UNIT 14

DEVELOPMENT OF FROG

Structure

14.1 INTRODUCTION

In Block 3 you were introduced to the basic concepts of the study of animal development. You learnt that though vertebrate development may vary among the different groups of animals, the early processes and stages of animal development are the same in all groups. You would recall that there are three main stages in early vertebrate development in which (1) the main body axes-dorsal/ventral and anterior/ posterior are to be set up; (2) the three germ layers-ectoderm, mesoderm and endoderm are formed and (3) patterning of the germ layers (early nervous system and mesoderm) takes place. Maternal genes provide factors in the form of RNAs and proteins in the cytoplasm eggs of most vertebrates (except mammals) as cues for the initial stages of development. Later the zygotic genes take over. These developmental stages shared by all vertebrates result in the formation of an embryo being born as either a larval stage which is different from the adult form and transforms into the adult form later, or as a young organism having the typical features of the adult of that species. You had also learnt that an organism's development is

ultimately the result of differential gene expression. This determines what proteins are formed and when they are formed so that they change the behaviour of cells at different stages of embryo formation.

In Unit 10 we discussed some animals that have been chosen as models to study and understand the stages and mechanisms of development. The African clawed frog *Xenopus laevis* is one organisms that has historically been used as an amphibian model due to its ease of procurement, its ability to reproduce the year round and its large sized eggs which are easy to observe and to manipulate surgically. Another advantage of using *Xenopus* as a model is that it is completely aquatic and is able to develop normally in tap water. The mechanisms involved in the changing of its larval form into an adult organism have been studied in depth and provides insights into the phenomenon of metamorphosis. Therefore, *Xenopus* and other species of frogs and some other amphibians have been used by early embryologists and present day developmental biologists to understand many mechanisms underlying development of vertebrates.

In this Unit we will study the various stages of development of *Xenopus laevis* starting from fertilisation, blastula formation, cell movements in gastrulation and formation of early nervous system in neurulation. We know that amphibians do not emerge from the embryonic stage as fully formed adults instead, they have a free swimming larval stage- the tadpole which undergoes metamorphosis to develop into a miniature adult. We will learn how the frog embryo develops from the fertilized egg to a stage in which it has distinct body axes. Another crucial process in early vertebrate development is the development of the three germ layers - ectoderm, mesoderm, and endoderm. In this unit we shall see how this is achieved in frog. After the initial patterning, the germ layers move into their appropriate positions in the body plan through the process of gastrulation. A key developmental event that accompanies gastrulation is the induction of the nervous system from the dorsal ectoderm by a complex cascade of signals from adjacent tissues, and we shall consider that here .You will see how the concepts you had learned in the previous units in Block 3 are exemplified in the various stages of frog development.

The 2012 Nobel Prize in Physiology and Medicine was awarded to John Gurdon for his pioneering work in nuclear transplantation experiments in frog eggs. His experimental techniques and studies became the forerunners of the modern stem cell research and cloning.

The process by which the larva transforms into an adult is called metamorphosis and this often involves radical biochemical and physiological changes in its form, usually controlled by hormones. We will learn about these changes in this unit. In some amphibians the larval stage also becomes the sexual stage and the larva does not undergo metamorphosis. This special stage of development is known as neotany, about which we will learn in the last section of this unit.

Objectives

After studying this Unit you should be able to:

- describe the various stages and processes in the development of frog;
- explain how body axes are formed in frog;
- describe the morphogenetic movements during frog gastrulation;

- ❖ correlate the fate map of frog with its outcome in development;
- ❖ describe the process of neurulation;
- ❖ explain the underlying process of metamorphosis and its hormonal control, and;
- ❖ define and discuss neotany in amphibians.

14.2 CLEAVAGE AND BLASTULATION

Before we discuss cleavage and formation of blastula in frog let us look into the structure of its egg and how the maternal factors that are already present in the cytoplasm of the egg control the laying down of the body axes before and after fertilisation. The anterior–posterior axis of the embryo though initiated after fertilization, is laid down only after gastrulation. We will first explore the relative roles of pre-existing maternal factors in the egg and the embryo's own developmental program in the formation of the body axes.

14.2.1 Setting the Embryonic Axes

The mature unfertilized egg of frog (in this unit we will use most of the times, unless specified otherwise the term frog to mean *Xenopus*) already has a distinct polarity (Fig.14.1) with a pigmented region referred to as **animal hemisphere** and a pale yolky region known as **vegetal hemisphere**(Refer again to unit 13).

