Study of frog embryonic development through models/ collection of frog spawns and observation of different developmental stages

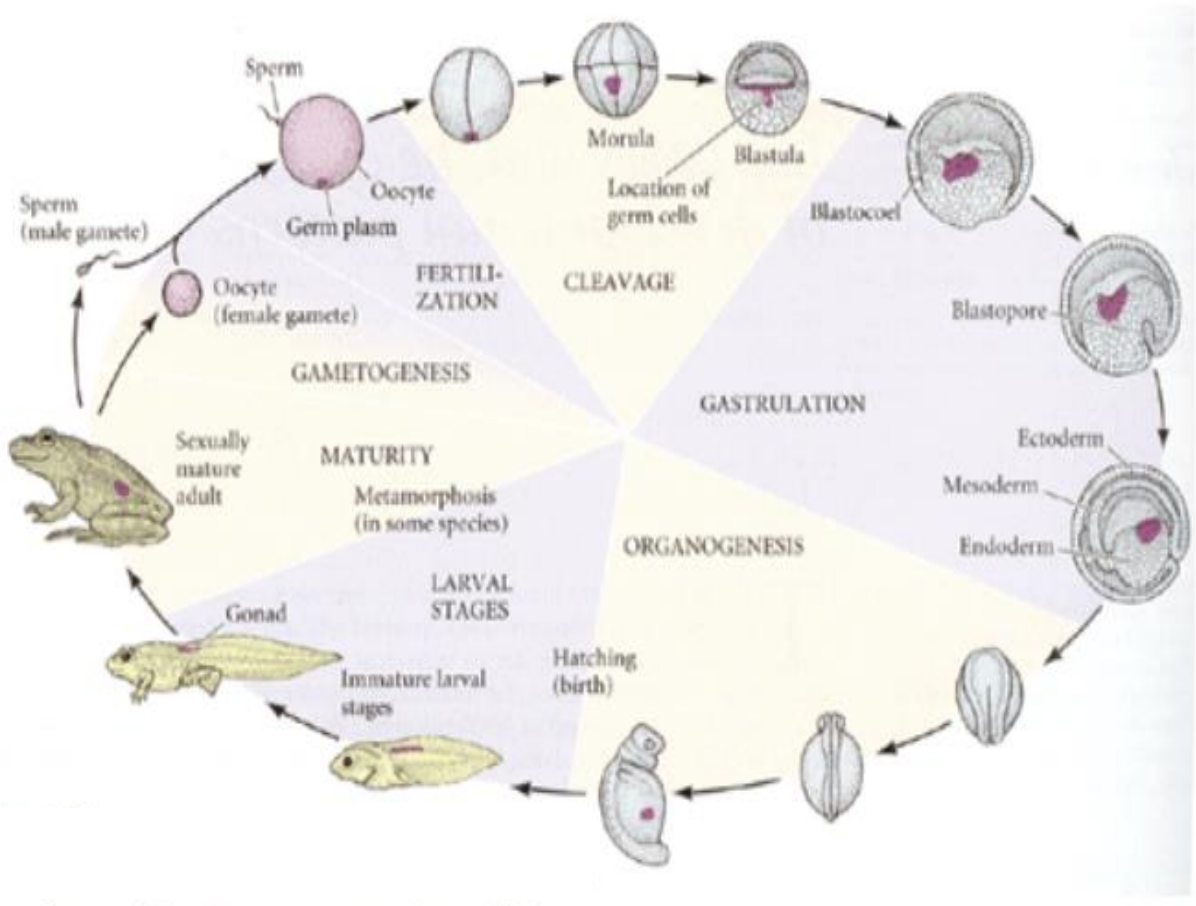


Fig: Study of embryonic development in frog

Embryonic Development of Frog

- When sperm fertilize the egg, streaming movements are set up in the egg and these results in distribution of materials. So that three regions can be seen, the upper animal hemisphere (pole) which is pigmented and lower white vegetal pole. Between the two hemispheres, there is a small are with no pigment called grey crescent.
- 2-3 hours after fertilization, the zygote begins to divide. The repeated division in the successive fashion is known as **cleavage or segmentation.**
- Division is mitotic
- The cleavage begins as a small depression at animal pole and gradually extends surrounding the zygote, dividing into two cell.
- The divisions are **holoblastic** and complete
- First cleavage is vertical; two celled stage
- Second cleavage is also vertical but right angle to the first one; forms 4 celled stage
- The cells are known as blastomere
- Third cleavage is horizontal but above the equatorial line forming unequal size cells. The upper 4 cells toward animal pole are small and pigmented known as micromeres or epiblast. The lower 4 large yolk laden cells are known as megameres or hypoblat.
- Fourth and fifth cleavage are also vertical forming 16 celled zygote. These division is followed by two horizontal cleacage, one toward animal pole and other toward vegetal pole, resulting in 32 celled stage.

