Principles of Incubation

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The artificial incubation of eggs is becoming an accepted feature of aviculture. As veterinarians are increasingly called on to solve problems in captive bird breeding, it is inevitable that they will be involved in incubation. This paper discusses the principles of incubation, so as to allow the veterinarian to analyse problems in this area.

Structure of the Egg

An egg can be thought of as a yolk and blastoderm, suspended in albumen, and encased in a shell for protection. (*See Figure 1*).

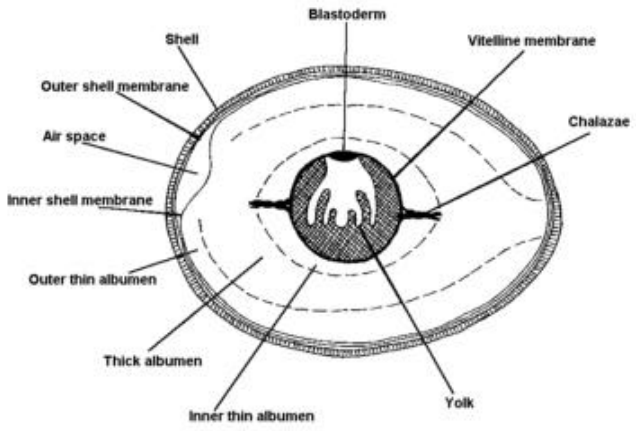

Figure 1:Structure of the egg (from Deeming, 1993)

The yolk consists of fats and protein, laid down in concentric layers, and encased in the inert vitelline membrane. On the top of the yolk lies the blastoderm (the non-developing embryo), a white disc 1 - 2 mm in diameter. As the density of the yolk beneath the blastoderm is less than the rest of the yolk, the blastoderm will always sit on the top of the yolk, regardless of the position the egg is in.

The yolk is suspended in the albumen by means of two chalazae (twisted fibres of albumen protein). Immediately around the yolk is a layer of thin albumen, then a layer of thick

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albumen, with a second layer of thin albumen adjacent to the shell membranes.

Enclosing the albumen are two shell membranes (inner and outer) and the calcitic shell. As the egg cools after laying, air is drawn through the shell, and forms a pocket between the two membranes at one pole of the cell, known as the air cell. The shell is generally 0.25 - 0.5mm thick, and serves two main functions; firstly, physical protection against trauma and microbial infection; and secondly, to regulate the exchange of water, oxygen and carbon dioxide between the egg and its environment. To achieve the second of these functions the shell is penetrated by numerous pores. Over all of this is a thin layer of amorphous organic material, the cuticle. The cuticle gives the shell its gloss, and it is thought it may have some antibacterial properties. Its importance is disputed.

With this structure, the egg is physically robust (ostrich eggs can withstand up to 55 kg of applied pressure) and capable of withstanding temperature fluctuations of short duration.

EMBRYONIC DEVELOPMENT

Initial activity of the embryo is temperature dependent. At 15ºC cell activity is suspended; at 25ºC there is some cellular activity, but no development; between 33º - 35ºC there is sub-optimal development that will lead to abnormalities; and at 36º - 37.5ºC development will proceed normally. Temperatures over 38ºC are poorly tolerated, with a subsequent increase in abnormalities and mortality. Embryonic development can be divided into three, roughly equal, phases: early; mid; and late.

The early phase is one of organ differentiation and early development. A guide to development (in poultry) in this phase is:

Day 1	Blastoderm becomes doughnut-shaped as cellular division occurs
Days 1-2	Brain, eyes and spinal cord begin to develop
Days 2-3	Heart and blood vessels develop, extra-embryonic membranes form.
Days 3-4	Body wall and viscera developing
Days 4-5	Limbs form

The extra-embryonic membranes that begin to form at days 2 - 3 are:

a) the amnion, which grows out from the body wall, and covers the embryo, cushioning it in amniotic fluid;

b) the yolk sac membrane grows out from the gut and envelops the yolk, and may later communicate with the albumen. For the first week this membrane also serves as the respiratory organ;

c) the allantois develops from the hindgut, and serves as a bladder;

d) after a few days the allantois and the chorion (an extension from the amnion) form the chorio-allantoic membrane, which acts as the primary respiratory organ till the chick pips. (*See Figure 2*).

