Avian Eggs

Introduction

Vertebrate animals are divided into 5 classes; fish, amphibians, reptiles, birds and mammals. All of these reproduce by laying eggs, with the exception of mammals, which bear live young. (Note that there are 2 species of mammals that do lay eggs - the duck billed platypus and the echidna, both native to Australia).

The egg-laying (oviparous) species may be divided according to the degree of development that occurs before the egg is laid by the female.

With **Fish and Amphibia,** the female lays unfertilised eggs, (ova), in water. These are then fertilised by male sperm externally and develop outside the female.

In **Birds and Reptiles,** fertilisation of the ovum occurs internally in the female, and the eggs that are subsequently laid contain the developing embryo. These eggs are laid on land, rather than in water. Between fertilisation and laying, the female secretes tissues around the embryo, which include food for nourishment and a flexible or inflexible outer shell, for protection and resistance to evaporation of moisture.

Bird Eggs

Birds lay a relatively small number of eggs, but each has a large concentrated yolk (macrolecithal) which provides sufficient food for the embryo to go through a full fetal development during incubation. Due to the large size of the yolk, the fetus develops as a plate-like structure on top of the yolk mass and only envelopes it at a later stage. A portion of the yolk mass may still be present as an external or semi-external yolk sac at hatching. The large volume to surface ratio requires structures to aid in the transport of oxygen and carbon dioxide, and for storage of waste products.

Most bird eggs are oval in shape, with one end rounded (the aerus) and the other more pointed (the taglion). However, cliff-nesting birds may have highly conical eggs, while many hole-nesting birds have nearly spherical eggs. The ostrich egg (1.5kg) is the largest bird egg, although some dinosaurs eggs were larger than this. The Bee Hummingbird produces an egg that weighs only half of a gram, whilst the eggs laid by some reptiles and most fish can be even smaller.

Humans have been eating eggs from birds since prehistoric times. Some birds will lay eggs even when they are not fertilised, and the chicken has been developed commercially over centuries to provide regular unfertilised eggs for human consumption. In 2009, an estimated 62.1 million metric tons of chicken eggs were produced worldwide from a total laying flock of approximately 6.4 billion hens. In

the UK we eat 30 million eggs a day – approximately 1 egg every other day per person. In addition to these, the eggs of many other bird species are also eaten.

There are many cultural traditions associated with eggs, and decorated ostrich eggs have been found dating back 60,000 years in Egypt. The decoration of eggs for Easter and other festivals is common, whilst eggs also have a long history of being used as a mild form of protest.

Chick Embryos as a Research Model

The developing chicken in the egg is first described by Aristotle, around 350 BCE. He opened chicken eggs at various time points of incubation and noted how the organism changed over time, recognising significant similarities between human and chicken development, and deducing the role of the placenta and umbilical cord in the human.

Chick embryo research in the 16th century significantly modernised ideas about human physiology. Various scientists used the chick embryo to demonstrate tissue differentiation, disproving the widely held belief of the time that organisms are "pre-formed" in their adult version and only grow larger during development. Distinct tissue areas were recognized that grew and gave rise to specific structures, including the blastoderm. Harvey also closely watched the development of the heart and blood and was the first to note the directional flow of blood between veins and arteries. The relatively large size of the chick embryo as a model organism allowed scientists during this time to make these significant observations without the help of a microscope.

Expanding use of the microscope unveiled the developing chick for close-up examination. By cutting a hole in the eggshell and covering it with another piece of shell, scientists were able to look directly into the egg while it continued to develop without dehydration. Soon studies of the developing chick identified the three embryonic germ layers: ectoderm, mesoderm and endoderm, giving rise to the field of embryology.

Host versus graft response was first described in the chick embryo. James Murphy (1914) found that rat tissues that could not grow in adult chickens survived in the developing chick, because the immune system of the chick is not functional until about day 14 of incubation.

In 1931, Ernest Goodpasture and Alice Woodruff developed a new technique that used chicken eggs to propagate a pox virus. The chick embryo was then used to isolate the mumps virus for vaccine development and subsequently the development of vaccines against influenza, chicken pox, smallpox, yellow fever, typhus and other diseases.

The ability of chick embryonic nerves to infiltrate a mouse tumor suggested to Rita Levi-Montalcini that the tumor must produce a diffusible growth factor (1952). She identified Nerve Growth Factor (NGF) leading to the discovery of a large family of growth factors, which are key regulators during normal development and disease processes including cancer.

The chick embryo is a unique model that overcomes many limitations to studying the biology of cancer in vivo. Because the chick embryo is naturally immuno-deficient, the chorioallantoic membrane, a well-vascularized extra-embryonic tissue located underneath the eggshell, readily supports the engraftment of both normal and tumor tissues. It has a successful history as a biological platform for the molecular analysis of cancer, including viral oncogenesis, carcinogenesis, tumor xenografting, tumor angiogenesis, and cancer metastasis.

The chicken genome was sequenced in 2004 and there are some fundamental similarities with the human genome. However, differences between human and chicken genomes help to identify functional elements: the genes and their regulatory elements, which are most likely to be conserved through time. Publication of the chicken genome enables expansion of transgenic techniques for advancing research within the chick model system.