Morula (mulberry shape stage):

- As the result of repeated and irregular cleavage, ball of cells is formed known as morula stage.
- One hemisphere of morula is composed of large number of small black and yolkless cells known as **micromeres** and other hemisphere is composed of fewer number of large white and yolk laden cells known as **megameres.**

Blastula stage:

- The micromeres dives more rapidly than megameres which results in formation of small fluid filled cavity known as **Blastocoel or segmentation cavity.**
- Blastocoel bearing stage is called **Blastula**
- The floor of blastocoel is composed of layer of yolk laden megameres while the roof is composed of micromeres.
- In this stage early Presumptive areas can be differentiated by staining technique.
- The entire animal pole of blastula represents the presumptive ectoderm, which is further divided into presumptive epidermis and presumptive neural plate
- A small area near vegetal pole is presumptive notocord

- Close to presumptive norocord there is a grey crescent region which is the presumptive mesoderm
- The remaining vegetal region is presumptive endoderm

Gastrula stage:

- Gastrula is the two layered embryo stage formed by migration and rearrangement of cells of blastula. The process of formation of gastrula is called **gastrulation.**
- Gastrulation involves some critical changes in the blastula such as- differentiation of cells, transformation from monoblastic to **diploblastic layer**, formation of **three primary germ layers.**

Gastrula completes in following stages:

1. **Epiboly:**

- In this step, micromeres at animal pole dives more repeatedly and rapidly enclosing the megameres except in the region of yolk plug. This overgrowth or spreading of micromere cells is known as Epibloy.

2. **Emboly or Intucking (Invagination):**

- In this step, small groove appears due to invagination of megameres near grey crescent region. The invagination gradually grows inward causing migration of cells.
- This stage is also known as Yolk plug stage.
- The narrowing of blastopore exerts pressure on underlying yolk laden megameres, result in protruding of some megameres cells as yolk plug.
- Contraction of lips of blastopore: contraction of lips from all side occurs so that blastopore become smaller and narrower.
- As invagination progresses archenteron increases in size and the blastocoel become reduced and finally obliterated.
- This groove is the beginning of archenteron and its anterior opening is called blastopore. The blastopore is guided by anterior margin called dorsal lip and backward projecting lateral lip.

3. **Involution:**

- Due to increase in size of archenteron as well as formation of yolk plug, there is rapid migration of presumptive areas within the embryo occurs. This movement of the presumptive areas is known as involution.
- Rotation of gastrula: gastrulation causes shift in the center of gravity of the embryo. In the blastula stage, embryo floats with animal pole upward. But formation of archenteron causes the embryo to rotate within the vitelline membrane so that blastopore comes near the vegetal pole.
- Gastrulation causes following changes-
- i) blastopore is presumptive gut
- ii) roof of archenteron is chordamesoderm

- iii) floor of archenteron is endoderm

4. Formation of three germ layer:

- The three layers are ectoderm, mesoderm and endoderm are known as primary germ layer. They are also called as germinal layers because entire organs and body are derived from these layer.

Fate of germ layers

1. **Ectoderm:** epidermis, cutaneous glands, eye lens, cornea, retina, conjunctiva, central nervous system (brain and spinal cord), pineal gland, pituitary gland, enamel of teeth etc are derived from primary ectoderm layer.
2. **Mesoderm**: notochord, pericardium, peritoneum, muscles, skeleton, connective tissues-blood, lymph, adipose tissue, dermis of skin, visceral organs, are derived from primary mesoderm layer.
3. **Endoderm:** epithelium of digestive tract, respiratory tracts, Eustachian tubes, gastric and intestinal glands, liver, pancreas, bile and pancreatic ducts, lining of urinary bladder are derived from primary endoderm layer.

