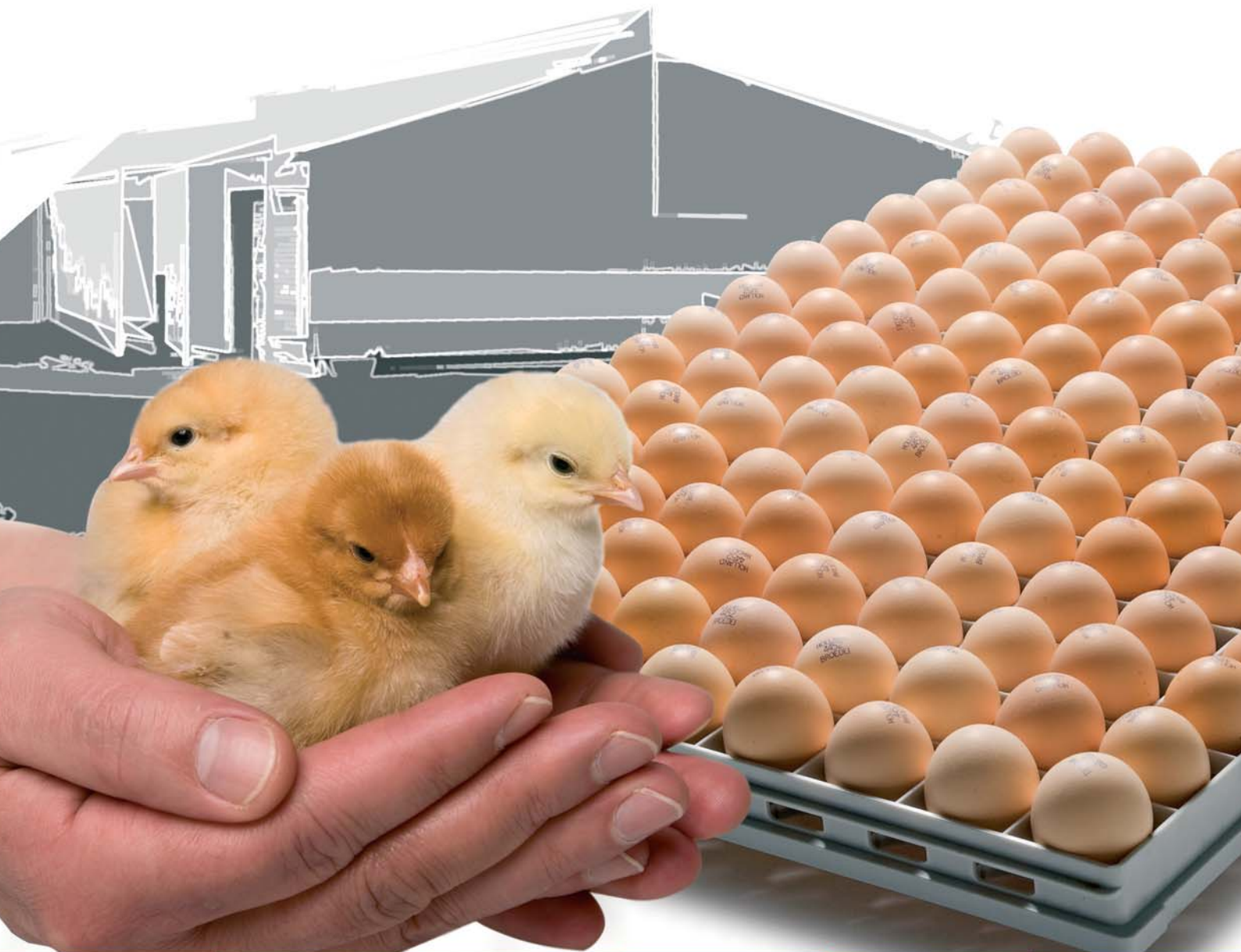


Incubation Guide



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Note: The performance data contained in this document was obtained from results and experience from our own research flocks and flocks of our customers. In no way does the data contained in this document constitute a warranty or guarantee of the same performance under different conditions of nutrition, density or physical or biological environment. In particular (but without limitation of the foregoing), we do not grant any warranties regarding the fitness for purpose, performance, use, nature or quality of the flocks. Hubbard makes no representation as to the accuracy or completeness of the information contained in this document.

THE HATCHING EGG

Day old chick quality depends for a large part on the quality of the hatching egg. It is therefore important during the whole reproductive period that all efforts are made to ensure optimum egg care and quality.

THE CHARACTERISTICS OF THE HATCHING EGG

➔ The composition of the egg and its influencing factors.

While the composition of the egg in terms of macro-ingredients (water, proteins and amino acids, total fats and macro-minerals) is slightly dependant on alimentary absorption, the trace elements, minerals and vitamins, and fatty acids from lipids, vary according to the nature of the nutrients ingested.

Therefore, unless a nutritional deficiency indirectly impairs the transfer of macro-nutrients to the egg, a feed with excess protein or calcium for example, does not necessarily improve chick or shell quality.

The situation is different for the micro-nutrients. The vitamin content of the egg (in particular the vitamins A, D and some of the B group) is directly linked to absorption from the alimentary tract. It is important to ensure that the nutritional requirements, especially for vitamins, are completely satisfied.

The same situation exists for the fatty acids. Feed with excess saturated fatty acids can lead to a reduced deposition of unsaturated fatty acids and compromise a good start to embryonic development.

Only flock age significantly impairs the level of macro-nutrients in the egg. As soon as the flock ages, the yolk size increases and the proportion of albumen in the egg decreases. The same applies to the macro-nutrients where pro rata increases and decreases occur depending on where they are concentrated in the egg.

This acts as an important reminder of managing the age of sexual maturity. If the start to production is too early this often leads to insufficient egg size, a reduced deposition of macro-nutrients in the egg and consequently, poor chick quality.

➔ Sanitary quality of the egg.

The sanitary quality of a hatching egg is a reflection of the sanitary status of the flock and of its environment from the moment the egg laid. It is therefore important that flocks are free from:

- ◆ Vertically transmissible diseases.
- ◆ Ovarian disease.
- ◆ Intestinal problems that can affect the absorption of vital nutrients from the intestine necessary for egg formation.
- ◆ Respiratory problems that can affect the blood pH and thereafter the transport and deposition of the nutrients in the egg.

The nest material condition and sanitation is critical:

In manual nests, nesting material:

- ◆ Should come from a secure source and be correctly disinfected on arrival at the store area.
- ◆ Should be stored in dry conditions away from direct sunlight and rain in a well ventilated area.
- ◆ Should be protected against all sources of contamination (wild birds, rats and other vermin, etc).

Once placed in the nests assure:

THE HATCHING EGG

- ◆ Disinfection (where permitted, 1 teaspoon – 5 to 10 grams – of para-formaldehyde powder each fortnight).
- ◆ Replacement of the nest material each 2-3 months or sooner if excessive faecal contamination occurs or if the material is allowed to get wet.

In automatic nests ensure:

- ◆ Protection from nest soiling at night time by an ejection or nest closure system.
- ◆ Regular washing and disinfection (including the collection belt).
- ◆ Replacement of the nest pads if there is abnormal damage.

➔ SELECTION OF HATCHING EGGS

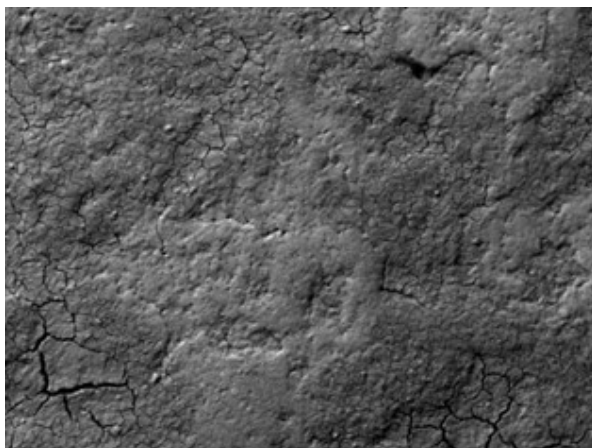
It will become apparent later in this guide when and how both shell quality and egg weight become important in determining the incubation parameters.

Although advocating the selection of eggs according to their weight and shell quality is not suggested, it is important to emphasise the importance of egg uniformity within the same flock. Egg uniformity and without doubt shell quality are directly related to the status of the donor flock.

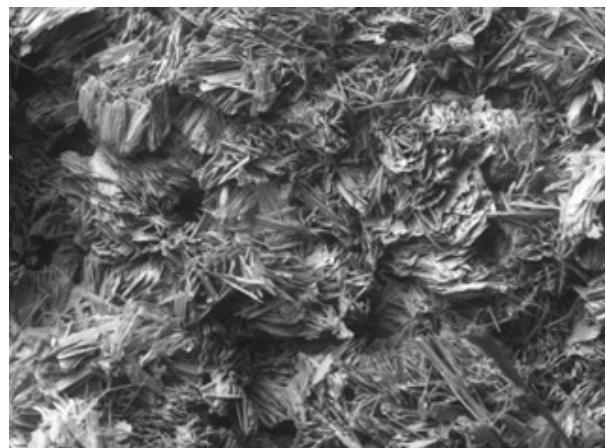
The choice of incubation parameters and profiles that correspond closely to each individual embryo's correct development requirements are easier when the eggs are uniform.

➔ The ideal hatching egg should:

- ◆ Have a dimension length to width of 1.4/1.0.
- ◆ Have a weight and size within the average of the flock.
- ◆ Be laid in a nest that is dry, clean and protected from dust.
- ◆ Come from a flock that is free of disease.
- ◆ Be free of faeces, or nest litter.
- ◆ Be clean and not soiled by albumen or yolk from other broken eggs.
- ◆ Be of uniform colour (dark or clear brown depending on the age of the flock) with a smooth shell exempt from roughness and calcium deposits.
- ◆ Have a solid shell, not broken, perforated or fragile and porous:



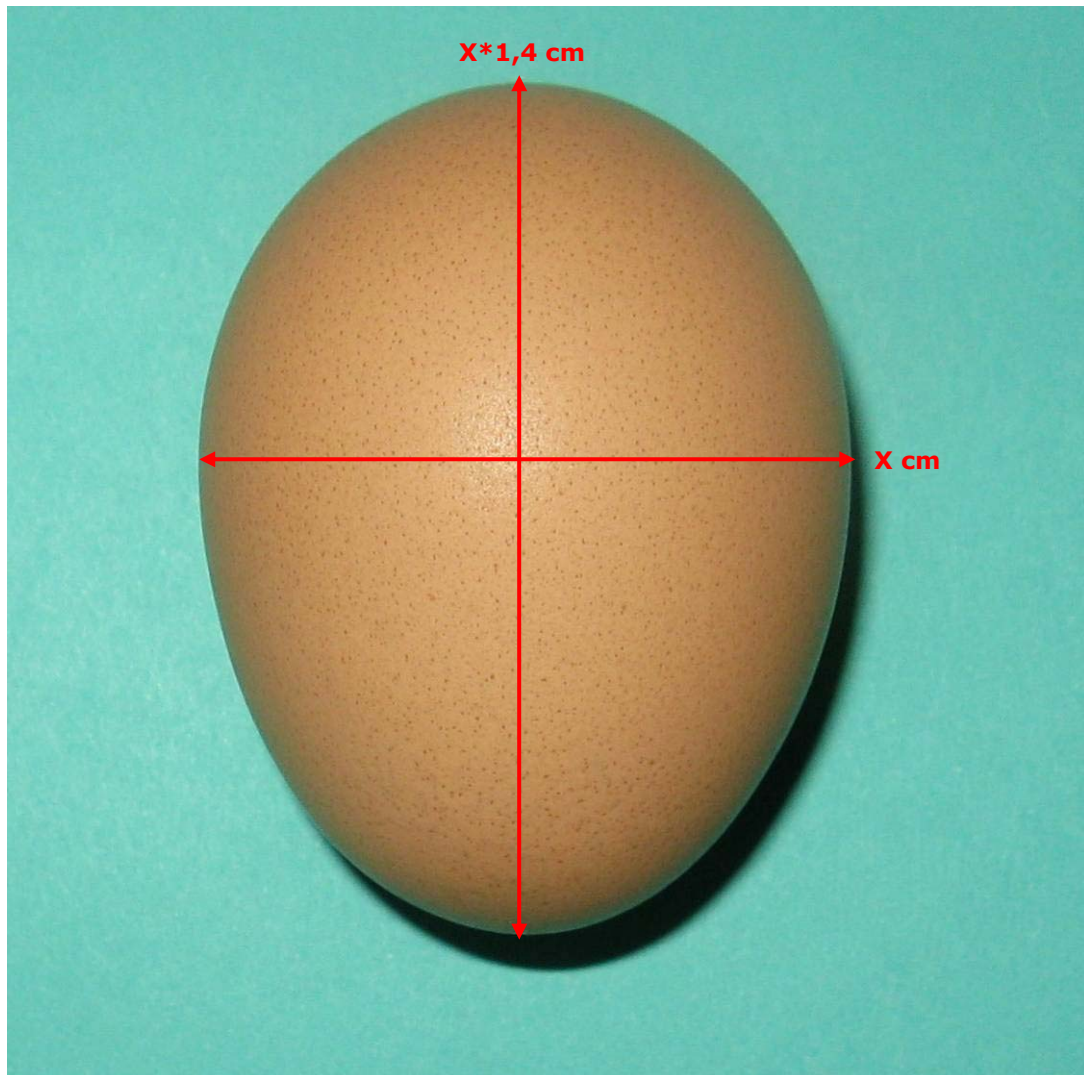
Smooth shell



Porous shell

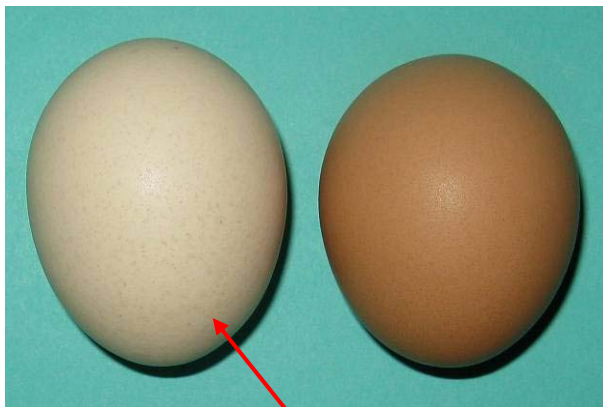
Selected eggs from the presentation by Dr. Eric Guinebert: From egg to chicken: miracle?, ITAVI, Rennes, SPACE 2004.

THE HATCHING EGG

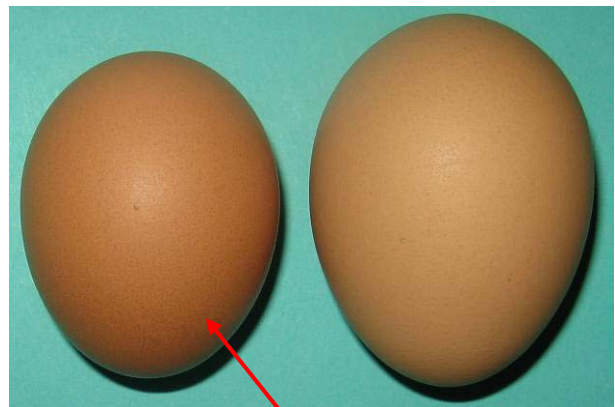


The ideal egg

Eggs that do not correspond to the above criteria should not be used for hatching eggs:

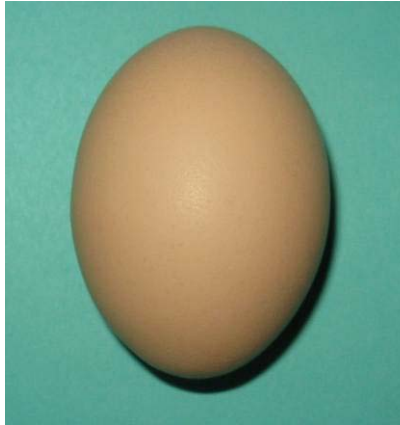


Pale shell



Small eggs

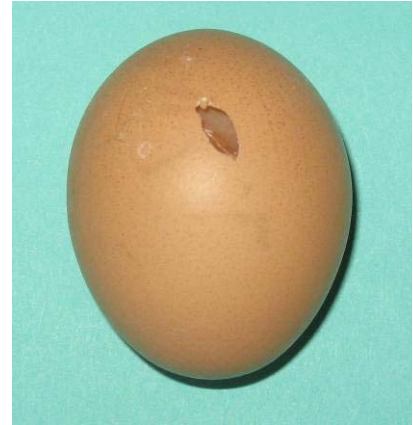
THE HATCHING EGG



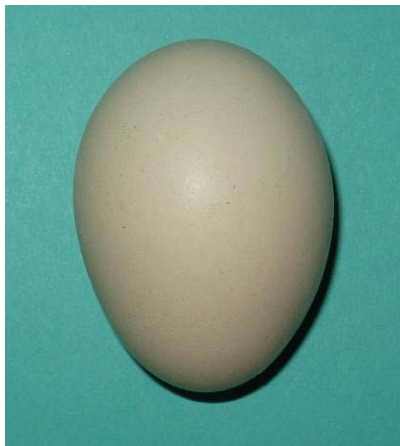
A long egg



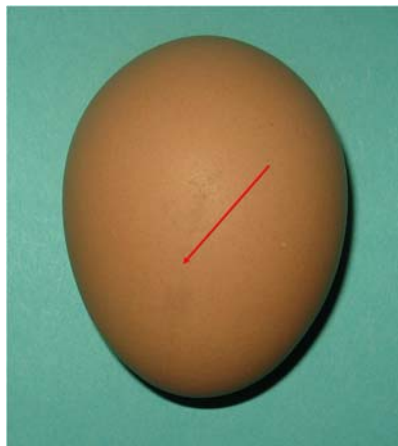
Calcification problems



Perforated egg shell



Deformed egg



Micro cracks



Dirty



Round egg



Stained egg shell



Wrinkled egg shell

If, for any reason, these eggs must be incubated, they should be identified, set and hatched separately.