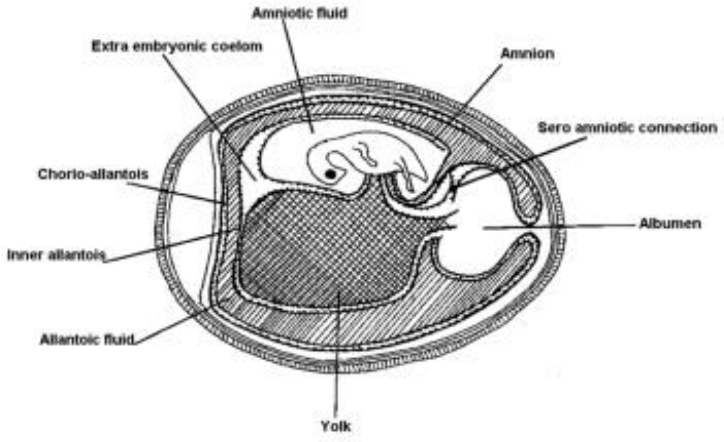


Figure 2: Developing egg (from Deeming, 1993)

The mid-phase is one of growth. The newly differentiated organs are simply growing in size without much complex cellular differentiation taking place. Feathers begin to develop.

The late phase is the final growth, the yolk sac is drawn up into the abdomen, the chick actively drinks albumen, the chick positions itself, pips internally, and finally hatches.

Of these three phases, the first and last are the most sensitive, easily disturbed with often fatal results. The ideal hatchability of artificially incubated fertile eggs is 90%. Of the 10% that normally fail to hatch, the distribution should be:

1)	Early	3 - 4 %
2)	Mid	1 - 2 %
3)	Late	up to 4 %

If embryonic mortality is exceeding these levels, a detailed investigation is called for.

Hatching

By the beginning of the late phase of embryonic development, the embryo is oriented along the long axis of the egg, head on the abdomen between the legs, and the back of the neck against the air cell.

During the last few days, the head turns to the right, so that it is under the right wing, with the right toe next to the beak. The chick may rotate up to 270^{o} in the shell, so that the right foot and head are uppermost. It is thought that the chick rubs the back of its neck against

the shell membranes, causing the air cell to move down towards the beak ("drawing down"). The beak then rubs against the inner shell membrane, rupturing it and allowing the beak access to the air cell ("internal pipping"). The chick can now start to breathe the limited amount of air in the air cell. The build up of CO_2 in this space then stimulates the chick to push with its neck and beak until it breaks a small window in the shell ("pipping"), through which the beak can often be seen. It can then breathe unlimited fresh air until it replenishes its strength for the next struggle. Muscle contractions turn the chick counter-clockwise, and as it turns the chick breaks off more shell. This continues until the top of the shell comes off. This total process can take hours, and even 1 - 3 days in some cases.

Artificial Incubation

There are three stages in the incubation process. They are:

1) Collection and storage of eggs;

2) Incubation; and

3) Hatching.

Collection and storage of eggs.

Many aviculturists like to leave the egg with the hen for the first 5 - 10 days of incubation, before removing it for artificial incubation. The drawback to this is when conditions in the nestbox are wet or unhygienic, or when the hen is excessively rough with the eggs. As the egg dries and cools, air is drawn into the shell, and if conditions are wet or unhygienic, water and bacteria may be drawn in as well, leading to infection. Hens with a history of floating air cells should be monitored closely, as this is usually due to rough treatment after laying, and the egg needs to be collected before any damage can be done. If collecting eggs as soon as possible after laying, it is advisable to allow the cuticle to dry (10 - 15 minutes) before collecting.

Although sterile towels and gloves are often used, they are probably not necessary unless the collector's hands are badly soiled. Once collected, the egg should be passed to the person who has responsibility for incubation. This person should have little or no contact with the rest of the flock. Any rough handling should be avoided.

There is considerable debate on how to sanitise eggs. Five major choices exist - dry brushing, washing, fumigation, UV lights, or a combination of any or all of the above. Sanitation serves two purposes. Firstly, to remove gross organic contamination, and secondly, to sterilise, as far as possible, the surface of the egg.

