Gastrulation, early somitogenesis and neurulation lab – 28 August

The chicken (*Gallus gallus*) is an excellent animal 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 (390C, no CO2 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 ~13-16ºC for up to 1 week prior to incubation. They are incubated at 38ºC-39ºC 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

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 (8th edition, 2013).

Gastrulation, early somitogenesis and neurulation lab – 28 August

EXPERIMENTAL PROCEDURES

Opening of the egg (https://www.youtube.com/watch?v=V06pm0fKaHA%7CVideo)

1. Eggs were incubated for several days at 38.5°C to generate embryos that undergo organogenesis.

2. Each student pair should have one egg and an egg holder, a pair of blunt forceps and a pair of scissors, petridishes, PBS, disposable pipet, syringe and 23G needle, dissection microscope.

3. Put the egg into the holder with the blunt end up (pointed end down).

4. Stick sticky tape to top and bottom of the egg.

5. Tap a small hole in the bottom narrow end of the egg. The hole should just fit the needle, but not allow the leakage of albumin.

6. Insert syringe and needle in the hole, and draw 4 mls of albumin. (This will lower the position of the embryo). Discard albumin in waste bin.

7. Use the pointy end of the scissors to tap a hole in the wider end of the egg into the air chamber, be very careful not to push the scissors too far into the egg.

8. Use the forceps to remove bits of shell. Do not remove egg shell beyond the air chamber. You will see the air chamber and the vitelline membrane.

9. Carefully remove the vitelline membrane at the top.

10. 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.

11. 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.

12. You may be able to see the heart beating without magnification. If not, then put the egg under the dissection microscope.

Gastrulation, early somitogenesis and neurulation lab – 28 August

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

5. Draw your embryo, indicate the Hamburger & Hamilton (HH) developmental stages using the guide on course manual pages 22-24, and annotate the embryonic structures that you have identified.

6. Hand your drawing in at the end of the prac.

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
- Neural tube and neural pores
- Head ectoderm
- Somites
- Brain vesicles
- Cardiogenic mesoderm
- Heart and vasculature

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.

Draw your embryo on this page

Identify the developmental stage and annotate the embryonic structures above.