Reproduction in Chickens

All birds are **oviparous** – they reproduce by laying eggs. Most fish, reptiles and amphibians, as well as insects and arachnids are also oviparous, but the only mammals that lay eggs are the *Monotremes* (the Platypus and Echidnas).

In birds, the fertilisation of the egg takes place internally, unlike many fish and reptiles, where it takes place externally.

Male anatomy

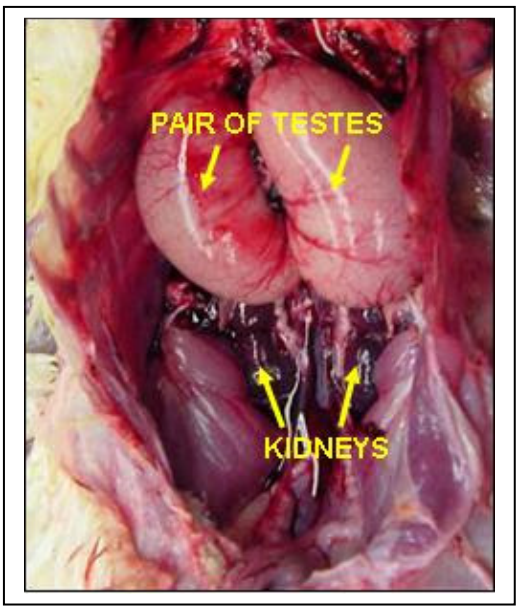
Unlike mammals, the sperm of all birds remains viable at body temperature, and the testes are located in the body cavity.

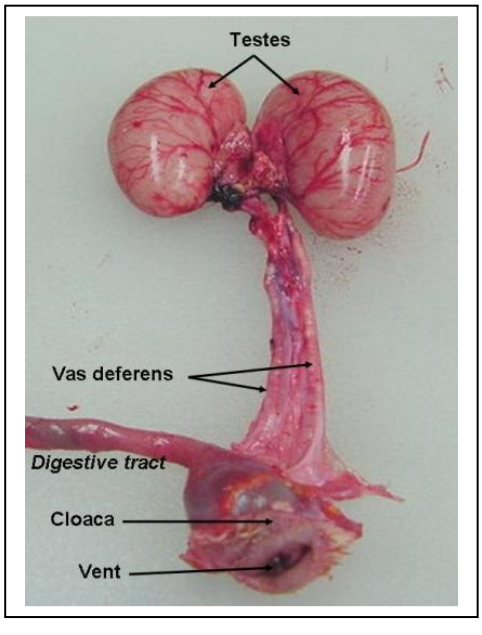
The male chicken has two testes, located close to the chicken's backbone, immediately in front of the kidneys. They are bean shaped and light yellow in colour and can change in size, becoming larger when the bird is in the mating season.

In addition to producing spermatozoa, the testes also produce the male hormones that influence the secondary sex characteristics of roosters; comb growth; the size of the tail feathers; the development of spurs and wattles; male aggression.

From each testis leads a *ductus deferens*, which opens into a small bump, or papilla, on the back wall of the cloaca. The ducts start out narrowly next to the testes and gradually widen as they near the papillae. The papillae serve as the mating organs. (Note that the rudimentary copulatory organ located on the middle and front portion of the cloaca is inaccurately named because the chicken does not use it for copulation, or mating. It is used by breeders to classify the sex of baby chicks.)

The *ductus deferens* is the main sperm storage organ in male chickens. Applying external pressure in this area results in ejaculation, and the collection of sperm in this way for artificial insemination of hens often is referred to as “milking the rooster”.



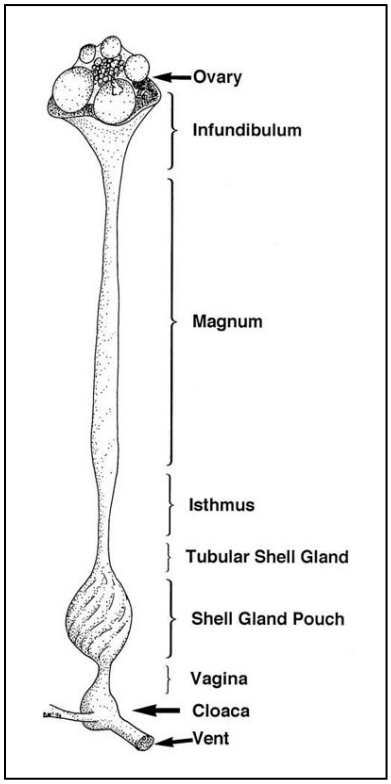


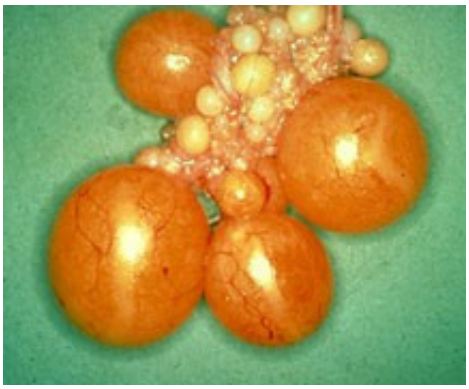
Female:

Female anatomy

The female chicken has only one functioning ovary and oviduct, the left. The right ovary stops developing when the female chick hatches, but the left one continues to mature.