The axis running from the animal pole to vegetal pole is referred to as **animal-vegetal axis**. The frog egg has many localised mRNAs and proteins that are laid down in the cytoplasm of egg while it is being formed. These 'maternal factors' control the development of the zygote till about 12 cleavages and then its own genes begin to be expressed. Of these a number of mRNAs are the signaling molecules that determine the animal-vegetal axis. ***Thus, the animal-vegetal axis is already laid down in the egg even before it is fertilized. The animal –vegetal axis of the egg is not exactly the anterior posterior axis of the tadpole but is related to it as the head will eventually develop from the animal region.*** Before fertilization you will recall that the egg is enclosed in a **vitelline membrane** surrounded by a jelly like coat (Fig.14.1). Within the first 90 minutes of sperm entry which can happen anywhere on the animal hemisphere, the rotation of the egg cortex with respect to inner cytoplasm is initiated. The cortex is a gel-like layer of actin filaments about 5 micrometres thick just under the membrane. The vegetal cortex opposite the sperm entry point moves towards the animal pole by about 30 degrees, relative to the rest of the cytoplasm. This cytoplasmic rearrangement is called the **cortical rotation** (Fig.14.2). Cortical rotation relocates some maternal factors that are originally located in the vegetal half of the egg to an area which will become the dorsal side. This leads to the formation of a 'signaling centre' opposite the site of sperm entry known as the blastula organizer or **Nieuwkoop Centre** (named after a Dutch embryologist Pieter Nieuwkoop who discovered the organising properties of the vegetal region). Signals from the Nieuwkoop center are required for the future development of all dorsal and

A signaling center is a localized region of the embryo that exerts a special influence on the surrounding cells and can thus determine how they will develop.

anterior structures. One of the main roles of the Nieuwkoop center is to specify another key signaling center, the **Spemann organizer,** which arises just above the Nieuwkoop center at the late blastula-early gastrula stage. Recall from Unit 10 that signals originating in the Spemann organizer are involved in further patterning along both the antero-posterior and dorso-ventral axes of the embryo, and in inducing the central nervous system. As a result of cortical rotation, there is reduction in the pigmentation of animal hemisphere opposite to sperm entry point (SEP) (Fig.14.2).

Fig.14.1: *Xenopus* egg with the pigmented animal hemisphere and light coloured vegetal hemisphere where the yolk is concentrated.

In most frogs (except *Xenopus*), thus, a crescent shaped surface area known as **gray crescent develops** on the surface of amphibian egg opposite to the point of sperm entry. The sperm entry point marks the **prospective ventral side**, while the region opposite to it becomes the **prospective dorsal side** of the embryo. ***Thus, the point of sperm entry lays down the initial dorsal-ventral axis of the larvae though it will become fixed only during gastrulation.*** During fertilization, the radially symmetrical unfertilized egg becomes bilaterally symmetrical.

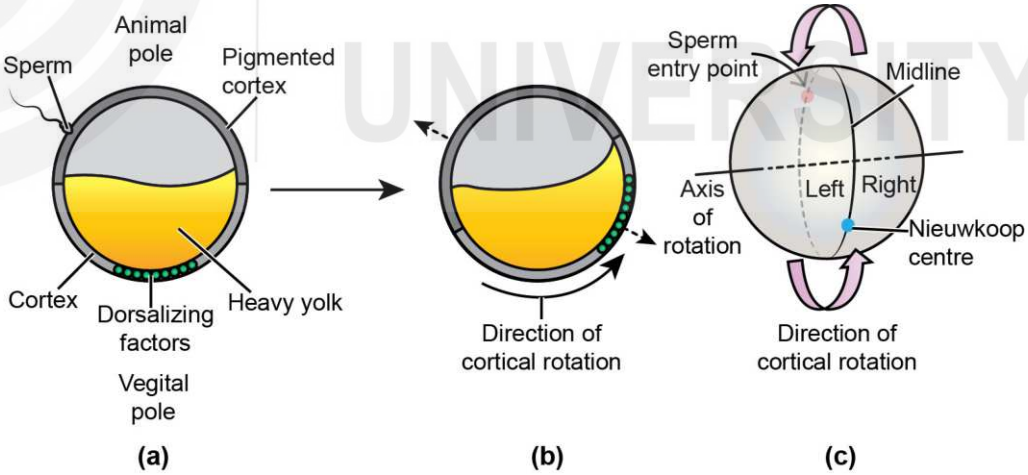


Fig.14.2: a) sperm entry point (SEP) and; b) cortical rotation that determines the (c) dorso-ventral axis of the embryo and establishment of Nieuwkoop Centre which is essential for normal development. Maternal factors are responsible for forming this organiser region in the egg.