STAGING CHICK EMBRYOS - HAMBURGER HAMILTON STAGES

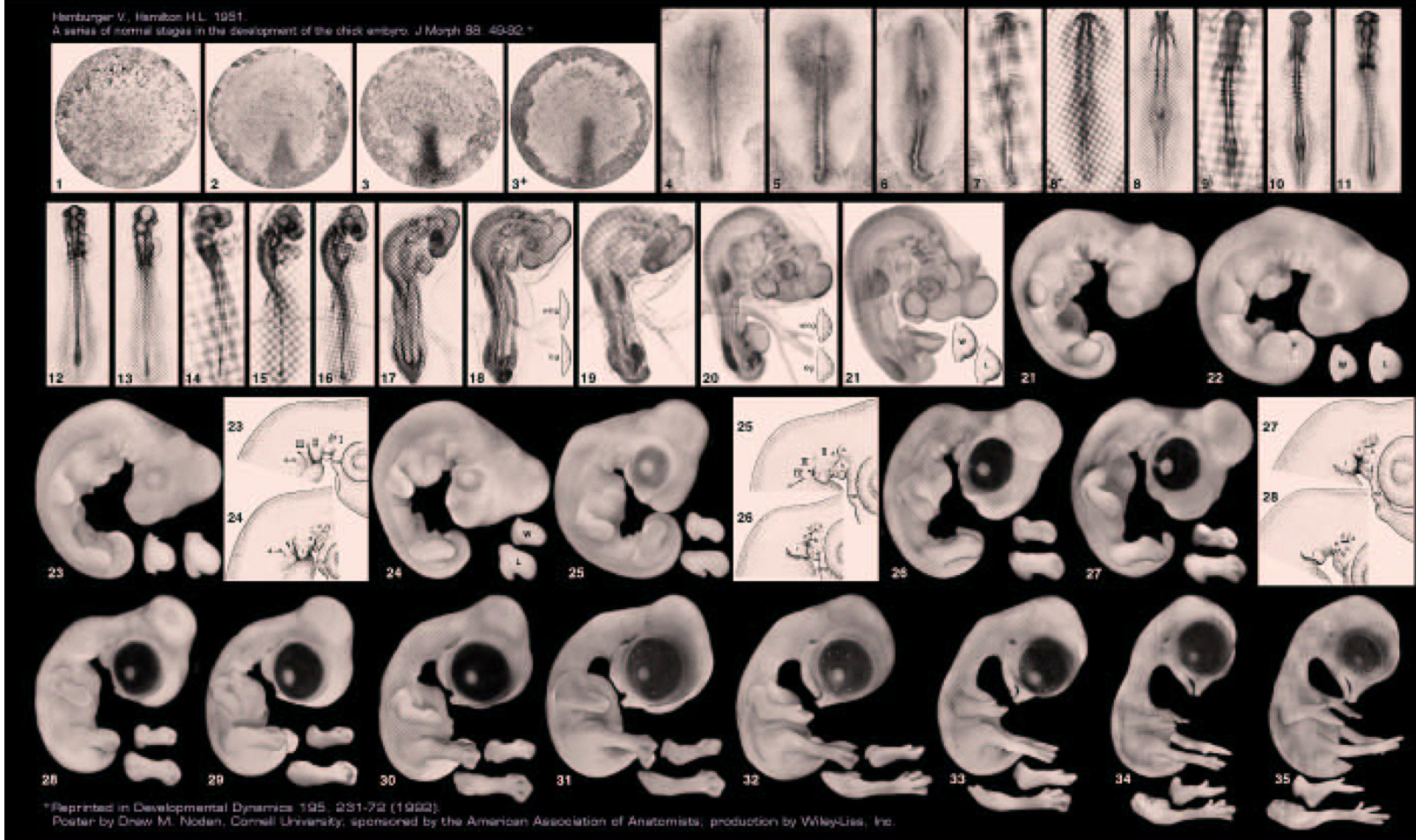
(Full tables available: http://www.ncbi.nlm.nih.gov/pubmed/1304820)

HH Stage	Time and Features
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, ±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±0.3mm; length of beak from anterior angle of nostril to tip of bill = 2.5mm; primordium of comb; labial groove; uropygial gland
37	11 da: Length of 3rd toe = 7.4±0.3mm; length of beak = 3.0 mm
38	12 da: Length of 3rd toe = 8.4±0.3 mm; length of beak = 3.1 mm
39	13 da: Length of 3rd toe = 9.8±0.3 mm; length of beak = 3.5 mm
40	14 da: Length of beak = 4.0 mm; length of 3rd toe = 12.7±0.5 mm
41	15 da: Length of beak from anterior angle of nostril to tip of upper bill = 4.5 mm; length of 3rd toe = 14.9±0.8 mm
42	16 da: Length of beak = 4.8 mm; length of 3rd toe = 16.7±0.8 mm
43	17 da: Length of beak = 5.0 mm; length of 3rd toe = 18.6±0.8 mm
44	18 da: Length of beak = 5.7 mm; length of 3rd toe = 20.4±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



The stage series of normal chick embryonic development is one of the most frequently cited and enduring biomedical articles ever published, and set the standards for amniote species-specific stage series that have followed. The coauthor of this paper, Viktor Hamburger, died in June, 2001, just a few weeks prior to his 101st birthday. In recognition of the tremendous importance of this stage series to countless research and teaching laboratories around the world, and in tribute to Prof. Hamburger's many contributions to developmental biology spanning seven decades, the editors and publisher of *Developmental Dynamics* commissioned the poster that accompanies this issue. Stages 1-35 (incubation days 0 through 8) were scanned from the original 1951 publication. Embryos have been resized and cropped for this poster format, and digitally enhanced to better highlight key features of each stage. Sketches of early limb bud and branchial (pharyngeal) arch development are also included; keys for these are in the original publication.

*Reprinted in Developmental Dynamics 195: 231-72 (1992).
Poster by Drew M. Noden, Cornell University; sponsored by the American Association of Anatomists; production by Wiley-Liss, Inc.

STAGING CHICK EMBRYOS - HAMBURGER HAMILTON STAGES