

# 26 STAINING AND MOUNTING OF BLASTODERM OF CHICK EMBRYO

## 26.1 INTRODUCTION

The eggs of birds and reptiles are laid on land. The egg of the hen is fertilised before it is laid and the embryonic development occurs outside the body of the mother. The hen's egg is covered by a shell and has no relationship with the external environment except for exchange of gases. Hen's egg is rich in nutrients in the form yolk to be utilised for the development of the embryo. The embryo is in the form of a flat disc, the blastoderm and overlies the yolk (Fig. 26.1). The yolk is surrounded by a thin transparent vitelline membrane. In this exercise you will isolate an early stage of developing embryo from the egg, stain it and make observations on development of various organs and organ systems.

### Objectives

After you have completed this laboratory exercise you should be able to:

- stain and mount the blastoderm of chick embryo
- identify, draw and label the various structures of a 33 hour chick embryo

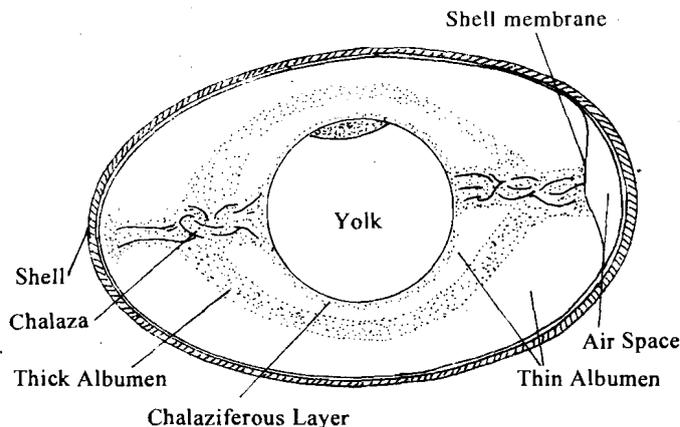


Fig. 26.1 : Hen's egg showing the location of blastoderm.

## 26.2 MATERIALS REQUIRED

fertilised eggs incubated for a period of 30 to 38 hrs. at 38°C

0.9% saline solution

chick ringer solution

finger bowl of 200 ml. capacity

watch glass

dissection microscope (a binocular stereoscopic dissection microscope is preferred)

lamp

filter paper

a pair of sharp, pointed scissors

strainer spoon

fine forceps

pasteur pipettes

neutral Red or Nile blue (0.1% solution)

## 26.3 PROCEDURE

- Crack a small area of the egg shell at the broad end with the blunt end of the forceps (Fig. 26.2a). Remove the pieces of the shell carefully so that you can see the opaque shell membrane (Fig. 26.2b). There are two shell membranes which are closely attached to each other except at the blunt end of the egg where they are separated from each other enclosing a small 'air-trapped' space. Because of the airspace at the blunt end, it is much more easier to open the egg from the broad end without damaging the embryo.

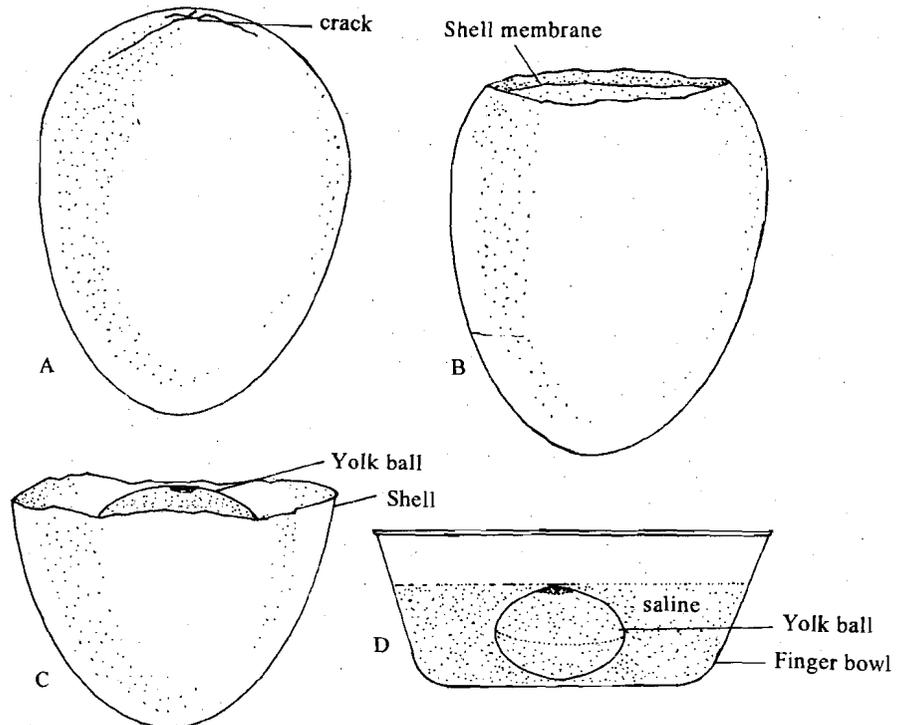


Fig. 26.2 : Procedure for the removal of the embryo from the egg.

