



PILA In Avian Eggs



1

PILA elements

- The law
- Biology and reproduction in the chicken
- Embryo development
- Incubation of chicken eggs
- Minor techniques
- Welfare and 'Humane End Points'
- Euthanasia

2

UK Egg Industry

- UK egg production is approx. 11.3 billion eggs per year
 - Import 1.4 billion eggs
 - Export 405 million eggs
 - Total UK Consumption: 13.5 billion eggs (92% self-sufficient)



Picture and information from: <https://www.egginfo.co.uk/egg-facts-and-figures/industry-information/data>

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UK Egg Industry



- UK produces 11.3 billion eggs/year (2021 data)
 - UK eats 37.1 million eggs per day (consumes 13.5 billion eggs per year)
- Each hen lays approximately 300 eggs/year
 - Culled after 12 months of laying when productivity declines
- The value of retail egg sales is over £1.2bn, which has risen by approximately £600m since 2004.

4

History of Chicken Embryos in Research

First described by Aristotle around 350 BCE

Able to view developing chick without needing a microscope

16th century – able to differentiate different developmental tissues

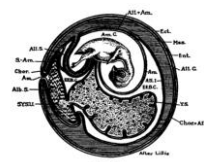
17th century – development of microscope

18th Century – embryology as a science

Immunology - host-graft response (1914)

Propagation of pox virus (1931)

Chicken genome sequenced (2004)



5

ASPA Act 1986 Amendment Regulations 2012 'Protected Animal'

- Sets out revised measures for the protection of animals used for scientific purposes.
- All living vertebrates, other than a human, **including certain immature forms**, and any living cephalopod.
 - However, the point of protection has changed from ½ way through incubation **to 2/3rd way** through incubation.
 - An embryo is protected if you manipulate it during the first two-thirds of the incubation period and then **allow it to survive into the final third** of the incubation period.

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Uses in Research

- Nobody knows how many embryonated chicken eggs are used annually
- The numbers are not reported to the HO
- Used to replace in vivo work previously done on 'protected animals'



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Code of Practice (CoP)

Section 3, Chapter 9: Birds
This chapter must be read in conjunction with the Introduction and Section 3 Chapter 1: Advice applicable to all animals.

1 Advice applicable to all birds
Additional details are provided for the commonly kept and used laboratory species in Part 2 onwards. It is essential that the housing and care of these categories, cover appropriate measures taken in respect to their behaviour, physiological and social requirements and protocols for such species should be comparable to the animal welfare code. Advice on requirements for other species of the subject should be sought from experts and care staff to ensure their individuality is maintained.

2 Additional advice applicable to chickens (*Gallus gallus domesticus*)
Chickens (domestic fowl, *Gallus gallus domesticus*) retain much of the biology and behaviour of the jungle fowl from which they were domesticated. Behaviours that are most important to the species are nesting (in burrows), foraging and caring for its offspring, socialising and dust bathing. Fowl are social and are preferably housed in groups of around 10-20 birds, with fewer males than females in adult groups. For example, a ratio of 1:6:2.

Attempts have been made to select strains of fowl for reduced feather pecking or aggressive behaviour. It is recommended to assess the suitability of appropriate strains of this type, including the feasibility of acquiring them, for each project.

2.1 Environmental conditions
2.1.1 Ventilation: See Part 1: Advice applicable to all birds.
2.1.2 Temperature: See Part 1: Advice applicable to all birds.

Where appropriate, birds should be provided with a range of nesting materials that meet their requirements. (Materials that domestic fowls, geese, turkeys and guinea fowls typically do not use and that are considered to be unsuitable for use by these species will suffice but heat should be within the temperature range. The species when housed are to be provided with suitable nesting materials that are available to them. The material should be available in a location accessible to the birds and should be made of a material that is suitable for use in a confined space. Nesting materials should be available in a location that is suitable for use in a confined space. Nesting materials should be available in a location that is suitable for use in a confined space.

