

## INTRODUCTION TO THE CHICK EMBRYO



The chicken (*Gallus gallus*) embryo is an excellent model for the study of early vertebrate embryogenesis and later organogenesis. The embryo is encased within a hardened eggshell which provides a natural incubator or culture dish. Through a hole in the eggshell, the embryo can be visualized and easily manipulated with microsurgical tools or gene constructs, then allowed to continue development *in ovo* to determine the consequence of the experimental manipulation.

Fertilized chicken eggs are readily available anywhere in the world and the equipment needed is minimal – a humidified incubator (39°C, no CO<sub>2</sub> required), a dissecting microscope, microsurgical tools that can be prepared in the lab or purchased, and either a hand-held mouth pipette or a manufactured micromanipulator and picospritzer.

Fertilized eggs can be held at ~13-16°C for up to 1 week prior to incubation. They are incubated at 38°C-39°C to the desired stage in a humidified incubator with the eggs placed on their side (horizontal). For long-term post-operative survival, it is best that the eggs be left in the incubator until experimental manipulation. However, eggs can be removed from the incubator and held at room temperature to slow development.

For staging of chicken embryos:

[https://embryology.med.unsw.edu.au/embryology/index.php/Hamburger\\_Hamilton\\_Stages](https://embryology.med.unsw.edu.au/embryology/index.php/Hamburger_Hamilton_Stages)

## SAFE WORKING PROCEDURES AND ANIMAL ETHICS

### Risks Associated with Practical

Eggs have the potential to be contaminated with Salmonella. Wear gloves throughout and students should wash their hands before leaving the lab.

Dissection implements are sharp, so students should take care not to cut themselves or other students.

Students should wear a labcoat, gloves and enclosed shoes to protect themselves from egg splatter.

### Animal Ethics Compliance

The procedures used in this practicum are in compliance with the UNSW Animal Care and Ethics Committee and the National Health and Medical Research Council 'Australian code of practice (8<sup>th</sup> edition, 2013).

## EXPERIMENTAL PROCEDURES

### **A. Opening of the egg**

1. Eggs were incubated for 1 up to 8 days at 38.5°C to generate embryos of several developmental stages.
2. Each student pair should have one egg and an egg holder, a pair of blunt forceps and a pair of scissors, 3 petridishes, PBS, disposable pipet, syringe and 23G needle, Indian ink solution, dissection microscope.
3. Put the egg into the holder with the blunt end up (pointed end down).
4. Use the pointy end of the scissors to tap a hole in the top of the egg into the air chamber, be very careful not to push the scissors too far into the egg.
5. Use the forceps to pick bits of shell out. Do not remove egg shell beyond the air chamber. You will see the air chamber and the vitelline membrane.
6. Carefully remove the vitelline membrane at the top.
7. If you do not see the embryo (a ring of blood vessels should be visible) then gently swirl the egg so that it floats up to the top of the yolk. If this doesn't work you will need to take another egg if available.
8. Use the forceps to break off pieces of shell down to the yolk so that the embryo (visible as a ring of blood vessels) is exposed and the rim of the shell is just above the surface of the egg white or albumen.
9. You may be able to see the heart beating without magnification. If not, then put the egg under the dissection microscope.

### **B. Early stage somitogenesis embryos**

1. Draw Indian Ink solution (if available) up into a 1 ml syringe fitted with a 23G syringe needle.
2. Open an egg as described above. Once the shell has been removed down to the level of the yolk and the vitelline membrane has been removed, slide the syringe needle under the embryo. It is easiest to insert the syringe needle vertically at the edge of the egg initially and then rotate the needle until it is almost horizontal using the edge of the egg shell as a support. The tip of the needle should end up just below the embryo in the centre of the egg.
3. Slowly inject the Indian ink solution to reveal the embryo and view under a dissecting microscope.
4. Identify the HH stage and the diverse embryonic structures. See last page or link below.  
[https://embryology.med.unsw.edu.au/embryology/index.php/Hamburger\\_Hamilton\\_Stages](https://embryology.med.unsw.edu.au/embryology/index.php/Hamburger_Hamilton_Stages)
5. Draw your embryo, indicate the developmental stage using the images on the last page, and annotate the embryonic structures that you have identified.
6. Hand your drawing in at the end of the prac.

