INQUIRY & INVESTIGATION

Ex Ovo Model for Directly Visualizing Chick Embryo Development

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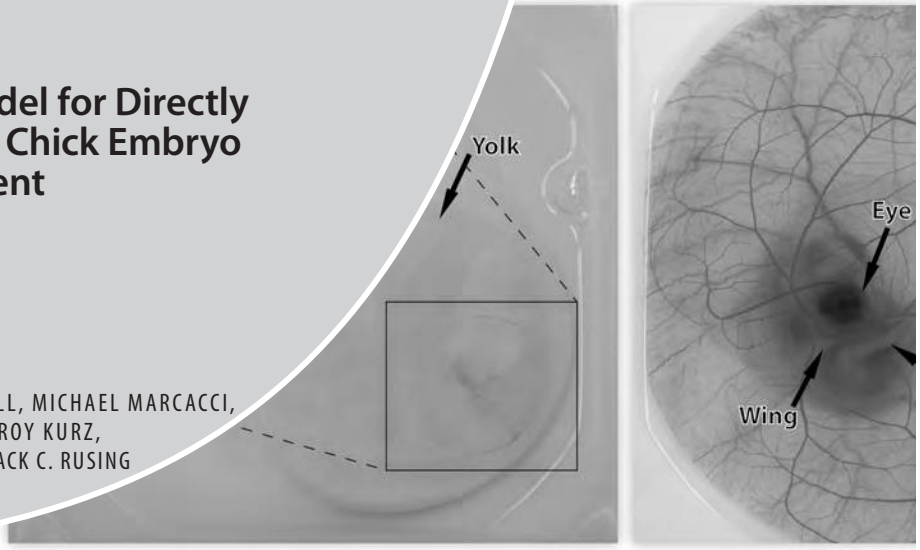

Abstract

We describe a technique for removing and growing chick embryos in culture that utilizes relatively inexpensive materials and requires little space. It can be readily performed in class by university, high school, or junior high students, and teachers of any grade level should be able to set it up for their students. Students will be able to directly observe the chick's development from 3 days post-fertilization to the point at which it would normally hatch. Observing embryonic development first hand, including the chick embryos' natural movements, gives students a full appreciation for the complexity and wonder of development. Students can make detailed observations and drawings, and gain understanding of important principles in developmental biology. Finally, we suggest various ways in which this project can be adapted to allow students in advanced classes to design and implement their own projects for investigating teratogenic effects on development using the ex ovo model of chick development.

Key Words: *Chick; embryonic development; scientific observations; teratogens.*

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To have a successful program in biology, students first need to experience excitement and the sense of amazement that inspires a love of biology. One biological phenomenon that can generate this sense of excitement in students, instructors, and scientists, both young and old, is embryonic development. The complex events that occur to create a higher-order organism from a single cell are simply remarkable. However, the wonder of embryology is largely concealed by the inaccessibility of the embryo in higher-order organisms. Here, we describe a method that permits visualization of the real-time events that occur during embryonic development using embryonated chick eggs.

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This project allows visualization of chick embryos as they develop from a tiny, almost indiscernible, fetus sitting atop the yolk. Within a day, the chorioallantoic membrane (CAM) vessels become visible, forming in a circle around the central embryo. At the same time, the tiny, beating, two-chambered heart becomes visible, forming the rudimentary cardiovascular system (Figure 1A; Video 1). As the chick continues to develop, it kicks, moves its head, and "swims" within the amniotic fluid (Figure 1B; Video 2), very similar to the known movements of human embryos developing in utero. Growth continues for about 19 days post-fertilization (the normal gestational period is 21 days) to a developed chick with down feathers (Figure 1C; Video 3). This project allows students to experience the wonder of embryonic development as their chick grows and transforms before their eyes. A time-lapse video of development using this ex ovo model is available (Video 4).

○ Learning Implications

Visualizing the small two-chambered heart beating as the live chick embryo is removed from the egg really brings to light the living organism that is developing inside the egg. Students can easily conceptualize the young chick emerging from the hatching egg at 3 weeks post-fertilization, but the concept of a developing, living embryo inside the eggshell is much more abstract and, therefore, harder to grasp, especially for younger students. Even when they understand what is happening inside the egg, direct observation is powerful for students in the elementary through undergraduate levels. In addition, a sense of accomplishment and autonomy is generated for students who are able to prepare their own ex ovo chick embryo to observe (generally junior high or older, although I have also helped elementary students perform this method). Various inquiry-based experiments can be designed for advanced students (see below for suggestions).

