# Temperature during the initial phases of incubation

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Introduction

For commercial and logistical reasons it is often necessary to store hatching eggs for several days or even weeks before setting. During egg storage, the survival rate of the embryos reduces, depending on age and quality of breeder flock, storage time and storage conditions. Through the manipulation of especially temperature but also other factors, the influence of the storage period and pre-incubation period on hatchability and chick quality can be manipulated.

Many factors influence the egg forming process and with it the quality of the egg. Health status and nutrition are without any doubt the most important factors, next to genetic background of the birds, but also fertility by itself might be considered as a factor influencing quality, as poor fertility is often observed in combination with poor chick quality.

The first 24 hours

As fertilization occurs within 15 to 20 minutes after ovulation (Howarth, 1970), the embryo has a chronological age of 24 to 26 hours after oviposition. The first cleavage division in the germinal disc is observed about 4 hours after ovulation (Perry, 1987), resulting in a blastoderm of approximately 40.000 to 60.000 cells at oviposition. Hayes and Nicolaides (1934) already observed differences in stages of embryonic development at oviposition. They reported that pre-gastrula and early gastrula stages were common in eggs from birds with poor hatching results, while eggs form birds with good hatching results contained embryos in an advanced gastrula stage. This indicates that there is an optimal stage of development at which eggs should be stored. Eyal Giladi and Kochav (1976) developed a classification method that distinguishes several stages of development between fertilization and start of the incubation process. The optimal developmental stage for storage of the eggs is reported to be stage 10-11 when this classification system is used.

Egg production

Once the egg is laid, especially temperature will influence the quality and survival rate of the embryo, next to storage length. If the temperature of the eggs is above the so-called physiological zero (approximately 26-27oC) development of the blastoderm will continue. Below the physiological zero, development and growth will not take place, but changes in the blastoderm can be observed, especially on cell level.

In modern poultry production, eggs are produced in laying nests. Traditionally these nests are small wooden boxes with a thick layer of litter material (oat hulls, rice hulls, straw, saw dust etc), in which the eggs are protected until they are collected by hand. More recently, a shift towards roll-away nests can be observed, in which the eggs roll away to a central belt after being produced. This brings the possibility to mechanize or even automate the collection process, which reduces labor and improves working conditions. A beneficial effect of automatic laying nests is a lower temperature pattern of the eggs, which has a positive effect on hatchability, especially when the breeder flock increases in age. Eggs in hand collected litter nests are buried in the isolating litter material of the

nest, and are warmed by every bird that comes into the nest to produce another egg (Meijerhof et al, 1994). This results in a further development of the embryos, past the optimal gastrula stage for storage, or the stage 10-11 of the Eyal Giladi classification system. This is confirmed by Fasenko et al (1991, 1999) who reported that embryos in eggs that were left in litter nests for 3.5 to 6.5 hours were more developed than those of eggs collected hourly. To avoid these negative temperature effect, eggs from litter nest should be collected at least 4 times a day, especially during warm periods (North, 1984).

Egg quality issues

One of the biggest risks for egg and chick quality is contamination of the eggs with bacteria. Chicks are very sensitive for bacterial contamination, and a reduction in hatch and increase in first week mortality will be observed if contamination levels increase, as well as an increase in potential risk of contamination with pathogens. When the egg is produced, it has the temperature of the hen's body. After leaving the body, the temperature reduces, and the egg content shrinks. This shrinkage of the egg content creates a negative pressure inside the egg, forcing air to go through the pores into the egg, forming a small air cell. If the egg is produced in a contaminated environment, bacteria will penetrate the egg because of this air flow, and the risk of bacterial contamination of the egg content will increase.

Eggs have a wide range of defense mechanisms against bacterial penetration. A rigid shell structure is a very obvious defense mechanism. Another important mechanism is the increase of the pH of the albumen in the first days after lay. Due to the release of carbon dioxide, albumen pH rises from 7 to 9.5 (Kosin and Konishi, 1973; Dawes, 1975), which is important for several developmental functions in the embryo (Stern, 1991), but also because it forms an effective protection against micro organisms, and. However, this increase in pH takes a couple of days, which means that directly after lay this defense mechanism is not so effective. Together with the fact that directly after lay, the egg reduces in temperature and forms an air cell with air entering the egg, this makes it very important to focus on producing eggs in a clean environment. Once the egg is older and stored at a low temperature, the risk of micro-organisms penetrating is reduced.