THE HATCHING EGG

➔ DISINFECTION OF EGGS

Even if all precautions have been taken to produce an optimum quality hatching egg the risk of contamination is ever present and cannot be ignored. The egg is particularly susceptible to contamination during the formation of the air cell.

The creation of the air cell starts from the moment that the egg is laid. The progressive cooling of the egg leads to the contraction of its components (in particular the albumen and the pores found at the small end), which causes a type of suction. Ambient air enters the egg and becomes trapped between the shell membranes.

If the air that enters the egg is contaminated because the environment is dirty for example or has been contaminated by soiling, shavings or straw that adheres to the shell surface bacteria or fungi can enter the egg and adhere to the external shell membrane.

The degree of contamination may be very slight or not detectable after testing the shell but any contamination is very dangerous as pathogens multiply very quickly at the moment the chicks start to hatch.

Disinfection of eggs while they are still warm and cooling down is the best time to prevent bacterial or fungal penetration into the egg. Further, disinfection of the egg shell surface has little effect on contaminants that have already penetrated the egg shell.

When the egg is laid its temperature is slightly less than the hen's body temperature, approximately 40°C (104.0°F). It will take the egg 4-6 hours (depending on the external temperature) to attain ambient temperature. It is during this period that the air cell is created and that eggs should be disinfected.

This underlines the importance of frequent egg collection (4 to 5 times daily) to take advantage of disinfection as the air cell is formed. Infrequent egg collection reduces disinfection efficiency.

However, good techniques during egg collection and disinfection do not guarantee clean quality eggs. Shell quality plays a major role in the prevention from contamination and it is essential to do everything possible to assure that disinfection programs are optimal.

Several studies have shown that the length of time that eggs are exposed to bacteria plays a less important role than the thickness of the shell in preventing contamination. It has been shown that a specific gravity of greater than 1.080 is optimal.

Shell quality and bacteria penetration

Specific gravity of the egg	Shell quality	% penetration after 30 minutes	% penetration after 60 minutes	% penetration after 24 hours
1.070	Bad	34	41	54
1.080	Average	18	25	27
1.090	Good	11	16	21

Sauter E.A. and Petersen C.F. (1974)

THE HATCHING EGG

➔ Disinfection methods.

Whatever method is chosen, efficient disinfection cannot take place unless the shell is clean. It is rare for disinfectants to be effective against pathogens that reside in organic material or dust.

The principal methods of disinfection are:

Spraying:

Spraying disinfectant is an effective way to reduce risks of bacterial contamination. This method is especially useful when hatching eggs are collected directly onto the incubator trays because it is possible to spray disinfectant onto the round/top part of the egg at the moment the eggs are collected.

Disinfectants that are most commonly used are those with a quaternary ammonium base, phenols, hydrogen peroxide, iodine or glutaraldehyde. Be aware that some disinfectants when applied can block the pores of the eggs, which will reduce the water loss during incubation and cause reductions in hatchability. Please check the suitability of a product with the supplier and follow their recommended application methodology exactly.

For maximum effectiveness, assure that the solution temperature is between 38°C (100.4°F) and 48°C (118.4°F). The disinfection process should take place in a clean environment free of dust.

Fumigation:

This is the most widespread method used. It is most effective against shell surface contamination. However, the gas which results from the ignition of the component chemicals or solutions used is poorly diffused over the shell pores. Therefore it is essential that fumigation occurs during the formation of the air cell.

The products that can be used for fumigation are numerous. The most frequently used and their dose rates are:

- ◆ Para-formaldehyde powder: 8-10 grams per m³.
- ◆ Formalin (37.5%) and potassium permanganate: 2 dose levels are recommended by the OIE:
 - 53 ml of formalin and 35 grams of potassium permanganate per m³.
 - 43 ml of formalin and 21 grams of potassium permanganate per m³.
- ◆ Mixture of 40% formalin and potassium permanganate: 45 ml and 30 grams per m³ respectively.

Conform to the local legislation, which can restrict even forbid the use of formalin.

The use of formalin and potassium permanganate requires specific precautions. Use a wide metallic receptacle that is resistant to heat. Always add the formalin to the potassium permanganate, never the reverse, and operators must wear a full protective mask.

The maximum germicidal effect of formalin is when the ambient temperature is between 24°C (75.2°F) and 35°C (95.0°F) and humidity is 85-90%. Contact time should be 20 minutes and the resulting para-formaldehyde gas must be extracted quickly, or neutralised by using ammonium (half the volume of formalin used into a container for 10 to 15 minutes).

THE HATCHING EGG

- ◆ Numerous other products, often containing formalin, quaternary ammonium, hydrogen peroxide are available. Check with the supplier for their recommended use.

Ultra Violet-C type radiation:

This is a method mostly used for water treatment but is not often used for disinfecting hatching eggs.

This is potentially due to the fact that the exact application methods have not yet been fully defined (the precise time for exposure, without damage to the embryo varies from 40 seconds to 5 minutes). It is also difficult to imagine how all sides of all eggs can be exposed to the UV-C even if eggs are directly collected onto the incubator trays.

Only an automatic system where eggs are collected and placed onto the incubator tray, followed by passage through a system for disinfection where the eggs are turned to expose all sides to the action of the UV-C is recommended.

The UV-C process may possess an efficient activity against the bacteria and fungus adhering to the shell membranes that can pass through the shell but UV-C may have difficulty penetrating dust and organic matter.

Only UV-C levels of 250-275 nm are bactericidal.

It is important to protect the eyes, as prolonged exposure to UV-C can damage the retina.

Ozone:

Ozone is often used to disinfect water supplies, in the food industry and for conservation of food.

Its molecular weight is similar to that of oxygen or carbon dioxide and can therefore easily enter the pores of the shell. This characteristic gives it a bactericidal quality on the shell membranes, but it remains unstable for both operators and the embryo.

Ozone is toxic, corrosive, combustible and its use requires strict security rules. At high dose rates (3%) there is a damaging effect on hatchability. Using levels of 100 times less still has a negative effect on the developing embryo and at the same time there is limited bactericidal activity.

Some researchers do not advise ozone as an alternative to formalin.

The optimal method of use is still not well defined but it is known that ozone has a tendency to naturally disassociate itself as dioxide and traverses the cell walls and attacks biological components by oxidation.

Its bactericidal and virucidal activities are known but neither optimal contact time nor ambient temperature are yet fully understood.

Washing:

This is without doubt one of the best methods, but equally one that requires attention. The automatic washing machines, usually positioned just after placing eggs onto the incubator trays, use high pressure nozzles to wash and sanitise and then spray and disinfect the entire shell surface at a temperature of 40-50°C (104.0-122.0°F).

THE HATCHING EGG

Most eggs, including dirty eggs or floor eggs, can be cleaned by this method. Special attention should be paid to the choice of sanitizer or disinfectant, some of which especially chlorine based sanitizers have a tendency to react with the shell cuticle and lose their effectiveness. Others due to their composition tend to block the pores and affect gas exchange. Please refer to the disinfectant supplier for more details.

For optimal efficiency it is important to ensure the active ingredient in the sanitizer or disinfectant concentration remains constant throughout the operation by regular monitoring and “top up” of the disinfectant. It is also critical that egg washing equipment is kept clean and any build up of organic material is regularly removed. Because they create a warm humid environment, egg washers are an ideal environment for many bacteria to grow such as *Pseudomonas sp.* Rather than sanitise eggs, egg washing may re-contaminate eggs so an egg washer protocol should be followed at all times.

Wiping, sanding and polishing:

Many farmers remove shavings, rice hulls, straw and faecal material from the shell by wiping, sanding or polishing. As long as the method is not abused this is a useful way to clean the shell surface.

When an egg requires only 1 or 2 wipes to be cleaned it can be considered as a hatching egg. On the contrary if it requires more than 2 wipes to remove the dirt it is not a hatching egg.

Use of glass wool, rock wool or polishing is not recommended since they destroy the cuticle and a part of the shell.

EGG STORAGE

Many comments and recommendations have been made since the 1980's about duplicating natural incubation conditions as modern single stage incubation has set new standards. Unfortunately though, we still do not understand all the physiological issues that are important for the survival of the embryo during egg storage.

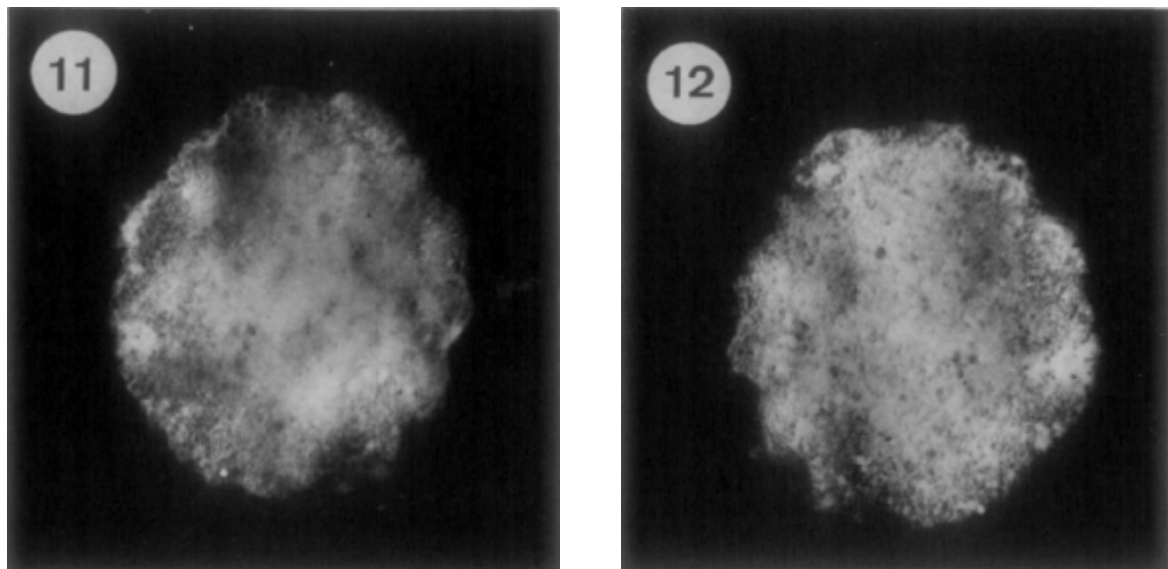
Storage, when it is longer than 7-8 days has a definite affect on hatchability, chick quality and future growth rate.

➔ FROM OVULATION TO OVIPOSITION

The egg is fertilised in the infundibulum shortly after ovulation. The first cleavage of the zygote starts approximately 5 hours after fertilization in the uterus and continues for a further 11 hours.

The cleavages of the embryo follow a model that is extremely variable, but they always start by the formation of a furrow in the central zone of the germ. The first 5 to 6 cell divisions follow this furrow vertically. The lower part of this furrow spreads laterally thus separating the central cells of the germ from the yolk. This is the start of the formation of the sub germinal cavity.

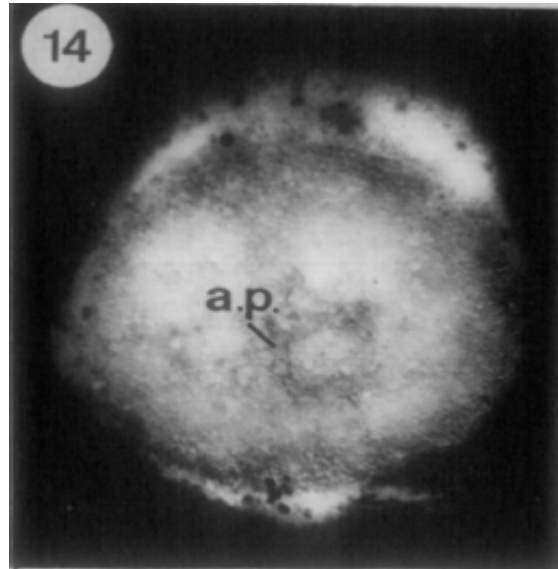
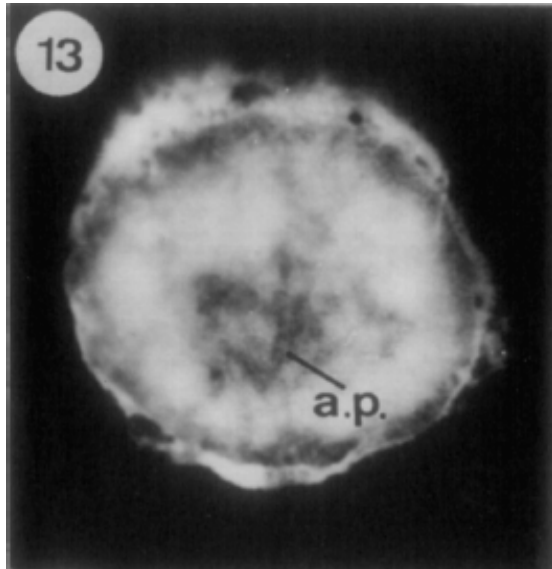
The rate of these mitotic divisions is extremely high during this period. They alternate from vertical and horizontal phases. After approximately 11 hours of cleavage, the cytoplasm disc at the top of the yolk, transforms itself into an opaque disc approximately 5 or 6 cells deep (Khaner O., 1993). This is stage VI of the classification determined by Eyal-Giladi H. and Kochav S. (1976):



Top view (11) lower view (12) of stage VI of embryonic development. The total cytoplasm mass of the germinal disc has divided, the cells are very small and form a uniform thick surface. It is at this stage that the germ can legitimately be called the blastoderm. (Eyal-Giladi H. and Kochav S., 1976).

The formation of the area pellucida starts at stage VII, approximately 12-14 hours after the egg enters the uterus. It is shown by the progressive migration of a certain number cells that face the sub germinal cavity, towards the bottom. From this moment the diameter of the germ increases regularly.

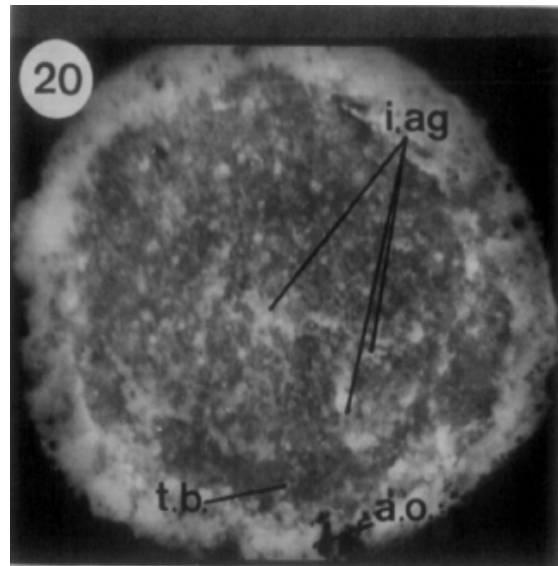
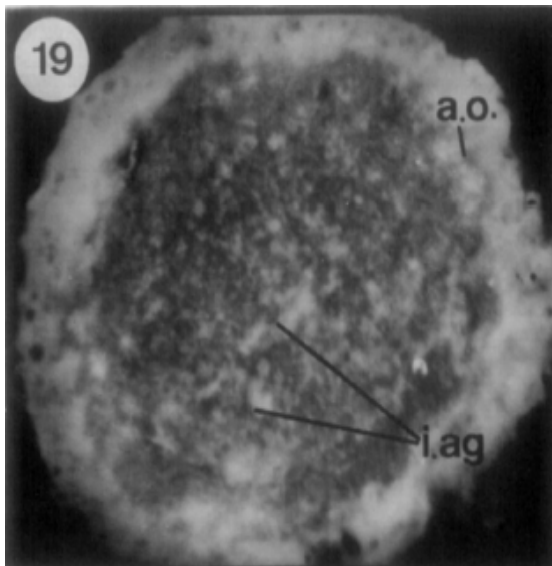
EGG STORAGE



Top view (13) lower view (14) of stage VII of embryonic development. While the top part remains intact, the lower part is subjected to cellular loss. This is the start of the formation of the area pellucida. (a.p.) (Eyal-Giladi H. and Kochav S., 1976).

The process continues for roughly another 8 or 9 hours to attain, at stage X of embryonic development, the area pellucida of one cell thickness and the area opaca is clearly defined. (Khaner O., 1993).

During this stage, within the lower part of the blastoderm, there is a visible formation of groups of cells and a further zone that is not part of this transformation. This starts the formation of the hypoblast.



Top view (19) lower view (20) of stage X of embryonic development. On the lower part isolated groups of cells appear (*isolated cell aggregates, i.ag.*), more numerous in the posterior part of the blastoderm. A *transparent belt (t.b.)* separates the groups of cells from the area opaca (Eyal-Giladi H. and Kochav S., 1976).

EGG STORAGE

It is generally at stage X of embryonic development that the egg is laid. The embryo consists of 40,000 to 60,000 cells, with a diameter of 3 to 4 mm and the anterior posterior axis is clearly defined.

However, some factors causing variation in cell number exist. Meijerhof R. (1992) showed in different studies that the age of the flock plays an essential role in the embryonic development at the moment of oviposition. Thus, the older the flock the more advanced the cellular development at oviposition.

Bodyweight appears to play an important role as well. Lines selected for increased body weights during rearing have a tendency to lay eggs with an advanced stage compared to ones selected for a lighter weight.