The *National Science Education Standards* (NSES) identify eight categories of content standards (National Research Council, 1996). This project addresses portions of three of these main categories: unifying concepts and

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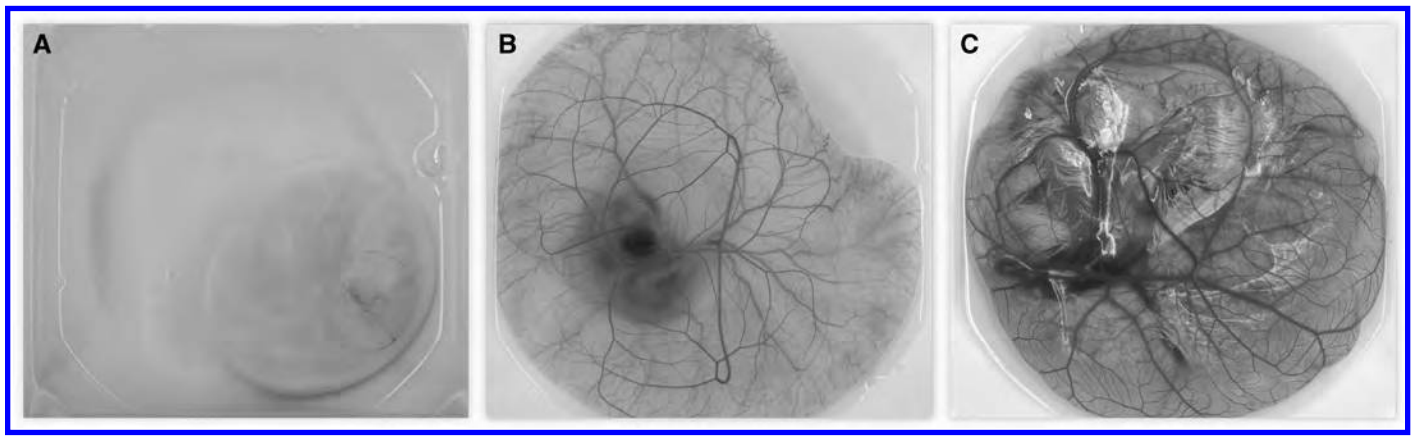


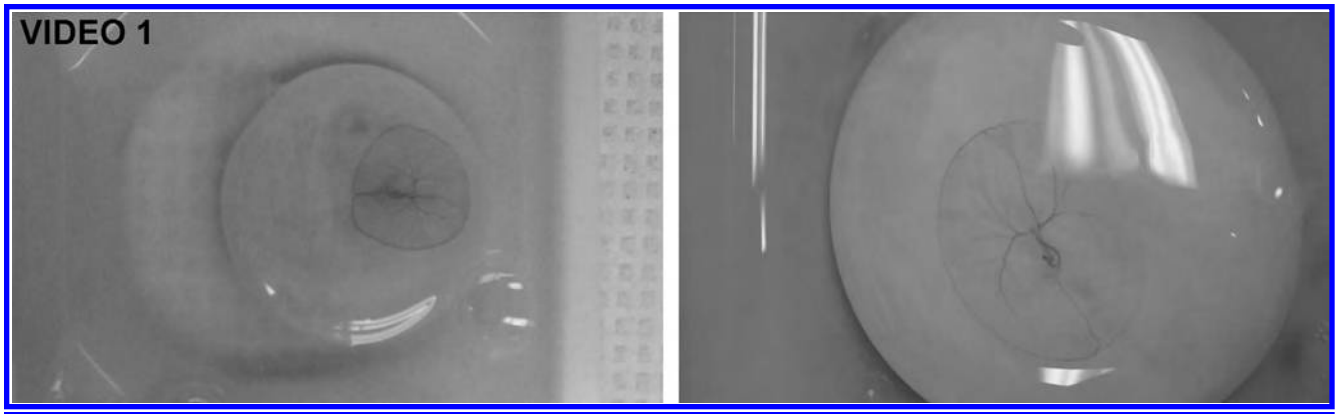
Figure 1. Image of the chick embryo at day 3 (A), immediately after removal from the shell. Dramatic changes are apparent over the course of the next week, and the chick's beak, limbs, eyes, and other prominent features become readily apparent by day 10 (B) and day 17 (C). The chorioallantoic membrane vessels, which normally grow just inside the eggshell, now extend throughout the top membrane of the ex ovo embryo. With a small dissection scope, individual erythrocytes can be observed moving through the capillaries and small vessels. By day 17 (C), the down feathers are visible. Movements become more subtle as the embryo begins to fill its space (just as it would in the shell), but kicking and other movements remain readily apparent. The real-time videos, in which the heartbeat and embryo movements were recorded, are available as Videos 1–3.