The ovary is fully formed when the chicken hatches and contains several thousand tiny ova, each ovum within its own follicle. As the female reaches maturity, these ova develop a few at a time into yolks.





Ovary

The oviduct is a tube like organ lying between the ovary and the cloaca. In a mature hen it is approximately 25 to 27 inches long, sub-divided into a number of sections, in each of which a specific development of the embryo occurs:

	Length	Time	Function
Infundibulum	2 inches	15 min	Picks up yolk, egg fertilized
Magnum	13 inches	3 hours	40-50% of white laid down-thick albumen
Isthmus	4 inches	1.25 hours	10% albumen shell membrane laid down, shape of egg determined
Uterus (Shell Gland)	4.2 inches	20.75 hours	40% of albumen, shell formed, pigment of cuticle laid down
Vagina/Cloaca	4 inches	-	Egg passes through as it is laid

Mating

When a rooster mates with a hen, he will climb onto her back and place a foot on each of her wings, forcing her tail feathers upward so he can press his cloaca to hers. The rooster ejaculates and transfers the sperm. Afterwards, he will often perform a dance, hopping around and strutting, while the hen un-ruffles her feathers, flaps her wings and walks away.

Fertilisation

Following mating, semen from the male bird enters the hen's cloaca and thence into the oviduct. It swims up the oviduct to the infundibulum, a process that can take several days, where it remains, awaiting the release of a yolk (oocyte) from the ovary.

When a yolk is fully developed, its follicle ruptures, releasing it from the ovary, where it enters the infundibulum of the oviduct. It is here that fertilisation takes place. A single ejaculation of sperm may remain viable in the infundibulum for some time and can fertilise successive oocytes over several days.

On the surface of every egg yolk there can be seen a tiny, whitish spot called the **blastodisc**. This contains a single female cell. If sperm is present when a yolk enters the infundibulum, a single sperm will penetrate the blastodisc, fertilizing it and the blastodisc becomes a **blastoderm**.

A membrane (vitelline membrane) is added to the yolk at this stage.

Development of the embryo inside the chicken

Shortly after fertilization, the blastoderm begins to divide into 2, 4, 8 and more cells and continues until the egg is laid, after which it subsides until the egg begins incubation.

Following fertilization, the blastoderm enters the magnum section of the oviduct, where the dense portion of the albumen (egg white) is added. This process takes about 3 hours. The egg then passes into the isthmus, where the two shell membranes form, over about 75 minutes. The shell membranes loosely contain the yolk and dense egg white until the rest of the albumen is added in the uterus.

The main part of the shell is added by the shell gland in the lower portion of the oviduct, over a period of about 20 hours. The shell is composed mainly of calcium carbonate, but this may be pigmented according to breed.

The chalazae, two cord-like structures which keep the yolk centered in the egg, first appear in the uterus. The chalazae also function as an axis around which the yolk can rotate and keep the germinal disc in hatching eggs uppermost at all times.

In the last portion of the oviduct, (the vagina), a thin coating called "bloom" is applied to the shell as a bacteriostat.

The whole process from fertilisation of the oocyte to egg laying only takes about 24 hours About 30 minutes after the egg is laid, another yolk is released and the process repeats itself.

For every breed of chicken there are guidelines for the optimum weight of the eggs (normally 50-75 grams).

Development of the embryo inside the egg

Once an embryonated egg has been laid, it needs to undergo further development over a number of days before the chick is sufficiently well developed to hatch. Further growth of the embryo inside the egg depends upon suitable conditions in terms of temperature and humidity. This developmental process is known as **incubation.**

However, in domestic chickens, it is not necessary for incubation to commence as soon as the eggs are laid, because embryo development can be suspended for a period, provided the eggs are kept cool. In nature, this will allow the hen to accumulate a clutch of eggs, which can then be incubated together, causing the young to hatch simultaneously.

Storage and Chilling:

In commercial situations, advantage can be taken of this phenomenon to "store" fertile eggs of domestic poultry (and several other species) for up to 14 days. Freshly laid fertilised hens eggs should be "stored" at 12-16°C and 75% RH in "chilling cabinets". Chilled eggs can be transported over long distances before incubation, provided this occurs within 14 days, (preferably 7 days), after which hatchability declines significantly.

Incubation

In most birds, the conditions necessary for successful incubation are provided by body heat from the parent bird. However, some birds, notably the *Megapodes*, use heat generated from rotting vegetable material, effectively creating a giant compost heap, while others may bury the eggs in sand burrows to make use of heat from the sun. In some desert climates, birds may actually need to keep the eggs cool during the heat of the day, and stand over them drooping their wings to provide shade.

In those birds that incubate their eggs by using body heat, the work can be divided differently between the sexes. It is usually the female that incubates the eggs, although in some species the male and the female take turns, whilst in the cassowaries, only the male incubates. The male Mountain Plover incubates the female's first clutch, but if she lays a second, she incubates it herself.

In domestic chickens, the act of sitting on a number (**clutch**) of eggs to incubate them is called **brooding**, whilst the behavioral tendency to sit on a clutch of eggs is called broodiness.