The position of the egg in the clutch can also influence the stage of development. Since the transit time is often longer, the first and last eggs laid in a clutch have a tendency to be laid with an advanced stage of development compared to those in the middle of the clutch.

The nest type also plays an important role. Embryos from eggs laid in manual nests are often at a more developed stage than those laid in automatic nests or in cages because they take longer to cool down after lay. This effect is due to the combination of the insulation value of the nest material and the presence of the hen that may sit on the nest for a long time period.

However, the stage of embryonic development at the moment of oviposition appears to be an essential factor affecting embryo survival during incubation:

The Stage of Embryonic Development at Oviposition and Hatchability

Pre-gastrula stage (< stage EG10*)	Advanced gastrula stage (≥ stage EG10*)	Hatchability
% embryos at this stage of embryonic development	% embryos at this stage of embryonic development	%
30	70	>55 to >84
62	38	<55

(*) Stage of embryonic development after classification by Eyal-Giladi H. and Kochav S. (1976).

Adapted from Reijrink I. *et al* (2008)

Furthermore, it seems to play primordial role in the capacity of the embryo to resist long storage periods. Numerous studies indicate that embryos at stage X of embryonic development resist storage stresses less than stages XII (Reijrink I., 2009) or XIII (Fasenko G.M. *et al*, 2003a) embryos.

It seems that the stages where the hypoblast and epiblast are still being formed (stage XII), or completely formed (stage XIII), can only be obtained by incubation.

➔ PRE-INCUBATION (PRESI)

Under natural conditions the hen does not tend to maintain eggs at a constant temperature. To the contrary, each time she lays a new egg, she reheats that egg and also the eggs she laid previously.

It is possible that this period of broodiness, which is short and intermittent, is intended to bring the embryos to a more advanced level and favours the regeneration of cells that have died during storage. Pre-incubation (PRESI - *pre-storage incubation* -) attempts to reproduce this phenomenon.

EGG STORAGE

This process consists of setting eggs in the incubator, direct from their arrival at the hatchery and before placing in the cool room, at a temperature of 37.7-37.8°C (99.9-100.1°F) for a period of 6 hours.

Tests that have been conducted in our hatcheries have always shown positive results (+4.1% on average for eggs stored between 4 and 13 days), but other researchers have obtained less conclusive results. The stage of embryonic development at the time of oviposition, pre-incubation temperature and the length of time since the egg was laid all seem to be factors that can greatly affect the success of this method.

Fasenko G.M. *et al* (2003b) found that pre-incubation a few days after being laid can have negative effects on hatchability.

This technique should be used with caution. The method is as yet not properly defined and beneficial effects may not always occur. The method is not always practical, as one incubator is permanently dedicated to this process.

➔ PHYSICAL-CHEMICAL CONSEQUENCES ON STORING EGGS

“Physiological zero” the temperature at which embryonic development ceases is also not well defined. Decuypere E. and Michels H. (1992) reviewed this subject in more detail. Some researchers found that it is 20-21°C (68.0-69.8°F), while others found it to be 25-27°C (77.0-80.6°F) with some even saying 28-29°C (82.4-84.2°F).

This variation in physiological zero temperature may be linked to different requirements and functions of the tissues concerned. This is indirectly shown by Wilson H.R. (1991) where a disproportionate development is observed when the embryo is maintained at a temperature varying between 27-35°C (80.6-95.0°F).

➔ **Water loss during storage.**

The organic cuticle that covers the shell at the pore level forms layers full of cracks and crevices that enlarge as the egg ages and allow the gaseous exchange between the egg and the ambient air.

Water loss is caused by evaporation, which is determined by the length of storage, temperature and humidity of the ambient air and the surface and porosity of the shell (Sauveur B., 1988).

Initially, evaporation starts from the shell membranes. This is followed by evaporation from the albumen. Although it has been suggested that water loss can have a negative effect on the albumen viscosity, no direct relationship has yet been established between evaporation, pH and albumen density.

Meijerhof R. *et al* (1994), reviewed by Reijrink I. *et al* (2008), showed that water loss during storage was largely unaffected by ambient humidity when this varied between 55 and 75%. In spite of this, it is generally agreed that water loss during storage should be limited and should range between 0.8 and 0.9% per week.

➔ **Affect of albumen.**

The “thick” albumen density results from the level of electrostatic links between ovomucin (and more particularly its sub unit β) and lysozyme. The divalent cations of the albumen (magnesium and calcium) are binding factors.

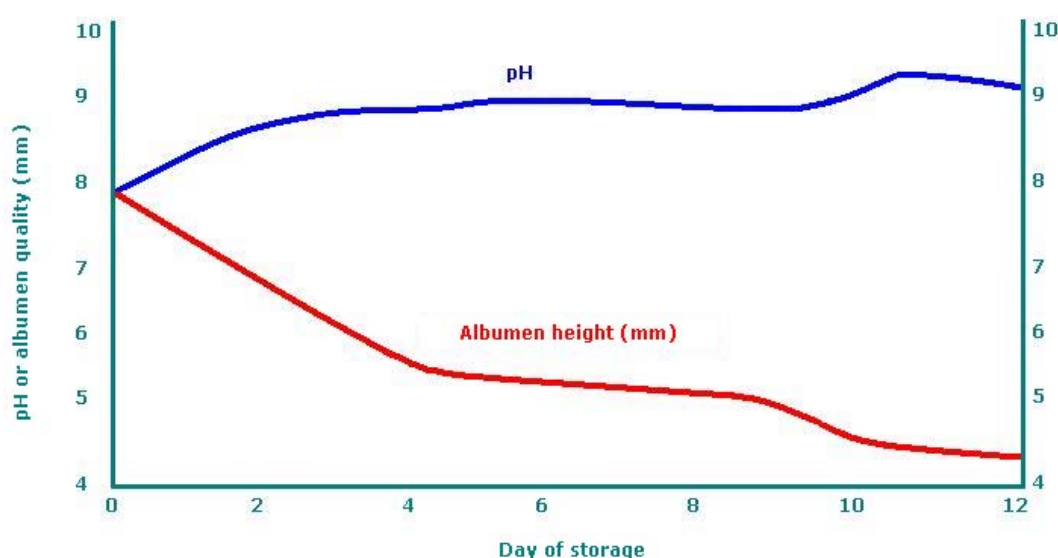
Albumen density is very dependant on pH, which naturally decreases regardless of the age of the flock or the increasing egg weight. It seems to play an essential role in gaseous exchanges and transport of nutrients to the embryo.

EGG STORAGE

After the egg is laid, the CO₂ in the albumen progressively escapes. The rate of loss of CO₂ will certainly depend on the buffering power of the albumen (which attains its minimum when the pH range is between 7.0 and 9.0), but also the ambient temperature, the shell conductance, storage time and the gas environment around the egg.

The points listed above lead to an increase of albumen pH, which starts at approximately 7.6 when the egg is laid and increases to 9.0 or 9.2 a few days later.

Evolution of pH and height of "thick" albumen during storage



Adapted from Van de Ven L. (2003)

The increase in pH is both important and necessary, not only because the early embryo development is governed by enzymes dependant on pH (Decuypere E. *et al*, 2001), but equally because the alkaline pH protects the embryo against potential bacteria contamination.

It is interesting to note that most of the pH increase happens during the first 3-4 days. This can explain the fact that eggs stored for short periods have a tendency to hatch better than those eggs incubated the same day as the egg is laid. The breakdown of the albumen, which causes the increase in pH, facilitates the gaseous exchanges and transport of nutrients to the embryo (Lapão C. *et al*, 1999).

This is especially true for eggs from young breeder flocks. Their albumen density is higher than eggs produced from older flocks, which is partly due to the fact that the pH of the albumen when the egg is laid is slightly lower:

Age of the flock (weeks)	pH of the albumen the day of laying	Thickness of the albumen the day of laying (mm)
32	8.08 ± 0.02	7.74 ± 0.29
42	8.19 ± 0.02	7.44 ± 0.29
54	8.23 ± 0.02	7.06 ± 0.29
59	8.30 ± 0.02	6.28 ± 0.29

Lapão C. *et al*, (1999)

EGG STORAGE

It is also interesting to note that whatever the pH at the start of storage, it tends to stabilize to the same level of approximately 9.0-9.2, 4 to 5 days after the egg is laid (Lapão C. *et al*, 1999). Long term storage does not lead to further increases in pH.

The best known effect of this increase on pH is “watery” albumen. The albumen height, as measured in Haugh units, reduces over time according to an exponential curve.

➔ The effects on the yolk.

At the moment the egg is laid, the yolk pH is between 6.0 and 6.3. It then increases progressively and stabilizes at around 6.5-6.8 (Reijrink I. *et al*, 2008).

During storage, the physical/chemical changes of the yolk are affected by the same criteria that apply for albumen. The increase in pH leads to:

- ◆ The vitelline membrane to become weak (similar to the composition of chalazae).
- ◆ A large amount of water being transferred to the yolk from the albumen, due to the high level of osmotic pressure between the albumen and the yolk, which contains hydrophilic proteins.
- ◆ The transfer of divalent cations to the yolk, which accelerates the process of “watery” albumen.
- ◆ A decrease in viscosity and a change in the yolk structure (relationship between yolk height and width causing the yolk to lose its shape).

If these changes occur more rapidly, due to ingestion of certain raw materials, coccidiostats and even some anti-parasitic medications, stains on the yolk surface can appear. This is known as “mottling” or “marbling” and can even be seen the moment the egg is laid (Sauveur B., 1988).

Observations after egg storage:

- ◆ An abnormally high level of water on the yolk.
- ◆ A loss of iron from the yolk to the albumen, which gives it a pink colour.
- ◆ Penetration of proteins into the yolk that gives it a salmon colour.

Since the density is lighter, the liquidification of the albumen also affects the placement of the yolk which moves to the top of the egg, where under normal storage conditions the air cell is located. The resulting exposure to the risks of dehydration and oxidation can compromise the survival of the embryo.

➔ Effects on the embryo.

During storage, the pH of the albumen changes rapidly from about 7.6 to 9.0 or 9.2. This leads to the progressive increase in the permeability of the vitelline membrane, which (including the chalazae), protects the embryo during storage and also the first 2-3 days of incubation up to the start of the embryo's appendages.

This weakening of the vitelline membrane, exposes the embryo to levels of high alkaline pH, which it is suggested, is responsible for early embryonic mortality (Reijrink I. *et al*, 2008).

Gillespie J. and McHanwell S. (1987), reviewed by Reijrink I. *et al* (2008), measured the pH in the extracellular space during the first few hours of incubation. They found varying values of 7.9 to 8.4. During previous studies the same researchers showed that migration of the fibroblast was optimal when pH was 8.2. It therefore appears that the optimal pH for the embryo development during the first few days of incubation is between 7.9 and 8.4.

EGG STORAGE

Research by Sauveur B. *et al* (1967) and Walsh T. (1993), reviewed by Brake J. *et al* (1997), showed similar results. For optimal embryonic development the albumen pH should be between 8.2 and 8.8.

It appears that the high pH that the embryo is subjected to (approximately 3 units of pH difference between the yolk and the albumen) is necessary for its development (Brake J. *et al*, 1997). This is not to say that the pH of the embryo evolves with time. Whatever level it is subjected to, the pH of the embryo remains sufficiently stable throughout storage.

It is at this moment that the level of embryonic development at the time of oviposition becomes important. When development is not too advanced, due to the ensuing metabolism and production of CO₂, embryos are incapable of maintaining an adequate level of pH. The embryos would be more comfortable with a more advanced stage of development.

The effects of embryonic development and pH are more complex though. Numerous studies have shown that pH levels close to those observed at the moment of oviposition, and obtained by exposure to an enriched CO₂ environment, have a negative affect on hatch results. It seems that the use of CO₂ is only beneficial if the levels used bring the levels of albumen pH close to those observed during the first 3 to 5 days of storage.

Temperature has an important affect on the embryo. Even if the egg is held at a temperature below "physiological zero", and even if no major morphological change is observed during storage, the incidence of apoptotic cells (which initiate the process of auto destruction) or necrotic cells seems to increase as soon as the length of storage and temperature increases.

Arora K. and Kosin I. (1968), reviewed by Reijrink I. *et al* (2008), observed that eggs stored for 21 days that the level of mitosis and necrosis were increased when storage temperature increase above 10°C (50.0°F). Also Bloom S. *et al* (1998), reviewed by Reijrink I. *et al* (2008), found that the percentage of apoptotic cells increased from 3.1% at the moment of oviposition to 13.9% after 14 days storage at 12°C (53.6°F).

➔ STORAGE CONDITIONS

➔ **Temperature.**

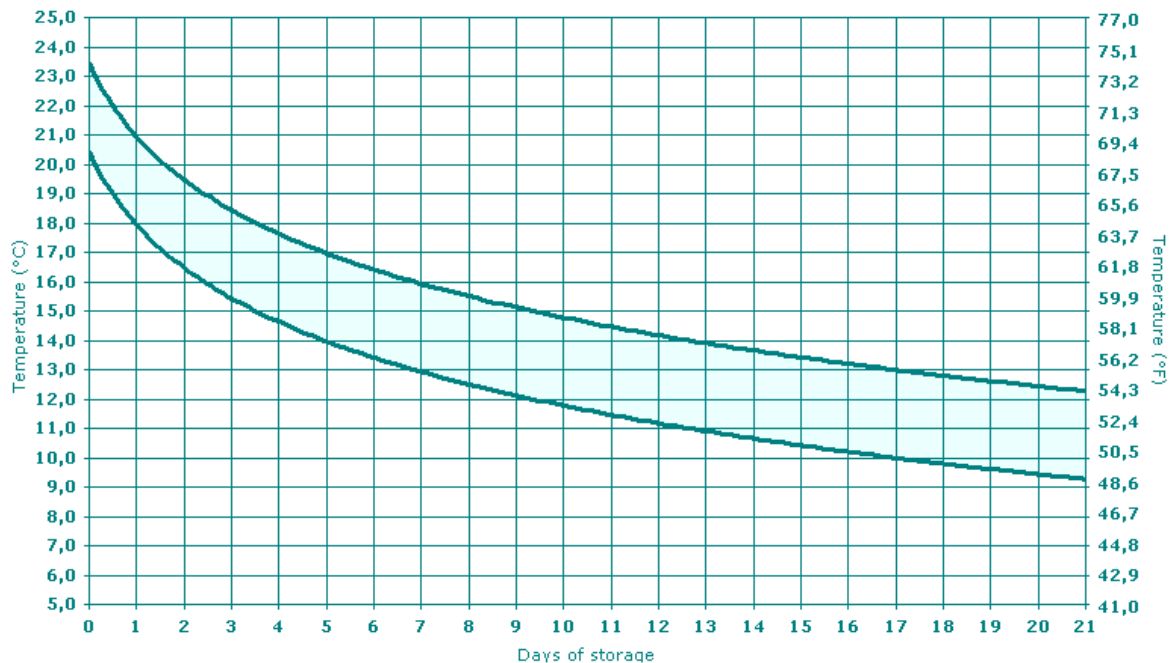
The previous paragraph shows that temperature plays a major role in embryonic survival during storage. Key points are:

- ◆ The progressive cooling down of eggs allows the embryo to attain a level of development able to sustain long term storage.
- ◆ For short term storage, temperatures slightly below "physiological zero" will allow some breakdown of the albumen and help transfer nutrients to the embryo, without greatly affecting the vitelline membrane.
- ◆ As soon as storage becomes longer, lower temperature allows for a substantial reduction in the number of apoptotic and necrotic cells.

This leads us to the following recommendations:

STOCKAGE DES ŒUFS

Evolution of temperature during storage



This is supported by the research compiled by Brake J. *et al* (1997). For eggs stored for more than 14 days, the best hatch results are obtained when storage temperature is about 12°C (53.6°F). However a temperature of 15°C (59.0°F) gives better results when eggs are only stored for 8 days and 18°C (64.4°F) is best for eggs stored for 2 days.

➔ Humidity.

It has been shown that humidity during storage does not appear to play a critical role in embryo survival. However there are two exceptions:

- ◆ Initially Walsh T. *et al* (1995) observed that for eggs stored at 23.9°C (75.0°F) over a period of 14 days, water loss is increased and hatch was reduced. The work of Jin Y. *et al* (2011) showed similar results; when eggs are stored at 29.0°C (84.2°F), water loss increases rapidly from 1.74% at 5 days storage to 3.67% after 10 days storage.
- ◆ Secondly, when storage temperatures are low from the 7th day, it can be more difficult to attain sufficient levels of humidity in the air to prevent excessive dehydration (Brake J. *et al*, 1997).

Practically, because shell conductance can sometimes be too high, or because the albumen quality is insufficient, only eggs from older flocks show increased sensitivity to low humidity (Brake J. *et al*, 1997).

In spite of all of this, it is recommended that water loss during storage should be limited. Ideally it should be between 0.8 and 0.9% per week.

➔ Turning eggs.

Turning eggs during storage is practiced by many hatcheries.

EGG STORAGE

Deeming D. (2000) suggested that turning of eggs allows the embryo to be exposed to new sources of nutrients and that this helps it to resist longer storage periods. Without turning, the embryo would be subjected to the same environment and perhaps more rapidly destroyed by embryonic metabolism.

Turning therefore allows the embryo to have access to new energy sources.

However, other theories exist. It is generally agreed that turning prevents excessive dehydration and oxidation of the embryo (the yolk is less likely to stick to the shell membranes).

Nevertheless, the effects of turning are still unclear. Proudfoot F. (1966) observed that eggs stored at an angle of 50° and turned daily 180°, showed better hatch than non-turned eggs. The longer the storage period then the effects of turning became more important with little or no effect after 14 days storage. Effects were noted from 21 days storage and above.

Elibol O. *et al* (2002) found that turning during storage was especially beneficial on eggs coming from old flocks but that the impact on eggs from young flocks, regardless of the storage period (3, 7 and 14 days used in the trial), was negligible.