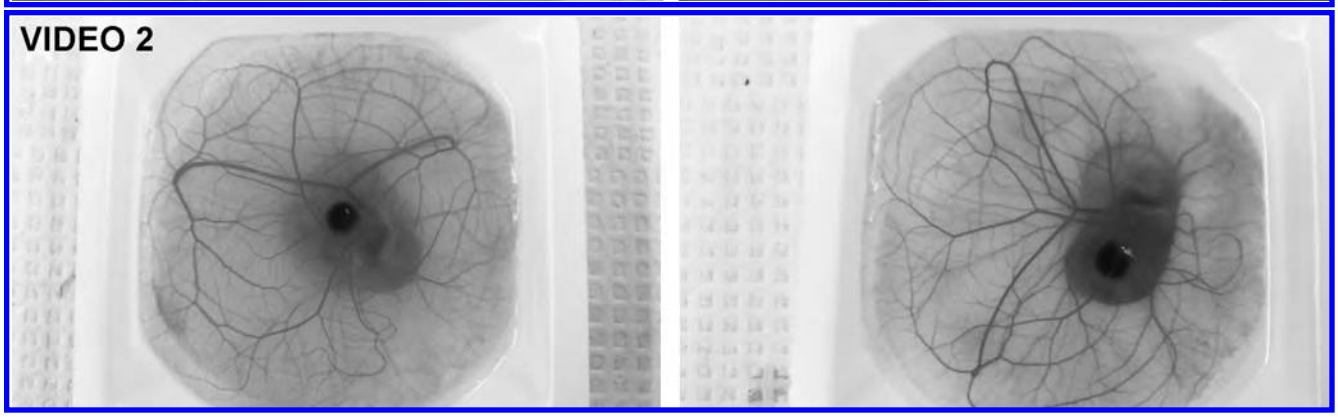
processes in science, science as inquiry, and life sciences. The "unifying concepts and processes" theme calls for projects that enable students to observe systems, order, and organization as the embryo develops, along with form and function (National Research Council, 1996). The Victorian poet and scholar Samuel Taylor Coleridge once said that "The history of man for the nine months preceding his birth would probably be more interesting, and contain events of a far greater moment, than the three score and ten years following it" (Gilbert et al., 2005). Students will learn, by direct observation, a central concept of developmental biology: that the organism must function as it builds itself. The development of an organism can be compared to building a complex machine such as a car. Although both are complex, with specialized parts that serve specific functions, the car does not have to function until it is completely assembled. By contrast, the embryo must respire before it has functioning lungs, it must digest before it has a stomach, build bones when it is only soft tissue, and form an array of neurons before it can think (Gilbert et al., 2005). Making detailed observations of the embryonic development of a complex organism helps students grasp these concepts. They will observe the cardiovascular system as the first to develop. The small beating heart with connected vessels from the yolk and the CAM (for gas exchange) is obvious very early in development, from the time the embryo contents are released from their shell. Students should relate this to the fact that the developing cells and tissues are living and functioning, thus requiring oxygen and nutrients distributed by the vessels connecting the embryo to the yolk and the CAM (Figure 1).