Dry brushing can be used if there is little or no organic contamination (e.g. eggs laid on dry, clean substrate).

Washing is advisable if there is any significant contamination. Several solutions are recommended, although none are registered for that use. The safest seem to be quaternary

ammonium compounds (e.g. ChickGuard) or tertiary halogenated compounds (e.g. Avi-Safe, Vetafarm). Iodine solutions have also been used. The washing and rinsing solution should be at 41ºC. This causes the air in the shell pores to expand, sealing the egg from penetration by liquid chemicals. After washing and rinsing, the egg should be left on a rack to dry.

UV lights are currently the "flavour of the month." Although they are successful in reducing bacterial numbers, they are ineffective on shaded parts of the egg. There may be health hazards for the operator if care is not taken.

Formaldehyde gas, produced either by adding formalin to potassium permanganate or by heating solid paraformaldehyde, is an effective means of sterilising the egg surface. This can be done alone, or in combination with any of the above. There are extreme health risks to the operator, and it should not be undertaken unless an effective exhaust system is in place.

Eggs can stored for up to 3 - 4 days at 15º - 20ºC (dry bulb) and 75% relative humidity (12.5ºC wet bulb). This will prevent cellular activity and prevent excessive weight loss. Storage over 7 days sees a marked increase in embryonic mortality. The eggs should be turned twice daily. Storage allows the batching of eggs, giving the aviculturist better control over hatching.

The eggs should be pre-warmed to 24ºC over the 24 hours before setting. This prevents both excessive cooling of the incubator, and temperature shock to the soon-to-be developing embryo. Immediately before setting, the egg's weight should be recorded.

Incubation.

The six key points to be examined when assessing an incubator are:

a. temperature settings and control;
b. relative humidity;
c. ventilation and gas exchange;
d. egg turning and position;
e. hygiene: and
f. monitoring and record keeping.

Incubators can be either single-staged (one batch of similarly aged eggs per incubator) or multi-staged (different batches of mixed ages). The multi-stage incubator is the most common in use.

a) Temperature control.

As stated earlier, the embryo develops between 36-37.5^{0}C. Consequently, most incubators are set at 37.3-37.5^{0}C, averaging out the embryo's requirements. This seems to work well in most cases.

Temperature has marked effects on the length of incubation and development of the embryo. Excessively high temperatures in the first few days of incubation will increase early embryonic death. Mildly elevated temperatures can reduce the incubation period; conversely, low temperatures (35ºC) can lengthen it. Both can have adverse effects on chick hatchability and subsequent survivability.

As the embryo develops, metabolic heat is given off, increasing the ambient temperature. Left unchecked, this could result in increased embryonic mortality. Incubators, therefore, work more by cooling than by heating to maintain a constant temperature. This cooling is achieved by bringing in cool air from outside the machine, and by placing cooler, fresh eggs alongside the more advanced eggs. For this reason, the incubator room should be maintained at 24ºC, with a 25% (or less) relative humidity. In a large capacity incubator full of eggs, power loss or fan failure may make it necessary to open the incubator door to avoid overheating the eggs. If the incubator has a large egg capacity, but has only a few eggs in it, air cooling will be greater than if the incubator was full. This may require setting the temperature 1º - 2ºC higher to compensate. Temperature should be monitored with a thermometer, rather than an LED display on the control panel. Additionally, it is wise to set several thermometers in different locations, to detect “hot spots”.

b) Relative Humidity.

All bird eggs lose water (and therefore weight) during incubation. This weight loss in parrots is ideally around 14% - 17%, varying between species.(see Table 1.) Too little weight loss, and the embryo is oedematous at hatch; too much, and the chick is dehydrated. Both conditions make hatching difficult, and chick survival unlikely.

The weight loss is determined by the porosity of the egg shell (defined as the rate of water vapour conductance) and the relative humidity of the ambient air.