The incubation period, the time from the start of uninterrupted incubation (**setting the eggs**) to the emergence of the young chick (**hatching**), is as shown below for the common poultry species:

Species	Chicken	Turkey	Duck	Goose	Quail	Ostrich	Pheasant
Incubation (days)	21-22	27-28	27-28	25-28	16-21	35-45	24

Note that whilst brooding, the chicken ceases to lay any more eggs, and following the hatch it may be some time before it re-commences laying. For these reasons, most commercial breeds of domestic chicken have had such "broody" behavior selectively bred out of them in order to increase egg production.

Artificial incubation

History

Around 3,000 years ago, the early Egyptians were incubating hens eggs, using large mud-brick buildings with a series of small rooms (ovens) located at each side of a central passageway. In the upper part of these “small incubation rooms”, there were shelves for burning straw, camel manure or charcoal in order to provide radiant heat to the eggs below. Vents were located in the roof of these chambers, which allowed smoke and fumes from the fires to escape and also provided some light. Thousands of eggs were placed on the floor of each incubator room and they were turned twice a day. Temperature control was achieved by controlling the strength of the fire and by regular openings of vents in the roof of the ovens and passageway. Humidity was controlled by spreading moist jute over the eggs when necessary. In this rudimentary incubation system, the temperature, humidity and ventilation were constantly checked and controlled by the hatchery workers, who lived inside the building.

Aristotle, writing about poultry at around 400BC, describes a similar method to Egyptian incubators, but the necessary heat for the eggs was provided by burying them in piles of decomposing manure. Artificial incubation of eggs using a similar method was also practiced in China as early as 246 BC and this eventually spread through South East Asia.

Attempts to introduce the Egyptian methods of artificial incubation to Europe were made in the mid 1600's, but they were not successful, probably due to the adverse winter climate in Europe at that time.

After the failure to introduce Egyptian incubators into Europe, the objective became to develop more sophisticated mechanical machines. In 1750, a French scientist, *de Beaumur*, published a book describing the use of fermentation to heat the incubator, as well as a rudimentary type of thermometer. Over the following 100 years, more experimental incubators were produced, some using hot water, some heated by charcoal and others by steam. However, very few of these machines were successful as they were unable to restrict the range of temperatures to the narrow range which was required.

In the second half of 19th century, Charles Hearson invented the first temperature regulated incubator, and from this point on, several models of small machines were developed and sold.

In the first decades of 20th century, electric “forced-draught” machines revolutionized the production of day-old chicks in the USA. These incubators allowed massive production of chicks with very much less labour, resulting in reduction of production costs and the commercial poultry industry was born.

From 1960 onwards, the progress of the poultry industry was extremely rapid. Huge flocks were reared in a single building and the capacity of incubators was increased to support this growth. Advances in technology led to electronic temperature regulation, automatic humidifiers and eggs turned automatically 24 times a day.

The 21st century has seen the development of systems for the automatic measurement of carbon dioxide, weight loss from eggs and even the temperature of the eggs *in situ.* The data are used to control heating, cooling, humidification and ventilation of the machines.

Artificial Incubation

Setting eggs

To start the incubation process, chilled eggs should be gently warmed to 20°C for 4-6 hours, before placing them in trays in an incubator. There are several types of incubator on the market, but for large numbers of eggs, forced air incubators are usual.

Eggs should always be set with the taglion, (pointed end), downwards and the air cell uppermost. Eggs that have been transported should be allowed to rest for 24 hours prior to setting, to allow the contents of the eggs to settle. The hatch rate of shipped eggs is usually lower than that of fresh eggs.

Four factors are critical for a successful hatch:

1) **Temperature**

The temperature at the centre of the egg should be maintained at 100^0F (37.7^0C), although the acceptable range is 97-102^0F. Mortality is seen if the temperatures falls below 96^0F or rises above 103^0F for a number of hours. As little as a 1^0F increase for a prolonged period will reduce hatch rates and a temperature of 105^0F for 15 minutes will seriously compromise the viability of the embryos. However, a slight reduction in temperature over the incubation period (0.5^0F/week) is acceptable. Low temperatures will retard embryo growth, delaying the hatch.

Note that as the embryos develop, they produce extra heat and just before hatching, this may be considerable. Generally speaking, overheating is worse than cooling, but fluctuations in temperature will also result in reduced hatch rates.

The temperature of the room in which the incubator is housed should be held between 55-65^0F, and be free from drafts and direct sunlight. An incubator should also be operated for several hours to stabilize its internal atmosphere before fertile eggs are set. Do not adjust the heat upward during the first 48 hours after eggs are set, as this may overheat the eggs. The eggs take time to warm to incubator temperature and in small incubators the temperature may drop below 98°F for the first 6-8 hours, or until the egg warm to 99°-100°F.

2) **Relative Humidity**

The relative humidity of an incubator maintained at 100^0F should be 60% (range 55-65%). If the humidity is excessive in the early stages, then the chick may be drowned in excess moisture. If the humidity is too low, then the the egg will lose water, the membranes will dry and the chick will fail to grow, be late hatching and may fail to break out of its shell.

