs were set 3 days after they were laid and a negative control where the eggs were not heat treated but were set with all the heat-treated groups after 21 days of storage. Twelve replicate setter trays of 162 eggs per treatment were used (1944 eggs per treatment). The broiler hatching eggs used were from a 33-week-old Ross 308 flock, and the positive control treatment hatched at 94.5%. The negative control eggs hatched at 28.1% – far worse than expected. This was probably because the first trial was carried out during a very hot August and the egg store could not keep the temperature down to the targeted 15.5°C. The three SPIDES treatments hatched at 75.3%, 79.4% and 75.1%, with treatment 2 being significantly ($P < 0.05$) better than the other two. Figure 7 shows hatchability and embryo mortality levels for all five treatments, showing that the SPIDES treatments improved both early and late mortality compared to the untreated stored control eggs.

The outcome of the heat treatment was very much better than expected, so a second trial was set up to make sure that they were repeatable. In this trial, sample eggs at the top, middle and bottom of the stack were fitted with thermistors attached to mini data loggers (Gemini Tinytag Talk 2 – TK 4023) to monitor internal temperature. The trial was expanded to look at the impact of heat treatment on eggs stored for 7 and 14 days, as well as the original 21 days. Eggs stored for 7 days were treated once on day 4 of storage. Eggs stored for 14 days were treated on days 4, 7 and 11 and eggs stored for 21 days were treated on days 5, 10, 15 and 19, repeating the best treatment in experiment 1.

The internal egg temperatures recorded in experiment 2 are shown in Fig. 8. It can be seen that the eggs at the top heated up the fastest and to the highest peak temperature. The eggs at the bottom of the trolley were next, and the ones in the middle were the slowest, only reaching just over 32.5°C.

In this second trial, the untreated eggs which were stored for 21 days hatched much better than before, achieving 62.6% rather than 28.1% hatchability. The trial was carried out in December, and the egg store was better able to hold temperature. Hatchability and early and late embryo mortality are shown in Fig. 9. SPIDES treatment improved hatchability by 2.3% after 7 days ($P < 0.05$), by 3.4% after 14 days ($P < 0.005$) and by 14.3% after 21 days ($P < 0.001$). For the 21-day storage period, SPIDES treatment reduced early embryo mortality by half and late embryo mortality by a third.

Despite the variability in peak temperature within the eggs due to their position on the trolley as they were heat treated, there was no correlation between peak temperature and hatchability of individual replicates, which suggested that all the trays had been able to exceed some undefined threshold temperature.
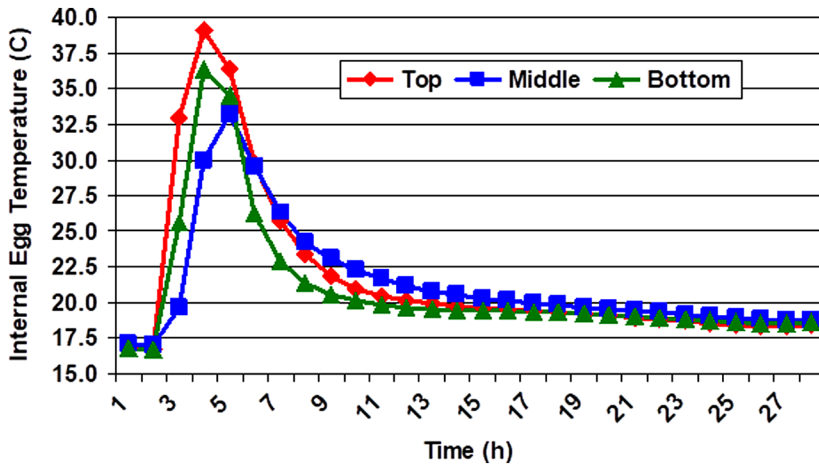
Figure 8 Internal temperatures of eggs in different positions – experiment 2.
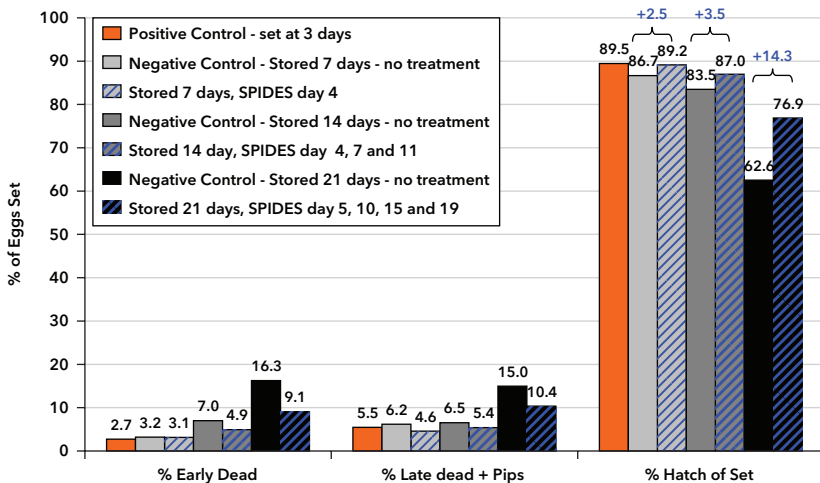

Figure 9 Experiment 2 – hatchability and early and late embryo mortality of eggs stored for 7, 14 or 21 days with or without heat treatment.