Relative humidity is the percentage of water vapour in the air, where saturation point has a relative humidity of 100%. Relative humidity is affected by temperature, altitude and absolute humidity (the actual amount of water vapour present). The relationship between absolute humidity, temperature and relative humidity is expressed in a psychrometric chart. (*See Figure 3*) Dry bulb temperature is that recorded by a standard thermometer. Wet bulb temperature is that recorded by a thermometer covered with a clean, wet wick, with one end of the wick inserted in a reservoir of distilled water. As water evaporates from the wick, it cools the thermometer, giving a lower temperature. The rate of evaporation (and therefore the amount of cooling) is dependent on the absolute humidity and rate of air flow.

By looking at a psychrometric chart, the effects of varying temperature and humidity can be seen. As dry bulb temperature decreases at a set absolute humidity, relative humidity increases, and vice versa. If the dry bulb temperature remains constant, and the absolute humidity decreases, so does relative humidity, and vice versa. At higher altitudes, the readings on the chart will change, and there will be a requirement for a higher absolute humidity at a constant dry bulb temperature to achieve the same relative humidity.

To obtain a 15% weight loss from eggs, relative humidity settings have ranged from 15% -

63%. On average, most incubators run at 35 - 50%. This should be adjusted by monitoring the average weight loss each week. Although most modern incubators have an LED display of relative humidity, this should be checked periodically using a wet bulb and dry bulb thermometer, and a psychrometric chart.

Water vapour to provide the absolute humidity arises from the water lost from eggs, the humidifier in the machine, and the ambient air in the incubator room. If a reduction in relative humidity is required, water loss from the eggs should not be reduced, so the reduction in humidity should ideally come from the ambient air. This can be done by air conditioning and dehumidifiers. Dehumidified, cool air entering the incubator will be further dehumidified as its temperature rises. This leaves the incubator humidifier free to control the relative humidity in the machine.

Daily weighing of eggs to measure weight loss should be mandatory. This can be analysed several ways. Firstly, the desired percentage weight loss can be plotted on a graph, and compared to the actual weight loss percentage. (*see Figure 4*).

$$\frac{setting\ weight - current\ weight}{setting\ weight} \times 100 = \%\ weight\ loss$$

Alternatively, the expected daily weight loss can be calculated, and then compared with actual daily weight loss.

$$\frac{setting\ weight \times desired\ \%\ wt\ loss}{length\ of\ incubation\ (days)} = expected\ daily\ weight\ loss$$