Table 3.9-1: Recommendations for mean temperature and relative humidity

Age (weeks)	Mean temp (°C)	Relative humidity (RH) (%)	Relative humidity (RH) (%)
0-1	25	20-30	60-70
1-2	25	20-27	60-65
2-4	25	18-24	40-50
4-6	25	16-21	40-50
6-8	25	14-19	40-50

https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/388895/COPAnimalsFullPrint.pdf

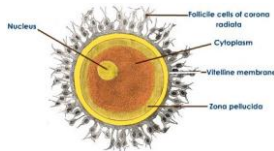
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Terminology - Oocyte vs Egg

Oocyte is an 'immature' female gamete (single cell)

Egg (Ovum) is a fertilizable gamete (comparable to an ovulated 'egg' in other animals)

- Biological definition: A mature, female, haploid, germ cell (ovum), which may divide to give rise to an embryo, usually after fertilisation by a haploid male cell (spermatozoa)



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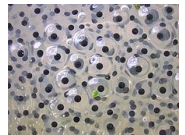
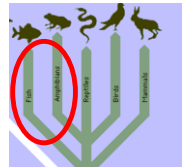
Fish and Amphibian eggs

Fish and Amphibians lay unfertilised eggs in water

Fertilisation and subsequent development takes place outside the female.

Most amphibians respire through gills as aquatic larvae, although the majority subsequently develop lungs.

However, they also use their skin as a secondary respiratory surface, which must be kept moist to function efficiently.



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Reptiles and Bird eggs

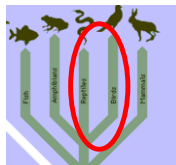
The eggs of birds are enclosed in a chalky shell.

The eggs of reptiles are enclosed in a leathery membrane.

Both can survive in dry conditions on land

Development of the embryo takes place within the shell/membrane.

This occurs partly inside the female, before the egg is laid and partly outside, during incubation



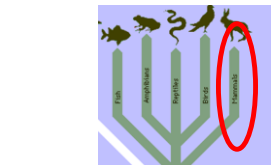
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Mammal eggs

Only 2 species of mammals lay eggs.



Echidna



Duck-billed platypus

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LO: 3.1.1

Reproduction in Chickens

Male anatomy

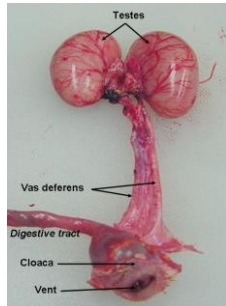
Abdominal testes

Deferent ducts (vas deferens) widen as they approach cloaca – act as main storage organ for spermatozoa

Open into papillae in cloaca

Papillae are mating organs

Sperm can remain viable in oviduct of female for up to 3 weeks



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LO: 3.1.1

Reproduction in Chickens

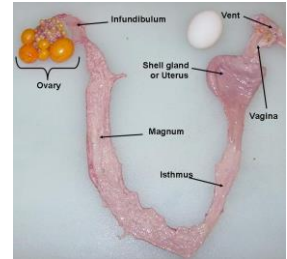
Female anatomy

Only one functioning ovary and oviduct (the left)

The right ovary develops until about day 7, and then regresses

Mature ovaries contain numerous follicles of various sizes

Oviduct produces albumen and shell to surround the egg as it passes down from ovary to cloaca



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LO: 3.1.1

Formation of the egg

The Follicle



- Each ovary contains about 500,000 oocytes (immature ova/egg) at hatching.
- The ovary of a mature hen contains follicles of different sizes and developmental stages.
- Most follicles degenerate, but about 2,000 will continue develop to reach approximately 6mm in diameter (**white follicles**)
- Under the influence of gonadotrophin and steroid hormones, white follicles are periodically initiated to accumulate yolk protein and grow in size to become **yellow follicles**.
- As they grow larger, the yellow oocytes are pushed toward the outer edge of the ovary, until remain connected by only a stalk.

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LO: 3.1.1

Formation of the egg

Yolk



Yolk is produced by the hens liver and transported to the follicular cells in the blood stream before passing to the oocyte.

The yolk contains lipids, proteins and polysaccharides.