### **C. Late stage organogenesis embryos**

1. Carefully tear a hole in the vitelline membrane.
2. Very carefully tilt egg contents in a large petri dish, holding the egg very close to the dish.
3. Find and dissect the embryo, transfer it to a clean petri dish and cover it with PBS.
4. Study embryo under a dissection microscope, determine the developmental HH stage (see last page of the manual or link below), and identify the embryonic structures.  
[https://embryology.med.unsw.edu.au/embryology/index.php/Hamburger\\_Hamilton\\_Stages](https://embryology.med.unsw.edu.au/embryology/index.php/Hamburger_Hamilton_Stages)
5. Draw your embryo, indicate the developmental stage (HH), and annotate the embryonic structures that you have identified.
6. Hand your drawing in at the end of the prac

### **D. Observation of developmental stages**

Move around the class to study the embryonic chicken stages of your colleagues. Identify the following structures:

- Amniotic sac
- Hensen's node
- Neural groove and folds
- Head ectoderm
- Somites
- Brain vesicles
- Cardiogenic mesoderm
- Heart and vasculature
- Optic vesicles
- Branchial arches
- Nasal placodes
- Nasal pits and nasal processes
- Otic vesicle
- Limb buds
- Autopod, stylopod and zeugopod
- Handplate and fingers

## **E. Mouse and chicken embryogenesis**

There will be one demonstration station with dissection and light microscopes where you can investigate mouse embryos from E7.5 (late gastrula) until late organogenesis (E15.5), skeletal preparations of mouse foetuses, and mounted and stained early stage chicken embryos.

Identify the following structures:

- Amniotic sac
- Hensen's node
- Neural groove and folds
- Head ectoderm
- Somites
- Brain vesicles
- Cardiogenic mesoderm
- Heart and vasculature
- Optic vesicles
- Branchial arches
- Nasal placodes
- Nasal pits and nasal processes
- Otic vesicle
- Limb buds
- Autopod, stylopod and zeugopod
- Handplate and fingers

Note that mouse foetuses mainly consist of cartilage (blue), and only some skeletal elements are ossified (red) including the calvaria.

## **F. Tidying up after the prac**

- Please discard egg waste and disposable consumables into the yellow bins (not the paper bins).
- Dispose of Syringes and needles in sharps bins
- Wash forceps and scissors with water, and wipe with ethanol, place in provided containers on the sink.
- Clean surfaces (microscope stages, benches) with water and ethanol, and wipe dry.

## STAGING CHICK EMBRYOS HAMBURGER HAMILTON STAGES

(Full tables available: <http://www.ncbi.nlm.nih.gov/pubmed/1304820>)