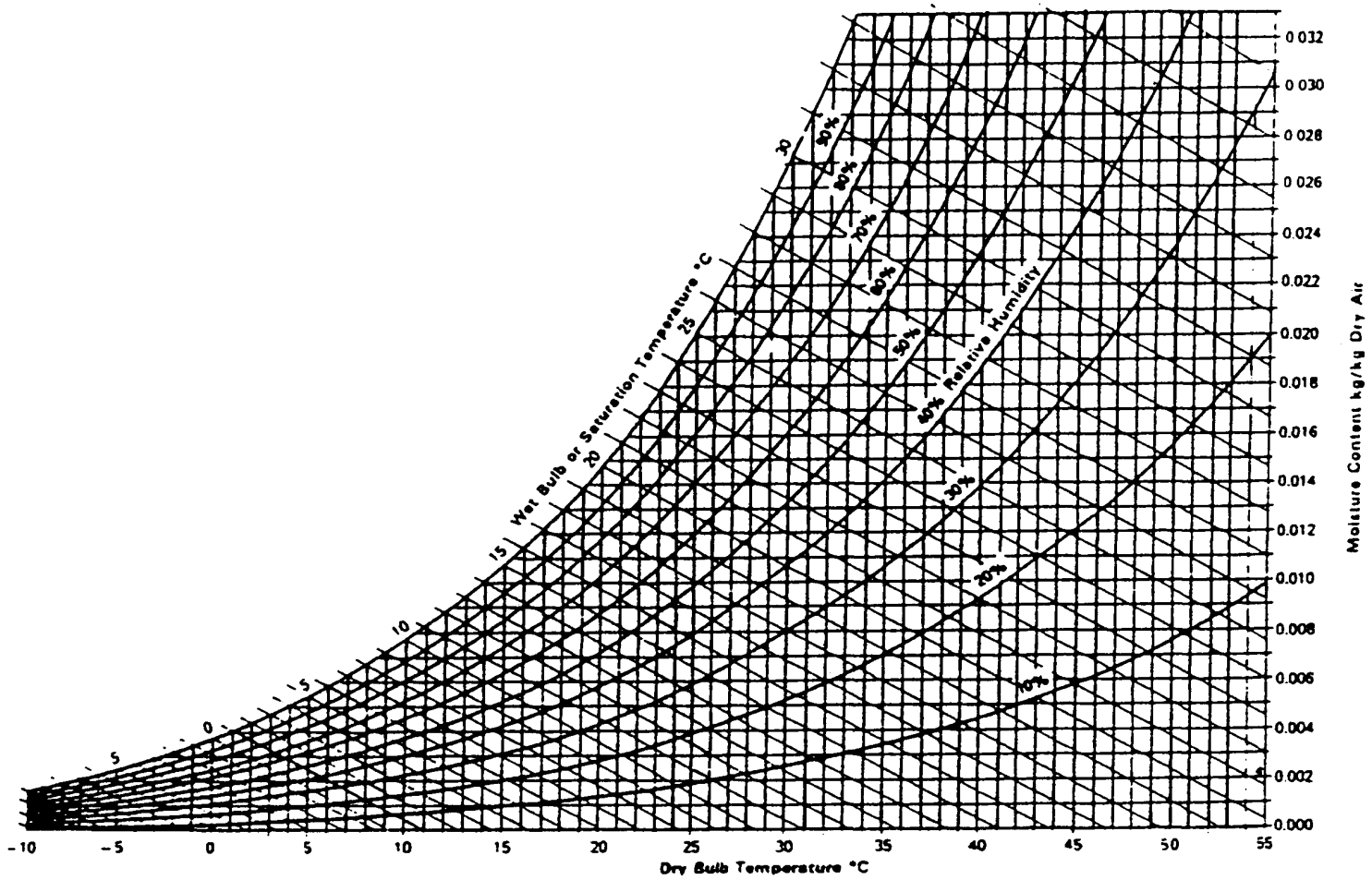


Figure 3: A standard psychometric chart

EGG CHART

Egg No _____ Egg Weight when collected _____

Time collected _____am/pm Date laid ___/___/___

Egg condition when collected: clean/wet, muddy/ other

_____

Weather conditions: _____

_____

Egg Fertile [ ] Date ___/___/___

Infertile [ ] Date ___/___/___

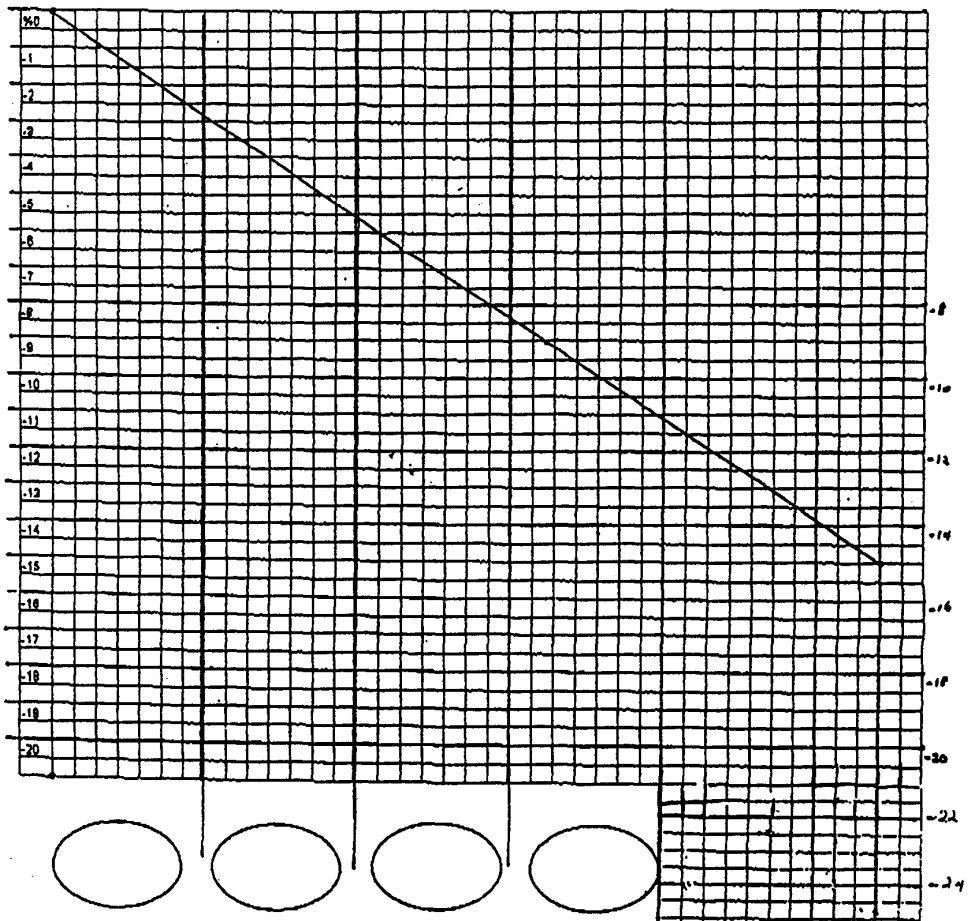
Days stored _____ Temp stored _____

Inoubator temp _____ F Room temp _____

Date into Incubator ___/___/___

Date out ___/___/___

Actual hatch date ___/___/___



Air Cell Size

Transfer to hatchet ___/___/___ Hatcher temp _____F

Pipped Air cell (yes/no) Time _____am/pm Day no _____

Pipped shell (yes/no) Time _____am/pm Day no _____

Chick helped out of egg (yes/no) Chick Weight _____

Yolk sac absorbed (yes/no)

Chick Condition: _____

Hatching Remarks: _____

Figure 4: Typical Egg Chart

Ideally, two or three incubators should be run, each at a different humidity. Very porous eggs can be kept in a high relative humidity (thus decreasing an otherwise excessive weight loss), thick shelled or low porosity eggs can run at a low relative humidity (thus increasing weight loss), and average eggs can be run at an average humidity. Some authors recommend placing an egg that is losing too much weight into a plastic bag or taping it up so as to reduce water loss. The drawback with this is the effect on gas exchange, which could result in a suffocated embryo. Therefore it must be done with care.

Species	Egg weight loss goal	Incubation period
Long-Billed Corella	14 - 16%	23-24 days
Galah	15 - 18%	23-25 days
Sulphur Crested Cockatoo	16 - 18%	25-27 days