Neurulation:

- It is the process of formation of neural tube or nerve cord.
- At the end of gastrulation the prospective neural plate comes to lie along the length of mid-dorsal region. Neural plate later forms central nervous system including brain and spinal cord.
- A pair of longitudinal ridges called neural folds appears along the edges of neural plate, which meet in a semicircle anteriorly.
- The neural folds increase in height and comes closer together the median line where they fuse to form neural tube, enclosing the neural canal.
- The closure of neural tube begins just in front of mid-region and proceeds both anteriorly and posteriorly
- At the front end neural tube remains open for short time through neuropore. But posteriorly it communicates for some time with archenteron by neurenteric canal.
- Finally closed tubular neural tube is formed which later form brain and spinal cord.

Notogenesis:

- It is the process of formation of notochord
- The meso-endodermal cell lying in mid dorsal region of roof of archenteron separates from mesoderm layer
- These cells become solid cylinder rod like structure along the median line and parallel to and just below the neural tube lies called notochord.
- Later, notochordal sheath develop around the notochord
- In adult notochord is replaced by vertebral column.

Formation of coelom:

- Coelom is the body cavity and it is mesodermal in origin
- Mesodermal layer split into two thin layers-outer somatic or (parietal) layer and inner visceral or (splanchnic) layer.
- Between these two layers a cavity is formed called splanchnocoel, which extend downward and continues to the outside below the gut
- Outer somatic layer combines with ectoderm to form body wall (somatopleure)
- Inner visceral layer unites with endoderm to form gut wall (splanchnopleure)
- Splanchnocoel continues to form coelom or body cavity between gut wall and body wall.
- The coelom is known as Schizocoel coelom.

Study of Spiral cleavage in snail eggs

- Spiral holoblastic cleavage is characteristic of several animal groups, including annelid worms, some flatworms, and most molluscs.

- The cleavage planes are not parallel or perpendicular to the animal-vegetal axis of the egg; rather, cleavage is at oblique angles, forming a “spiral” arrangement of daughter blastomeres.

- The cells touch one another at more places than do those of radially cleaving embryos.

- Spirally cleaving embryos usually undergo fewer divisions before they begin gastrulation, making it possible to follow the fate of each cell of the blastula.