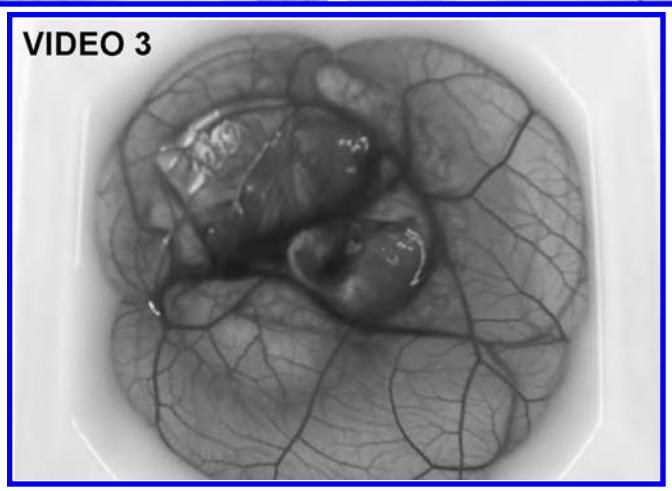
This project also facilitates student learning within the "evidence, models, and explanation" category of the NSES guidelines. Students learn "how we know" what we know. Much of our understanding of developmental biology was generated by detailed observations and drawings. As students are guided to make their own detailed observations and drawings at various days during chick embryonic development (Figure 2), the project can be related to historically important biological observations made by Aristotle, William Harvey, Marcello Malpighi, and others (Gilbert, 2010).

○ Suggestions for Advanced, Inquiry-Based Studies

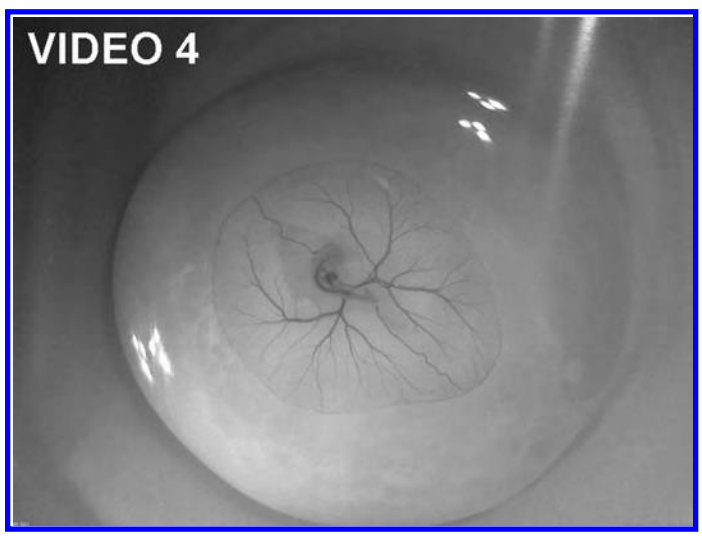
In addition to the experience of generating their own ex ovo embryo transplants and making detailed observations and drawings of development, students also gain specific knowledge and skills regarding sterilization procedures and techniques, and the order and timeline of events during embryonic development. Furthermore, accessibility to the developing chick embryo opens the door to several inquiry-based experiments. Undergraduates or advanced high school students can design and test the effects of various teratogens on normal chick development. Most students have heard of things that pregnant women should avoid. Students can research why these "avoided substances" are teratogenic and what threshold concentrations are thought to become problematic. Students learn the importance of controls and struggle with the concept of identifying physiologically relevant concentrations; most substances can be teratogenic at high enough levels. Although it is an inexact calculation, students in my undergraduate developmental biology course generally realize that mammalian embryos are maximally exposed to the levels of substances that would be found in the mother's bloodstream. Determining the blood concentration levels that result from various doses of teratogenic substances, such as 800 mg aspirin or a bottle of wine, is a rewarding and informative challenge for students. Examples of successful experiments include the effects of second-hand smoke, alcohol, or excessive aspirin use, along with many others. Importantly, constraints may be needed regarding which teratogenic reagents can be tested, and how these are applied, due to availability and safety. However, many embryonic teratogens are harmless to students and adults, and are readily available at standard grocery and drug stores. Identifying







Videos 1–3. Real-time videos, in which the heartbeat and embryo movements were recorded, are available. See *ABT* online for video clips.



Video 4. Time-lapse video of ex ovo chick embryonic development. Images are taken every minute over the course of 14 days of growth. The video is "jumpy" because of the constant movement of the chick as it grows and develops. It is interesting to note that this particular embryo developed micro-ophthalmia in the right eye; as the chick develops, the right eye stops growing and would normally be quite large, consistent with avian embryos and the left eye (underneath). See *ABT* online for video clip.

interesting teratogens to test, and determining creative methods for their application and dosing, can be a great learning experience for high school and college students.

