# Avian Eggs

1



# Programme

The law

Biology and reproduction in the chicken

Embryo development

Incubation of chicken eggs

Minor techniques

Welfare and “Humane End Points”

Euthanasia

2



# The Law

The 2010 EU Directive (2010/63/EU) does not protect bird or reptile eggs.

But some stages of embryonated eggs remain protected in the UK, under the revised ASPA of 2012.

However, the point of protection has changed from ½ way through incubation to 2/3$^{rds}$ 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.

3



# Biology of Avian Eggs

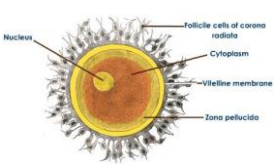
4



# What is an egg?

Common usage
The oval or round reproductive cell laid by a female bird, reptile, amphibian, fish or invertebrate.

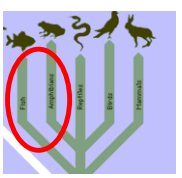
Biological definition
A mature, female, haploid, germ cell (ovum), which may divide to give rise to an embryo, usually after fertilisation by a haploid male cell (spermatozoa)




5



# Fish and Amphibian eggs

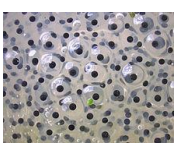



Fish and Amphibians lay unfertilised eggs (ova) in water

Fertilisation and subsequent development takes place outside the female.

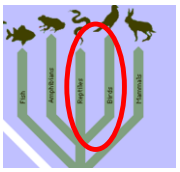
Most amphibians respire through gills as aquatic larvae, although the majority subsequently develop lungs.

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



6

## 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

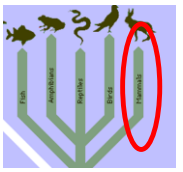
Development of the embryo takes place within the shell/membrane.

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

7



## Mammal eggs



Only 2 species of mammals lay eggs.

Echidna

Duck-billed platypus

8



## Commercial importance of poultry eggs

Over 30 million egg laying chickens in UK

Each hen lays approximately 300 eggs/year

UK produces 10 billion eggs/year (approx 30 million eggs /day)

Average person consumes 170 eggs/head/year

9



## Research importance

How many embryonated chicken eggs are used in research every year in the UK?

### Nobody knows

The numbers are not reported to the Home Office.

However, 1.2 billion eggs are used worldwide annually, to produce 3 billion doses of human influenza vaccine.

10



## 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)

11



## Reproduction in Chickens

### Male anatomy

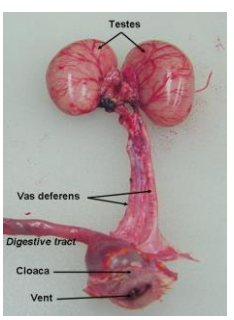
Abdominal testes

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




12

LO: 3.1.1

## Reproduction in Chickens

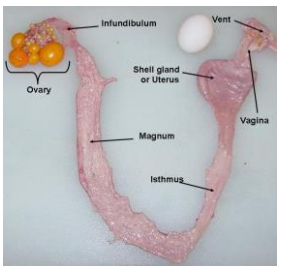
### Female anatomy

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




13

LO: 3.1.1

## Formation of the egg

### The Follicle

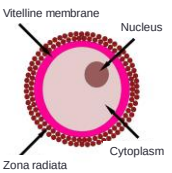
Each ovary contains about 500,000 oocytes (immature ova) at hatching.

Each oocyte is enclosed in a cell membrane, which is covered by an extra-cellular protein coat – the vitelline membrane

The vitelline membrane is surrounded by a layer of follicular cells - the zona radiata

The follicular cells of the zona radiata pass nutrients from capillaries of the ovary to the growing egg

This structure is called a follicle




14

LO: 3.1.1 15

## Formation of the egg

### The Follicle

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.

15

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, whence it passes to the ooctyte.

The yolk contains lipids, proteins and polysaccharides.

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

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.

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

16

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

17

LO: 3.1.1

## Formation of the egg

### Ovulation

The follicle ruptures along a line with few, if any, blood vessels (the stigma).

If by chance any blood vessels are ruptured at ovulation, a small drop of blood (blood spot) may be deposited on the yolk.

Once released from the follicle, the oocyte, surrounded by the vitelline membrane, passes into the thin, funnel-like lips of the infundibulum of the oviduct.