The conclusions of Mahmud A. and Pasha T. (2008) were similar: turning eggs during storage (6 to 8 times daily) brought no benefit on eggs coming from flocks of 32 weeks and stored for 5 days.

Sauveur B. (1988) went further when he stated that turning of eggs during storage has never been shown to lead to significant improvement in results.

It is therefore unlikely that turning can be widely recommended. Nevertheless, it seems that results from Elibol O. *et al* (2002) are logical and turning eggs from older flocks may be worth further consideration.

➡ **Storage with the small end up.**

Studies on this are rare. Sauveur B. (1988) observed that storing with the small end up is favourable for long term storage. Deeming D. (2000) observed that the yolk remains in contact with the albumen which keeps the embryo away from the shell membrane.

Tests carried out in our own hatcheries have always shown positive results and it is a technique that can be considered when eggs are stored on paper or plastic trays for egg storage for longer than 14 days.

In order not to damage the air space care should be taken when handling the eggs.

➡ **Control of the atmosphere.**

Techniques that aim to alter the gaseous environment for the eggs during storage include:

- ◆ Techniques to partially replace oxygen by nitrogen to reduce the risk of oxidation, or
- ◆ Techniques to increase the levels of ambient CO₂ and limit the loss of CO₂ from the albumen.

EGG STORAGE

The affects of nitrogen on the survival of the embryo during storage are uncertain. Proudfoot F. (1965, 1972), reviewed by Reijrink I. *et al* (2008), found that using nitrogen may reduce the level of oxygen in the air to approximately 4%, but also tends to stabilize the pH of both the yolk and albumen. This may also have a positive effect on the hatch. Reijrink I. *et al* (2010a) found no positive effect when they tested eggs stored for 14 days at a temperature of 16°C in an environment of 95.8% nitrogen.

The effects of CO₂ are more controversial. Meijerhof R. (1992) quoted numerous researchers who not only found no positive effect using CO₂, but who moreover slowed down the negative effects. Reijrink I. *et al* (2008) observed the same effect. Each time researchers tried to maintain the pH level of the albumen at the same level as at oviposition, no positive effect on hatch was observed.

Placing eggs inside plastic cryovac bags has often been shown to be beneficial. The alteration of the gaseous environment occurs by increasing the levels of humidity and CO₂ and lowering of the level of oxygen. This maintains the pH of the albumen close to that normally observed after 3-5 days of storage (see page 18).

➔ RECOMMENDATIONS

Duration and conditions for storage

	Period of storage						
	1-2 days	3-4 days	5-6 days	7-8 days	9-12 days	13-16 days	17-20 days
Temperature	19.0°C (66.2°F)	17.0°C (62.6°F)	15.5°C (59.9°F)	14.0°C (57.2°F)	12.5°C (54.5°F)	12.0°C (53.6°F)	11.5°C (52.7°F)
Relative Humidity (%)	70.0	80.0	85.0	90.0	90.0	90.0	90.0
Turning	No	No	No	No	Yes	Yes	Yes
Small end up	No	No	No	No	No	Yes	Yes
Bagging	No	No	No	No	No	Yes	Yes

➔ EFFECTS OF STORAGE ON INCUBATION TIME

It is generally agreed that storage increases the total incubation time. This is essentially due to a delay in the development of the embryo (on average 45 to 50 minutes for each day of storage) and to a slower growth rate of the embryo during the first 48 hours of incubation (Sauveur B., 1988).

Research by Fassenko G.M. (2007) observed the same effect. Arora K. and Kosin I. (1966) showed that eggs stored for long periods do not start development immediately after being elevated to incubation temperatures. Mather C. and Laughlin K. (1977) showed that the embryonic development of eggs stored for 14 days compared to fresh eggs was delayed 12.2 hours. They also observed that embryo development during the first part of incubation is much slower.

During her study, Fassenko G.M. (2007) observed that the embryonic development of eggs stored for 14 days starts about 6 hours later than for fresh eggs. This implies that the individual response to incubation temperature can be variable; some embryos start development quickly while others are significantly delayed.

EGG STORAGE

The causes of this variability are not well known but it is suggested that the embryonic stage of development at oviposition is the most likely reason (more advanced growth, so a quicker response to the incubation temperature).

Storage not only affects the length of incubation but also has an effect on embryonic mortality and chick quality.

However, the effect of egg storage relative to the age of the donor flock has not yet been clearly identified. Yassin H. *et al* (2008) and De Lange G. (2009) found that prolonged storage has more of an affect on eggs from young breeders. Meijerhof R. *et al* (1994), reviewed by Reijrink I. *et al* (2010b), Lapão C. *et al* (1999), Elibol O. *et al* (2002) and Tona K. *et al* (2004) showed the results were worse as the age of the donor flock is older. This is attributable to the thick albumen being less dense.

Egg storage not only interferes with the hatch results but chick quality and subsequent growth as well. Therefore, everything possible should be done to ensure storage and subsequent incubation conditions are perfectly managed.

INCUBATION

➔ PREHEATING

From the previous section, it is clear that the conditions and length of storage play a major role in changes to the physical-chemical properties of eggs, the development and survival of the embryo and therefore the hatch results.

Initial thoughts may be that preheating compensates for the effects of egg storage. In fact the reverse may be more correct because preheating minimizes the adverse impact of storage. This is achieved in 3 ways:

- ◆ To favour the regeneration of cells that die during storage.
- ◆ To better co-ordinate embryo development before incubation starts.
- ◆ Reduce the hatch window and thus improve the chick quality.

The preheating methods used can vary from hatchery to hatchery but they are all based on a progressive increase in temperature to a level that allows regeneration of cell growth. Funk E. and Biellier H (1944), reviewed by Reijrink I. *et al* (2010b), have shown that the morphological development of the embryo continues when the internal egg temperature is more than 27°C (80.6°F).

The goal of preheating is therefore to bring the eggs to a temperature that will promote cell growth during a period that is sufficiently long to permit the majority of embryos to arrive at the same level of development:

Conditions for preheating

	Temperature	Humidity	Duration
In front of the incubators (controlled temperature, but minimal air movement)	25-27°C (77.0-80.6°F)	50-55%	Minimum 12 hours
In the Incubator	25-27°C (77.0-80.6°F)	50-55%	Minimum 8 hours

Studies by Reijrink I. *et al* (2010b) investigated the preheating conditions in further detail. By comparing two methods of preheating they only found beneficial results when the storage time was consistent.

Duration of storage	Method of pre-heating	Fertility (%)	Hatch (%)	Hatch of fertile (%)	Total embryonic mortality (%)
4 days	From 19.0 to 37.8°C in 4 hours	95.6	88.6	92.7	7.13
	From 19.0 to 37.8°C in 24 hours	95.0	88.9	93.5	6.34
13 days	From 19.0 to 37.8°C in 4 hours	93.6	68.5	73.2	26.68
	From 19.0 to 37.8°C in 24 hours	92.1	72.6	78.9	20.87

Adapted from Reijrink I. *et al* (2010b)

INCUBATION

The results shown above are similar to those obtained by Mahmud A. and Pasha T. (2008). These researchers found no benefit to preheating when egg storage was short.

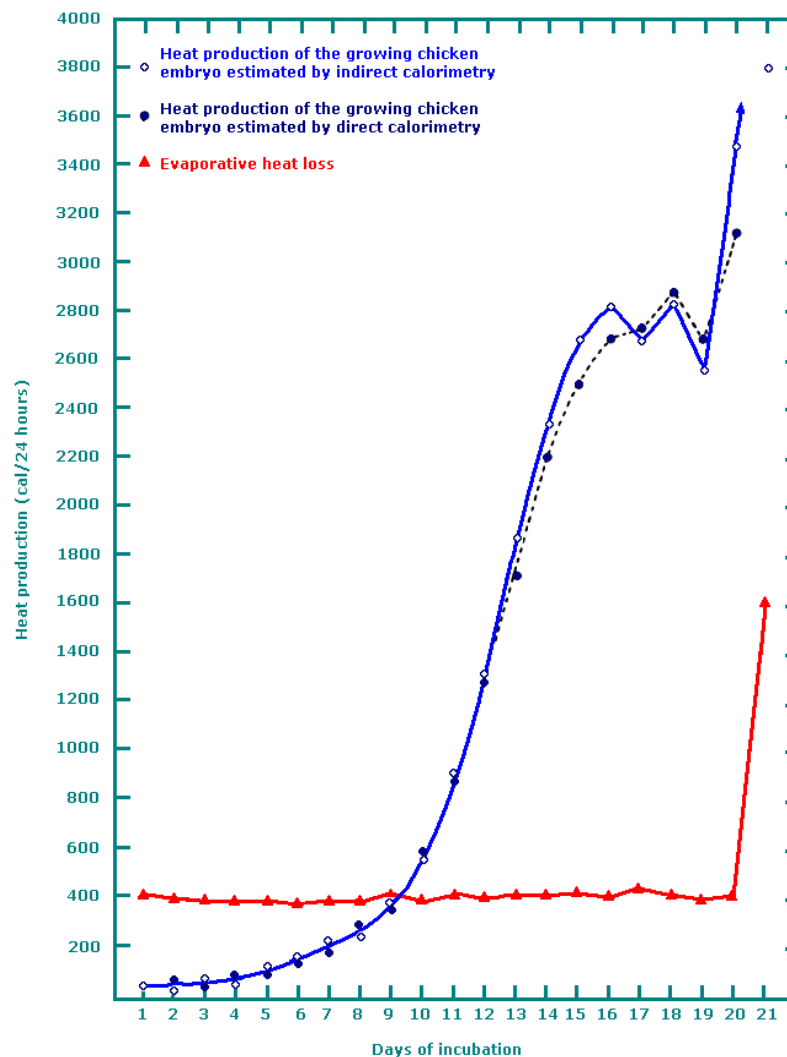
➔ INCUBATION TEMPERATURE

Embryonic development is essentially controlled by temperature. It is one of the most important parameters in determining the incubation conditions.

➔ Heat production from the embryo.

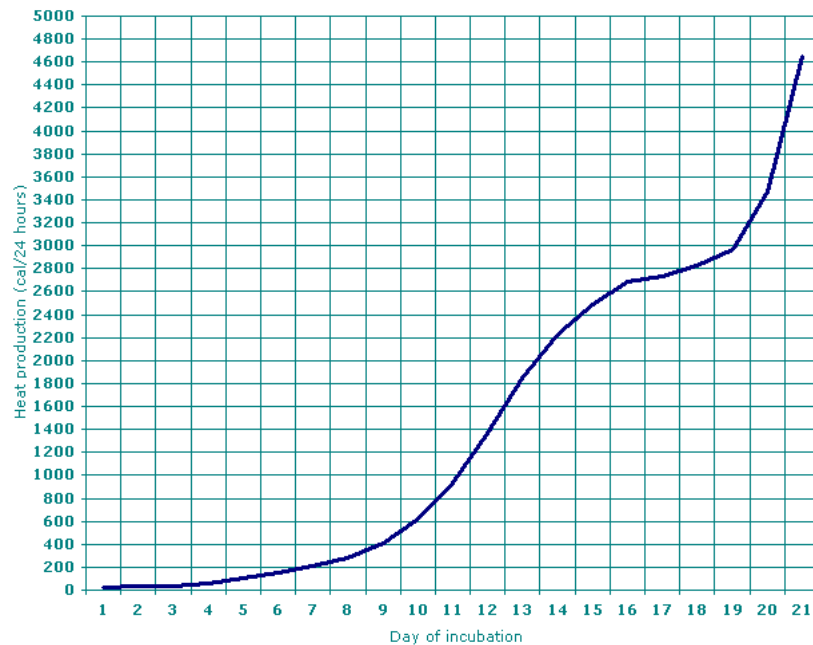
It is generally agreed that during embryonic development there are two important periods; endothermic at the start of incubation, lasting about 8-9 days and exothermic at the end of incubation, lasting about 7-8 days. Between the two, a stage called isothermic which is often very short is also sometimes mentioned.

Romijn C. and Lokhorst W. (1960) were the first to determine the heat production from the embryo:



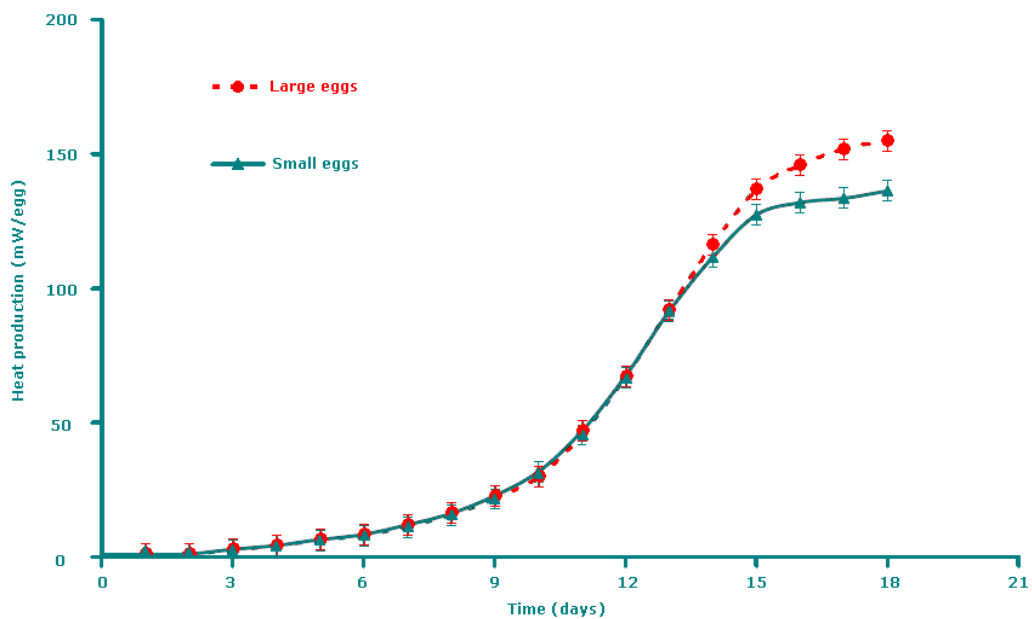
INCUBATION

Their observations are in agreement with those of Sauveur B. (1988), based on part from work done by Romanoff A.L. (1967):



Hardly 7 years separates these 2 graphs and the scales and values are comparable. Nearly 40 years later, however, Lourens A. *et al* (2006) suggested two other major factors affecting the production of heat from the embryo:

- ◆ The growth potential of the breed.
- ◆ The egg weight.



INCUBATION

The scales are different (1 cal/24 hours = 0.048425925 mW/egg) and it is possible to conclude that commercial breeds produce at the end of incubation approximately 20% more heat than breeds described as "traditional". This is confirmed by studies made by Boerjan M. (2005):

Metabolic heat production (W/1000 eggs) from commercial broilers, layers and North Holland Blue "traditional"

Days of incubation	Fast growth (broiler)	Egg layers (white)	Traditional breed
17	151.2	133.2	130.0
18	156.6	130.2	137.0
19	164.4	127.2	124.0
20	252.0	130.8	169.0

Adapted from Boerjan M. (2005)

It appears that incubation programmes should take into account the growth potential of the breed and that it is impossible to satisfy the needs of all embryos of breeds with different growth potentials if they are incubated in the same machine.

However, Lourens A. *et al* (2006) and other earlier studies have clearly identified the importance of egg weight. By maintaining a constant shell temperature during incubation, they observed that large eggs (70 g average weight in the trial) need less adjustment of machine temperature compared to smaller eggs (56.1 grams average weight in the trial) during the second part of incubation.

This suggests that the growth of embryos within large eggs accelerates from 14-15 days of incubation.

These observations are in agreement with those of Wilson H.R. (1991), who observed that the embryo weight is not correlated to the egg weight during the first part of incubation.

It appears therefore that large eggs have different requirements and cannot be incubated with smaller eggs.

➔ Heat perceived by the embryo.

The concept of heat perceived is different to that of heat produced; the first being very dependant on the surrounding environment of the egg (including shell quality) while the second is intrinsic to the embryo metabolism.

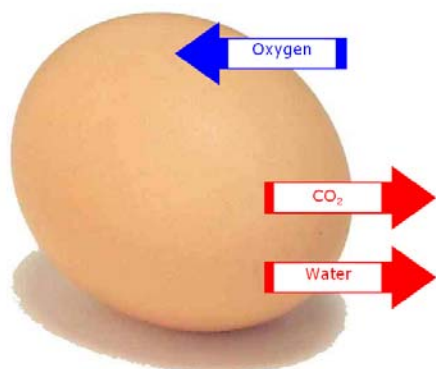
It has already been explained that embryonic development encompasses two phases; one endothermic and the other exothermic. Heat perceived by the embryo depends on the capacity of the machine to heat (increase temperature) or exhaust (reduce temperature). There are 4 main factors:

- ◆ Shell conductance.
- ◆ The difference in temperature between the egg and the environment.
- ◆ The caloric capacity of the air.
- ◆ Air speed.

Shell conductance:

Under aerobic conditions, the principal source of energy for the embryo comes from the lipids contained in the yolk. Their metabolism may provoke the production of water and CO₂ which must be evacuated through the shell pores.

INCUBATION



Conductance is no more than the capacity of the shell to diffuse the necessary gasses for the metabolism of O₂ and its by-products (H₂O and CO₂).

This depends on the functional surface of the pores and shell thickness and is independent of the incubation conditions.

It is not an expression of the speed at which gas can be exchanged. This only depends on the gradient differences in concentration between the egg and its environment.

Shell conductance is very important in determining how easily the embryo can eliminate heat at the end of incubation. Eggs whose shell conductance is high can support higher incubation temperature levels compared to those eggs whose conductance is low. Low conductance eggs should be incubated separately using a lower temperature.