14.2.2 Cleavage and Blastula Formation

The amphibian egg is moderately telolecithal. The yolk is concentrated in the vegetal hemisphere which creates hindrance in cleavage. The cleavage is radially symmetrical and unequal holoblastic (recall the various types of

cleavages from Unit 13). The first cleavage furrow is vertical and occurs along the animal – vegetal axis. It begins to form at the animal hemisphere and slowly extends towards the vegetal hemispheres. It divides the egg into two equal sized daughter cells and *also forms the prospective left and right sides of the developing embryo*. The cells derived from the cleavage division as you know, are called blastomeres. The first cleavage cuts through the site of the sperm entry and the Nieuwkoop Centre or the gray crescent in case of *Rana* species (see Fig.14.2 c and 14.3).

The second cleavage is also vertical along the animal – vegetal axis but at right angles to the first. It starts while the first cleavage is still going on in the vegetal hemisphere, producing four blastomeres (2 with the Nieuwkoop Centre that is the dorsal side and 2 without on the ventral side). The third cleavage is equatorial. This cleavage furrow does not pass exactly through the equator but is displaced towards the animal pole because of the vegetally placed yolk. It divides the embryo into four small animal blastomeres (micromeres) and four large blastomeres (macromeres). This way the animal and vegetal halves get separated (Fig.14.3).

If the 4 cell embryo is divided into two halves, one dorsal and one ventral, the half with the Nieuwkoop Centre develops fairly normally with only some ventral structures not developing. But the half without the Nieuwkoop Centre develops without dorsal or anterior structures. This shows that the Nieuwkoop centre is required for normal development and also explains Roux's experimental result where he had destroyed one cell at the 2 cell stage and got half and embryo.

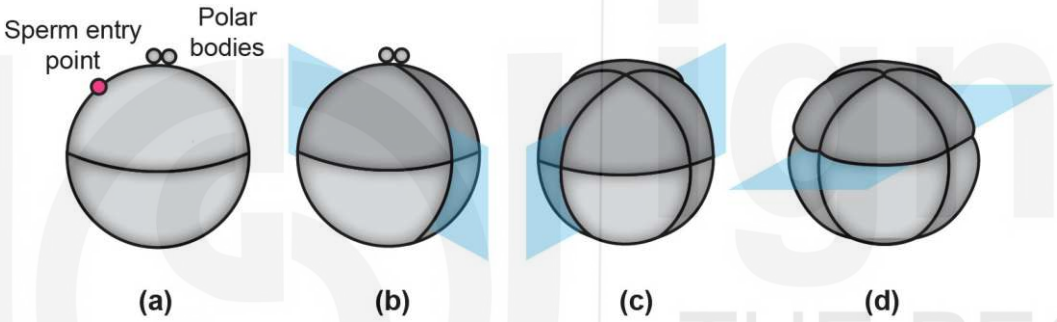


Fig.14.3: a) Fertilized egg and (b, c & d) first three cleavage divisions that separate the animal and vegetal valves.

The fourth cleavage is vertical, holoblastic and unequal. It produces sixteen blastomeres. Further divisions result in rapid multiplication of micromeres as compared to macromeres (Fig.14.4 a).