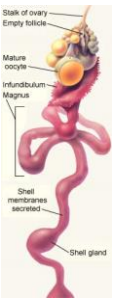
Ovulation in chickens will first occur between 18-24 weeks, depending on breed, feeding, light intensity and other management factors.

18

LO: 3.1.1

## Formation of the egg

### Meiosis




The released oocyte (egg) is a single cell.

Shortly after release from the follicle, the nucleus of this cell will undergo meiosis to produce a haploid gamete.

If fertilisation occurs, the fused male and female gametes will become a zygote and thence 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 enclosed in a shell.

19

LO: 3.1.1 20

## 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.

20

LO: 3.1.1

## Formation of the egg

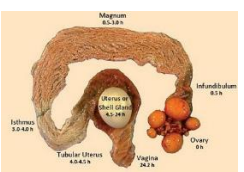
### The Magnum

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




21

LO: 3.1.1

## Formation of the egg

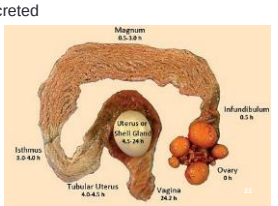
### The Isthmus

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.




22

LO: 3.1.1

## Formation of the egg

### Uterus (Shell gland)

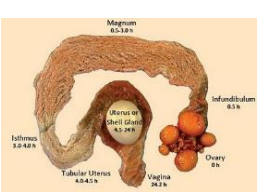
The egg remains in the uterus for 20 hours

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




23

LO: 3.1.1 24

## The egg at time of laying


24

## Egg laying

Chickens usually lay a clutch of about 5 eggs over 5 days

Clutch size varies between species

Chickens need >14 hours of light for egg production

Generally lay within 6 hours of sunrise (or light exposure in artificial light)

Ovulate the next egg within 1 hour of laying

But if laying occurs in the afternoon, a chicken will not usually ovulate that day, but miss a day and ovulate the following morning.

25



## Embryo development

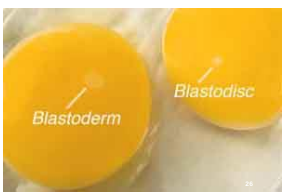
Fertilisation

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




26



## Embryo development

In the oviduct

The blastoderm will spend about 25 hours in the warmth of the hen's body (about 106° F (41$^0$ 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 80$^0$ F (27$^0$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 ).

27



28



## Amniotes

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

Amniote embryos possess 4 extra-embryonic membranes.

AMNION
CHORION
ALLANTOIS
YOLK SAC

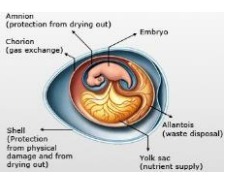
These extra-embryonic membranes allow birds and reptiles to lay eggs on land. They shelter and sustain the embryo against the harsher conditions of a terrestrial environment.

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

29



## Embryonic membranes




The extra embryonic membranes ensure the following:

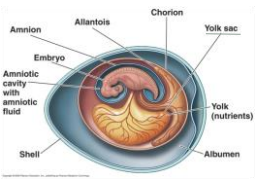
- Prevent desiccation
- Enable gas exchange between the embryo and its environment (respiration)
- Allow waste materials to be disposed of while the embryo develops; preventing waste products from building up to toxic levels within the egg
- Enable the embryo to receive the nutrition it needs to develop

30

## 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

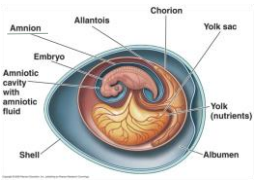
31



## Embryonic membranes

Amnion

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.

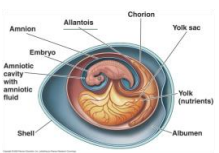
32



## 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.

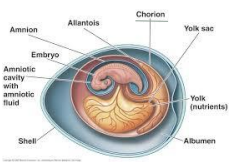
33



## 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.

34



## Incubation

35



## Incubation

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 13-21$^{0}$C and 70-80% RH

They may also be transported under the same conditions

Warming eggs to commence incubation is known as setting.

36

## Incubation

Setting Eggs

If eggs are kept at 13-21$^0$C, they will remain fertile for 14 days, but are best set before 7 days following laying.