Since shell conductance does not increase in a linear relationship with egg size, it follows that large eggs may always have difficulty eliminating heat that is produced by the embryo (French N.A., 1997).

The factors that control shell conductance are not well understood. Within the same flock under the same conditions of feed and environment, results can still be variable. Visschedijk A. *et al* (1985), reviewed by Molenaar R. *et al* (2010), showed that the CV% of shell conductance can be up to 22% on eggs coming from the same flock on the same day which is approximately 3 times greater variability than is observed for egg weight.

Nevertheless, optimizing flock uniformity is a helpful tool to help optimise shell quality.

The difference of temperature between the egg and its environment:

The speed at which heat is exchanged essentially depends on the difference between the temperature of the egg and its immediate environment. Since the production of heat from the embryo is inferior to the loss of heat by evaporation during the first 8-9 days of incubation (see page 24), the set point temperature of the incubator should be higher than that of the embryo.

Inversely, from the 9th to 10th days of incubation, the heat production from the embryo is greater than the loss of heat by evaporation. The set point temperature of the incubator should therefore be lower than embryo temperature.

The calorific capacity of the air:

The calorific capacity of the air is determined by relative humidity. When the air is dry, it is a poor conductor of heat. Humid air allows a more uniform distribution of temperature within a machine.

It is better to use slightly higher humidity at the start of incubation to distribute heat more evenly, which produces a more uniform development of the embryos. As an alternative to the permanent use of humidifiers, the increase in humidity is best achieved by closing the ventilation outlets, which forces passive evaporation from the eggs themselves.

INCUBATION

Air speed:

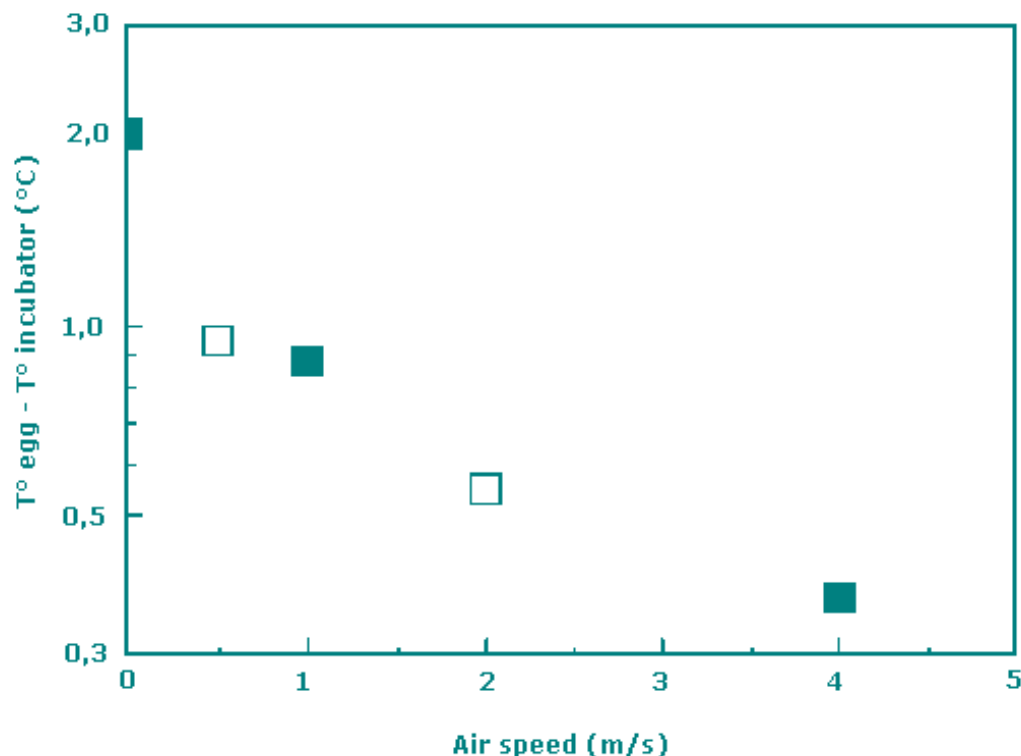
The capacity of the egg to exchange heat with its environment not only depends on its mass and shell conductance but equally from the air temperature surrounding it. This is known as the thermal conductance of the egg.

This is extremely dependant on the air speed around the egg and is also influenced by the stage of development of the other eggs in the machine (the same level of development in single stage machines and different stages of development in multi stage machines).

Sotherland P. *et al* (1987), reviewed by French N.A. (1997), showed that the air that surrounds the egg can also be a barrier to thermal exchanges. This barrier may be up to 100 times more effective than the egg itself. It is therefore important that the air speed surrounding the eggs is sufficient to disrupt the air barrier around the egg.

Since air speed has a negligible effect on water loss during incubation, air speed has in theory no upper limit. Within the same incubator, however, air speed is variable (from 0.2-0.3 m/s up to 3-4 m/s) and it appears obvious that the differences in temperature between the egg and its environment are more considerable when air speed is low (French N.A., 1997).

The effect of air speed on differences in temperature between the egg and the machine



Logarithmic scale. Estimations based on models by Sotherland P. *et al* (1987, ■) and Meijerhof R. and van Beek G. (1993), using 50 grams hatching eggs and metabolic heat production of 100 mW (French N.A., 1997).

Uniformity of air speed inside the incubator depends on the obstacles that the air encounters inside the machine. Most restriction to air speed comes from the eggs themselves, the flats the eggs are in, and also from the space between each of the incubator trays.

French N.A. (1997) showed that the air speed required to maintain a uniform shell temperature was inversely proportional to the distance between two incubator trays (placed horizontally but also with turning at 45°).

INCUBATION

In addition, this research showed that the requirement for air speed was more critical when the stage of development of two comparable incubator trays was similar (single stage or multiple setting trolleys, compared to fixed-tray multiple setting).

➔ Temperature requirements:

The previous section shows that the set temperature of the machine does not truly indicate the temperature perceived by the embryo and that it is necessary to review other indicators of perceived temperature.

Shell temperature is a good indication of the temperature perceived by the embryo (differences between the shell temperature and the embryo are often not more than 0.1-0.2°C). It is possible to adapt the incubator temperature according to the recorded shell temperature.

French N.A. (1997) highlighted the conclusions from research by Lundy H. (1969) and Wilson H.R. (1991):

- ◆ For most chicken species, the optimum incubation temperature is found between 37.0-38.0°C (98.6-100.4°F), although it is even possible to hatch under temperatures that vary between 35.0-40.2°C (95.0-104.4°F).
- ◆ Embryos are more sensitive to high rather than low temperature.
- ◆ The effect of sub-optimal temperature depends on the intensity and duration during the period that it occurred.
- ◆ Embryos appear more sensitive to sub-optimal temperature at the start rather than the end of incubation.

Observations by Decuyper E. *et al* (2001) agreed with these points. Based on work by Barott H.G. (1937) they established that incubation temperature for maximum hatch is between 37.0-38.0°C (98.6-100.4°F); the optimal value being 37.8°C (100.0°F).

Lourens A. *et al* (2005) achieved better hatch results and chick quality when shell temperature was maintained at 37.8°C (100.0°F) during the complete incubation period. According to these same researchers insufficient temperature during the first week (36.7°C - 98.1°F - trial temperature) delayed embryonic development and can also compromise the chick's thermoregulatory systems during the first 7 days after hatching.

In contrast, high temperature at the end of incubation (38.6°C - 101.5°F - trial temperature) appears to increase the chick's thermo-tolerance, thus increasing their tolerance to heat stress later in life (Hulet R. *et al*, 2007).

These observations are in agreement with those of French N.A. (1997). Embryos are poikilothermic during most of the incubation period so they do not increase their oxygen intake when temperature is low. Inversely, they increase oxygen intake and improve their metabolism when temperature is high.

It is important to stress that the preceding statement in no way implies that oxygen intake by the embryo depends solely on temperature. Indeed it seems that with a similar stage of development, the cumulative oxygen intake remains the same whatever is the perceived temperature. Even if compensatory growth mechanisms have been observed by some researchers, only the speed of embryo development appears to be affected (French N.A., 1997).

Molenaar R. *et al* (2010) noted that a shell temperature of 37.5-38.0°C (99.5-100.4°F) during the complete incubation period gives the best hatch and chick quality results.

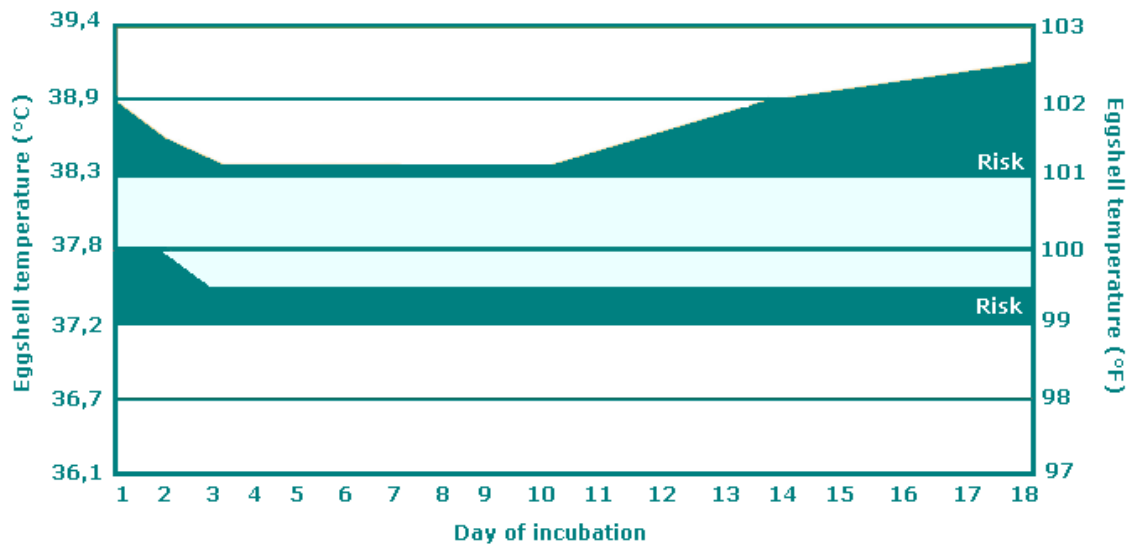
INCUBATION

Despite our knowledge about the qualitative effects of temperature, there is an insufficient quantitative understanding of the thresholds of embryo tolerance to extreme temperature. Barott H.G. (1937), reviewed by Decuypere E. and Michels H. (1992), noted that the incubation temperature should not deviate from $\pm 0.3^{\circ}\text{C}$ from the set point ($37,8^{\circ}\text{C} - 100,0^{\circ}\text{F}$).

This rather tight tolerance between minimum and maximum temperature may be too simplistic for what is a more dynamic situation. It may be possible to allow greater temperature fluctuations at specific stages of incubation. This is because the effects of either high or low temperature during a specific period of incubation may have variable effects on hatching results, growth or other characteristics (Decuypere E. and Michels H., 1992).

Based on more recent work, French N.A. (2010) defined a wider margin of tolerance and identified "risk zones", which can influence hatch results, chick quality or future growth:

Shell temperature during incubation



The graph shows the ideal shell temperature and also the risk zones (above the ideal temperature, risks on hatch performance and ulterior sub-optimal performance; below the ideal temperature risk of delayed hatch).

Adapted from French N.A. (2010)

Measuring shell temperature:

- ◆ Use an infra red thermometer (hand held as per photo).
- ◆ Take the shell temperature at the centre of the egg, on 15 eggs in the middle of the incubator tray.
- ◆ Repeat this on 3-4 trays from different places in the machine.
- ◆ If the incubator does not have a central corridor to do the work inside the machine, work quickly.
- ◆ Do not record temperature on infertile eggs or dead embryos.
- ◆ Calculate the average and uniformity.
- ◆ Adapt the incubator temperature set point based on the results.



INCUBATION

➔ Recommendations.

Single stage:

Single stage machines that permit specific set temperature profiles to be applied during incubation with the ability to adjust the ventilation settings, may successfully allow the embryo's requirements to be met during the early part of incubation. These machine environments can create serious problems towards the end of incubation however if recorded temperatures are too high, or if the air speed between the trays is insufficient.

It is important to be sure that the incubators have sufficient ventilation and cooling ability.

The previous statement leads us to the following recommendations:

Day	Set temperature		Shell temperature (°F)	Ventilation
	Minimum (°F)	Maximum (°F)		
-8/12	77.0	81.0	-	0%
0	100.4	100.5	-	0%
1	100.4	100.5	100.0-100.2	0%
2	100.2	100.3	100.0-100.2	0%
3	100.2	100.3	100.0-100.2	0-10%
4	100.0	100.1	100.0-100.2	0-10%
5	99.9	100.0	100.0-100.2	10-20%
6	99.9	100.0	100.0-100.2	10-20%
7	99.8	99.9	100.0-100.5	20-30%
8	99.8	99.9	100.0-100.5	20-30%
9	99.7	99.9	100.0-100.5	30-40%
10	99.5	99.8	100.0-100.5	30-40%
11	99.2	99.6	100.0-101.0	40-50%
12	98.8	99.2	100.0-101.0	40-50%
13	98.5	99.0	100.0-101.0	40-50%
14	98.3	98.8	100.0-101.0	50-60%
15	98.0	98.5	100.0-101.0	50-60%
16	98.0	98.5	100.0-101.0	50-60%
17	98.0	98.5	100.0-101.0	60-70%
18	98.0	98.5	100.0-101.0	60-70%

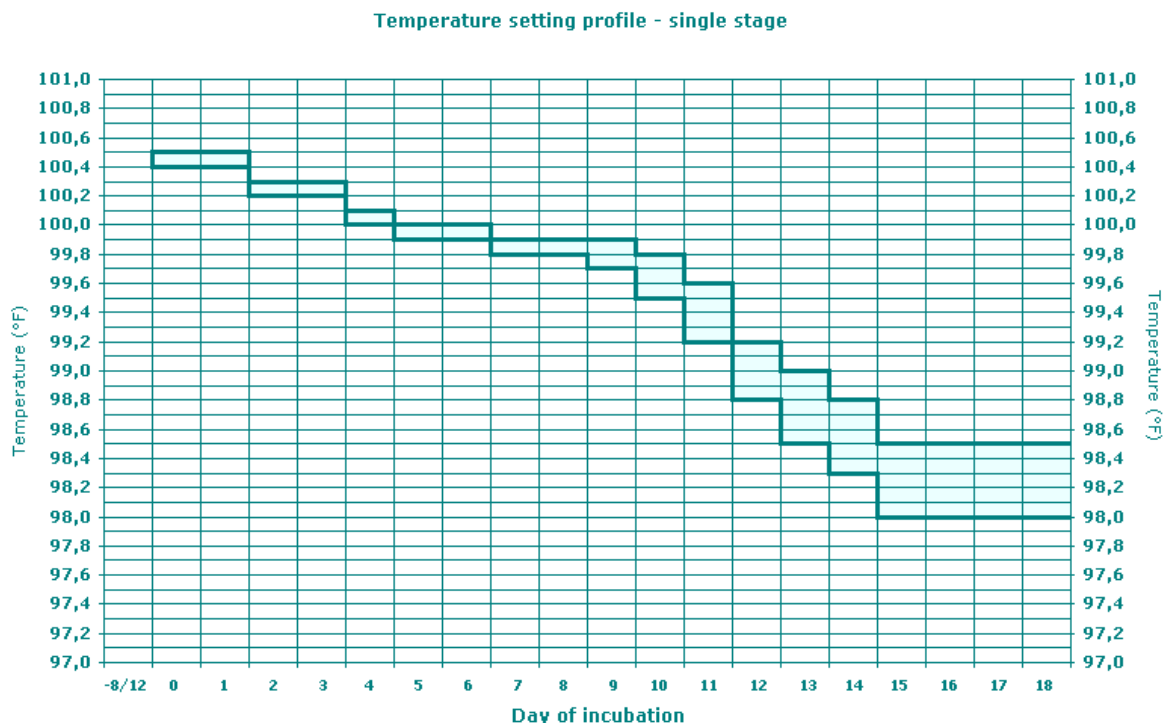
N.B.: The machine type, its capacity, loading method, incubator room ventilation including above the machines can influence the settings to apply. Check with your incubator supplier.

Higher temperatures are best for eggs from young breeder flocks, when the breed is of a slow growing type or when the shell conductance is high. Inversely, lower temperatures are recommended for eggs from older breeder flocks, breeds with fast growth, or when the shell conductance is weak.

The above shell temperatures apply to all breeds and all types of eggs.

INCUBATION

The graph shown below shows the evolution of set temperature during incubation:



Multistage:

Since it is possible to adapt the set temperatures relative to the stage of embryo development, only single stage machines allow constant shell temperature throughout incubation.

For multistage machines, it is established that the heat produced by the embryos at the end of incubation is used to heat the eggs and embryos at the start of development. Since this is the case the set point is fixed and does not always meet the individual embryo's optimum requirements. Shell temperatures will often be below the embryo's requirements at the start of incubation and above at the end (Molenaar R. *et al*, 2010).

Rather than being based on individual embryo requirements, the recommendations below emphasise the incubator egg capacity and its ability to retain or remove the heat that is produced:

Incubator capacity	Set temperature		Ventilation
	Minimum (°F)	Maximum (°F)	
0-25 000 eggs	99.8	99.9	30-40%
25 000-50 000 eggs	99.7	99.8	40-50%
50 000-75 000 eggs	99.6	99.7	40-60%
75 000-100 000 eggs	99.5	99.6	40-70%
+ 100 000 eggs	99.4	99.5	40-70%

N.B.: The above recommendations assume that the incubation room environment, including the incoming air to the machines is perfectly managed (temperature, humidity and volume).