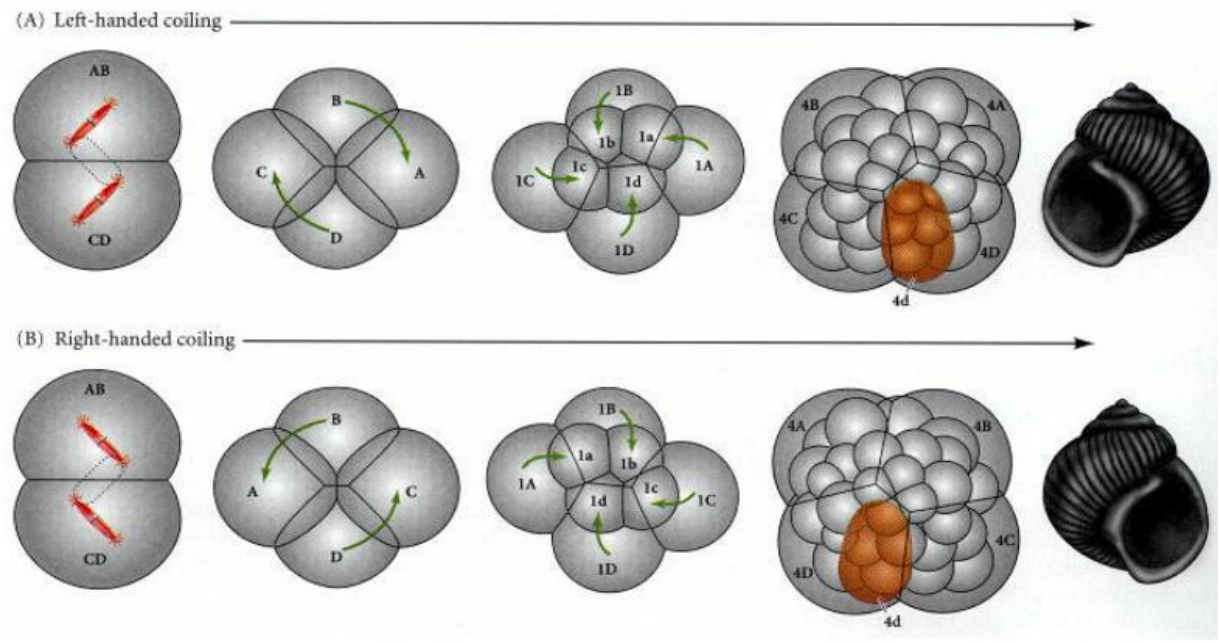


Fig: Looking down on the animal pole of left-coiling and right-coiling snails. The origin of sinistral and dextral coiling can be traced to the orientation of the mitotic spindle at the second cleavage. The left-coiling (A) and right-coiling (B) snails develop as mirror images of each other.

Study of embryonic development in chick embryo:

1. W.M. 18 Hours Chick Embryo:

1. At this stage the dark peripheral area opaca and central translucent area pellucida are distinctly visible.

2. In the anterior part is present the pro-amnion, which is a small and comparatively more translucent region of area pellucida and is characterised by the absence of mesoderm.

3. In the middle of area pellucida, in the posterior half, runs a primitive streak having a primitive groove through its centre. The primitive groove is being bound by primitive folds.

4. In the anterior half of area pellucida, in the middle, runs a neural groove bound by neural folds.

5. The primitive streak and neural groove is separated by a thickening-the Hensen's node having a small depression in the centre-the Hensen's pit.

6. The primitive streak gives rise to an out-growth, the notochord immediately below the primitive groove.

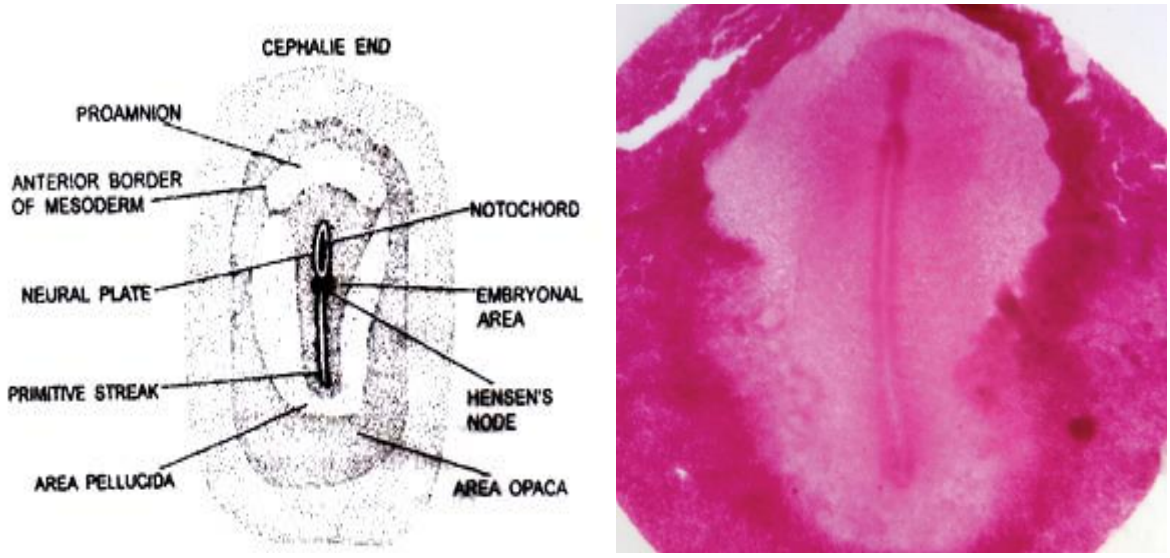


Fig: Whole mount of 18h chick embryo

2. Whole mount of 24 Hours or 4 Pairs of Somites Stage of Chick Embryo:

1. At this stage the dark peripheral area opaca and central translucent and colourless area pellucida are distinctly visible.

2. In the anterior part is present the proamnion, which is a small and comparatively more translucent region of area pellucida and is characterised by the absence of mesoderm.

3. In the middle of area pellucida, in its posterior half runs a primitive streak with a primitive groove in its centre. The primitive groove is bound by primitive folds.