Chilled eggs should be gently warmed to 20$^0$C for 4-6 hours before placing in the incubator (setting).

Eggs should always be set with the taglion (pointed end) downwards.

37



## Incubation

Critical factors

Temperature

Relative humidity

Ventilation

Turning

38



## Temperature

Correct temperature is critical

Eggshell temperature correlates to internal temperature

Optimum temperature is 100-101$^0$F (37.8 – 38.3$^0$C)

Use a medical infrared ear thermometer on side of shell (equator)

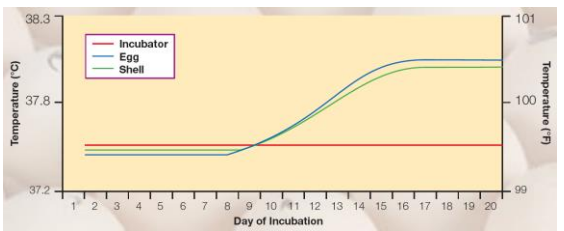
Eggs in middle of trays will be hottest

39



## Temperature

If no temperature adjustment possible




40



## Relative Humidity

Controls weight loss

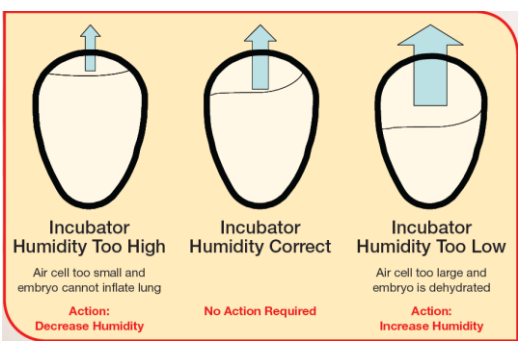
Should routinely monitor the air cell

Lose 11-12% of weight from day 1-18

41



## Relative Humidity




42

| 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 | 8201 | 8364 | 8175 | 8191 | 8242 | 8336 | 8089 | 8263 | 8307 |
| 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 Deads | 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 |

43



## 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

44



## 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.

45



## Days 18 - 21

Three days before hatching, eggs are normally moved into a separate hatcher.

The temperature is lowered to 36-37$^0$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

46



47



## Candling

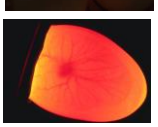
Determines viability

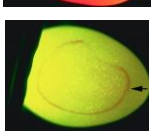
Uses a bright light source behind the egg to show details through the shell.

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.

Infertile eggs may show a "blood ring"





48

49



## Health monitoring and SPF chicken flocks

50



## 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

51



## 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.

52



| 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 orthoreoviruses | 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 |

53



## Biosecurity

### 1) 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

54

## Biosecurity

### 2) Equipment

- Check before use
- Is it clean and sterile?

### 3) Inocula

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

### 4) Transport

- sterile conditions
- filter topped boxes

55



## 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.

56



## 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.

57



## Minor Procedures

58



## 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

59



## 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?

60

## 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

61



## 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.

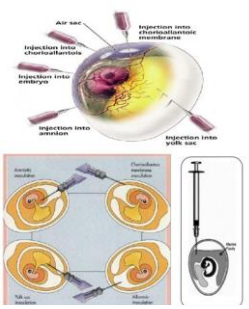
Sterilization: The site of innoculation is sterilised using an iodine swab, or similar.

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

62



## INOCULATION PHASE

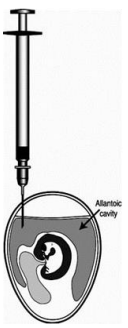



63



## 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




64



65



## 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.

67

LO: 5.2 68

## Welfare and Humane End-Points

- Do chicken embryos feel pain, suffering and distress?
- Many people believe that they do not have the capacity to suffer
- 'The issue of the development stage at which there is sufficient anatomical development to permit sentience, and therefore to warrant inclusion in the controls, requires a better scientific analysis.' (Bioscience coalition)
- EU Directive 2010/63 and the UK Animal Welfare Act 2006 do not apply to embryonated eggs.
- ASPA protects them from 2/3rds incubation, but does not require you to assess the actual suffering experienced by an avian egg.

68

LO: 1.12 69

## 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

69

LO: 1.12 70

## 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

70