INCUBATION

➔ HUMIDITY DURING INCUBATION

The concentration of water in the egg and the chick are similar; 74-75% for eggs (shell excluded, Sauveur B., 1988), and 72-73% for a day old chick (Medway W. and Kare M.R., 1957). Water loss during incubation must therefore correspond more or less to the quantity of water produced by the metabolism of lipid contained in the yolk (see page 26). It is also true that lipid metabolism requires as much water as it produces (Ar A. and Rahn H., 1980, reviewed by Baggott G.K., 2001).

In 1974, Rahn H. and Ar A. indicated that during incubation water loss is normally 18%.

Ar A. (1991), reviewed by Baggott G.K. (2001), noted that in most chicken species, total water loss of 20% relative to the initial egg mass results in a similar water concentration in the chick as in the egg.

Romanoff A.L. (1968), reviewed by Baggott G.K. (2001), showed that throughout the incubation period, 28.6 grams of water disappeared from the albumen and 7.2 grams from the yolk. Inversely, 24.7 grams appeared in the embryo's body tissue and 2.5 grams in the residual yolk. The balance is therefore -8.6 grams. These values are close to those described by Sauveur B. (1988) who indicated that the production of water is 8.54 grams during 18 days of incubation, and 10.31 grams up to 21 days of incubation.

Meijerhof R. (2009a) indicated that production of metabolic water represents 12 to 14% of the initial egg mass and that at least 9 to 10% of this water should be eliminated to allow for the formation of an air space sufficient to engage pulmonary respiration.

Hays F.A. and Spear E.W. (1951), reviewed by Molenaar R. *et al* (2010) observed that chicks are able to hatch when the cumulative losses at the moment of internal pipping varied between 6.5 and 12.0%.

Tona K. *et al* (2001a) obtained the best hatch results when cumulative losses during 18 days of incubation were between 10.9 and 11.1%. They observed that higher losses lead to fewer hatch problems compared to when the losses were lower. They also found direct relations between the flock age, egg weight and water loss (in grams). Nevertheless, they did not observe a relationship between the flock age, level of hatch or embryonic mortality and the percentage of water loss.

When water loss before internal pipping is less than 6.5%, the resulting air cell size is insufficient to engage pulmonary respiration. Inversely, when losses are more than 14.0%, the risks of dehydration increase (Molenaar R. *et al*, 2010). According to Meijerhof R. (2009a), risks of dehydration appear when losses are close to 17-18%.

The fact remains that egg weight loss during incubation is essentially linked to water loss (Tona K. *et al*, 2001a) and that this is only dependant on the shell conductance and ambient humidity. No other factor is involved.

Since shell conductance varies strongly from one egg to another, rather than using an optimal loss, a margin for loss is better. Molenaar R. *et al* (2010) valued this at between 6.5 and 14.0%.

These same researchers suggest that since the essential goal for water loss is to create an air cell of sufficient size, when the water is lost is not critical provided that the cumulative loss allows pulmonary respiration to start.

INCUBATION

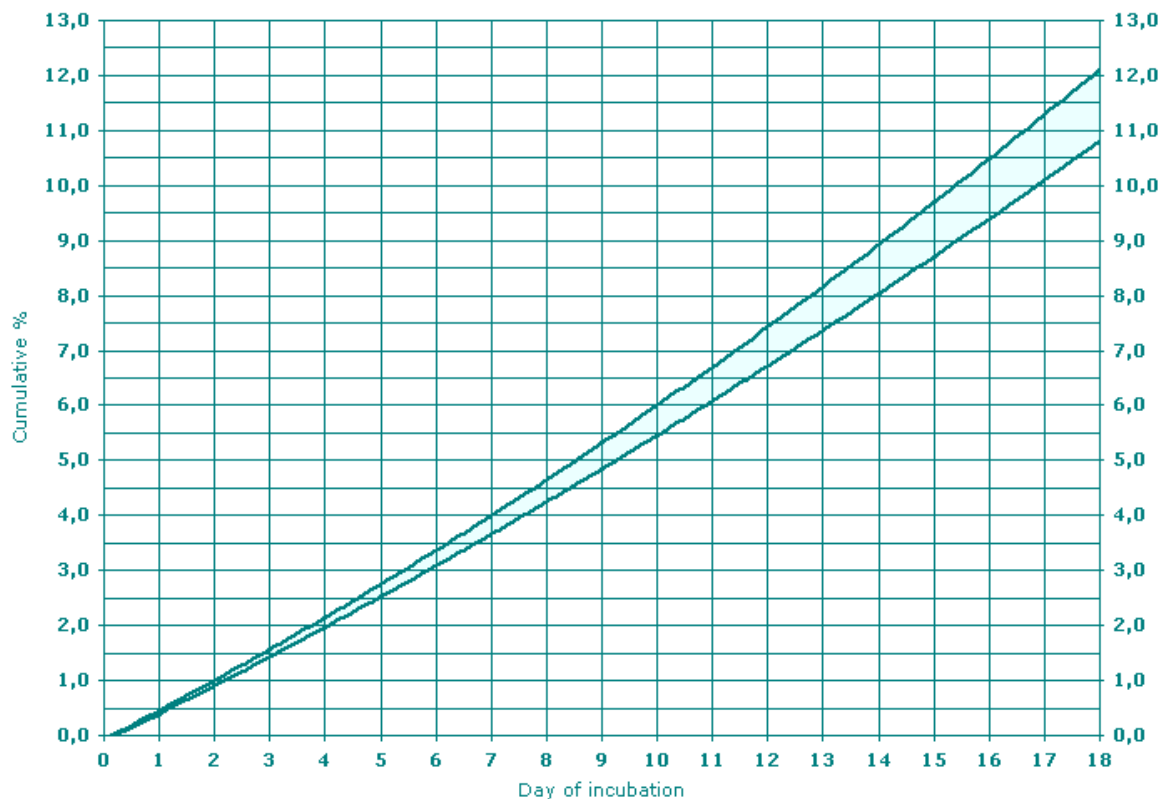
Robertson I. (1961a) has however found that excessive levels of humidity (75-80%) provoke an increase in embryonic mortality during the first 10 days of incubation. He also observed that the hatchability remained satisfactory when the humidity varied between 40 and 70%, with an optimum level of 50%.

➔ Recommendations.

Water loss during incubation only has negative effects on hatch results if the humidity exceeds the optimum level and is closer to the extremes of the margins of water loss cited above (Molenaar R. *et al*, 2010). The levels of humidity should therefore be adjusted to be within the recommended water loss range.

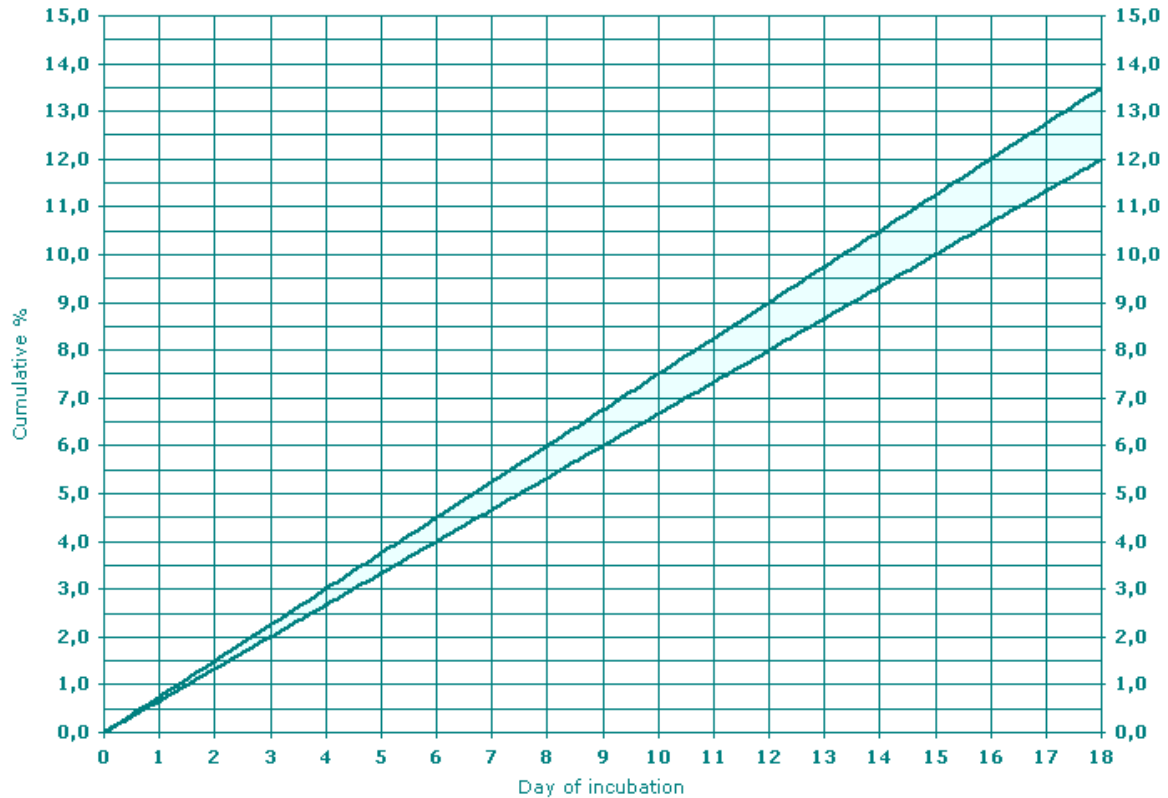
In practical terms, whether using single stage or multistage machines, humidity should be set between 50 and 55%. The method of water loss is little affected by the type of loading:

- ◆ In single stage machines, the adjustment of the ventilation vents is possible so water loss increases slightly exponentially during incubation:



INCUBATION

- ◆ Mostly linear for multistage machines:



➔ TURNING

Turning eggs plays a positive role in preventing either the yolk becoming stuck to the shell membrane (Sauveur B., 1988), or the allantoic does not become stuck to the embryo. It also allows for the development of the *area vasculosa* and of the chorioallantoic membrane (Cutchin H.R. *et al*, 2009) and facilitates the inclusion of the albumen into the allantochorion (Sauveur B., 1988).

Turning eliminates the chance that a part of the albumen stays outside the allantochorion, that it does not intervene between this and the shell membranes and does not reduce the gas exchange (Decuyper E. *et al*, 2001).

Eggs that are not turned often endanger embryos via insufficient oxygen pressure in the arteries and high levels of hematocrit (Decuyper E. *et al*, 2001).

Turning equally facilitates the timely complete closing of the chorioallantoic membrane at the small end of the egg, the accumulation of proteins in the amniotic fluid and a better utilisation of the albumen (Tona K. *et al*, 2005).

Turning prevents embryonic malpositions at the end of incubation (Tona K. *et al*, 2003).

Despite these known benefits, factors such as the angle, frequency and the period during which turning should be carried out still remain unclear.

INCUBATION

Cutchin H.R. *et al* (2009) showed that an angle of turning of 15° (relative to the vertical) leads to embryonic mortality in the second part of incubation 10 times greater than when eggs are turned at an angle of 45°.

In the same study, it was also found that the incidence of embryos showing excess residual albumen at 18 days of incubation, increased to nearly 20 times. Both hatch and intermediate embryonic mortality was observed when eggs were turned at an angle of 30°.

Funk E.M. and Forward J.F. (1953), reviewed by Elibol O. and Brake J. (2006a), found that hatch levels increased as soon as the turning angle is above 20 to 45°. They did not however, observe any real difference between turning at 40 and 45°.

Wilson H.R. (1991) mentions that eggs must be turned at an angle of 45 to 70° (relative to the vertical). Yet, Funk E.M. and Forward J.F. (1960), reviewed by Elibol O. and Brake J. (2006a), when comparing angles of 30, 45, 60 and 75°, obtained the best results with 45°.

While Elibol O. and Brake J. (2006a) did not find any effect on the level of hatch when the turning angle varied between 35 and 45°, they demonstrated an inversely proportional relationship between the turning angle and the incidence of malpositions. Further, they observed an increase in the frequency of turning can offset the effects of insufficient angle.

Robertson I. (1961b) found that the turning frequency (using a base angle of 45°) had a notable effect on hatch and determined that a turning frequency of 96 times per day compared to fewer number of turns per day gave the best results. Turning as often as 480 times daily only slightly decreased hatch results.

Elibol O. and Brake J. (2003) showed similar results. By comparing different frequencies of turning between the 3rd and the 11th day of incubation, they found that a turning frequency of 96 times per day gave the best results compared to frequencies of 2 or 4 times less per day.

Wilson H.R. (1991) mentions that maximum hatch is obtained when eggs are turned 96 times daily but that 24 times daily is more practical. Deeming D. (1990), reviewed by Decuypere E. *et al* (2001), considered that the most important period for turning is between the 3rd and 7th day of incubation and that beyond the 13th day the effects of turning are negligible.

In another study, however, Tona K. *et al* (2005) concluded that turning is necessary until 12 days of incubation but that it is convenient not to stop before the 16th day. Furthermore, they observed that the embryo weight seems to be influenced by when the last day of turning occurred and they formed a hypothesis that turning may have an effect in stimulating growth.

These researchers demonstrated in 2003 that turning up to the 18th day of incubation had a beneficial effect on hatch results. They observed that the periods when eggs were turned (12, 15 or 18 days in the trial) did not appear to have any effect on the level of corticosterone in the blood (indicator of the level of stress in the embryo). Nevertheless, the period when eggs were turned affected the concentration of CO₂ in the air cell. Thyroid hormones in the blood (an indicator of metabolic activity) increased when turning was maintained until 18 days. These same researchers previously demonstrated that elevated levels of thyroid hormones (in particular T₃, triiodothyronine) had a positive effect on the hatch level.

Tona K. *et al* (2001b) observed that the hatch level increased when the time turning was stopped was delayed (15, 16, 17 and 18 days in the trial) and that this was most beneficial for eggs from old flocks.

INCUBATION

Elilol O. and Brake J. (2006b) did not observe big differences in hatch when the turning was stopped at 8, 10, 12 or 14 days of incubation. They therefore concluded that turning can be stopped after the 8th day of incubation.

In the same study though, they found a strong interaction between the age of the donor flock and the frequency of turning (the older the flock then turning for a longer period is more beneficial).

➔ Recommendations.

There is a consensus that the angle of 45° is optimal but when to stop turning and the frequency of turning per day still leaves many unanswered questions. Trials made in our own hatcheries clearly indicate that an increase in the frequency of turning had a beneficial effect on hatch percentages. It is advisable, wherever possible, to turn eggs every quarter or half hour and certainly more than every hour.

To stop turning early can only be considered providing it does not provoke hot spots in the machine. French N.A. (1997) showed that the air speed required to eliminate the heat produced by the embryo is reduced when the incubator trays are horizontal. This may promote the formation of hotter zones in the machine, particularly where the air speed is sub optimal.

Maintaining turning until transfer and increasing the turning frequency more than normal can help to reduce the risk of hot spots and thus indirectly reduce the requirements for air speed.

➔ CO₂

Gas exchange during incubation plays a fundamental role in the development and viability of the embryo, hatch results, growth and physiology of the chick.

Molenaar R. *et al* (2010) described tolerance levels to CO₂ for the embryo. They stated embryos can tolerate only 1% CO₂ during the first 4 days, up to 3% from the 5th day of incubation and even up to 5% from the 9th day. Although these levels do not appear to have negative effects on hatch results, it is not well understood how CO₂ concentration may alter embryonic development.

The embryo's consumption of oxygen increases exponentially during the first two weeks of incubation. Since this is in direct relation with the surface of the *area vasculosa* and the speed of growth of the chorioallantoic membrane, it is suggested that hypoxia ^{and/or} hypercapnia during the first part of incubation favours vascular development (Decuypere E. *et al*, 2006).

Similarly, since the ideal pH for the start of embryonic development varies between 7.9 and 8.4 (see page 17) or between 8.2 and 8.8 (see page 18), relatively high levels of CO₂ during the start of incubation improves embryo growth. Molenaar R. *et al* (2010) in some studies showed that a concentration of 2 to 4% CO₂ during the first 48 hours of incubation reduced the albumen pH and favoured development of the embryo and of the embryonic annexes. In further studies the same individuals found equal levels of CO₂ had a negative effect on hatch.

In turkeys, work has shown that levels of CO₂ of 0.3% during the first 10 days of incubation increased the hatch level by 5% compared to levels of 0.1% CO₂.

Inversely, in chickens, it has been shown that levels of 0.7-0.8% CO₂ during the first 5 days can have a negative effect on hatch and the speed of embryonic development.

INCUBATION

Molenaar R. *et al* (2010) described many trials which demonstrated a progressive increase in CO₂ levels during the first 10 days of incubation (up to a level of 0.7-1.5%) increased embryonic development. The effect on hatch remains unclear though.

Decuyper E. *et al* (2006) showed that a progressive increase in CO₂ during the first 10 days (up to a level of 1.0-1.5%) stretches the lumen of the aorta, increases the CO₂ pressure in the air space, accelerates the embryonic development and therefore promotes tighter hatch windows.

These individuals also observed an increase in the levels of corticosterone and the hormone T₃ in the blood. Since corticosterone is implicated in the metabolism of thyroid hormones and glucocorticoids, and thyroid hormones are implicated in the preparation for pipping and hatch, this would explain the observation of shorter incubation times.

Sauveur B. (1988) maintains that the level of oxygen in the incoming air must never be less than 20.5%. This is the threshold below which oxygen uptake by the embryo becomes too difficult. Dorn D.J. (2010) noted however, that hypercapnia induced during the first days of incubation (by closing the ventilation vents) provokes a moderate hypoxia, with about 19% O₂ in the incubator.