Students can also learn about the common application of this model system to the study of tumor vascularization, where tumor

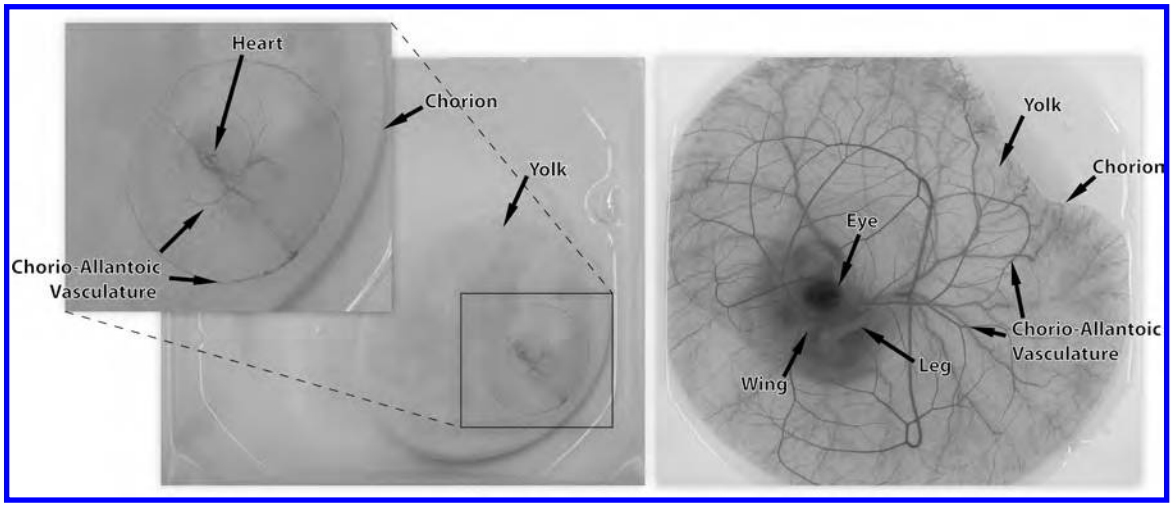


Figure 2. An example of labeled pictures from students, included in their project lab reports. Some students made detailed drawings, whereas other took pictures with their phones or digital cameras.

onplants are added to the CAM vessels. The tumors, which require a blood supply, just like all other living cells, stimulate vascular growth from the CAM vessels into the tumor. In this manner, tumor growth, tumor vascularization, tumor metastasis, and anti-angiogenic (blocking blood vessel growth) treatments are studied. See a good review article by Deryugina and Quigley for more information on the use of this model for pre-clinical tumor studies (Deryugina & Quigley, 2008).

Materials

- Fertilized eggs. We obtain ours from a local farm (McIntyre Poultry and Eggs, 10542 Vista Camino, Lakeside, CA 92040-1604; ~$2 per fertilized egg). They ship; if you are in the area, you can pick up the eggs to save on delivery costs. You may be able to find a local poultry farm near you. There are also several online sources that sell fertilized eggs for educational purposes.
  - **Note:** When you purchase the fertilized eggs, they are at day 0 (time of egg laying). This is a time when embryonic development is naturally paused until incubation begins. You can maintain this "pause" by keeping the eggs cool, preferably around 10–15°C. The development is initiated when the eggs are incubated at 37°C. Thus, you can plan ahead to initiate development such that important timepoints correlate well with other scheduled classroom activities.
  - **Note:** Avian embryos are not considered live animals by U.S. regulatory agencies and, thus, are not covered under standard mammalian research protocol guidelines. Therefore, you do not need special permissions or animal protocols to work with this model, particularly since the embryos cannot hatch.
- Air-flow thermal incubator: Any incubator that can hold 37°C (98.6°F) will suffice. You need to keep it humidified and can do so by placing a small cup or bowl of sterile water in the incubator. Various options are available for ~$60.
- Square weigh boats: 3.5 × 3.5 × 1 inches (Fisher Scientific, catalog no. 08-732-113; VWR International, catalog no. 89106-766; case of 500, ~$100)
- Square Petri dishes: 100 × 100 × 15 mm (Fisher Scientific, catalog no. 08-757-11A, pack of 500, ~$300; Carolina Biological, catalog no. 741470, pack of 10, ~$10)
- 70% ethanol/30% water for sterilization purposes
- Dremel drill (optional, but recommended) with heavy-duty cut-off wheel no. 420
- Large cardboard box(es) to create a clean area for the egg cutting
- Stand to hold the Dremel drill (Carolina Biological, catalog no. 707194)
- Three prong extension clamp (to hold Dremel drill to the stand)
- Paper towels, lab goggles, lab coat (optional), lab gloves
- Waste basket for unsuccessful attempts