The colour of an egg yolk is from xanthophyll carotenoids - lutein and zeaxanthin

Component	%
Water	48
Protein	17.5
Fat	32.5
Carbohydrates	1
Other	1

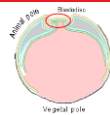
Yolk makes up about 33% of the liquid weight of a newly laid hen's egg and contains approximately 60 calories, three times the calorific content of the egg white.

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LO: 3.1.1

Formation of the egg

Yolk



- Over a period of about 10 days, the oocyte accumulates so much yolk that the nucleus of the cell and most of the cytoplasmic contents are pushed to one side of the cell at the equator.
 - This side of the egg is referred to as the **animal pole**.
 - The opposite side, where the yolk is stored, is referred to as the **vegetal pole**.
- When the oocyte has reached a suitable size (40mm), by accumulation of yolk, the follicle will rupture and release the oocyte into the hen's oviduct (ovulation).
- Ovulation in chickens occurs approximately every 24-27 hours

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LO: 3.1.1

Formation of the egg

Ovulation



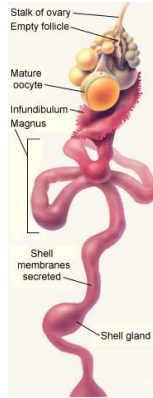
- The follicle ruptures along a line with few, if any, blood vessels (**the stigma**).
 - **Blood spot** – rupture of tiny blood at ovulation, so a small drop of blood may be deposited on the yolk.
- Once released from the follicle, the oocyte, surrounded by a transparent casing (the vitelline membrane), passes into the thin, funnel-like lips of the infundibulum of the oviduct.
- Ovulation in chickens will first occur between 18-24 weeks, depending on breed, feeding, light intensity and other management factors.

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LO: 3.1.1

Formation of the 'egg' - Meiosis

- The released ovum (egg) is a single cell.
- Shortly after release from the follicle, the nucleus of this cell will undergo **meiosis** (divide) to produce a haploid gamete.
- If fertilisation occurs, the fused male and female gametes will become a **zygote** and then a chick embryo.
- Whether the oocyte is fertilised or not, it will continue down the oviduct to be covered by layers of albumen ('egg white'), and be enclosed in a shell.

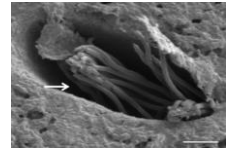


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LO: 3.1.1

Formation of the egg - Fertilisation

- Inside the lower portion of the oviduct are special crypts (sperm storage tubules) where sperm from a rooster can be stored and remain viable for up to three weeks.
- Ovulation causes the walls of the oviduct to contract and cilia lining the walls to move, causing an upward current in the oviduct.
- If sperm are present in the storage tubules of the lower oviduct, some of these will be swept up the oviduct toward the oocyte, and fertilisation will take place in the infundibulum.



Sasanomi et al. 2013

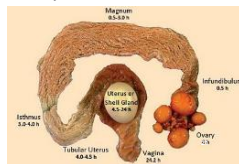


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LO: 3.1.1

Formation of the egg - The Magnum

- Whether or not the oocyte has been fertilised, it will leave the infundibulum and enter the **magnum** section of the oviduct within about 30 minutes.
- The dense portion of the albumen is added in the magnum
- 50% of the total albumen is added here.
- The egg remains in the magnum for approximately 3 hours

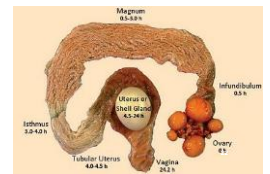


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LO: 3.1.1

Formation of the egg - The Isthmus

- The magnum is separated from the uterus by the **isthmus**, a narrow ring without glands.
- The egg remains in the isthmus for approximately 1 hour, where a further 10% of the total albumen is secreted
- The 2 shell membranes are also formed here
- The shape of the egg is largely determined in this section.