Humidity values are less critical in the early stages of incubation than in the later stages. The degree of evaporation can be assessed by candling, (to view the size of the air sac), or by measuring weight loss. As incubation proceeds, the egg will normally become lighter, and the air sac will shrink, owing to evaporation.

Towards the end of incubation, the humidity must be raised, which will help the chick to hatch.

3) Ventilation

Air circulating in the incubator will bring oxygen to the egg and remove carbon dioxide. This must be done without causing draughts and removing too much moisture. From day 12 the chick embryo relies on oxygen in the atmosphere and is susceptible to build-up of carbon dioxide.

The concentration of oxygen should be above 20% and CO_2 should be below 0.5%. Air movement past the egg should be 12 cubic feet/ minute. Airflow rates and CO_2 levels should be monitored.
Older eggs will require increased ventilation.

4) Turning

The broody hen will turn her eggs twice an hour. If the eggs aren't turned, the embryo may stick to the shell and can hatch with physical defects.

For best results, artificially incubated eggs should be placed with the pointed end down and turned at least three times per day, until day 18 (three days before hatching). Most modern machines will turn once/hour.

Days 18-21

Three days before hatching (day 18-19), eggs are moved to a "hatcher" where the temperature is lowered to 36.1-37.2°C and the relative humidity increased to 75%. This keeps the membrane around the hatching chick from drying out once the chick cracks the shell. They are not turned in the hatcher.

Many commercial incubators are industrial-sized with shelves holding thousands of eggs at a time.

Fertility and Candling

Normal fertility may vary from 55% - 95%, depending on the season, condition and type of bird. Throughout incubation, the fertility of the eggs can be checked by candling. This involves shining a bright light through the egg shell, to display the presence or absence of the embryo and the network of blood vessels surrounding it. Candling is normally carried out at about day 7. If there is no sign of development by day 10, then "clear" eggs should be discarded.

Candling a chicken egg

The Air Cell

Soon after an egg is laid, a small air bubble forms in the large end under the shell, between the inner and outer shell membranes. This air bubble relieves stress and pressure on the embryo resulting from changes in temperature and will expand as fluid is lost from the embryo. The lower the relative humidity of the incubator, the more fluid is depleted and the larger the air cell grows.

By the time of hatching, the air cell should have enlarged to the point where the chick can reach its beak through the membrane wall, allowing it to breathe, before it pips through the shell, after which it will "zip" around the shell. If humidity has been excessive, the chick may pip internally into the air cell and drown in excess fluid. On the other hand, if humidity has been too low, the air cell will be oversized and the chick may be "shrink wrapped" in the inner membrane and unable to hatch.

The Egg Membranes

During the incubation process, the 4 membranes of the chicken egg play a vital role in maintaining life:

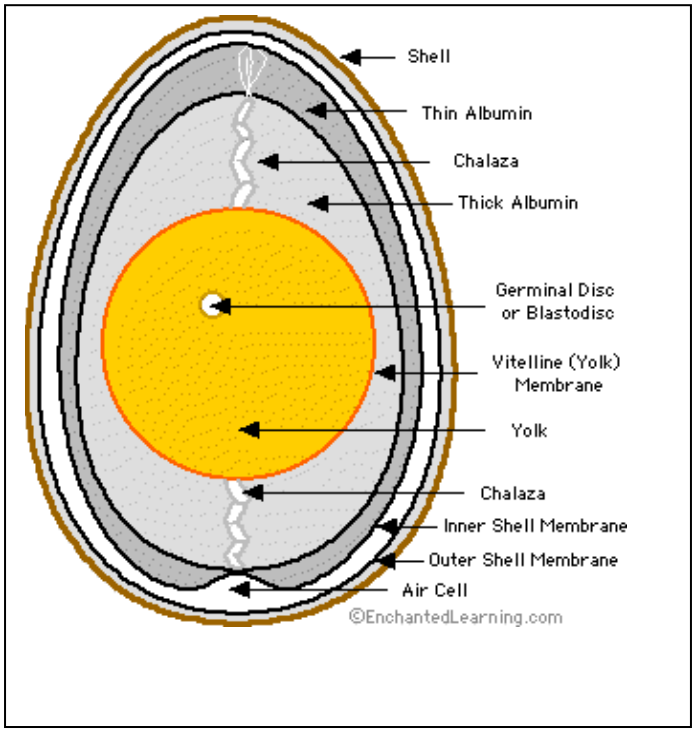
Yolk sac: Envelopes the yolk, which acts as a food source during incubation. Any remaining yolk at hatching is drawn into the abdomen of the chick and used as food for the first 2-3 days after hatching.

Amnion: a protective fluid filled sac that surrounds the embryo for protection.

Allantois: develops a circulatory system that connects to the embryo and serves 3 functions:

a. Respiratory
b. Excretory
c. Digestive

Chorion: This membrane fuses the inner shell membrane to the allantois.



Influenza virus growth in eggs

Before the development of cell culture, many viruses were propagated in embryonated chicken eggs. Today this method is most commonly used for growth of influenza virus. The excellent yield of virus from chicken eggs has led to their widespread use in research laboratories and for vaccine production. The majority of influenza vaccines – both inactivated and infectious – are produced in chicken eggs.