Having shown that the results of the first experiment were repeatable, and that heat treatment was effective in eggs as fresh as 7 days, the third experiment explored the duration and frequency of heat treatment on Ross 308 eggs stored for 21 days. As with the previous trial, there was a positive control set at 3 days storage and a negative control set after 21 days with no heat treatment. The SPIDES treatments explored exposure times of 2, 4 or 6 h, each of which was repeated 3, 4 or 6 times.
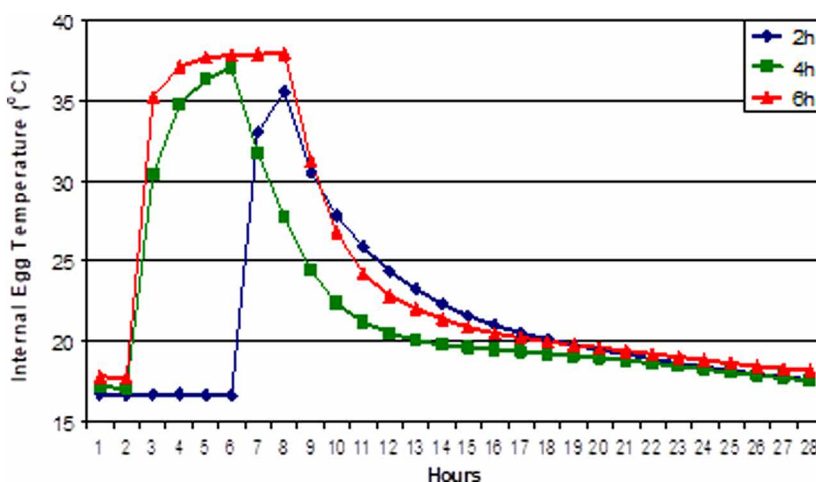
Figure 10 Internal egg temperature of eggs held for 2, 4 or 6 h in the corridor of a Chickmaster fixed-rack multistage setter.

Figure 10 gives the internal egg temperatures of eggs heated in the corridor of a Chickmaster fixed-rack multistage setter for 2, 4 or 6 h. It shows that the longest treatment allowed the eggs to reach incubation temperature and be held there for 3 h. Four-hour exposure just allowed the eggs to reach incubation temperature, but they were over 35°C for 2 h. The shortest exposure just reached 35°C before the eggs were moved back to the egg store. Temperature variability between top, bottom and middle trays was greatest in the 2-h treatment and least in the 6-h one.

Once again, the hatchability of the untreated, stored eggs was poor (Fig. 11). Of the SPIDES-treated eggs, the longest treatments gave worse hatchability – catastrophically so for the six treatments of 6 h, which hatched at 1.1%. Six repetitions gave the worst hatch for all three exposure times, but the losses were less with 4- and 2-h exposures.