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LO: 3.1.1

Formation of the egg - Uterus (Shell gland)

- The egg remains in the uterus for 20 hours
- Final 40% of (thin) albumen is secreted here
- Shell formed of crystalline calcium carbonate
- Pigmentation occurs at a late stage
- Shell is finally covered in a muco-protein layer which protects the egg from micro-organisms



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LO: 3.1.1

The egg at time of laying



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LO: 3.1.1

Egg laying



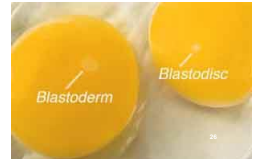
- Chickens usually lay a clutch of about 5 eggs over 5 days
- Clutch size varies between species
- Chickens need at least 12 hours of light for egg production
 - 14 to 16 hours of light for peak production
- Generally lay within 6 hours of sunrise (or light exposure in artificial light)
- Ovulate the next egg within 1 hour of laying
 - If laying occurs in the afternoon, a chicken will not usually ovulate that day, but miss a day and ovulate the following morning.

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LO: 3.1.1

Embryo development - Fertilisation

- If fertilisation occurs, the nucleus of the oocyte (germinal disc or **blastodisc**), which contains the genetic material of the hen, is fertilized by a sperm.
- About three hours after fertilization the newly formed single cell divides into two cells. Then 4, 8, 16 etc.
 - Division of the cell results in the formation of a **blastula**.
 - The blastula consists of a layer of cells known as the **blastoderm**



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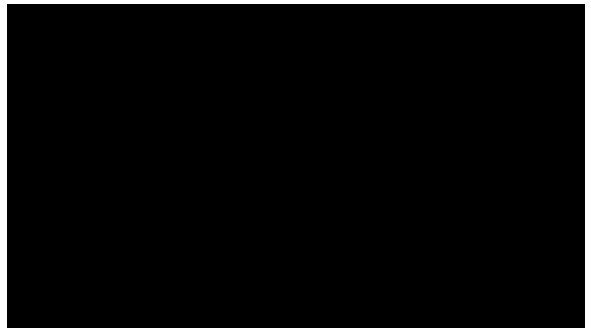
LO: 3.1.1

Embryo development - In the oviduct

- The blastoderm will spend about 25 hours in the warmth of the hen's body (about 41° C), while the egg passes down the oviduct.
- Cell division continues until the egg is laid.
- Once the egg is laid, its temperature will drop below 27°C, and embryo development will cease.
- The cooling of the egg will cause contracture of the internal tissues, which results in the formation of a small air sac at the blunt end (**the aerus**).

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LO: 3.1.1



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LO: 3.1.1

Amniotes

Reptiles, birds and mammals are all **AMNIOTES**

Amniote embryos possess **4 extra-embryonic membranes**:

- **Yolk Sac**
- **Amnion**
- **Allantois**
- **Chorion**



These extra-embryonic membranes allow birds and reptiles to lay eggs on land. They shelter and sustain the embryo against the harsher conditions of a terrestrial environment.

Mammals retain these extra-embryonic membranes, even though the embryo develops in the female until birth.

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LO: 3.1.1

Embryonic membranes

The extra embryonic membranes function to:

- Prevent desiccation
- Enable gas exchange between the embryo and its environment (respiration)
- Allow waste materials to be disposed of while the embryo develops; preventing waste products from building up to toxic levels within the egg
- Enable the embryo to receive the nutrition it needs to develop



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LO: 3.1.1

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Embryonic membranes - Yolk sac

- This sac envelops the yolk and produces an enzyme that changes the yolk material into a form that can be used as a food source by the developing embryo.
- Any remaining, unused yolk material in the yolk sac when the chicken hatches from the egg is drawn into the abdomen for use as food by the chick for the first two to three days after hatching



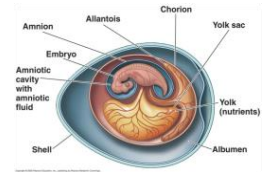
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LO: 3.1.1

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Embryonic membranes - Amnion (Amniotic cavity with amniotic fluid)

- The amnion is a transparent sac filled with a colourless fluid - amniotic fluid.
 - Surrounds and protects the embryo.
 - This provides the embryo with a stable fluid environment which acts as a shock-absorber and permits the developing embryo to move and exercise.



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LO: 3.1.1

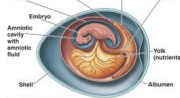
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Embryonic membranes - Allantois

The allantois develops an extensive circulatory system connected to that of the embryo which is driven by the embryonic heart.