On average it takes one or two eggs to produce a single dose of annual flu vaccine. To produce a reassorted vaccine strain in the face of an epidemic, eggs may be inoculated with a mixture of the epidemic influenza virus strain (red) and a standard strain (green). Both strains replicate themselves, but as they do so their genetic material becomes mixed, producing hybrids. The reassortants are analyzed, and those with the epidemic strain surface proteins but other genes of the standard strain will be selected. These are injected into different eggs to replicate before harvesting.

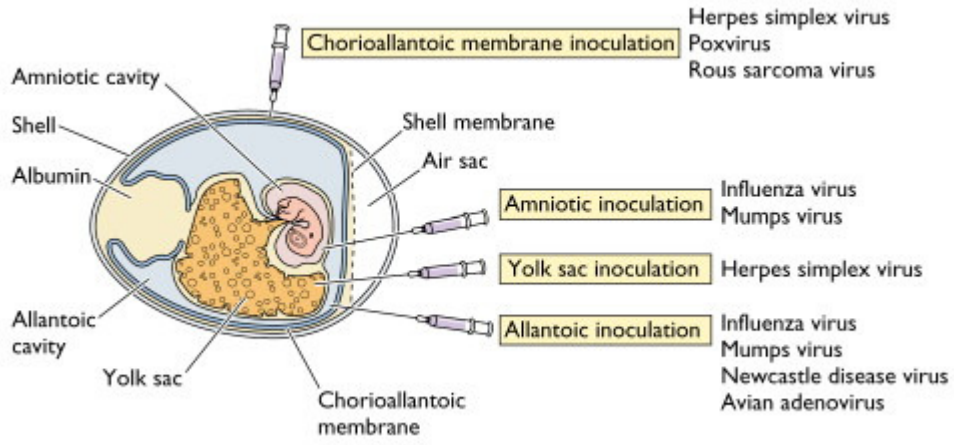
Since influenza virus vaccines are cultivated in eggs, people with egg allergies cannot be given influenza vaccines.

Virus inoculation in embryonated eggs

Viruses are obligate intracellular parasites. They depend totally on host cells for their existence. For this reason, they have to be cultured in a cellular environment.

Viruses may be cultured in embryonated eggs. They may be cultured by inoculation into the chorioallantoic membrane (CAM), allantoic cavity, amniotic cavity, or yolk sac, depending on the virus. They may also be grown in cell lines, tissue primary cultures, or by inoculation into suckling mice.

Routes of Injection



Different viruses can be injected into an egg at different sites, and the egg can be easily observed for viral replication throughout the development of the chicken embryo.

Virus inoculation into the chorioallantoic membrane (CAM) is used for e.g. Variola or Vaccinia viruses. As the viruses replicate they produce visible lesions called pocks. Each infectious virus particle forms one pock. The CAM can also be used for culture of some fungi.

Inoculation into the allantoic cavity provides a rich yield of influenza and some paramyxoviruses. Allantoic inoculation is employed for growing the influenza virus for vaccine production. Other allantoic vaccines include Yellow fever (17D strain), and rabies vaccines. Duck eggs are bigger and

have a longer incubation period then hen's egg. They therefore provide a better yield of rabies virus and have been used for the preparation of the inactivated non-neural rabies vaccines.

The amniotic cavity is the site used for primary isolation of influenza A virus and the mumps virus.

The yolk sac is the preferred site for culture of Chlamydia, Rickettsia & some viruses.

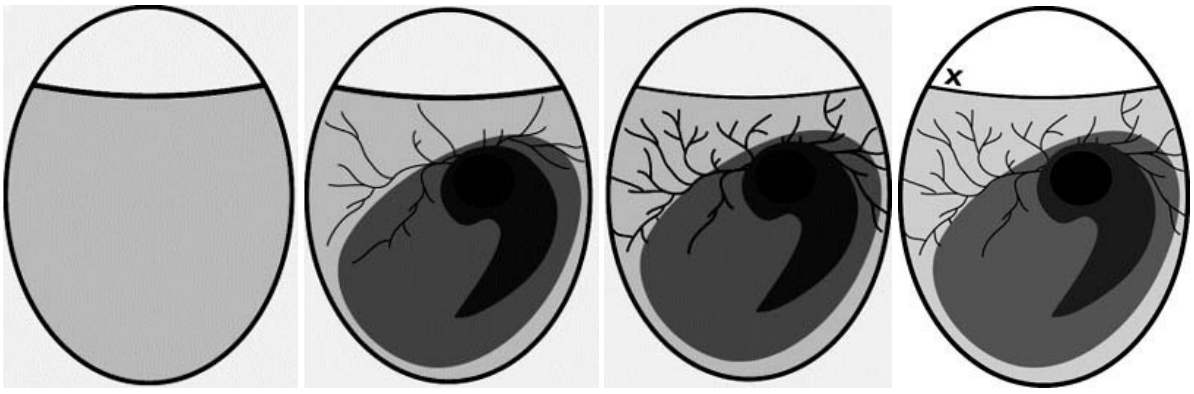
Eggs are used for mass vaccine production in influenza. Fertile chicken eggs provide a convenient, space-saving incubator. Eggs are sterile, low cost, easy to maintain, and readily available. As well as being free from bacteria and many latent viruses, they are also free from exposure to specific and non-specific elements of the immune system.