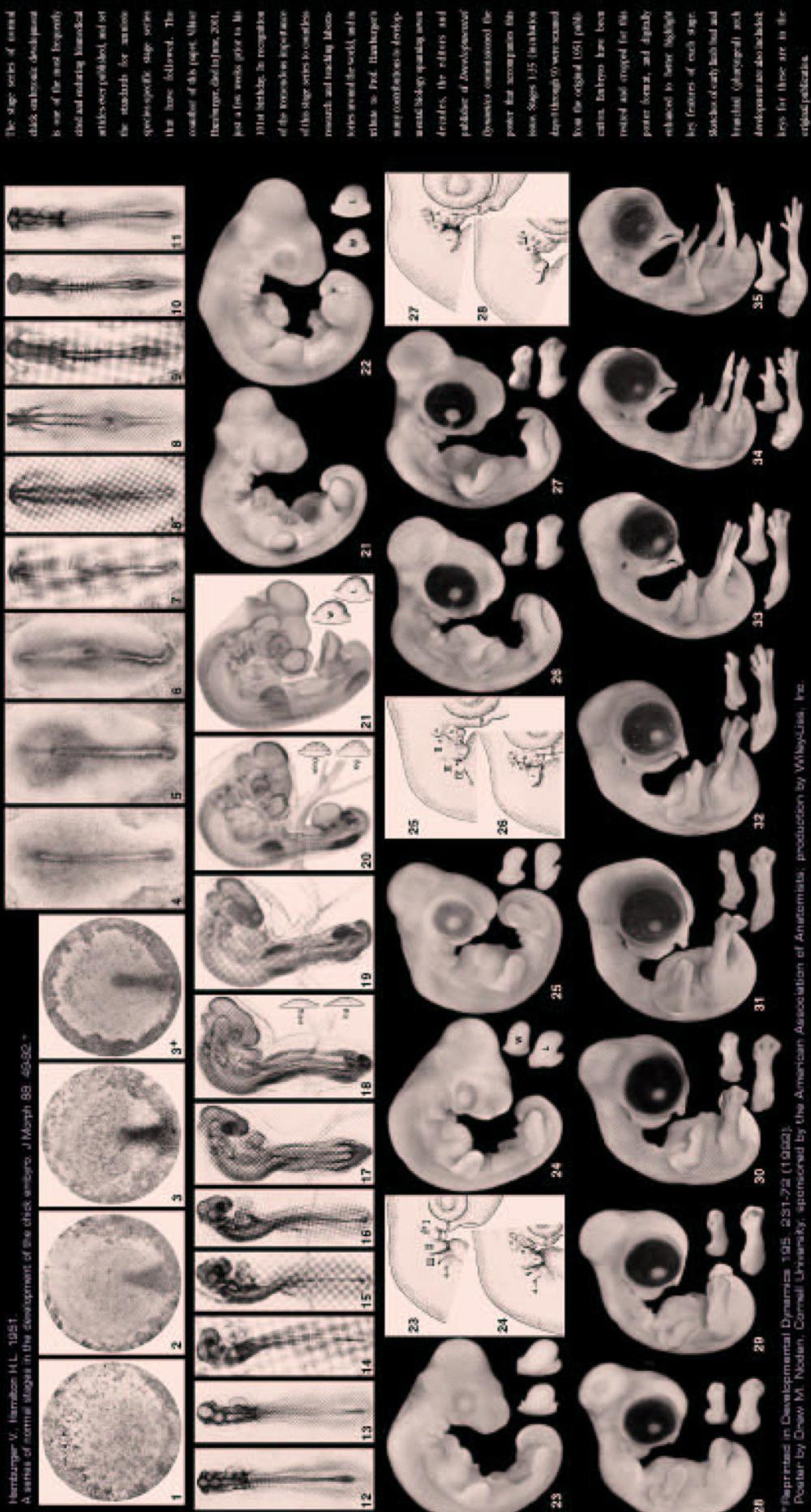
- 2 6-7 hr: Initial primitive streak, 0.3-0.5 mm long
- 3 12-13 hr: Intermediate primitive streak
- 4 18-19 hr: Definitive primitive streak,  $\pm 1.88$  mm long
- 5 19-22 hr: Head process (notochord)
- 6 23-25 hr: Head fold
- 7 23-26 hr: 1 somite; neural folds
- 7 to 8- ca. 23-26 hr: 1-3 somites; coelom
- 8 26-29 hr: 4 somites; blood islands
- 9 29-33 hr: 7 somites; primary optic vesicles
- 9+ to 10- ca. 33 hr: 8-9 somites; anterior amniotic fold
- 10 33-38 hr: 10 somites; 3 primary brain vesicles
- 11 40-45 hr: 13 somites; 5 neuromeres of hindbrain
- 12 45-49 hr: 16 somites; telencephalon
- 13 48-52 hr: 19 somites; atrioventricular canal
- 13+ to 14- ca. 50-52 hr: 20-21 somites; tail bud
- 14 50-53 hr: 22 somites; trunk flexure; visceral arches I and II, clefts 1 and 2
- 14+ to 15- ca. 50-54 hr: 23 somites; premandibular head cavities
- 15 50-55 hr: 24-27 somites; visceral arch III, cleft 3
- 16 51-56 hr: 26-28 somites; wing bud; posterior amniotic fold
- 17 52-64 hr: 29-32 somites; leg bud; epiphysis
- 18 65-69 hr: 30-36 somites extending beyond level of leg bud; allantois
- 19 68-72 hr: 37-40 somites extending into tail; maxillary process
- 20 70-72 hr: 40-43 somites; rotation completed; eye pigment faint grey
- 21 3.5 da: 43-44 somites; pharyngeal arch IV, pharyngeal cleft 4; eye pigment faint
- 22 3.5 da: Somites extend to tip of tail; eye pigmentation distinct
- 23 3.5-4 da: Dorsal contour from hindbrain to tail is a curved line
- 24 4 da: Toe plate in leg-bud distinct; 4th pharyngeal cleft reduced to small pit
- 25 4.5 da: Elbow and knee joints; 3rd & 4th pharyngeal clefts reduced to small pits
- 26 4.5-5 da: 1st 3 toes; 3rd and 4th pharyngeal clefts no longer visible
- 27 5 da: Beak just barely recognisable
- 28 5.5 da: 3 digits, 4 toes; beak outgrowth distinct in profile
- 29 6 da: Rudiment of 5th toe; beak more prominent; no egg tooth
- 30 6.5 da: Feather germs; scleral papillae; egg tooth
- 31 7 da: Web between 1st and 2nd digits; feather germs dorsally continuous from brachial to lumbosacral level
- 32 7.5 da: Anterior tip of mandible has reached beak
- 33 7.5-8.0 da: Web on radial margin of wing and 1st digit
- 34 8 da: Nictitating membrane
- 35 8 - 9 da: Phalanges in toes