4. In the anterior half of area pellucida, in the middle, runs the neural groove bound by neural folds.

5. The primitive streak and neural groove are separated by Hensen's node having a small depression in the centre-the Hensen's pit.

6. Immediately below the primitive groove the primitive streak gives rise to a small out-growth, the notochord and on either side to mesoderm.

7. In the area pellucida embryonic and extra embryonic regions also become distinguished.

8. In the anterior- most part the ectoderm has given rise to head fold, which is a pocket-like extension of neural folds. The underlying endoderm is also transformed into a pocket-like foregut. The proamnion is greatly reduced.

9. In front of Hensen's node the mesoderm of embryonic area differentiated into 3-4 pairs of mesodermal somites.

10. The neural canal, in the region of head fold, gives rise to forebrain.

11. The foregut extends on either side into an amino-cardiac vesicle.

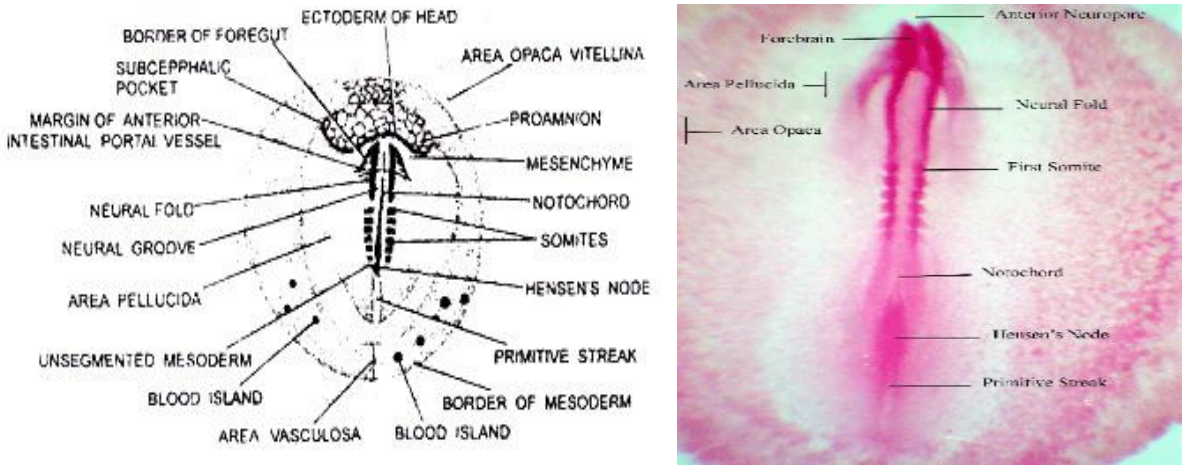


Fig: Whole mount of 24 h chick embryo

3. Whole Mount of Chick Embryo of 13-14 Pairs Somites or 36 Hours:

1. At this stage the dark peripheral area opaca and central translucent and colourless area pellucida are not visible.

2. The extra embryonic area has grown in size.

3. The primitive streak is comparatively reduced because of great lengthening of neural canal and neural folds. The notochord has extended from behind the brain up to the end of body.