NB All procedures involving the manipulation of infectious materials should be conducted within biological safety cabinets, specially designed hoods, or other physical containment devices, or by personnel wearing appropriate personal protective clothing and equipment according to the relevant ACDP containment level.

Eggs used for cultivation must be sterile and the shell should be intact and healthy. Eggs should be candled prior to inoculation to ensure they are viable, then the inoculated eggs candled daily to check the embryos inside. See figure 1. For propagation of influenza virus, pathogen-free eggs are used 7-12 days after fertilization.

Figure 1. Determination of the viability of the embryo


Candling



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Under the candling lamp, the embryo appears as a dark shadow with the head as a dark spot. Healthy embryos respond to the light by moving. Sometimes this movement is very sluggish and it can take 30 to 40 seconds for the embryo to move. This indicates the embryo is not healthy and the egg should be discarded.

Look carefully at the blood vessels. They are well defined in a healthy embryo. After an embryo has died, the blood vessels start to break down. They then appear as streaks under the shell when viewed under the candling lamp. Candling will also reveal cracks in the eggshells. Eggs with cracked shells should be discarded.

1. **Infertile eggs:** These are easy to detect, as the egg is clear. Discard

2. **Early deaths:** The embryo has developed for several days and then died. Candling will reveal a small dark area and disrupted blood vessels. Often deteriorating blood vessels will appear as a dark ring around the egg. If the embryo is small and does not move, and the blood vessels have broken down, discard these eggs.

3. **Late Deaths:** These are often difficult to tell apart from a viable embryo at the same stage of development. Look for the absence of movement and the breakdown of the blood vessels. Discard these also.

4. **Viable Embryos:** These have strong healthy well defined blood vessels and the embryo moves in response to the light. Mark the air sac and the inoculation site and then return the eggs to the incubator ready for inoculation.

Materials needed:

- Eggs: sterile 7-12 day embryonated eggs (usually 9-10 days).
- Egg rack.
- Egg shell punch. A dental drill can be used if it is available. Otherwise an egg shell punch can be improvised from e.g. a needle (20 gauge) and a rubber stopper. Insert the needle into the rubber stopper so that only 1 mm of the tip is showing (see figure 1).
- Cotton wool.
- A 70 % alcohol solution in water.
- Syringe 1 mL.
- Needles (25-27 gauge, 16 mm).
- Sellotape or melted wax to seal the inoculation site.
- Inoculum. This must be free of microbial contamination.
- Discard tray.

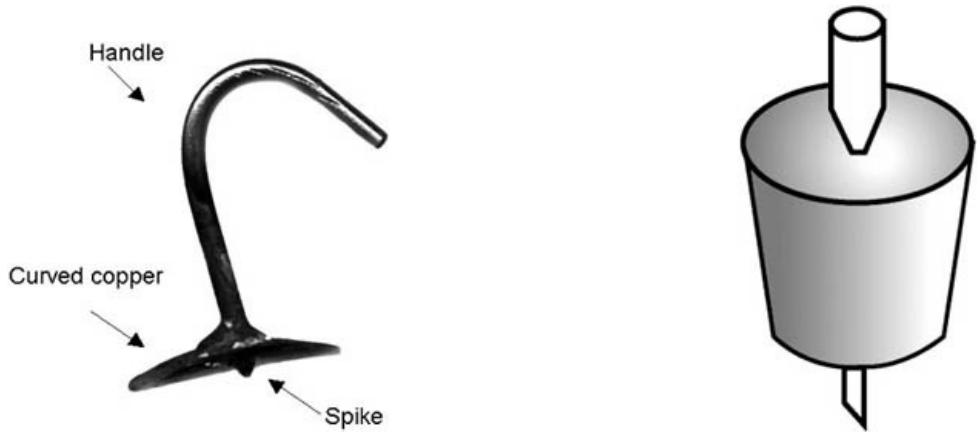


Figure 2. **a) Egg shell punch** **b) Improvised egg shell punch**

***Procedure*:**

1. Determine the location of the air sac

Candle the eggs and identify the air sac. This will usually be at the larger end. In some eggs the air sac will have not developed on the blunt end but half way down the egg. These eggs are not suitable for vaccine production.

2. Mark the inoculation site

Hold the blunt end of the egg against the aperture of the candling lamp and note the position of the head of the embryo. Turn the egg a quarter turn away from the head. Draw a line on the shell marking the edge of the air sac, and draw an X approximately 2mm above this line. This X marks the inoculation site.

Alternatively a non-veined area of the allantoic cavity just below the air sac is located and marked with a pencil. A hole will be made in the shell at this position for the inoculation. Another hole should be drilled at the top of the egg, otherwise when virus is injected, the pressure in the air sac will simply force out the inoculum.