Suggested Schedule

Start incubating the fertilized eggs at 37°C (98.6°F) two days prior to the first day of lab. This way the embryos will be at day 3 when removed from the egg.

First day of lab: Students receive instruction on developmental biology, including cell division, cellular differentiation, and embryo growth. The level of information can be dependent on the class level. Students also receive instruction on maintaining sterile conditions. Have students sterilize the weigh boats with 70% ethanol in preparation for opening the eggs the next day. Students can also be given information regarding the developmental events that occur in the chick. The developmental timecourse for the chick is shown in Figure 3. *Note:* The chick embryo will not be able to live past its development because it requires the process of hatching to become a viable chick, despite development proceeding normally. They generally live until about day 19.

Second day of lab: Open the chick eggs at day 3 (3 days after incubation was begun). See procedures for details.

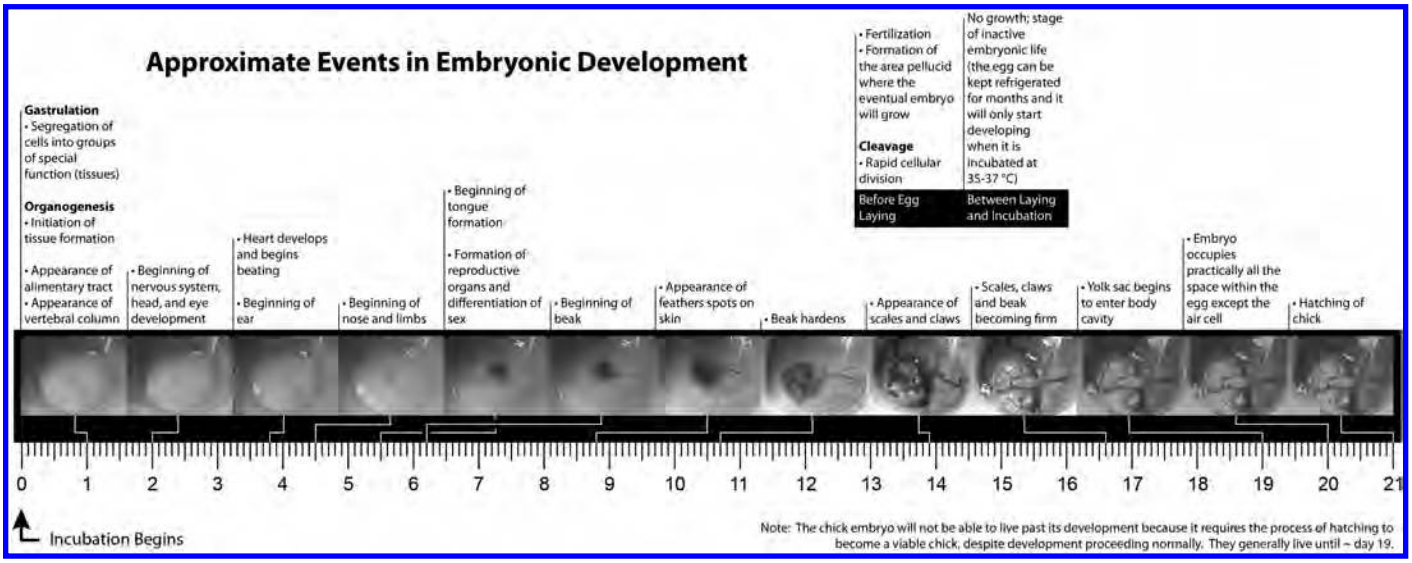


Figure 3. Timecourse of chick development.