Egg storage

It is well documented that egg storage reduces hatchability (Becker, 1964; Fasenko et al 2001; Yassin at al; 2008) and reduces chick quality (Byng and Nash, 1962; Tona et al, 2003; 2004). Not only the percentage of malformed embryos increases with storage time (Mather and Laughlin, 1977, 1979), but also post-hatch growth performance reduces (Becker, 1960; Tona et al, 2003; 2004) and post hatch mortality increases (Merrit, 1964; Yassin et al, 2009). Although there are substantial genetic differences between lines and breeds, normally it can be expected that hatchability will go down after 5 to 7 days with approximately 0,5% per day. If storage time exceeds, the drop in hatchability per day will increase even more. Besides that, egg storage also add to the time needed for the eggs to hatch (Mather and Laughlin, 1976; Tona et al., 2003). The general assumption is that one day of storage adds one hour to the incubation process, probably due to a weaker embryo that needs more time to start up the developmental process (Reijrink et al, 2008), as storage causes a delay in the initiation of development (Kaufman, 1939; Arora, 1965; Mather and Laughlin, 1976).

Setting the eggs immediately after production reduces hatchability as well (Asmundson and MacIlraith, 1948). Although the negative effect of very short storage times on hatchability is limited to maximum 1-2%, it is advisable to store the eggs for at least 24 hours but preferably 48 hours before setting (Benton and Brake, 1996). This is probably related with a minimum increase in pH of the albumen that is needed for optimal embryo development. A rapid increase of the pH of the albumen by keeping the eggs for a short period in ammonium gas reduces the negative influence of very short egg storage times.

Storage time and temperature

Already in 1902, Edwards reported that eggs should be stored at a temperature at below "physiological zero", to maintain dormancy of the embryo (Proudfoot and Hulan, 1983). Although dormancy of the embryo is maintained below this temperature level, the morphology of the embryo is not static. Arora and Kosin (1966) reported a series of recognizable regressive changes in the structure of the blastoderm when eggs were stored at 13oC. Already in 1944, Funk and Biellier reported a shrinkage of the blastoderm when eggs were stored at 13oC. Arora and Kosin (1966) did not observe gross morphology changes in the embryo when stored for 21 days at 7.2oC, 12.8oC or 18.3oC, but they did report changes in cellular activity. With increasing storage time, the number of mitotic and necrotic indexes increased in the embryos for all 3 temperatures, but more in the 12.8oC and 18.3oC storage group than in the 7,2oC storage temperature. They hypothesized that many of the mitotic cells died during storage. At a storage temperature of 7,2oC the cellular activity of the embryo was marginal, and they concluded therefore that it is advisable to reduce the temperature if eggs are stored for a longer period, to limit the negative effects of storage. Reduction of storage temperature reduces the rate of cell death in the embryo, resulting in a more viable embryo once incubation starts.

Many authors, as reviewed by Mayes and Takeballi (1984) and Wilson (1991) reported that optimum storage temperature should be decreased with increasing storage time. In general, suggested temperatures are 20-25oC for storage of eggs less than 4 days, 16-17oC for eggs stored 4 to 7 days, and 10-12oC for eggs stored for more than 7 days. In fact, this temperature profile is lower for prolonged storage than what is used in the field at this moment, and what is also recommended by for instance incubator manufacturers. In practical conditions, a storage temperature of 20-22oC is usually recommended for eggs stored for maximum 4-5 days. From 4-5 days up to 8-10 days, eggs are usually kept at 16-18oC, while storage temperatures should be reduced to 15-16 degrees if eggs are stored up to 14 days. If egg storage exceeds 14 days, it is advised to reduce the temperature to 13-14oC. So although research shows that the embryo is probably better preserved at lower temperatures, storage temperatures in practical conditions are usually kept higher. It is also questionable why temperatures for short term storage of eggs are advised to be relatively high. It is doubtful if the embryo will benefit from a reduction of cell death due to a lower storage temperature if storage time is short, but it will probably not harm it anyway, so why would it be advisable to keep short egg storage temperatures high?