- 36 10 da: Length of 3rd toe from tip to middle of metatarsal joint =  $5.4 \pm 0.3$  mm; length of beak from anterior angle of nostril to tip of bill = 2.5 mm; primordium of comb; labial groove; uropygial gland
- 37 11 da: Length of 3rd toe =  $7.4 \pm 0.3$  mm; length of beak = 3.0 mm
- 38 12 da: Length of 3rd toe =  $8.4 \pm 0.3$  mm; length of beak = 3.1 mm
- 39 13 da: Length of 3rd toe =  $9.8 \pm 0.3$  mm; length of beak = 3.5 mm
- 40 14 da: Length of beak = 4.0 mm; length of 3rd toe =  $12.7 \pm 0.5$  mm
- 41 15 da: Length of beak from anterior angle of nostril to tip of upper bill = 4.5 mm; length of 3<sup>rd</sup> toe =  $14.9 \pm 0.8$  mm
- 42 16 da: Length of beak = 4.8 mm; length of 3rd toe =  $16.7 \pm 0.8$  mm
- 43 17 da: Length of beak = 5.0 mm; length of 3rd toe =  $18.6 \pm 0.8$  mm
- 44 18 da: Length of beak = 5.7 mm; length of 3rd toe =  $20.4 \pm 0.8$  mm
- 45 19-20 da: Yolk sac half enclosed in body cavity; chorio-allantoic membrane contains less blood and is "sticky" in living embryo
- 46 20-21 da: Newly-hatched chick

# In Memory of Viktor Hamburger: 1900-2001

## NORMAL STAGES OF CHICK EMBRYONIC DEVELOPMENT

Hamburger V, Hamilton HL. 1951.

A series of normal stages in the development of the chick embryo. J Morph 88: 465-502.



The stage series of normal chick embryonic development is one of the most frequently cited and enduring classical and continuing standards in this very published, and set the standard for numerous species-specific stage series that have followed. This volume of this series (Hamburger, Hamilton, 1951) was just a few weeks prior to the 1951 death. In recognition of the continuing importance of this stage series to valuable research and teaching laboratories around the world, and in tribute to Prof. Hamburger's contributions to developmental biology, spanning seven decades, the editors and publishers of *Developmental Dynamics* considered the power that summarizes this issue. Stages 1-15 (available upon request) were selected from the original 1951 publication. The others have been revised and arranged for this poster format, and digitally enhanced to better highlight key features of each stage. Stochasticity both initial and terminal (climacteric) chick development also make keys for these are in the cited publications.