Science Education Collection

Chick *ex ovo* Culture

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Abstract

One strength of the chicken (*Gallus gallus domesticus*) as a model organism for developmental biology is that the embryo develops outside the female and is easily accessible for experimental manipulation. Many techniques allow scientists to examine chicken embryos inside the eggshell (*in ovo*), but embryonic access can be limited at later stages of development. Fortunately, chicks can also be cultured *ex ovo*, or outside of the eggshell. The major advantage to *ex ovo* culture is greater access to tissues that might otherwise be obstructed by the shell or the orientation of the chick within the egg, especially for embryos in later stages of development.

There are two principle strategies to *ex ovo* culture: whole yolk culture and explant culture. During whole yolk culture, the eggshell is cracked and the contents are transferred to a simple housing vessel. However, in explant culture methods, the embryo is excised from the yolk and mounted in the housing vessel to maintain membrane tension, which is important for normal development.

Basic protocols for whole-yolk and explant techniques will be provided in this video, along with a discussion of the pros and cons of culturing chicks outside of the shell. Finally, experimental applications of *ex ovo* culture will be discussed, demonstrating how this approach is used to improve access to the embryo for microscopy and genetic manipulation of late stage embryos.

Transcript

The chick is a versatile model organism for the study of developmental pathways because most of its development takes place outside of the mother. Nonetheless, the eggshell prevents access to the embryo for some forms of experimentation. Fortunately, chicks can be incubated outside of the shell, or "*ex ovo*," using some commonly available lab supplies. In this video, you will learn about the principles of *ex ovo* culture, step-by-step procedures for two different culture methods, and applications of the technique in developmental studies.

Before talking about how to raise chicks *ex ovo*, let's go over some principles of the technique. Within the egg, the chick develops in close association with the vitelline membrane surrounding the yolk. The remaining volume is filled with albumin, or egg white, which protects the embryo and serves as a source of protein.

Culture outside of the shell can be performed via whole yolk culture, using all of the egg contents in a simple container. Alternatively, embryonic tissues can be excised and grown in *ex ovo* explant culture. Both of these techniques have distinct advantages over the use of windowed eggs.

First, windowing later stage chicken embryos is complicated due the presence of many crucial blood vessels, which develop within the membranes that surround the chick. This makes the *ex ovo* method preferable for working with chicks at later stages.

Second, because they can fit within imaging rigs, *ex ovo* culture setups are more amenable to high-resolution imaging.

Furthermore, removing embryos from the shell exposes a greater number of tissues for experimental manipulations like microsurgery and microinjection.

Since the chick relies upon the eggshell for protection and essential minerals, embryo survival without this structure requires some extra care. For instance, housing must be designed to maintain a sterile, humid environment, and nutrients must be provided in the form of albumin or culture medium. For long-term culture, crushed shell is also required as a calcium source. Furthermore, since tension on the membranes supporting the embryo is important for normal development, successful *ex ovo* culture additionally depends upon housing that maintains normal membrane morphology.

Now that you know the basics, it's time to chicken out! To prepare eggs for whole yolk *ex ovo* culture, begin by incubating them at 37.5 °C until just before the desired stage.

While you're waiting, prepare the housing. Petri dishes, weigh boats, and hammocks are commonly used to hold the egg contents. First, sterilize all components by treatment with UV light or ethanol.

Additionally, prepare an outer chamber filled with sterile water to maintain humidity during incubation.

When the eggs are ready, set them in a horizontal position for a few minutes so the embryo rises to the top. Then crack the bottom of the shell and carefully transfer the egg contents into the housing. Finally, cover the housing vessel to maintain humidity, and place the setup into an incubator until the desired age is reached for downstream processing.

An alternative *ex ovo* strategy, explant culture, requires a few additional steps after removing the embryo from the shell.

In this technique, the vitelline membrane is gently separated from the yolk, carrying the developing embryo with it.

Then, the membrane is mounted in order to maintain tension, which can be done by pulling the tissue tight around a glass ring or by simple adherence to filter paper.

After mounting, culture medium is added to cover the tissue, and the embryo is placed in a humidified chamber.

The explanted embryos can now be returned to an incubator for up to 24 hours of further development.

After learning the principles and methods of *ex ovo* culture, we're ready for some fowl play.

ex ovo culture is particularly useful when you need to alter gene expression in older embryos. One approach to this is electroporation, in which an electric current is used to permeabilize cell membranes for the delivery of genetic material. Since the *ex ovo* cultured embryos are also highly accessible for imaging, the uptake of genetic material can easily be validated through fluorescent imaging of whole embryos.

Real-time imaging of cell dynamics is also possible using *ex ovo* culture. Human cancer cells can form tumors by co-opting blood vessels of the chick chorioallantoic membrane, or CAM. After tumor formation, fluorescent particles can be injected directly into to the bloodstream and tracked in real time. Accumulation of particles in tumor tissue can be used as an indicator of angiogenesis, or the formation of new blood vessels.

Although whole embryos can be cultured using explant techniques, some experiments require that embryonic tissues be dissected prior to culture. For example, developing neural tissue can be excised and grown on glass cover slips. After a period of incubation, a specific population of cells known as the neural crest can be observed migrating away from the tissue. Treatment with experimental agents and timelapse imaging can then be used to test factors controlling cell migration.

You've just watched JoVE's guide to chick *ex ovo* culture. This video covered the principles of *ex ovo* culture, the basics of whole-yolk and explant methods, and some of the ways these techniques are used in labs today. Thanks for watching!