The reason for this is probably more related with the machines than with the eggs. Keeping eggs at a low temperature makes it more difficult for the incubators to warm up the eggs uniformly and at the correct speed. Although this influence seems to be limited, one should realize that a modern incubator can contain over 100.000 eggs, which means that often the egg load in the machine

exceeds 7000 kg. As eggs have thermal properties that can be compared with water (Meijerhof and van Beek, 1993), it means that 7000 liter of water has to be warmed up by air, in a uniform and fast way. Reducing the temperature of the eggs more than needed makes this process more difficult. Another negative effect of reducing the temperature of the eggs more than necessary is the risk of condensation ("sweating") of the eggs. When egg temperatures are below the dewpoint of the room in which they are brought, condensation will occur which will lead to an increase of bacterial contamination levels. This should always be avoided. It is therefore advisable to not cool the eggs more than is strictly necessary to keep the embryos at a viable state.

Eggs should be stored under the physiological zero, the temperature at which no development occurs. Once incubation has started, it should not be stopped, at least not for the first 10-12 days. As the embryo considers only temperature as the crucial factor for embryonic development, it is of utmost important to respect this physiological zero and to keep the eggs below that level, as only a few hours above it are enough to give the embryo the impression that incubation has started. If eggs are not cooled fast enough, or for instance are kept in the sun for a few hours, this can already happen. If the embryo afterwards is cooled down again, the early mortality rate of these embryos will increase dramatically. It is therefore very important to control the temperature continuously during storage, but also to cool the eggs uniformly towards the air temperature level. This can be done by creating air velocity in the egg storage room, which will increase the heat transfer. Once all eggs are on temperature, no additional air velocity is needed anymore, other than to keep the air temperature uniformly distributed.

Egg storage and humidity

During egg storage, it is often recommended to increase the relative humidity levels, to avoid moisture loss of the eggs. Although several researchers reported a better hatchability when R.H. during storage was maintained at a high level (90% vs 60-80%) (Cooney, 1943; Proudfoot, 1976), results are not always consistent. Kaufman (1939) concluded from experiments with extended moisture loss by artificially lowering of air pressure, that dehydration is not the main cause for the increase of embryo mortality after prolonged storage. As moisture loss during storage and moisture loss during incubation most probably is additive (Meijerhof, 1994) and moisture loss during prolonged storage at low temperatures is approximately 0,05% per day (Becker et al., 1968; Meijerhof and van Beek, 1993), it is questionable if a high relative humidity during storage is necessary for maintaining hatchability. It is however important if a minimum weight is set for eggs to be considered hatching eggs. If moisture loss during storage is increased, this will reduce the number of eggs meeting the weight requirements. However, it can be questioned if the moisture loss during storage is of influence on hatching results or chick quality. Moisture loss is created by the difference in the so-called water vapor pressure across the egg shell (the water vapor pressure deficit) (Meijerhof and van Beek, 1993). This water vapor pressure is the result of the combination of temperature and relative humidity. As long term storage conditions normally operate at lower temperature conditions, this will by itself lower the water vapor pressure deficit across the egg shell.

The function of moisture loss during incubation is to release enough moisture to create an air cell that allows the embryo to develop lung ventilation before hatching. In the majority of modern machines, the moisture loss as a percentage until internal pipping is not above optimum, and often

enough even below what is reported as an optimum moisture loss. In this situation, an increased moisture loss might even be beneficial, as it helps to create a big enough air cell at internal pipping.

Creating an increase in relative humidity during storage by spraying water in the storage room is not always advisable, as it might result in droplets of water on the eggs, which will increase the risk of bacterial contamination. In that respect it is advisable to store the eggs in a closed environment with as little ventilation as possible, to maintain the evaporated water of the eggs in the room and increase the relative humidity in that way. As eggs in storage do not have a significant sized embryo which is actively developing, no oxygen is needed and no metabolic carbon dioxide needs to be removed, and therefore no ventilation is required during storage.

Pre-storage incubation

Although in general it is not recommended to store eggs above the temperature at which growth and development can occur, there are some situations where it can be beneficial. Research has shown that although the optimal developmental stage for storage of the embryos is the gastrula stage, or stage 10-11 when the Eyal Giladi classification system is used, some eggs are produced in a stage before the optimum stage for storage or the pre-gastrula stage. Becker and Bearse (1958) and Kosin (1956) already reported that pre-incubation heating of eggs for 1 to 5 hours at a temperature of 37oC prior to storage can result in an improvement of hatchability after long term storage. Also temporary heating of stored eggs to incubation temperature for 1 h (Kosin, 1956) or for 2-4 h (Nikolaeva, 1958) on a daily base resulted in an increase in hatchabilty, especially after prolonged storage.