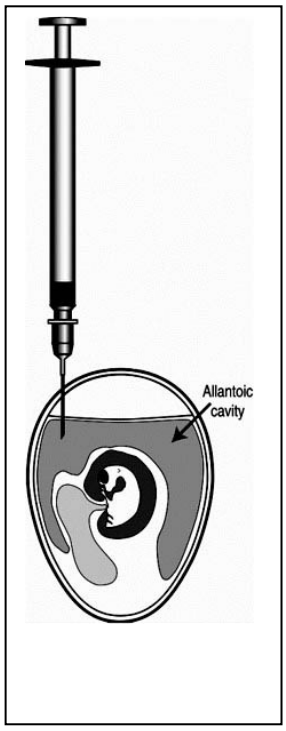
3. Inoculation procedure

Place the egg with the air sac uppermost in the egg rack. Use cotton wool and 70 percent alcohol to swab the end of the eggs to be inoculated. Allow the alcohol to evaporate. Use a sterilised eggshell punch or needle, and/or swab it with alcohol solution. Pierce a hole in the end of the egg at the marked inoculation site.

Draw the inoculum into a 1 mL syringe. Keeping the needle and syringe vertical, place the needle through the hole in the eggshell. The needle passes through the hole in the shell, through the chorioallantoic membrane, and the virus is placed in the allantoic cavity. The needle will need to penetrate approximately 16 mm into the egg to reach the allantoic cavity. Inject 0.1 mL of inoculum into the egg (see figure 3).

Withdraw the needle from the egg and seal the hole in the shell with tape or melted wax.

Place the inoculated eggs into a second incubator at 37 degrees C for 48 hours.



4. Harvesting the virus

During incubation, the virus replicates in the cells that make up the chorioallantoic membrane. As new virus particles are produced, they are released into the allantoic fluid. To harvest the virus, the top of the egg shell covering the air sac is removed. The shell membrane and chorioallantoic membrane are pierced with a pipette, and the allantoic fluid withdrawn – usually about 10 ml per egg. Sufficient virus may be produced in one or two eggs (depending on the viral strain) to produce one 15 microgram dose of vaccine.

Windowing eggs

For some studies, it is necessary to open the egg shell to allow access to the embryo for manipulation and then to close it again afterwards, without minimal disturbance to the growth and viability of the developing embryo. If a flap of shell is created to cover the window, then it may also be possible to open this periodically throughout incubation to observe or further manipulate the developing embryo. This can be done from as early as day 2 of incubation, although windowing at the very early stages of incubation will substantially reduce hatchability. At day 1, there may be less than 5% survival, although this figure will rise to over 50% at day 8-10.

There are several techniques for creating a window in the shell, but the same principles apply to all…..

1) **Candling**
   It is essential to candle the eggs before use, as this will reveal infertile eggs and any damaged shells (cracks etc). Discard these.

2) **Sterility**
   An SPF chick embryo is essentially sterile until it hatches, and it is vital that infection is not introduced during the procedure. Wipe the egg shell with 70% alcohol before commencing and always use sterile instruments.

3) **Removing albumen**
   It is necessary to remove 3-4ml of albumen from the egg before a window is cut. This will allow the chorio-allantioc membrane to fall away from the shell and avoid damage when cutting the shell. Cover the blunt end of the egg (aerus), with a small piece of scotch tape and make a small hole through this and the egg shell, into the air sac. Use an egg shell punch, or the point of a sharp pair of scissors, or a pointed scalpel blade. The hole needs only to be small – enough to introduce a 19 gauge 1½ inch needle through. Holding the egg horizontally, so the embryo is at the top, angle the needle downwards (away from the embryo) at 45^0 to the horizontal, and remove 3-4 ml of albumen. Re-seal the hole by covering with tape and discard the albumen.

4) **Creating the window**
   Lay the egg on its side such that the embryo will be at the top and apply a larger piece of scotch tape to the side of the egg, over the animal pole. Taking a sharp pair of scissors, or a dental cutter, remove a window of shell approximately 1cm square from the equator of the egg, but leaving a “hinge, so that the shell is not completely detatched. Should the shell crack during this procedure, then the egg should be discarded.

5) **Repairing the window**
   Once the procedure has been carried out, the window should be replaced and re-sealed using more scotch tape. There will be room air trapped under this, which is believed to be one of the factors that reduce viability of embryos using this technique.

Humane methods of killing avian eggs

Schedule 1 methods

TABLE B - Embryonic forms

1) Overdose of anaesthetic, using a route and anaesthetic agent appropriate for the size and stage of development of the bird.
2) 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.
3) Decapitation (birds up to 50 grams)

FURTHER READING

1) Poultry Health and Management: David Sainsbury ISBN: 0-632-05172-8

2) Poultry Diseases: Pattison, McMullin, Bradbury, Alexander ISBN: 978-0-7020-2862-5

3) The Management and Welfare of Farm Animals: J Webster ISBN 978-1-4051-8174-7

4) Animal Health – Health, Disease and Welfare of Farm Livestock: D. Sainsbury

Blackwell ISBN0-632-03888-8

5) Laboratory Animal Anaesthesia: Flecknell P, Elsevier Academic Press ISBN 0-12-260361-3

6) Handbook of Lab Animal Management and Welfare, 4th Edition: Wolfensohn and Lloyd

Wiley Blackwell ISBN 978-4706-5549-8

Useful web sites

- www.thepoultrysite.com General information on poultry diseases and management
- www.defra.gov.uk/ - Information on import/export, and notifiable diseases
- http://www.wikihow.com/Candle-an-Egg Information on how to candle eggs including a video clip