The allantoic membrane functions for:

- Respiration** – Oxygen and carbon dioxide are absorbed through the eggshell and are exchanged in the allantois.
- Excretion** – The allantois stores excretions (urea).
- Digestion** – Absorbs albumen (used as food by the embryo), and calcium from the shell for the needs of the embryo.



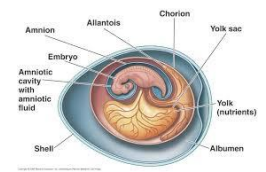
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LO: 3.1.1

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Embryonic membranes - Chorion

- The chorion surrounds both the amnion and the yolk sac.
- Initially the chorion has no apparent function, but later it fuses with the allantois to form the chorio-allantoic membrane.
- The chorio-allantoic membrane lines the inside surface of the egg and is connected to the blood vessels of the embryo.



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Incubation

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LO: 4.2

Incubation - 'Chilling' Eggs



- Chicken embryos can remain viable for up to 2 weeks after laying, provided the temperatures are low.
- They will only begin to develop again if they are warmed by natural or artificial incubation.
- Fertile eggs can therefore be "stored" for a limited time in chilling cabinets.
- Usually held at 15-18°C and 70-80% Relative Humidity (RH)
- They may also be transported under the same conditions
- Warming eggs to commence incubation is known as **setting**.

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Incubation - 'Setting' Eggs

- Chilled eggs should be gently warmed to 20°C for 4-6 hours before placing in the incubator (setting).
- If eggs are kept at 15 to 18°C, they will remain fertile for 14 days, but are best set before 7 days following laying.
- Eggs should always be set with the **taglion** (pointed end) downwards.

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Incubation - Critical factors

- Temperature
- Relative humidity
- Ventilation
- Turning

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Temperature



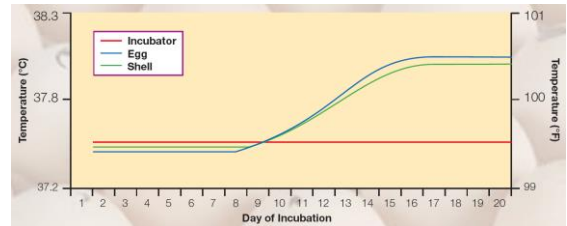
- Correct temperature is critical
- Eggshell temperature correlates to internal temperature
- Optimum temperature is 100-101°F (37.8 – 38.3°C)
- Use a medical infrared ear thermometer on side of shell (equator)
- Eggs in middle of trays will be hottest



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Temperature

If no temperature adjustment possible



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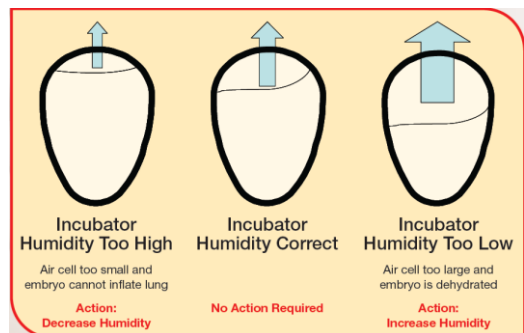
Relative Humidity

- Controls weight loss (reduces evaporation of fluids)
 - If too high, chick may drown due to excess fluid (small air sac)
 - If too low, egg will lose too much fluid, the membranes will dry and chick will not be able to break through shell
- Should routinely monitor the air cell
- Lose 11-12% of weight from day 1-18