Major Mitchell Cockatoo	12 - 15%	24-26 days
Blue & Gold Macaw	15 - 19%	27 days
Green-Wing Macaw	17 - 20%	27 days
Scarlet Macaw	15 - 19%	27 days
Eclectus Parrot	14 - 15%	28 days

Table 1. Desired Egg Weight Losses (from Jordan, 1989), and Doneley (1996)

c) Ventilation and gas exchange.

Oxygen is utilised by the embryonic cells, which then produce carbon dioxide as a waste product. The exchange of these two gases between the egg and its environment occurs initially through the yolk sac membrane, but predominantly across the chorio-allantoic membrane. Without an effective exchange, the embryo will quickly die. The rate of this exchange is determined by the porosity of the egg shell, and the concentration gradient of the gases across the shell. Low O_2 levels are thought to contribute to anasarca. This is seen in chicks where egg shell porosity is poor, leading to decreased weight loss. By reducing the relative humidity, the weight loss can be increased, but the chick is still oedematous. This may be due to hypoxia causing tissue and blood vessel damage, with subsequent oedema.

In late incubation the embryo's oxygen consumption rises, leading to low O_2 levels and high CO_2 levels, a strong stimulus to hatching.

The air in the incubator is drawn from the incubator room. Although requirements for temperature and relative humidity have been discussed, a flow of fresh air into this room should not be disregarded in the quest for control of other factors. Most modern incubators are forced air ventilated (i.e. driven by a fan). Older types are still air, working by convection and air vents. These are more difficult to run.

d) Egg turning and position.

