ANAT2341/EMBRYOLOGY - PRACTICAL CLASS 3 – T3 WEEK 4

Please note the different location for this week's practical class:
Wallace Wurth Teaching Lab 120.

PRACTICAL CLASS PROGRAM:

- Weekly Quiz + revision (15 minutes)
- Practical class activities (90 minutes)
- Practical Class Revision (15 minutes)

PRACTICAL CLASS ACTIVITIES

1. Fertile egg dissections
2. Whole body regeneration in planarians
3. (There will be back up activities available if required)

LEARNING OBJECTIVES:

- Understand the process of gastrulation, and being able to identify the defining structures in the chicken embryo.
- Understand early neurulation, mesoderm and heart development, and being able to identify the defining structures in the chicken embryo.
- Understand the regeneration process of planarians following amputation and being able to identify blastema formation, antero-posterior (A/P) and dorsal-ventral (D/V) axes conservation, eye regrowth, re-pigmentation of the regenerated tissues, and worm behavior.

Activity 1: Fertile egg dissections

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^{o}C, 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 between 13-16^{o}C 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

EXPERIMENTAL PROCEDURES

Opening of the egg

1. Eggs were incubated at 38.5°C and if fertilization was successful, they will contain very early embryos.

2. Each student pair should have one egg and an 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.

Early stage somitogenesis embryos

10. Open an egg as described above. Remove the shell down to the level of the yolk and the vitelline membrane.

11. Identify the HH stage and the diverse embryonic structures. See last page or link: https://embryology.med.unsw.edu.au/embryology/index.php/Hamburger_Hamilton_Stages

12. Draw or photograph your embryo, indicate the Hamburger & Hamilton (HH) developmental stages using the guide, and annotate the embryonic structures that you have identified.

13. Post your annotated embryo images to Padlet.

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

Activity 2: Whole body regeneration in planarians

Planarians are wonderful creatures that can be the superhero of your fantasies or the villian in your nightmares. Cut them into many pieces and each piece grows back into a complete worm! You might think growing an entire animal from a piece would take a long time for this inconspicuous worm to do. But you would be wrong. In fact, it takes only about two weeks for all the missing pieces to grow back to make a complete animal. Wow, a piece of a tail can grow a new head including its brain! Now all the new worms that have grown back are nearly identical clones of each other. What an amazing organism!!! These little worms are helping scientists around the world to unravel the mysteries of regeneration and can help you explore fundamental biological questions, such as growth, regrowth, aging, and even stem cell biology (Cutting Class educational resource).

EXPERIMENTAL PROCEDURES

1. Put the desired number of worms in a Petri dish.
2. Wipe the blade with ethanol
3. Cut the worms transversally in two (or more) pieces: the first cut is made between the eyes and the pharynx, the second cut between the pharynx and the tip of the tail. Three fragments are obtained: head, trunk and tail. (You are welcome to also try other directions - including sagitally if you feel adventurous and have steady hands! ;-))
4. Wipe the blade after every 2-3 cuts to remove planarian mucus.
5. Monitor the immediate formation of the blastema.
6. Transfer regenerates to 6 well-dishes
7. Monitor regenerates (amputated worms) at 1 and at 2 weeks post-amputation. There will be a dissection microscope available in the Anatomy Teaching Class.

TIDYING UP AFTER THE PRACTICAL CLASS

- 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.

SAFE WORKING PROCEDURES AND ANIMAL ETHICS

Risks Associated with Practical

Eggs have the potential to be contaminated with the bacteria Salmonella. Wear gloves throughout and students should always 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 lab coat, 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^{th} edition, 2013).

BACK UP ACTIVITIES

1. Histology exercises of practical class 1
2. Smartsparrow exercises