- Remove the shell membranes with forceps. You will observe the yellow yolk with the blastoderm floating in the thin albumen.
- With the help of a pasteur pipette remove as much of the albumen as possible.
- Broaden the shell opening (Fig. 26.2c) so that you can easily pour the entire contents of the egg in the finger bowl containing Ringer solution (Fig. 26.2d).
- Place a drop of 0.1% neutral red or Nile blue solution on the blastoderm. After 5 minutes wash off the excess stain by carefully squirting Ringer solution with the help of a pasteur pipette.
- Observe the structure of the blastoderm under a dissection microscope, preferably under a binocular stereoscopic dissection microscope.
- To isolate the blastoderm from the rest of the yolk, follow the following procedure.
  - Prepare a petri dish by placing a watch glass with the convex side up in the middle and pour warm (37°C) Chick Ringer solution until the fluid just covers the top of the watch glass.
  - Lift the vitelline membrane (the membrane covering the yolk) with your forceps and gently cut it in a circle of about 3 mm beyond the edge of the blastoderm. This will leave the blastoderm intact and will enable you to handle it easily.
  - Hold the cut edge of the membrane with your forceps and carefully float it from the surface of the yolk into the strainer spoon. Holding onto the membrane lift the spoon out of saline and transfer the membrane and blastoderm to the prepared petridish.
  - Carefully and gently flush some Ringer solution onto the blastoderm to remove excess yolk by grasping the cut edges with the forceps. Place the

petridish under the dissection microscope. Slide the blastoderm up onto the top of watch glass which is already covered by saline. If the blastoderm tends to slide off the watch glass, it can be anchored with a small piece of filter paper placed on the top of one edge.

## 26.4 OBSERVATIONS

1. Examine the blastoderm under the microscope. For the best view of the beating heart, place the embryo with its ventral side up. To turn your embryo over, gently slide the blastoderm off the watch glass, grasp one edge with your forceps and with a quick flip turn it over. Then slide it back up on to the watch glass.
2. The embryo is surrounded by a relatively clear area called area pellucida which itself is surrounded by area opaca.
3. You will observe the vitelline circulation. Trace the blood vessel carrying nutrients from the yolk to the embryo and then back to the capillary network overlying the yolk.
4. Study the embryo and identify the following structures with the help of Fig. 26.3.

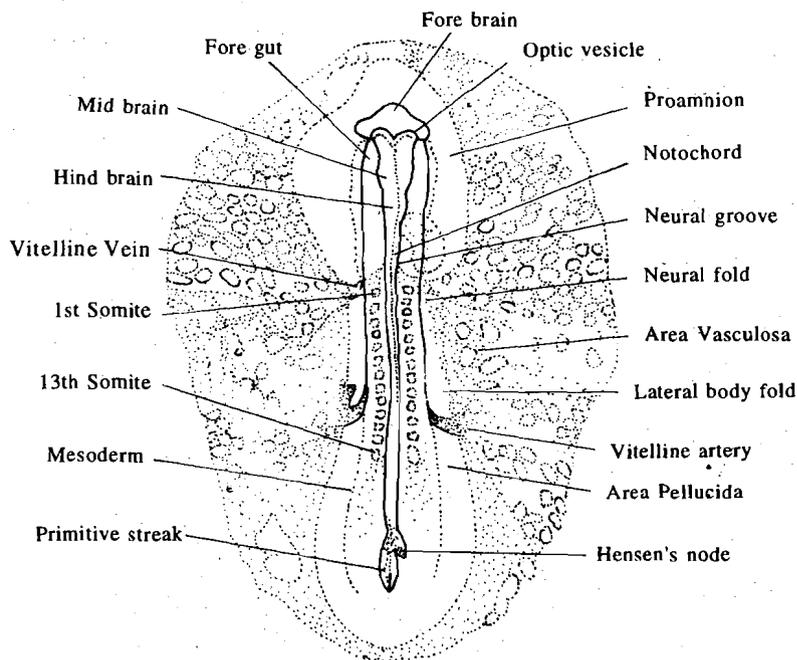


Fig. 26.3 : A 33 hour chick embryo.

- a) Observe that the head region of the embryo is divided into three segments an anterior forebrain with optic vesicle, a median enlargement the midbrain and finally the hind brain which continues as neural tube throughout the length of the embryo.
- b) Observe that the neural folds have not fused posteriorly. The remnants of primitive streak can be seen posterior to the open neural folds.
- c) Observe notochord as a thick streak in the middle portion of the embryo, lying to the neural tube and entering forward to the forebrain as a solid rod.
- d) Beneath the brain and anterior part of neural tube you will observe the tubular heart which has not yet completed its morphogenesis.

- e) Observe the bilateral pair of large omphalomesenteric veins entering into the heart at the posterior region. These veins bring blood to the heart from the vitelline vessels developing in the area of the yolk.
- f) Observe the somites in the middle region of the body. They are derived from mesoderm. They will give rise to the various types of muscles. Count the number of somites that are developed at the age of the embryo.
- g) Also observe the outline of the gut which is clearly visible on either side of the midbrain area.

Draw and label the diagram of the embryo you have mounted.

**Note:** If you do not observe a blastoderm when you open the egg and only a white spot in the egg yolk, then it means that the embryo has failed to develop. Discard this egg and use another incubated egg. However check with your counsellor before you discard the egg.