The commonly held belief that turning an egg is necessary to stop the embryo sticking to the shell appears to be unfounded. However, failing to turn an egg can result in poor growth of the extra-embryonic membranes, a reduction in the amount of extra-embryonic fluid produced during development, and poor growth rates of the embryo during the second half of incubation. It is now considered necessary to ensure that all the albumen is utilised by the embryo.

The position of the egg during incubation can have an effect on the incidence of malpositioning. Eggs that are incubated vertically or horizontally for the entire incubation have a higher incidence of malpositions. Best results seem to be obtained when the air cell is slightly elevated off the horizontal.

Turning can be done either manually or automatically. Automatic turners have to be checked for vibration. They usually turn the egg through 45^0 each turn. Turning can be set for hourly, 2 hourly, or less. Good results have been obtained with only one or two turns daily, but most prefer more frequent turning.

e) Hygiene.

Hygiene is essential in an incubation facility. Microbial contamination of the environment through either poor hygiene practices or a contaminated egg can potentially cost hundreds of dollars. Operators should maintain strict hygiene, with changes of clothing and footwear for anyone entering the facility. Ideally, personnel responsible for the flock management should not enter the facility at all. Incubators need to be designed so that they can be easily cleaned and disinfected. Fumigation with formaldehyde is not recommended if there are eggs in the incubator, as this has been associated with embryonic mortality.

Veterinary Pathology Services (Brisbane, Sydney and Adelaide) now offer an incubation monitoring service. Swabs from the incubator are plated out and colony counts performed at 24 and 48 hours. This should be performed on a regular basis, and cleaning carried out if the results indicate contamination.

f) Monitoring and record keeping.

Monitoring of the egg through candling and weighing is an essential part of the incubation process, and should be done on a daily basis. With training and experience, it is possible to detect fertility early in incubation, and detect dead embryos shortly after death. Malpositioned chicks can be detected at hatch.

Without accurate records of weight loss, incubator temperature and relative humidity, management changes, etc. it is difficult, if not impossible, to successfully investigate incubation problems. This must be impressed on aviculturists, as some think it “too much effort.”

Hatching.

Trials have shown that the incidence of malpositioning and late embryonic mortality can be reduced if eggs are not turned for the last 2 - 3 days of incubation. For this reason, eggs are removed from the incubator at this time, and transferred to a hatcher.

With the amount of heat being produced by the eggs at this stage, the hatcher should be run at 36.4^{o}C to prevent excessive heating of the eggs and to reduce heat stress on the chicks. Larger hatchers with low numbers of eggs can be run at the same temperature as the incubator.

Decreasing the dry bulb temperature will automatically increase the relative humidity. Further increases in absolute humidity to reach 70% relative humidity may be necessary. This higher humidity is necessary to avoid dehydration of the chick and membranes as the chick pips. Eggs that have lost insufficient weight during incubation will not lose it at this stage, as the excess moisture has already been stored in the chick's thigh muscles and pipping muscle.

Good ventilation is essential at this stage. Stale air must be ducted from the room.

Hygiene is essential, as the potential for contamination and subsequent infection (particularly of the yolk sac) is high.

Conclusion

Although this paper has not discussed troubleshooting incubation problems, it is hoped that the principles outlined will be a good starting point for those veterinarians involved in aviculture.

References

Black, D. (1995) *How to deal with incubation failure*. in Ostrich Odyssey 95: The Proceedings of the Fifth Australian Ostrich Association Conference.

Deeming, D.C. (1993) *The incubation requirements of ostrich (Struthio camelus) eggs and embryos* in Bryden, D.I. (Ed.) Ostrich Odyssey: Proceedings of the meeting of the Australian Ostrich Association Inc. (Vic.) Post-Graduate Foundation in Veterinary Science, University of Sydney.

Doneley, R.J.T. (1996) *Control and Therapy of Diseases of Birds*. Vade Mecum Series A, No. 21, University of Sydney Post Graduate Committee in Veterinary Science.

Jordan, R. (1989).*Parrot Incubation Procedures*. Black Cockatoo Press, Prahran, Vic.

Joyner, K.L. (1994) *Theriogenology*. in Ritchie, Harrison and Harrison, *Avian Medicine: Principles and Application*. Wingers Publishing, Lake Worth, Florida