4. The mesoderm, in front of Hensen's node, has given rise to 13-14 pairs of somites.

5. The brain is differentiated into fore brain, mid brain and hind brain.

6. In the fore brain region optic vesicles and in the hind brain region optic vesicles have developed.

7. The area opaca has changed into area vasculosa.

8. Proamnion has disappeared.

9. Anterior omphalomesentric vein and vitelline artery have developed.

10. The cardiac vesicle has given rise to heart.

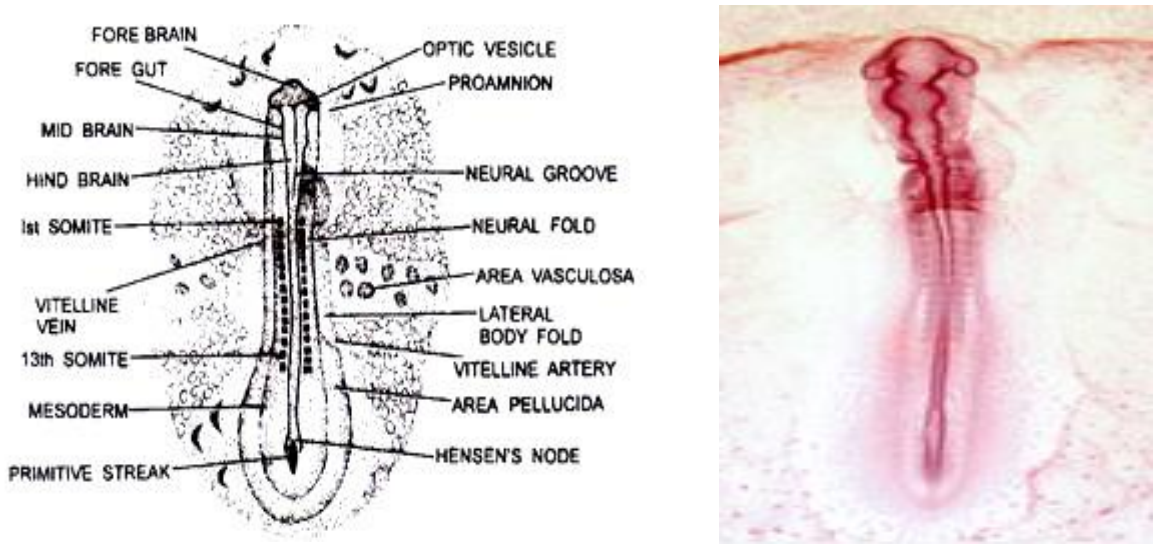


Fig: Whole mount of 36 h chick embryo

4. W.M. of 48 Hours Chick Embryo of 26-28 Pairs of Somites:

1. At this stage the area opaca and area pellucida are not visible.

2. The extra embryonic area has grown in size.

3. Primitive streak has disappeared.

4. The mesoderm, in front of Hensen's node, has given rise to 26-28 pairs of somites.

5. The brain has differentiated into telencephalon, prosencephalon, mesencephalon, metancephalon and mylencephalon.

6. The heart has been differentiated into ventricle and atrium. Sinus venosus and truncusarteriosus have also started developing.