Video 5. In this video, we provide a step-by-step illustration of the method for removing the chick embryo contents from the egg to prepare the ex ovo model. Several hints and suggestions are given throughout. See *ABT* online for video clip.

Over the course of the next 16+ days: Observe the embryos at various stages of development. Students can be instructed on making detailed observations, either by drawing or taking digital images, and labeling the images at various timepoints during development (Figure 2). This should be related to early developmental biology studies when scientists such as Malpighi made remarkable leaps in our understanding of developmental biology through observations, even without the advantage of fancy microscopes and modern scientific equipment.

Procedures

A video tutorial of this procedure is available (Video 5).

1. Obtain fertilized eggs and incubate with turning (180 degrees, once a day) until the morning of day 3 when the ex ovo embryo will be prepared. At this point, make a mark on the top of the egg and ensure that the egg remains in this orientation throughout the rest of the procedure so that the embryo remains at the top.
2. You will keep the embryos in 4 × 4 square weigh boats (weigh boats from VWR International, catalog no. 12577-027) covered with square Petri dishes (square dishes from Fisher, catalog no. 08-757-11A).

   Sterilize containers by washing thoroughly with 70% ethanol (prepare by mixing 300 mL of water with 700 mL of ethanol). Thoroughly dry before removing the chicks from their shells. If you prepare the weigh boats in advance, wrap the stacked weigh boats with Saran wrap or parafilm until use to minimize exposure.
3. Removal of the embryo contents from the shell can be a messy process, so it is important to set up in a location where clean-up is relatively simple. We generally use a box set-up to catch albumin that may splatter during this process (Figure 4; Video 5). The embryo should be removed from its shell at day 3 post-fertilization. The key to this is to prevent the yolk from catching on the eggshell as you crack and open the egg. This is why it is good to use a Dremel drill to score the bottom of the egg in a single long cut so that there are fewer jagged edges of shell as the embryo's egg contents are removed from the shell.
   a. Keep the egg upright so that the embryo will be on top of the yolk as the egg is opened from the bottom.
   b. Using a Dremel drill, carefully score the bottom third of the eggshell in a single line to make it easier to open. Use just enough pressure to allow the Dremel to cut the shell without penetrating the albumin too deeply. You do not want to cut the yolk.
   c. After scoring the bottom third, hold the egg close to the weigh boat. Firmly press inward, pulling up and out from the bottom. Once the crack begins to extend from the scored section, quickly pull the shell apart and out of the way, releasing the contents quickly so that the yolk is not broken.