Analysing the results further, the hatchability for each treatment was recalculated as the percentage of lost hatchability due to storage that was recovered after SPIDES treatment, which in this experiment had a range between 60% recovery at the upper end and 60% detriment for the worst treatment (i.e. the treated eggs were substantially worse than the negative control). Logging the internal temperature made it possible to calculate the cumulative time above key temperatures for all nine treatments and examine its relationship with hatch performance. The amount of time the eggs were above 30°C and 32°C was identified, including the time accumulated as the eggs were warming up and as they were (more slowly) cooling down. Plotting the time spent above 32°C against the percentage recovery of the hatch lost due to storage as a second-order polynomial curve gave an R^2 value of 0.967,
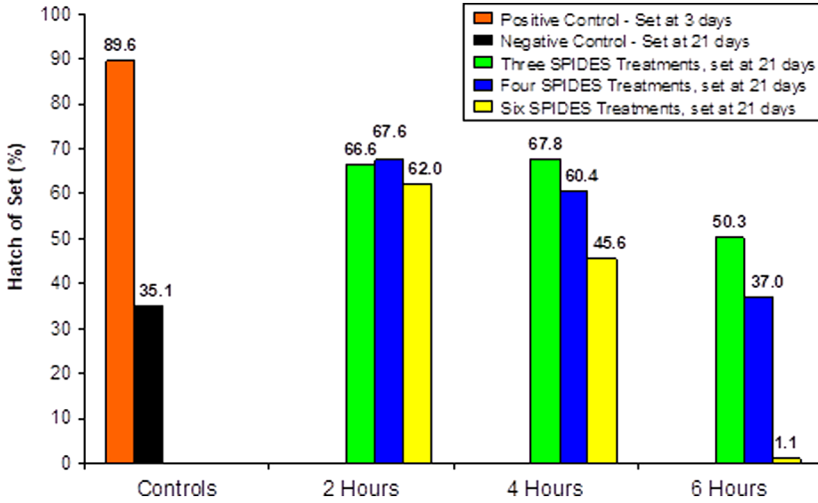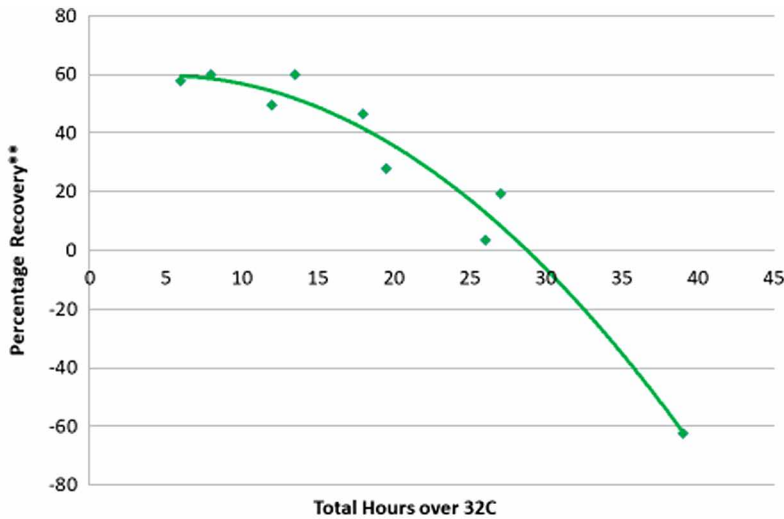
Figure 11 Experiment 3 – hatchability of fresh and stored eggs given heat treatments of 2, 4 or 6 h 3, 4 or 6 times.

Figure 12 The relationship between cumulative exposure to temperatures above 32°C and the percentage of the hatchability lost due to storage recovered after SPIDES treatments.

marginally higher than the R^2 of the fitted curve when the baseline was set at 30°C. The results of this trial showed that for the best outcome, the total amount of time that the eggs spent above 32°C should not exceed 15 h (Fig. 12).

When Funk and Biellier (1944) explored rates of growth in early-stage embryos at different temperatures, they not only looked at the low temperatures that allow little or no development, but they also looked at the temperatures approaching incubation temperature (37.8°C). They showed that while at 26.7°C the blastoderms increased in size from 4.9 mm to 6.3 mm in 7 days, at 29.4°C, they grew from 4.8 mm to 32.1 mm. Higher temperatures further increased the growth rate, to a point where normal incubation temperature caused the embryo to reach 27.2 mm after 3 days. It would seem that for SPIDES, this upper physiological zero is a key indicator.

One final experiment was carried out in the UK. Having relied so far on an incubator which could heat the eggs relatively fast, it was necessary to test a slower warm up, to make sure that SPIDES would work in large single-stage setters. Experiment 4 was performed in two 12-tray Bristol trial setters, which could be programmed to heat up at different speeds. For this experiment, they were programmed to reach 37.8°C in either 4 h or 8 h, with heat treatments given at 9, 12, 16 and 19 days of age. The eggs were all set on the same day, when individual trays were between 22 days old and 25 days old. The number of trays of each egg age was the same in each treatment, and data were collected to setter tray. The two heating speeds gave identical hatchability, 52.9%, having recovered 70% of the hatchability lost due to storage.

Concurrent with the experiments carried out in Aviagen facilities, Dymond et al. (2013) compared various heat treatment regimens with eggs set fresh or after 21 days. The SPIDES programme involved treating eggs for 4 h on days 5, 9, 15 and 19 prior to set on day 21 post oviposition. Additional treatments gave 6-h or 12-h heat treatment on day 5 only, with the eggs again set on day 21. The egg shell temperature during the 4-h heat treatment showed that the eggs heated up rather faster than in the Chickmaster setters, and the total time above 37.5°C was about 100 min per treatment, rather than only just reaching 37.5°C in the larger incubators. Starting from a base of EGK 10.5 in the untreated eggs, each 4-h SPIDES treatment increased the embryo stage by 1.5 to 2 stages, such that after four treatments the embryos had reached H&H stage 3. The single, longer heat treatments of 6 or 12 h delivered 5.2 h and 10.1 h above 37.5°C respectively, with embryo stage reaching EGK 14 and H&H 3.5. As with the internal trials, the eggs set fresh hatched very well, at 92%. Storage for 21 days without heat treatment caused hatch to drop to 71%, more than 60% of which was recovered after SPIDES treatment. The single, longer treatments, on the other hand, were both associated with hatchability worse than that of untreated eggs. It would seem that a single, early treatment is not sufficient to restore hatchability in eggs stored for 21 days, although it is also possible that the 12-h treatment, especially, was sufficiently long to exceed the optimal time above 32°C suggested in experiment 3. The results of the third internal experiment made it very clear why hatcheries had found SPIDES such a hit or miss technique