The response by embryos to moderate levels of hypoxia, particularly during the second week of incubation, essentially depends on their metabolism and speed of growth. Dorn D.J. (2010) observed that moderate hypoxia provokes cardiac hyperplasia and hypertrophy of the embryo only in fast growing strains.

Furthermore it was noted that the embryo is particularly sensitive to hypoxia during the period 6 to 12 days of incubation (period of strong growth).

➔ **Recommendations.**

The concentrations of CO₂ required during the first part of incubation are not yet well defined but they clearly appear to depend on the breed's growth potential.

Nevertheless, it is probable that a progressive increase up to a level of 0.5-0.7% may be beneficial to the development of the *area vasculosa* and the embryo itself.

INCUBATION

Single stage:

Day	Level of CO ₂ in the incubator	Ventilation
-8/12	-	
0	-	0%
1	0.1-0.2%	0%
2	0.1-0.2%	0%
3	0.3-0.5%	0%
4	0.3-0.5%	0-10%
5	0.5-0.7%	0-10%
6	0.5-0.7%	10-20%
7	0.5-0.7%	10-20%
8	0.3-0.5%	20-30%
9	0.3-0.5%	20-30%
10	0.3-0.5%	30-40%
11	0.3-0.5%	30-40%
12	0.2-0.4%	40-50%
13	0.2-0.4%	40-50%
14	0.2-0.4%	40-50%
15	0.2-0.4%	50-60%
16	0.2-0.4%	50-60%
17	0.2-0.4%	50-60%
18	0.2-0.4%	60-70%

Multistage machines:

Since it is not possible to adjust ventilation rate, the level of CO₂ in the incubator should be maintained at 0.3% throughout the whole incubation period.

➔ LOADING THE MACHINES

The previous sections have demonstrated that during incubation, within a small degree of tolerance, the embryo requires special environmental conditions. Factors such as the potential growth of the breed, egg weight and shell conductance can modify these conditions. It is also recommended that the eggs are incubated uniformly.

However, the environment surrounding the egg depends on two main factors:

- ◆ The level of fertility.
- ◆ Loading the machine.

While the embryo encounters both endothermic and exothermic stages during its development, an infertile egg does not require or produce heat. Its temperature will always be less than that of the developing embryo and one or more infertile eggs on the same incubator tray can affect the conditions perceived by surrounding embryos.

The infertile egg tends to absorb the heat produced and thus make the surrounding embryos cold. Since the water loss of the infertile egg is less than that of a developing embryo, it can affect the level of humidity in the machine. Finally, as it does not produce CO₂, infertile eggs may reduce the concentration of CO₂ in the machine.

Optimising the environment during incubation will provide consistent hatch results. However, as breeds vary in their requirements and incubators in their designs, it is important to fully understand the guidelines from the breed supplier and incubator manufacture.

INCUBATION

Although on a different scale, the guidelines for loading eggs in the machines follow the same principle: only machines that are filled to at least 75-80% of their capacity can provide uniform environmental conditions.

If, for planning purposes the number of eggs to be set leaves parts of the machine empty, the distribution of trays and trolleys (even eggs on the same tray) around the machine should be as uniform as possible.

If this principle is not followed then air speed and consequently the conditions for temperature, humidity and CO₂, will be adversely affected.

➔ DISINFECTION DURING INCUBATION

Several years ago, fumigation of eggs at the time of setting and even disinfection during incubation was normal practice in many hatcheries.

Today, fumigation is undertaken before setting the eggs and disinfection during incubation is used less frequently. This is probably linked to the risks of cross contamination within the same incubator being low and therefore it is not necessary to have a permanent disinfection programme.

Trials undertaken in our own hatcheries have shown neither positive nor negative effects of disinfection during incubation.

Although the risks of cross contamination during incubation remain low, in no way does this justify a relaxation in the standard of overall biosecurity and hygiene especially the general cleanliness of the incubator rooms.

Disinfection inside the machines is possible though. When this is carried out by evaporation most frequently formalin diluted at a rate of 5-6% is used.

When disinfection is administered by fogging, follow the recommendations supplied by the machine manufacturer. Pay particular attention to the type of disinfectants utilized because disinfectants can plug nozzles which will prevent proper results.

➔ INCUBATION ROOM ENVIRONMENT

Incubator room environments are only important when the incubators take their fresh air requirements from the room, or when pre-heating is done in front of the machines. Guideline conditions are:

Temperature	Humidity	Air volume
25°C (77.0°F)	50-55%	1.0-1.5 m ³ /hour/100 eggs

When air is introduced directly into the machines, or from a plenum, follow the instructions given by the machine manufacturer.

TRANSFER

Transfers are normally completed during the 18th day of incubation unless *inovo* machines are utilized. If *inovo* machines are utilized transfer should occur at 19-19½ days. They can be done manually or automatically, but they should be done quickly. Take care when handling the incubator and hatcher trays during the transfer of eggs from one to the other. The “suction pad” should be properly adjusted and the transfer machine should operate smoothly.

Candling may occur at the same time as transfer and the “clear” eggs (infertile and early dead embryos) can be removed. However, if infertility is more than 15%, it is better to fill up the trays to the normal level with fertile eggs. This leads to a more uniform dispersal of heat and reduces the chance that eggs can become cold.

If the level of infertile eggs justifies backfilling empty spaces in trays with fertile eggs then the empty trays left over should be placed on the bottom of the hatcher trolley.

Wash and disinfect the egg transfer vacuum pad once the transfer is complete to eliminate the chance of future contamination. Be sure that both the vacuum pad device and the hatch trays are completely dry before the next transfer.

➡ TRANSFER ROOM ENVIRONMENT

The conditions for this room are the same as for the incubator room:

Temperature	Humidity
25°C (77.0°F)	50-55%

HATCHING

At the time of oviposition the egg (shell included) contains approximately 65.6% water, 12.1% protein, 10.5% fat, 0.9% carbohydrates and 10.9% minerals. These nutrients complete what is available to the embryo for its development.

Protein is essentially used for growth. From the initial quantity, approximately 48% can be found in the chick, 47% remains in the residual yolk, 2.5% can be found in the allantoids and only 2.5% is lost, likely due to catabolism (Molenaar R., 2010).

As discussed earlier, lipids found mostly in the yolk are the main source of energy for the embryo (approximately 90% of the energy used comes from lipids). Their oxidation increases from the 9th day of incubation, at the same time that embryo growth accelerates. From the initial quantity, approximately 20% is found in the chick, 40% in the residual yolk, and 40% is catabolised (Molenaar R., 2010).

The levels of carbohydrates in the egg are very low and remain so during most of the incubation period. Most of the simple carbohydrates are used during the first week when the chorioallantoic membrane is not yet in place and cannot supply the oxygen required to metabolise lipids.

A second peak of glucose metabolism is observed at the end of incubation. Extra energy sources are required for hatching when the availability of oxygen is low so the embryo must switch from lipid to carbohydrate metabolism.

It is therefore essential during its development that the embryo has stored sufficient carbohydrate reserves. Glucose is essentially stored in the form of glycogen in the liver, muscles, heart and the peri-vitelline membrane (Molenaar R., 2010). When the chick prepares for hatch, it prioritises the mobilisation of liver glycogen and the resulting anaerobic glycolysis increases the level of lactate in the blood. (Molenaar R. 2010).

Based on these changes it follows that temperature and oxygenation are the two essential factors for hatching and that sub-optimal incubator conditions interfere with viability and development of the embryo.

➔ HATCHER TEMPERATURES

The graphs on pages 24 and 25 clearly show that embryonic heat production achieves a plateau on the 16th-17th day of incubation. Contrary to many assumptions, this plateau is not an expression of the maximum heat production from the embryo but more likely a deficiency of oxygen.

Decuyper E. and Michels H. (1992) noted that the maximum oxygen flow is achieved at the end of incubation. This, as discussed earlier, is dependant on the permeability of the shell cuticle, the shell and shell membranes.

Christensen V.L. *et al* (2001) investigated oxygen consumption in more detail. In turkeys, oxygen consumption increases in an exponential way until approximately the 25th-26th day of incubation. At this time, the oxygen requirements are more than the maximum oxygen flow to the embryo and the embryo activates other energy sources. These have been prepared during development. The embryo stores glycogen in a number of organs but in chickens a small amount is stored in the heart and therefore it has a limited glycogen activity.

Liver glycogen is supplied the heart and this is controlled by the thyroid hormones.

HATCHING

Due to poikilothermia, any increase in temperature perceived by the embryo leads to an increase in oxygen demand. This places the chick under metabolic stress and the embryo moves rapidly towards carbohydrate metabolism. This explains under extreme stress the observations of eventual cardiac arrests and the level of residual yolk, as described in earlier sections.

It has also been shown that high temperature at the end of incubation reduces the level of maltase in the jejunum (an indicator of a maturing intestine) (Wineland M.J. *et al*, 2001). This impairs the development of chondrocytes (indicators of bone mineralisation) (Yalçın S. *et al*, 2007).

The discussion of the effects of high temperature above do not apply to specific new techniques which provide "heat shocks" at the end of incubation. Since the period of heat stress is short, the embryo is preconditioned to tolerate stresses it may experience during rearing without major effects on embryo metabolism.

➔ Recommendations:

There is no justification for increasing hatcher temperatures. On the contrary, some researchers have found that relatively low temperatures may prolong the total incubation period but were favourable to improved grow-out performance.

Day	Set temperature		Ventilation
	Minimum (°F)	Maximum (°F)	
19	98.0	98.5	30-50%
20	98.0	98.5	30-50%
21	97.0	98.0	50-70%

N.B.: The type of machine, its capacity, method of loading, ventilation in the incubator room and above the machines can influence the recommendations for specific hatcheries. Check with your machine manufacturer.

The higher level is preferable when eggs are from young donor flocks, breeds with slow growth, or when the shell conductance is high. Inversely, the lower temperatures are suggested when eggs come from older donor flocks, breeds with fast growth or when the shell conductance is low.

➔ HUMIDITY DURING HATCHING

It has been observed that as long as humidity is within the limits for adequate moisture loss, it is less important than some other incubation factors.

During actual hatch, the adjustment of humidity will depend essentially on weight loss observed at transfer and to limit the risks of excessive dehydration.

Day	Recommended Humidity
19	50-55%
20	55-60%
21	60%

N.B.: From the start of pipping, and above all at peak of hatch, the real levels of humidity can be higher than those recommended. Ensure that the high humidity alarm does not come on before 70-75%.

➔ LEVELS OF CO₂

Relatively high levels of CO₂ during the first part of incubation have been shown to favour the development of the *area vasculosa* and the chorioallantoic membrane.

HATCHING

In contrast, since CO₂ penalises the development of some organs, high levels of CO₂ at the end of incubation may not be beneficial. Wineland M.J. *et al* (2001) showed that insufficient oxygen pressure leads to a lower heart weight, without affecting the level of cardiac glycogen.

However, it has been demonstrated that hypoxia (including incubation at altitude) or hypercapnia shortens the incubation time and favours tighter hatch windows (Decuyper E. *et al*, 2006).

Embryos subjected to weak oxygen pressure at the time of hatching are more likely to develop hypertrophy of the right ventricle and be more sensitive to ascites (Decuyper E. *et al*, 2006).

Molenaar R. *et al* (2010) noted that oxygen pressure in the air cell only reaches 14.2% just before hatching. Inversely, CO₂ reaches about 5.6%. These are the pressures that initiate pipping and higher levels of CO₂ in the environment can force some chicks to hatch even when they are not ready.

➔ Recommendations.

The effects of different levels of CO₂ in the atmosphere are still not defined but it seems clear that the main effect of CO₂ largely depends on the breed's potential for growth.

Although CO₂ seems to have a beneficial effect on the eventual resistance of the heart to hypoxia, CO₂ can force chicks that are not yet ready to hatch, reducing the overall chick quality.

Day	Level of CO ₂ in the hatcher	Ventilation
19	0,2-0,4%	30-50%
20	0,4-0,6%	30-50%
21	0,2-0,4%	50-70%

➔ THE HATCH WINDOW

This is the period between the hatching of the first and last chicks. It provides a good indicator of the incubation conditions, including pre-heating for the hatch.

There is one overriding rule that must not be forgotten; egg uniformity must be as high a percentage as possible as outlined in earlier sections.

Chick quality and uniformity can be enhanced by a hatch window that is as short as possible. A short hatch window avoids first, chicks hatching too early reducing the risks of dehydration and secondly, chicks that hatch late are not ready when pulling occurs and which are lazy on placement.

The objectives are:

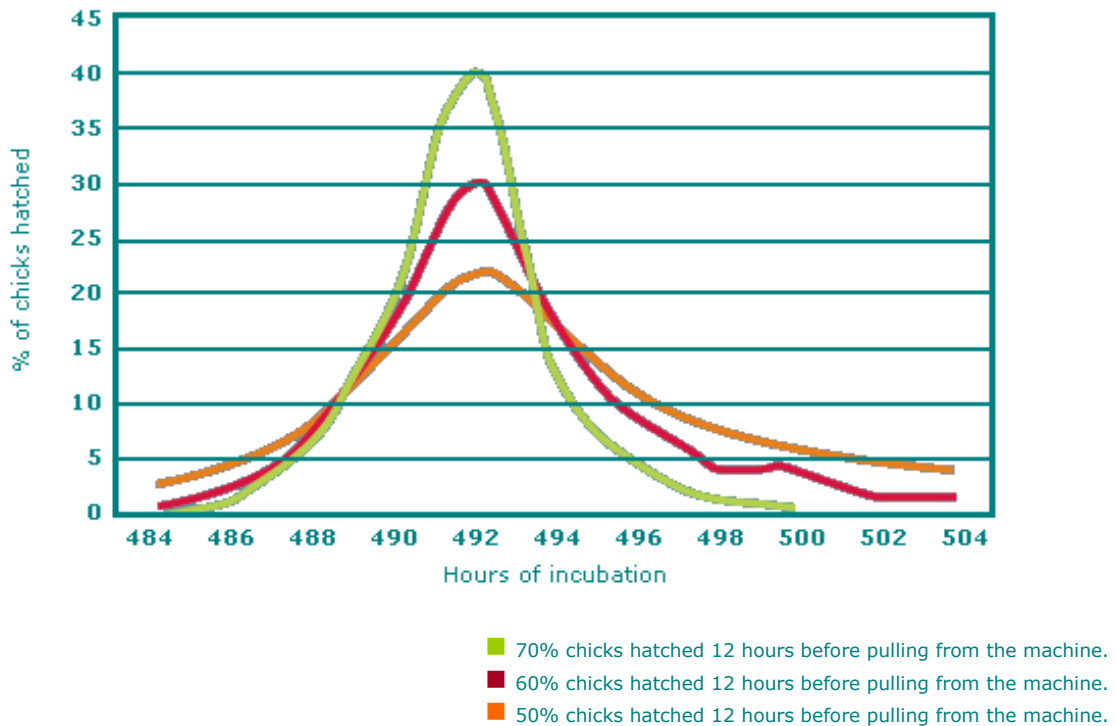
Hatch window	Time
Very good	< 24 hours
Good	24-30 hours
Average	30-36 hours
Bad	> 36 hours

HATCHING

A short hatch window is not the only factor to consider. The time chicks are pulled must correspond to the total incubation period calculated for the given hatch. It is pointless to have a short hatch window when the total incubation time is 15 or 20 hours less than optimal!

The graph below shows the evolution of hatch window relative to the percentage of chicks hatched:

Evolution of types of hatch windows relative to the percentage of chicks hatched 12 hours before being pulled



Adapted from Thornton G. (2011)

A realistic objective for the hatch window is to achieve 60% of the chicks hatched 12 hours before pulling. In the same way, French N.A. (2010) noted that 30 hours before pulling the chicks, no more than 2% should have hatched.

➔ TOTAL INCUBATION TIME

It has been shown earlier that embryonic development depends almost exclusively on temperature and its uniformity. However, another factor can be added; the growth potential of the breed.

Breeds with fast growth potential produce more metabolic heat. Not only do they tend to hatch earlier but they are also more sensitive to high temperatures.

In contrast, breeds with slower growth produce less metabolic heat, have a tendency to hatch later and are less sensitive to high temperatures.

In theory, by incubating different breeds in different machines, it is possible to have a coordinated hatch of two different types of breed at the same time. This may explain why longer incubation times are tending to be used today.

HATCHING

However, it is not always easy to define precise incubation times and we will not limit ourselves here to exact incubation times when the perceived temperatures by the embryo correspond to those described previously:

Breed's potential for growth	Total incubation time
Fast	500-508 hours
Slow	504-512 hours

➔ DISINFECTION DURING HATCHING

Whatever efforts have been made during incubation to maximise the sanitary status of hatching eggs, the risk of contamination remains during incubation. The risks are particularly high during hatching.

In practice, few effective alternatives to formalin (or products with a formalin base) have been found. It is not possible to recommend the use of formalin without all the necessary safety precautions including:

- ◆ Use shallow pans with a diameter of 30-40 cm.
- ◆ Place one in each hatcher, just inside the door or under one of the hatcher trolleys.
- ◆ Pour 500-600 ml of a solution of 18-20% formalin into the pan (250-300 ml formalin 36-40% strength and 250-300 ml of water).
- ◆ Leave it to evaporate.

For maximum effectiveness, the formalin should be placed when 5 to 10% chicks have hatched (towards the middle to end of the 20th day of incubation).

Only one application is required. Earlier applications have little effect on contamination. Frequent repeat treatments risk over exposure to formalin.

Excess use of formalin is easily identifiable. Chicks show a deep yellow colour and in extreme cases may show signs of respiratory stress at hatching.

Formalin irritates the respiratory tract and an overexposure may cause lesions in the trachea which may subsequently become infected and affect growth and livability.