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Relative Humidity



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LO: 4.2 43

Tray No.	1	2	3	4	5	6	7	8	9
No. of Eggs	132	132	132	132	132	132	132	132	132
Weight of Empty Tray	1205	1210	1205	1208	1206	1208	1212	1201	1205
Weight of Full Tray	2201	2364	2175	2191	2242	2336	2089	2263	2307
Transfer Weight	7382	7499	7324	7451	7510	7637	7113	7183	7206
No. of Chicks Hatched	120	116	123	122	115	118	109	104	106
Total Chick Weight	4268	4238	4384	4395	4193	4371	3748	3667	3724
Culls and Deaths	1	0	1	1	2	1	2	3	2
Unhatched Eggs	11	16	8	9	15	13	21	25	24
Egg Weight Loss (%)	11.7	12.1	12.2	10.6	10.4	9.8	14.2	15.3	15.5
Mean Egg Weight (g)	53.0	54.2	52.8	52.9	53.3	54.0	52.1	53.5	53.8
Mean Chick Weight (g)	35.6	36.5	35.6	36.0	36.5	37.0	34.4	35.3	35.1
Chick Yield (%)	67.1	67.4	67.5	68.1	68.4	68.6	66.0	65.9	65.3

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LO: 4.2 44

Ventilation

- Air circulating in the incubator will bring oxygen to the egg and remove carbon dioxide.
- This must be done without causing draughts or removing too much moisture.
- Air flow in cabinets and carbon dioxide levels should be monitored.
- As eggs mature, they will require increased ventilation

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LO: 4.2 45

Turning

- If eggs aren't turned fairly regularly, there is a danger the embryo may adhere to the shell and become deformed.
- Eggs should be turned at least 3 times a day.
- Larger incubators will turn eggs once/hour.
- Eggs should not be turned in the last 3 days of incubation when they are moved into the hatcher.

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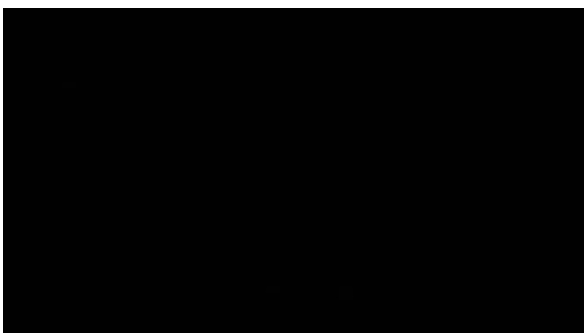
LO: 4.2 46

Days 18 - 21

- Three days before hatching, eggs are normally moved into a separate hatcher.
- Eggs are no longer turned, they are placed with pointed end down so the air sac is above the chick
- The temperature is lowered to 36-37°C
- The RH is increased to 75%
 - This keeps the membranes around the chick from drying out once the chick cracks the shell.
 - Avoids "shrink-wrapping" the chick in membranes

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LO: 4.2

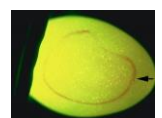
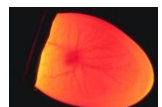


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LO: 3.1.1

Candling

- Determines viability
- Uses a bright light source behind the egg to show details through the shell.
- LEDs are very bright, also put out a cool light that doesn't heat and damage the embryos.
- Being able to identify and remove non-viable eggs (infertile or early death) avoids the risk of a bad egg exploding and contaminating your hatch.
- Nonviable eggs may show a "blood ring"



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LO: 4.2



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LO: 4.2

Microbiological status of eggs needed in vaccine production

- If chicken eggs are to be used as incubators for disease, then it is essential that they start free of disease.
- Embryonated eggs for live vaccine production must be Specified Pathogen Free (SPF).
- This is a requirement of the **European Pharmacopoeia EP7.0.5.2.2.** and the **United States Department of Agriculture Veterinary Services Memorandum 800.65.**
- However, embryonated eggs for research and some veterinary vaccines may be produced from "clean" (not SPF) flocks



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LO: 4.2

Microbiological status of eggs needed in vaccine production

European Pharmacopoeia EP7.0.5.2.2 requirements

- Two generations prior to the producing generation must be free of listed agents.
- The producing generation must maintain the status throughout lay and be monitored through extensive testing performed by certified veterinary laboratories.