7. The eye has been differentiated into optic cup and lens and optic vesicle has also developed sufficiently.

8. The head region has curved on right side due to cranial flexion.

9. Three pharyngeal gill-slits have also been differentiated.

10. Behind Hensen' node a tail bud has also developed.

11. Lateral amniotic folds, anterior omphalomesentric vein and vitelline artery have appeared.

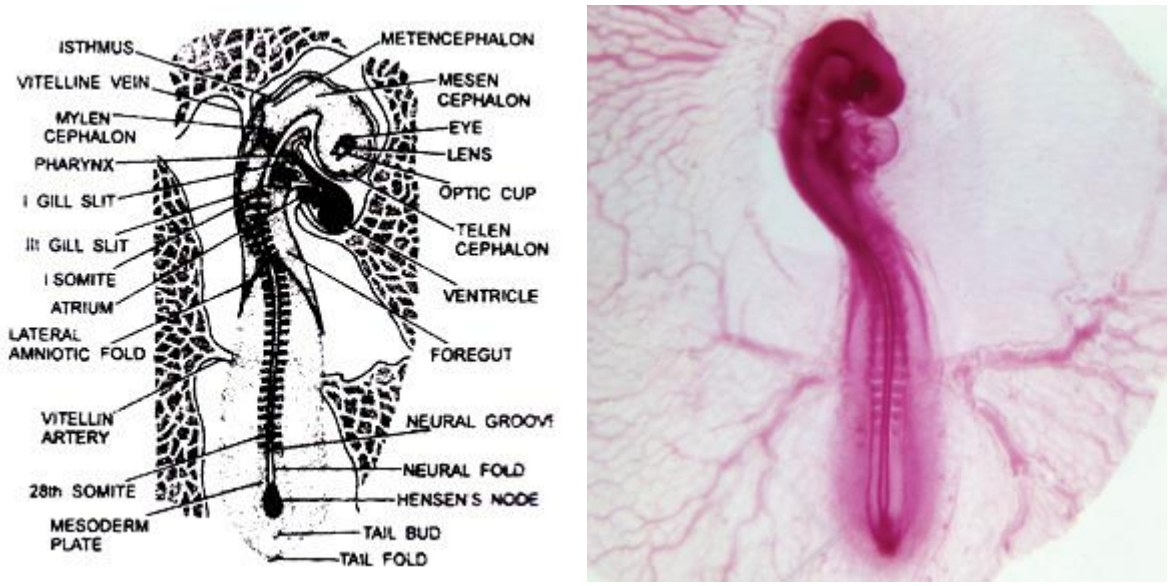
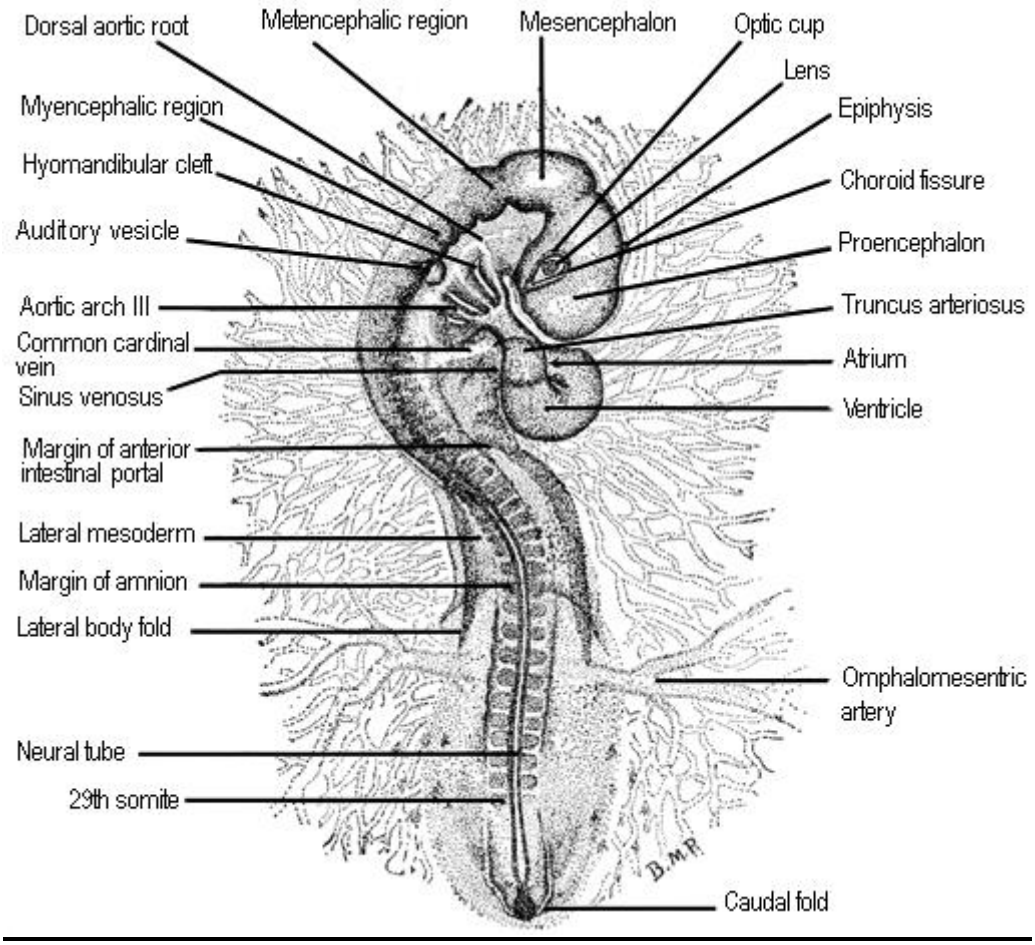


Fig: Whole mount of 48 h chick embryo

6. W.M. of 55 Hours Stage of Chick Embryo:



6. W.M. of 72 Hours or 36 Pairs of Somites Stage of Chick Embryo:

1. At this stage area opaca and area pellucida are not visible.

2. The extra embryonic area has grown in size.

3. Primitive streak has disappeared.

4. The mesoderm, in front of Hensen's node, has given rise to 36 pairs of somites.

5. The brain has differentiated into telencephalon, mesencephalon, metancephalon and mylencephalon.

6. The heart has been differentiated into ventricle and atrium.

7. The eye has differentiated into optic cup and lens and optic vesicle has also developed sufficiently.

8. The head region has bent on right side due to cranial flexion.

9. Four pairs of gill-slits have been differentiated.

10. Tail bud is greatly developed and has given rise to allentoic stalk and tail.

11. Lateral amniotic folds, vitelline artery and anterior omphalomesentric vein have developed.

12. In the middle region a pair of fore limb buds and in front of tail a pair of hind limb buds have developed, which will give rise to fore and hind limbs.