More recently, Reijrink et al (2009) confirmed these results, but also showed that pre-storage heat treatment or temporarily heating during storage can occasionally result in a too advanced developmental stage of the embryo, resulting in a reduced hatchability. When the embryo is already in the optimal stage of development at egg collection, a heat treatment bares the risk that the embryo will develop into the next stage, which is less optimal for storage. As it the developmental stage at the moment of egg collection and storage depends on factors as type of nest, frequency of collection, temperature in the house, age of the flock etc, variation in stage of development can occur and is not always easy to predict in practical situations. This makes pre-incubation heat treatments less suitable in field conditions, and should only be applied when accurate control of these factors is in place.

Onset of incubation

The most important and most sensitive period of incubation is the first few days. In this period not only the embryo starts to develop, but also membranes and compartments are formed that serve and protect the embryo during development (Nechaeva et al. , 2004). Sub-optimal conditions as alterations in temperature in this period can have dramatic effects on the embryo and final results of the incubation process. As in the first few days a whole sequence of developmental stages in the embryo are following up rapidly, a problem in this period might influence crucial processes in the formation of the embryo. Temperature plays an important role in this. Too high temperatures will influence the developmental process and show at hatch as brain hernia and posterior duplications. Too low temperatures will increase the incidence of abnormal development (Wilson, 1991) and will result in an increase in early mortality rates in the incubation process, but unless eggs are opened,

will be often recognized as  infertiles instead of early deads. However, also problems in chick quality, for instance open navels and string navels at hatch, can be caused by too low temperatures in the initial stages of incubation.

Pre-heating

As most machines have limited heating capacity, it will take relatively long for eggs to warm up to incubation temperature, as the heat transfer to the eggs has to be done by air. Although the egg shell warms up reasonably quick and the machines might be able to reach the desired temperature after a few hours, it takes much longer before the content of the last egg is on incubation temperature. This might even take more than 24 hours.

Kaufman (1938) and Steinke (1972) found that prolonged egg storage delayed the onset of embryonic development at the onset of incubation. A delayed onset of development has a negative effect on hatchability and chick quality, as it results in incomplete and suboptimal developmental processes in the embryo (Reijrink et al, 2008). To shorten the warming process and avoid a delay in onset of lay, as well as making the warming process more uniform for all eggs in the machine, the batch of eggs can be pre-heated. With pre-heating the eggs are brought to a temperature level just on or below the physiological zero, to get maximum energy in the eggs without starting growth and development. After the pre-heating period, the load of eggs can be more easily and more uniformly heated by the machine to reach incubation temperature. Another advantage of pre-heating is that the process of condensation is avoided. At a incubation temperature of 37.8oC and 55% relative humidity, the dewpoint (the temperature at which condensation will occur) is approximately 27oC. This means that pre-heating at 27oC will bring the embryos just at the physiological zero, and at the same time will prevent condensation of the eggs at that temperature are placed in the machine and warmed to the normal incubation temperature.

Little information is available in the literature regarding the effect of the rate of preincubation warming on hatchability and chick quality. Reijrink et al (2010) warmed the eggs from storage temperature to incubation temperature in 4 or 24 hours and found an interaction with storage duration. When eggs were stored for 4 days, the pre-incubation temperature profile had no effect, but eggs stored for 13 days had better hatchability when the warming process took 24 hours instead of 4 hours. Mayes and Takeballi (1984) reviewed the results of several studies that examined the rate of preincubation warming, and concluded that most authors warmed the eggs for 18 to 24 hours at room temperature. Meijerhof et al (1994) preheated eggs after storage in 16 hours to 20oC and 27oC. Although no differences were found for eggs from younger flocks, older flocks showed a reduced hatchability when the eggs were preheated to the higher temperature, suggesting that embryos from older breeders are more sensitive for temperatures close to or over the physiological zero than embryos from younger breeders. Although the reason for the difference between breeder age is not clear, care should be taken to limit the pre-warming period when a temperature close to or above the physiological zero (27oC) is used.

The positive effect of pre-heating is dependent on the type of machine that is used. Machines that have more heating capacity or are for instance used in a multi-stage program with air moving from the eggs in the later stages of incubation to the fresh eggs, tend to benefit less from pre-heating the eggs than eggs that have more limitations on warming the eggs rapidly at the onset of incubation.

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