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LO: 4.2

Agent	Vertical transmission
Avian adenoviruses, group 1	Yes
Avian encephalomyelitis virus	Yes
Avian infectious bronchitis virus	No
Avian infectious laryngotracheitis virus	No
Avian leucosis viruses	Yes
Avian nephritis virus	No
Avian orthoroviruses	Yes
Avian reticuloendotheliosis virus	Yes
Chicken anaemia virus	Yes
Egg drop syndrome virus	Yes
Infectious bursal disease virus	No
Influenza A virus	No
Marek's disease virus	No
Newcastle disease virus	No
Turkey rhinotracheitis virus	No
Mycoplasma gallisepticum	Yes
Mycoplasma synoviae	Yes
Salmonella pullorum	Yes

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LO: 4.1

Biosecurity - Personnel

- People act as vectors for diseases: organisms survive in hair, on skin, in respiratory tract, on clothing
- Quarantine policy – exclusion times between units (24, 48, 72h, 7 days)
- Protective clothing – dedicated clothing, air shower, wet shower, overshoes, face mask, hair net etc



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LO: 4.1

55

Biosecurity

Equipment

- Check before use
- Is it clean and sterile?



Inocula

- Sterilise or screen inoculum before use
 - If cannot screened, then treat as if contaminated



Transport

- sterile conditions
- filter topped boxes

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LO: 4.1

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Qualities looked for in SPF eggs

Egg fertility: Fertility can be affected by genetics, as well as the production system and the age of the flock.

Embryo viability: Embryo viability is a key factor affecting the average yield of virus production. A higher quantity of vaccine virus can be harvested if an embryo survives the infection of the seed virus for a longer period of time. This may also be influenced by genetic selection.

Egg shell quality: The egg shell quality is a factor of the birds' ability to metabolize calcium and the way that calcium is constructed in the shell. The egg weight curve also has an effect. All of these factors are may be influenced by genetics, as well as the feeding and age of the flock.

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LO: 4.1

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Qualities looked for in SPF eggs

Quantity of allantoic fluid produced

The quantity of allantoic fluid is largely predetermined by the genetics of a bird. Higher volumes of allantoic fluid are obviously desirable.



Pre-incubation selection program

It is possible to measure the speed of cell replication in the fertile egg which will affect embryo development and egg weight loss. Both of these can influence the yield of allantoic fluid.

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LO: 4.8 & 8.1

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Why use chick embryos?

- Chicken embryos are not immune-competent until day 14 of incubation. They can therefore be used for studies in tissue grafting and immune responses.
- Viruses are obligate intracellular parasites and can only be cultured in a cellular environment. An avian egg provides a very good live tissue in which to culture and harvest some viruses (i.e. influenza A).
- The genome of the domestic chicken was sequenced in 2004. Comparisons between the chicken and human genomes have helped to elucidate functional and regulatory genes.
- Transgenic techniques are possible

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LO: 4.8 & 8.1

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Why use chick embryos?

- Readily available, low cost and easy to maintain - don't need feeding
- Can be purchased as Specific Pathogen Free (SPF)
- They maintain their sterility within the shell
- Have natural resistance against contaminating bacteria.
- Free from exposure to specific and non-specific elements of the immune system
- Provide a constant environment for culture
- Less sentient than more mature animals?

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LO: 4.8 & 8.1

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Prior considerations

- Check health of egg
 - Shell is intact and healthy
 - Candle prior to inoculation to ensure viability
 - Candle daily after inoculation to check the embryos
- Appropriate protective clothing
- Ensure clean uncluttered environment
- Get equipment ready
- Check dose/sample volume
 - Determined by route and size/species
 - As small as practical
- Careful handling
- Use a sterile technique

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LO: 4.8 & 8.1

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Egg Inoculation

Candling: the fertile eggs are candled. Cracked, infertile and dead embryos must be discarded.

Marking: The inoculation site is marked on the shell.

Sterilization: The site of inoculation is sterilised using an iodine swab, or similar.

Drilling the shell: A small hole is drilled in the shell at the selected point using a dentist drill or egg shell punch.