13. Olfactory pit, visceral arches, amnion, allantois and amniotic cavity have also developed.

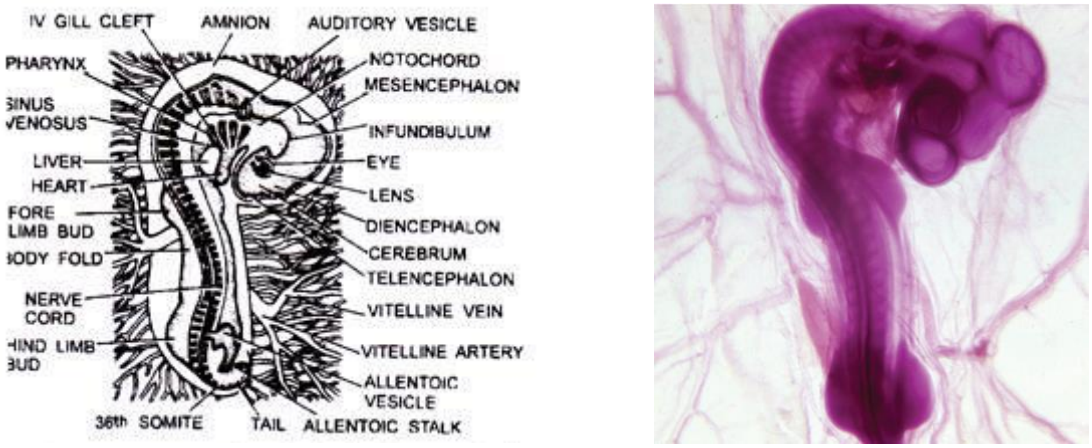


Fig: Whole mount of 72 h chick embryo

Preparation of Sperm Slide:

- Take a drop of Bull's semen on a cleaned, prewashed microscopic slide.
- Spread the semen evenly over the slide using needle and let it air dry.
- Fix using methanol and let it air dry.
- Stain with eosin for 10 minutes and wash off the excess stain.
- Examine under microscope.

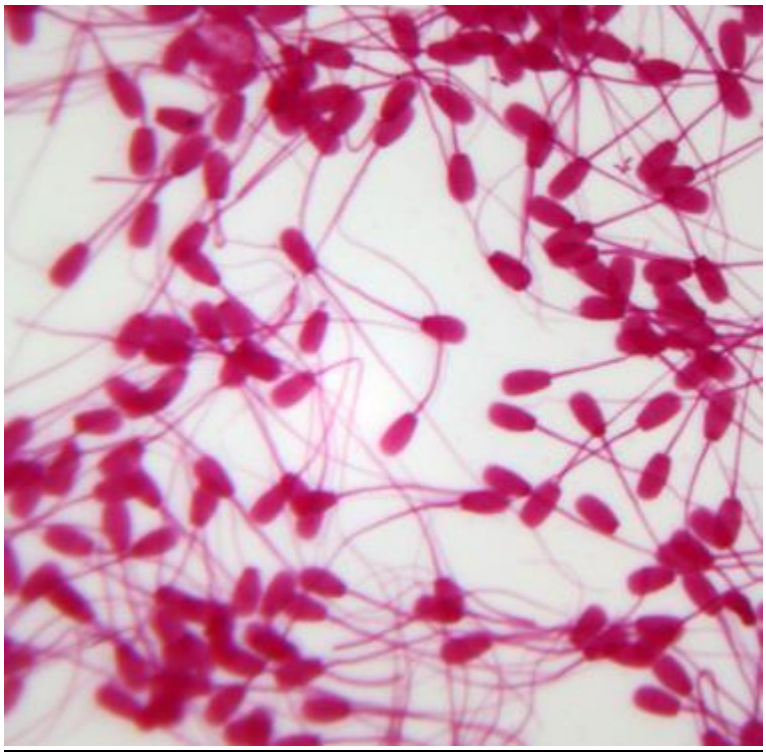


Fig: Bull sperm under microscope