For other disinfection products, follow the instructions of the supplier and ensure that they can be safely used for embryos and chicks.

➔ ENVIRONMENT IN THE HATCHER ROOM

The hatcher room environment is only important when the hatchers take their air from this room. The recommendations for the hatcher room are:

Temperature	Humidity	Air volume
25-26°C (77.0-78.8°F)	55-60%	3.0-3.5 m ³ /hour/100 eggs

When the air is directly introduced into the machines, or if a plenum is used, follow the instructions of the machine manufacturer.

CHICK QUALITY

While the success of incubation may be monitored by recording hatchability, this approach is too limited, especially for integrated producers. Incubation conditions affect not only the hatch results but also the chick quality. The economic impact of chick quality is far more important than the cost of a small shortage or excess of chicks.

Meijerhof R. (2009b) noted that temperature plays an important role in the utilisation of nutritional reserves in the yolk and on the complete closure of the navel. Further research showed differences of 2°F in embryo temperature resulted in significant differences in terms of growth and FCR on broilers at 6 weeks of age. These same temperature differences provoked differences in chick development and some organs.

Hulet R. (2001) showed that by adapting the incubator temperature to the actual production of metabolic heat it was possible to improve the level of hatch by about 2% compared to normal incubation programmes.

Since large eggs have more difficulty to eliminate heat, a deterioration in chick quality is often observed with an increase in residual yolk as the flocks become older. Lourens S. *et al* (2006) showed that when shell temperature was constant, the embryos coming from small or large eggs were both as efficient in transferring nutrients from the yolk to their body.

Two main methods are used today to measure chick quality:

- ◆ Measuring chick length.
- ◆ The Pasgar[®] Score, a simplified version from the Tona Score developed by the University of Leuven (Belgium) in the 1990's.

➔ CHICK LENGTH

Since embryonic development is regulated by temperature, all changes to the environment will modify the embryo growth. It has been outlined earlier that high temperatures accelerate development enhancing hypoxia and impairing the utilisation of lipids as a principal source of energy. The embryo switches more rapidly and intensely towards carbohydrate metabolism and in some cases protein metabolism.

It therefore seems logical that high temperatures may affect the rate of growth of some organs (particularly the heart) and the residual yolk. This was first shown by Romanoff A.L. (1960) reviewed by Leksrisompong N. *et al* (2007), then confirmed by many other researchers.



Residual yolk, size and view of the heart of two chicks having perceived different temperatures during incubation:

- ◆ Left: high temperature.
- ◆ Right: normal temperature.

CHICK QUALITY

In a large scale test, Hill D. (2001) observed that the chick length measured from the head to the rump, increased with flock age. Chick length appeared longer in single stage machines and varied relative according to the position of the egg in the machine. Furthermore, chicks coming from old flocks were often shorter than chicks from adult flocks. Rearing mortality was higher when chicks came from hatcheries producing chicks that were shorter. It was concluded that chick length was a good tool for predicting future performance.

Chick length from the head to the rump was always the most perceptible indicator of chick quality. Nevertheless, it was found that results were not always repeatable. Therefore, it was proposed to use a more repeatable measure of chick length measured from the point of the beak to the end of the middle toe.

➔ Methodology.

- ◆ Take 20 chicks at random from each origin.
- ◆ Measure their length, from the point of the beak to the middle toe (nail excluded).
- ◆ Calculate the average and the uniformity.
- ◆ Record the results relative to the age of the donor flock, egg weight and incubation conditions.



Chick length from young donor flocks varies between 18.5 and 19.5 cm., between 19.0 and 20.0 cm. for chicks from adult flocks and between 19.5 and 20.5 cm. for chicks from old flocks. It is important to note that chick growth continues after the hatch and that in order to compare information, it is important to undertake measurements at the same time.

➔ THE PASGAR[©] SCORE

This method in addition of being a quantitative measure, it is also a more qualitative method than chick length and aims to evaluate the overall incubation conditions. It is questionable whether it is able to determine future performance (Meijerhof R. 2009b).

➔ Methodology:

- ◆ Take at random 50 chicks from each origin.
- ◆ Evaluate the following parameters:



Chick vitality:

- ◆ Lying on its back, it sits up immediately (score = 0).
- ◆ It takes more than 3 seconds to sit up (score = 1).

CHICK QUALITY



Navel:

- ♦ The navel is normal when it is completely closed and all the yolk is absorbed (score = 0).
- ♦ If the navel is open ^{and}/or one can see a dried cord (score = 1).



Hock joint:

- ♦ The hock joint is not inflamed and have a normal colour (score = 0).
- ♦ The hock joint is inflamed ^{and}/or red (score = 1).



Beak:

- ♦ The beak is clean and the nostrils are closed (score = 0).
- ♦ The beak is dirty ^{and}/or has a red dot (score = 1).



Abdomen:

The size of the abdomen depends on the size of the yolk sac and is essentially linked to temperature and humidity during incubation.

- ♦ Soft abdomen (score = 0).
- ♦ Hard abdomen, skin stretched (score = 1).

CHICK QUALITY

- ◆ Record the scores for each of the parameters for each chick.
- ◆ For each individual, add up the different scores and then deduct the results from the maximum score of 10.
- ◆ Calculate the average.

Optimum incubation conditions should give results on average of a minimum of 9 (Pas Reform, 2006).

ANALYSIS OF THE CAUSES OF EMBRYONIC MORTALITY

Numerous publications exist on this subject. The observations and possible causes detailed below are mainly based on research by Wilson H.R. (2004):

➔ General problems.

Observations	Possible causes
Clear eggs found at transfer: No signs of embryonic development, eggs are infertile	- Immature males - Ratio females/males is incorrect (too many or not enough males) - Extreme climatic conditions - Old breeder flock - Health problem - Males or females too heavy - Excess or deficiency of nutrients, feed control too strong - Leg problems, especially in the males - Some drugs, pesticides, toxins or mycotoxins - Parasites - Inappropriate stocking density - Unsuitable light programme (intensity or duration) - Poor management
Clear eggs found at transfer: Signs of embryonic development (enlarged germinal disc), eggs are fertile	- Storage period too long - Inappropriate storage conditions (temperature too high or too low, fluctuating temperatures) - Inappropriate fumigation (over dose or fumigation during the period 12-96 hours of incubation). Incorrect application of disinfectant on the eggs - Heat shock - Pores blocked - High temperature at the start of incubation - Flock too young or too old - Health problems - Wash water temperature too high - Drugs, toxins, pesticides - Frequency of egg collection is insufficient or incomplete
Clear eggs found at transfer: Presence of the blood ring or a dead embryo before 3 days of incubation, no black eye visible	- Egg storage too long or wrong temperature - Inappropriate fumigation (over dose or fumigation during the period 12-96 hours of incubation) - High temperature at the start of incubation - Insufficient temperature at the start of incubation - Health problem - Flock too old - Severe nutritional deficiencies (biotin, vitamin A, copper, vitamin E, boron, pantothenic acid) - Drugs, toxins, pesticides - Contamination - Weak embryo development at the time of oviposition
Dead embryos: 3 to 6 days of incubation, presence of the circulatory system, embryo lying on its left side, absence of egg-tooth	- Read the previous section - Insufficient ventilation or blocked pores - Inadequate turning - Inadequate turning angle - Vitamin deficiencies: vitamin E, riboflavin, biotin, pantothenic or linoleic acid
Dead embryos: 7 to 17 days of incubation, presence of egg-tooth and nails, feather follicles (8 days) or feathers (11 days)	- Inadequate temperature, humidity, turning or ventilation during incubation - Contamination - Nutritional deficiencies: riboflavin, vitamin B₁₂, biotin, niacin, pyridoxine, pantothenic or linoleic acid, phosphorus or bore

ANALYSIS OF THE CAUSES OF EMBRYONIC MORTALITY

Observations	Possible causes
- Dead embryos: >18 days of incubation	- Inadequate temperature, humidity, turning or ventilation in the incubator - Inadequate temperature, humidity or ventilation in the hatcher - Contamination - Excessive or prolonged fumigation - Eggs cooled during transfer, or transfer done too late - Broken eggs before setting, during incubation or during transfer - Nutritional deficiencies: vitamin D, vitamin A, folic acid, pantothenic acid, riboflavin, vitamin E, selenium, vitamin K, biotin, thiamine, vitamin B₁₂, calcium, phosphorus, manganese or linoleic acid - Malposition of the embryo - Hatcher opened too frequently during pipping or hatching - Poor shell quality - Health problem

➔ Specific problems :

Observations	Possible causes
Non pipped eggs, embryos completely formed, excessive residual yolk, a part of the yolk is not completely absorbed, presence of albumen	- Inadequate turning - Humidity too high during incubation or after transfer - Insufficient temperature during incubation - Hatcher temperature too high - Eggs cooled during transfer - Nutritional deficiencies - Health problems - Inadequate ventilation - Prolonged storage
Eggs pipped, embryos completely formed, dead in shell	- Insufficient humidity or temperature during long periods - Insufficient humidity in the hatcher - High temperature in the hatcher - Nutritional deficiencies - Health problems - Insufficient ventilation - Inadequate turning during the first 12 days - Shocks during transfer - Prolonged storage
Eggs partially pipped, embryos dead or alive	- Excessive fumigation during hatching - Eggs incubated small end up
Early hatch, noisy chicks	- Small eggs - Difference between breeds - Incubator temperature too high - Incubator humidity too low
Delayed hatch	- Big eggs - Old flock - Prolonged storage - Insufficient temperature during incubation - Weak embryos - Humidity too high during incubation
Hatch window is too long	- Mixing of eggs stored for different lengths of time in the same incubator - Mixing of eggs from both young and old flocks - Mixing of both small and big eggs - Incorrect egg handling - Hot or cold spots in the incubator or hatcher - Incubation or hatch temperature too high or too low
Poor uniform hatch between the different hatch trays	- Mixing of small and big eggs - Mixing of eggs from both young and old flocks - Mixing of eggs from different breeds - A part of the eggs were stored for too long - Inadequate ventilation in either the incubator or hatcher - Health problem in one or more flocks - Different storage conditions

ANALYSIS OF THE CAUSES OF EMBRYONIC MORTALITY

Observations	Possible causes
Sticky chicks, traces of albumen on the fluff	- Insufficient temperature during incubation - Humidity during incubation too high - Inadequate turning - Old eggs - Eggs too big
Chicks stuck to the shell, chicks with part of the shell stuck to their fluff	- Humidity too low during storage, incubation and/or hatching - Inadequate turning - Eggs broken or poor shell quality
Early hatch, umbilical buttons	Temperature too high during incubation or hatching
Small chicks	- Small eggs - Insufficient humidity during storage or incubation - Temperature too high during incubation - Porous or weak shells
Navel not closed, dry fluff	- Temperature high during incubation or variation of temperature - Insufficient temperature during hatching - Humidity during hatch too high or insufficient ventilation at the end of hatching - Inadequate breeder nutrition
Navel not closed, wet, smelly. Big chicks, lethargic, soft abdomen	- Omphalitis - Insufficient temperature in the incubator - Humidity high in either the incubator or hatcher - Inadequate ventilation
Weak chicks	- Temperature high in the hatcher - Ventilation insufficient in the hatcher - Excessive fumigation - Contamination
Malposition	- Eggs incubated with the small end up - Inadequate turning - Temperatures during incubation high or low - Humidity high - Old flock - Eggs too big - Nutritional deficiencies, particularly vitamins A and B₁₂ - Poor transport and storage conditions
Malformation	- Inappropriate storage conditions - Poor hatching eggs transport conditions - Nutritional deficiencies (biotin, riboflavin, zinc or manganese) - Inadequate turning - Poor orientation of the eggs (eggs small end up) - Temperatures during incubation too high or low - Health problems - Inadequate ventilation or thick shells
Curled toes, splayed legs	- Temperatures during incubation high or low - Nutritional problems - Wet hatcher tray surface
Short fluff, dry, rough	- Nutritional deficiencies, particularly riboflavin - Mycotoxins or other inhibiting factors that provoke nutritional deficiencies - High temperature during the first 14 days of incubation
Eyes closed, fluff stuck to the eyes	- Temperature too high in the hatcher - Low humidity in the hatcher - Improper function of the dust recuperation system - Chicks left too long on the hatcher after they are ready - Excessive air circulation in the hatcher
Dwarf like chicks, insufficient growth	- Contaminated eggs - Contamination of the hatchery, particularly during hatching - Health problems - Nutritional deficiencies - Thyroid anomaly

ANALYSIS OF THE CAUSES OF EMBRYONIC MORTALITY

Observations	Possible causes
Exploding eggs	- Dirty eggs or nests - Floor eggs - Inadequate egg washing, eggs dried or cleaned with dirty cloths - Dust in the breeder house, storage room or transport vehicles - Condensation on the egg shell surface - Using a contaminated solution to spray the eggs - Eggs contaminated by other exploding eggs - Handling eggs with dirty hands
Missing one or both eyes	- Temperature high during the first 6 days of incubation - Insufficient oxygenation during the first 6 days of incubation
Exposed brain	- Temperature high during the first 3 days of incubation - Insufficient oxygenation during the first 3 days of incubation
Ectopic viscera 	Temperature high in the incubator
Haemorrhages 	- Sub cutaneous haemorrhages: temperature high in the incubator or hatcher - Haemorrhages of the chorioallantoic membrane: inadequate egg handling during transfer - Nutritional deficiencies (vitamins K or E) - Dead embryos between the 11th and 15th day of incubation and which exhibit a deep red colour: bacteria of fungal contamination
Reddening of the joints on hatched or pipped but not hatched chicks	- Difficult hatch - Vitamin deficiencies - Hard shells - High humidity during incubation ^{and/or} high temperature in the hatcher
Small air cell, big pipping area, membranes intact, redness of the joints, oedema, residual albumen, yolk sac not absorbed, water loss less than 10%	- High humidity in the incubator - Hard shells - Insufficient temperature in the incubator

ANALYSIS OF THE CAUSES OF EMBRYONIC MORTALITY

Observations	Possible causes
- Micromelia (shortening of the long bones, parrot beak or bowed legs), chondrodystrophy - Short beak, no beak, anomalies of the face	Nutritional deficiencies (biotin or manganese)
Swollen neck and head (exudative diathesis) 	- Temperature high during the first 5 days of incubation - Nutritional deficiencies (niacin) - Nutritional deficiencies, vitamin E or selenium

➔ Nutritional deficiencies and toxins.

Nutrient	Effects of a deficiency ^{and/or} an excess
Vitamin A	- Abnormal development of the venous system - Skeletal abnormality (particularly the skull and the backbone) - Degenerative changes of the brain, spinal column and nerves - Early embryonic mortality (during the first 2-3 days of incubation) - Chicks which hatch can exhibit runny eyes or closed eyelids - An excess of vitamin A can also provoke skeletal abnormalities
Vitamin D₃	- Late embryo mortality (from the 17th day) - Growth problems during rearing - Insufficient skeletal development
Vitamin E	- Problems of the circulatory system - Exudative diathesis - Haemorrhages - Encephalomalacia - Eye abnormalities - Oedema of the neck and legs - Embryonic mortality between 2 to 5 days of incubation - Muscular weakness after hatching
Vitamin K	Haemorrhages of the embryo and the membranes slightly after or during hatching
Thiamine	- Polyneuritis - Early and late embryonic mortality (from the 19th day) - Numerous dead chicks in the hatcher trays
Riboflavin	- Short legs - Disorganisation of the circulatory system - Oedema - Twisted toes - Micromelia - Anaemia - Brown or deep green liver - Embryonic mortality between days 3 to 5, 10 to 15 and 21 of incubation

ANALYSIS OF THE CAUSES OF EMBRYONIC MORTALITY

Nutrient	Effects of a deficiency ^{and/or} an excess
• Niacin	• Hyperplasia of the muscles • Oedema • Short upper beak • Abnormalities of the venous and nervous systems • Embryonic mortality during days 8 to 14 of incubation
• Vitamin B ₆ (Pyridoxine)	• Growth inhibition • Embryonic mortality during days 8 to 14 of incubation
• Pantothenic acid	• Sub cutaneous haemorrhages • Oedema • Hydrocephaly • Lack of feathers • Twisted toes • Embryonic mortality during days 2 to 4 and 11 to 15 of incubation
• Biotin	• Chondrodystrophy • Micromelia • Syndactyly • Haemorrhages on the embryo and membranes • Embryonic mortality during days 3 to 4 and from the 17th day of incubation
• Folic acid	• Syndactyly • Bowed bones • Dish head, small eyes, ectopic viscera • Parrot beak, other problems of the beak • Embryonic mortality from the 17th day of incubation
• Vitamin B ₁₂	• Oedema (particularly around the eyes) • Haemorrhages • Twisted toes • Short beak • Weak leg muscle development • Malposition (head between the legs) • Embryonic mortality during days 8 to 14 and 16 to 18 of incubation
• Manganese	• Chondrodystrophy • Deformed skeleton • Shortening of the long bones • Parrot beak • Micromelia • Oedema • Embryonic mortality from the 18th day of incubation • Incoordination of the chicks
• Zinc	• Skeletal abnormalities (particularly of the backbone) • Small eyes • Ectopic viscera • Abnormalities of the beak and head • Weak chicks
• Calcium	• Indirect effects: insufficient shell quality, weight loss too high, higher risks of contamination • Insufficient growth
• Magnesium	• Trembling, convulsion • Gasping
• Phosphorus	• Bone malformation • Embryonic mortality during days 14 to 16 of incubation
• Copper	• Abnormalities to the blood and circulatory system • Early peak of mortality (before the 3rd day of incubation)
• Selenium	• Exudative diathesis • Excess selenium provokes oedema of the head and neck, twisted legs, necrosis of the brain and marrow, a shortened upper beak and an increase in the incidence of malposition

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