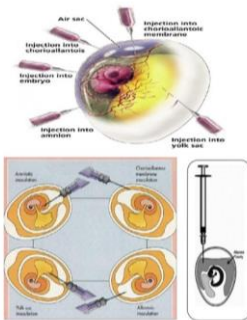


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LO: 4.8 & 8.1

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INOCULATION PHASE



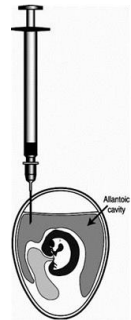
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LO: 4.8 & 8.1

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Allantoic cavity inoculation

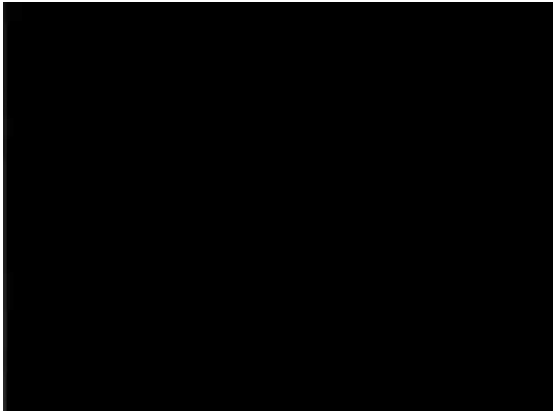
- Determine the location of the air sac
 - mark the opposite side to embryo
- Mark the inoculation site
 - away from embryo, 2mm above air sac
- Disinfect top of egg
- Drill hole in shell (using sterile technique)
- Inoculate
 - Insert needle 16mm or so to penetrate the allantoic cavity
- Seal hole with wax or tape
- Replace in incubator



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LO: 4.8 & 8.1

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LO: 4.8 & 8.1

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Harvesting

- As new infective particles are produced, they are released into the egg (allantoic fluid or yolk sac).
- To harvest the agents, remove the top of the egg shell covering the air sac.
- Use sterile equipment.
- Pierce shell membrane and chorioallantoic membrane with a pipette, remove the embryo if required, and withdraw the infected fluid.

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LO: 8.2

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Welfare and Humane End-Points

- Do chicken embryos feel pain, suffering and distress?
 - 2015 article states that pain starts to develop at day 7 of breeding
- EU Directive 2010/63 and the UK Animal Welfare Act 2006 do not apply to embryonated eggs.
 - 'The issue of the development stage at which there is sufficient anatomical development to permit sentience, and therefore warrant inclusion in the controls, requires a better scientific analysis.' (Bioscience coalition)
- **ASPA protects embryonated forms from 2/3^{ds} incubation**, but does not require you to assess the actual suffering experienced by an avian egg.

10.1016/j.jcp.2015.05.001
Ethical evaluation and short-term anaesthesia of the chick embryo
 J. C. P. 2015, 10(10), 1016-1017
 doi:10.1016/j.jcp.2015.05.001

Abstract
 Chickens have long been regarded as an animal with a sensitive capacity. The fundamental question of when the chick embryo is able to experience pain, suffering and distress, and therefore warrant inclusion in the controls, requires a better scientific analysis. The issue of the development stage at which there is sufficient anatomical development to permit sentience, and therefore warrant inclusion in the controls, requires a better scientific analysis. (Bioscience coalition)

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LO: 1.12

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Humane Methods of Killing

Schedule 1 methods for embryonated eggs

- **Embryos of all animals**
 Overdose of anaesthetic using a route and agent appropriate for the size, stage of development and species of animal -
- **Embryos of birds and reptiles only**
 Refrigeration, or disruption of membranes, or maceration in apparatus approved under appropriate slaughter legislation, or exposure to carbon dioxide in near 100% concentration, until they are dead
- **Embryos of mammals & birds up to 50g**
 Decapitation

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LO: 1.12

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Confirmation of death

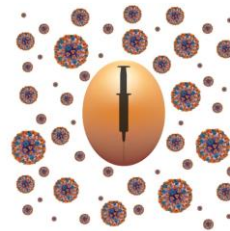
The Schedule 1 methods killing are appropriate only if the process is completed by one the methods listed below:

- **Confirmation of permanent cessation of the circulation**
- **Destruction of the brain**
- **Dislocation of the neck**
- **Exsanguination**
- **Confirming the onset of rigor mortis**
- **Instantaneous destruction of the body in a macerator**

The animal must be killed before one of these "completions" is applied

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Questions?



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