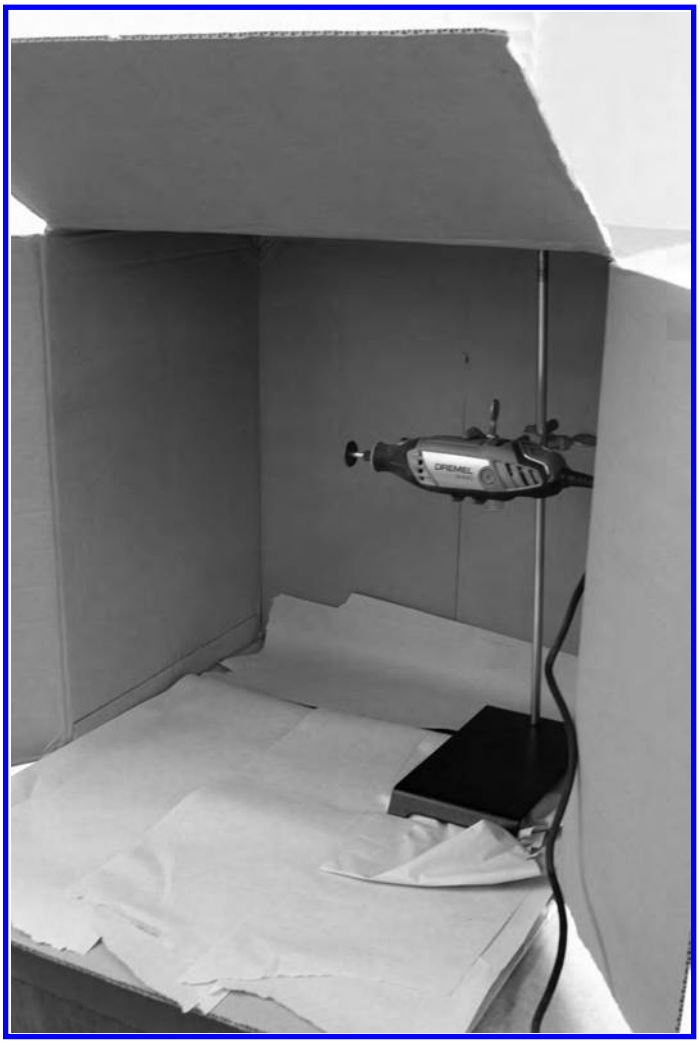


Figure 4. Picture of the setup for the ex ovo preparation.

Note: It is not necessary to use the Dremel drill if the egg can be cracked and the embryonic contents removed in a "sunny-side-up" fashion. However, the yolks of fertilized eggs are more fragile than your standard breakfast eggs.

Note: The embryos tend to be more robust, with a higher percentage developing close to their normal hatching stage if the embryo is released from the shell at day 3 post-fertilization. However, it is somewhat easier to remove the chick embryo contents from the shell at day 2. If you are unsuccessful trying this at day 3, you should attempt it at day 2 instead.

4. Maintain in a 37°C incubator (98.6°F). You should place a shallow but wide sterilized bowl of boiled water (recooled) at the bottom of the incubator to maintain humidity in the chamber. The embryos are stronger than you might think and can be removed from the incubator for brief periods for observation, etc. However, you should be gentle when moving the embryos (minimize shaking), and minimize the amount of handling and the time removed from the incubator as much as possible.

○ Important Thoughts & Conclusions

This ex ovo development method was not devised for rearing the chick embryos in culture, but to provide a model that was less expensive than mice and adaptable for undergraduate research on tumor angiogenesis (Deryugina & Quigley, 2008). However, this model has also been extremely valuable for my developmental biology (Bio400) course and has been extended to other environments, ranging from my own son's second-grade classroom to high school outreach classes, and groups of secondary-school science teachers that attend our master's in science education program. The reaction to this model is tremendous at all levels. However, there are a few important aspects that you should know and prepare the students for in advance.

Although they can develop fully without the shell, the chick embryos will perish prior to the point of "hatching," just before 3 weeks of gestation. This is at least partially due to the requirements of the embryo's preparation for hatching that occurs within the shell, and the process of hatching itself, that are critical to the survival of the chick. Because they lack a shell, the ex ovo chick embryos will not be able to "hatch" and become full-grown chicks. On one hand, this prevents logistical issues with regard to finding a home for several newly hatched chicks, but it does present an issue for students, particularly young students, who often become attached to their developing embryo. This should be addressed ahead of time (day 1) such that there are no false expectations. I use this as an opportunity to address and discuss the bioethical issues of animal research. It is important to note that, because the embryos do not hatch, they do not fall under the animal research guidelines and, thus, you do not need special permissions or protocols to work with the chick embryos (San Francisco State University, 2011). It is generally accepted that the embryo cannot feel pain until approximately day 19. Thus, an important point of consideration may be to gently euthanize the chick embryos at day 18 by hypothermia, typically by placing the embryo in a –20°C freezer (standard freezer temperature). Another consideration is that, no matter how well this procedure is performed, not all embryos will make it to day 18. Many will be sacrificed during the opening process (especially the first time) and others will perish throughout the following 2 to 3 weeks. As Veronica Van Heyningen once said, "The amazing thing about development is not that it sometimes goes wrong, but that it ever succeeds" (Gilbert et al., 2005). This is particularly true when you remember that, in this classroom model system, you are growing a chick embryo outside of its protective shell. Fortunately, there is no noticeable event when the chick embryos die. A simple lack of movement, pale coloration, and slow regression of the CAM vessels is all that indicates that an embryo has passed. If you prepare several ex ovo embryos, a few are likely to survive most of the embryonic period and this can continue to be a valuable science project, particularly if you consider every event to be a teachable moment. Even the most apprehensive students, both young (K–6) and older (7–university), and those that become quite attached to their embryos, still appreciate the project and give it very high reviews. Every student has emerged fascinated by developmental biology and more knowledgeable about this complex biological process.

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