to implement. Working from small-scale experiments where exposures were defined in terms of time meant very different things depending on the type and capacity of the setters being used to deliver the treatments. Access to Tinytag data loggers allowed us to measure the actual egg temperatures achieved. Help from Murray Bakst and James Wade allowed us to see what those temperatures were doing to embryo development. The combination was a very powerful one.

8.2 Field trials

After the second experiment, SPIDES was discussed widely within Aviagen, and managers of individual hatcheries encouraged to try it. Most of the hatcheries saw some improvement, and plotting hatch lift versus egg age at set showed a very similar relationship to that seen in experiment two. Generally, the poorer the storage conditions, the more the hatchability was improved, to the extent that recoveries of over 60% can probably be assumed to indicate that storage temperatures were either too high or were fluctuating. Results improved once it was possible to give guidance in terms of internal egg temperatures and cumulative time above 32°C.

This stage of the investigation made it very obvious that giving only a treatment duration, without considering its impact on egg temperature, was one of the reasons that heat treatment had taken so long to be adopted. If the first Aviagen experiment had used a large, single-stage setter to heat the eggs, the heat treatment would probably not have improved hatchability.

Feedback from the Aviagen hatcheries told us as follows:

- SPIDES can only work if the eggs are well separated in setter trays and on racks. Bulk packed eggs will not heat or cool evenly.
- Putting warm eggs straight back into the egg store causes the temperature of neighbouring eggs to fluctuate and they hatch worse than they should. Eggs needed to be cooled to a maximum of 24°C before they were returned to the egg store.
- Most single-stage setters delivered SPIDES treatments more effectively if only half full, with alternate tray racks empty.
- SPIDES consistently gave a small benefit even in eggs of only 7 days old.
- The hatchery manager needed to see the benefit in order to tolerate the extra planning required to keep up regular treatments in a full egg store.

8.3 Dedicated short periods of incubation during egg storage machines

The information gathered in the experiments reported here was sufficient to work with incubator manufacturers to design a dedicated SPIDES

machine, which could be used in egg stores remote from the hatchery which had no incubators on site. Most incubator companies now offer dedicated SPIDES machines; the most effective use egg surface temperature to control the process and have significantly increased heating, cooling and fan capacity to ensure that the heating and cooling are rapid and evenly distributed.

8.4 Future trends in research

There has been a steadily increasing body of publications investigating the aspects of how SPIDES affects embryos since the early publications by French et al. (2011) and Nicholson et al. (2013), with an increasing number of trials reported which were not initiated by Aviagen.

Collaborative trials with the University of Ankara have shown, among other things, that the effect of SPIDES and egg turning are additive (Özlü et al., 2021), suggesting that the two techniques target different processes which both cause hatchability to fall with egg age. Most recently, Brady et al. (2022) compared the activation of transcriptome processes in blastoderms in fresh eggs, in eggs which had been stored for 21 days and others which had been both stored and SPIDES treated. Compared to the fresh eggs, the blastoderms in stored eggs showed enrichment of pathways associated with cell stress and death. SPIDES treatment, despite the eggs being the same age as the stored ones, was associated with enrichment of pathways associated with basic cell and anti-apoptotic functions. This is probably the start of an understanding of why heating to incubation temperature makes such a big difference to hatchability and also why it seems to work particularly well when storage conditions are suboptimal. Further work should be both fascinating and valuable.

9   Acknowledgements

10   Conclusion

Chicken hatching eggs will not hatch as well if they are stored for more than a week than they do if set fresh. Poultry researchers have investigated the causes of hatchability deteriorating and many methods that might affect the rate of change, and there are excellent and exhaustive reviews of the literature. Techniques which are helpful on a small scale in the laboratory are helpful but only a starting point. Some may be impossible to multiply up at current levels of technology and others may need more work to explore the limits. At a practical commercial level, egg age probably changes faster than an organisation is able to change its egg storage protocols. Hatcheries which are able to manage egg storage temperatures, turn eggs from older flocks, and deliver SPIDES treatments will usually cope best with fluctuating egg temperatures.

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