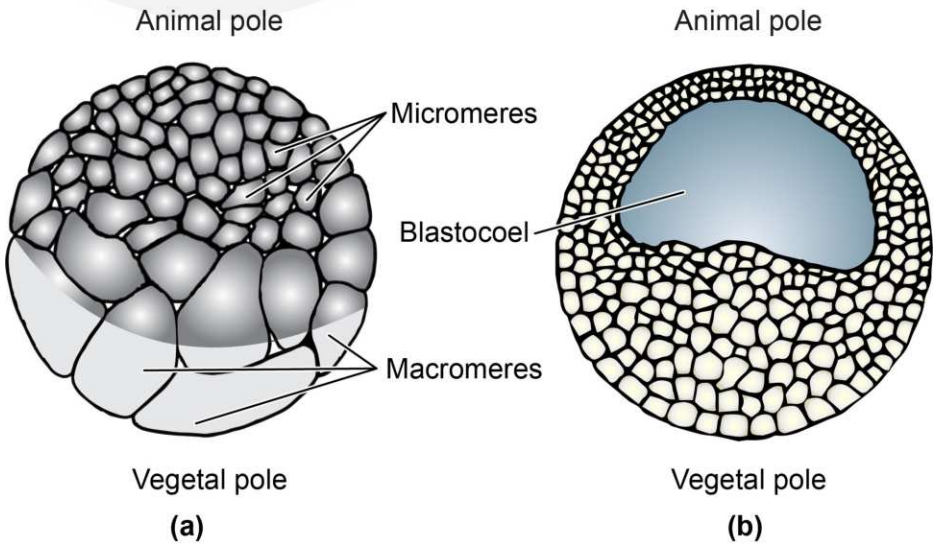


Fig.14.4: a) Rapid division in the animal half results in blastomere called micromeres and the slowly dividing blastomeres in the vegetal half are called macromeres; b) section of the blastula showing the blastocoel.

An amphibian embryo consisting of 16 to 64 cells is commonly called a **morula** (resembles the shape of mulberry). The continued cleavage results in formation of smaller and smaller blastomeres since there is no growth between cell divisions. Cells at the vegetal pole are larger than those at the animal pole as rate of division is slower in comparison to the cell division in the animal hemisphere. At the 128 cell stage a fluid filled cavity called **blastocoel** clearly develops in the animal region. The **blastocoel** is eccentric in position (see Fig. 14.4 b). The embryo at this stage as you know is called a **blastula.** The blastomeres adhere to each other strongly by cell adhesion molecules mainly by EP-cadherins. The mRNA for this molecule is already present in the maternal cytoplasm.

Blastocoel serves two major functions:

1. It permits cell migration during gastrulation
2. It prevents premature interaction of cells beneath and above it.

The amphibian egg at the end of blastula formation has gone through 12 cell divisions. At this stage the embryo consists of many thousand cells.

14.2.3 Mid-Blastula Transition

After the 12th cleavage division, the embryo enters the stage of mid blastula transition (MBT) during which it prepares itself for the next stage namely, gastrulation. The zygotic genes now begin to get expressed and prepare the blastomeres for movement. MBT demarcates the transition from maternal cell fate determinants like mRNAs and proteins (already present before fertilization) to the embryo producing its own mRNAs and proteins produced by the developing zygote.

The characteristic features of mid blastula transition (MBT) stage are the following:

1. Rate of cleavage slows down.
2. Synchrony of cell division is lost.
3. The embryo's own RNA synthesis starts and different genes are transcribed in different cells, e.g. Veg T protein is formed in the vegetal cells from the localized maternal m RNA. Vegetal cells under the influence of Veg T protein become endodermal cells. They begin secreting factors that induce the cells above them to become mesodermal.

SAQ 1

Fill in the blank spaces in the statements given below:

i) The Nieuwkoop Centre's role is to specify another signal centre the …….. which in turn leads to the development of embryo's ……

ii) Sperm entry results in the specification of the …… side, opposite the SEP.

iii) Egg structure determines the animal ……..…/…… ……..….. axis and the first cleavage also determine the prospective ……. and ……., ……. sides of the embryo.

iv) The cells of the ……... hemisphere divide at a faster rate than cells in the ……. hemisphere.

v) Mid blastula transition marks the expression of …… genes.

14.2.4 Fate Map of Amphibian Blastula

You already know that a fate map gives the information about regions of the blastula that will give rise to the future cells and tissues of the embryo. By studying the fate map, one can easily understand the complex morphogenetic movements involved in gastrulation. The external appearance of the blastula at 32 cell stage gives no indication of what those cell will become at later stage as the cells are largely unspecified. If some cells are removed during the blastula stage, others will be able to take over the fate of the missing cells. This implies considerable developmental flexibility at this early stage of development. The actual fate of cells is heavily dependent on the signals they receive from neighboring cells. The fate map of amphibian blastula had been first constructed by vital staining method of Vogt (1929). According to the fate map the surface of the amphibian blastula can be divided into three major areas or zones (Fig.14.5.) namely, presumptive ectoderm, presumptive mesoderm and presumptive endoderm.

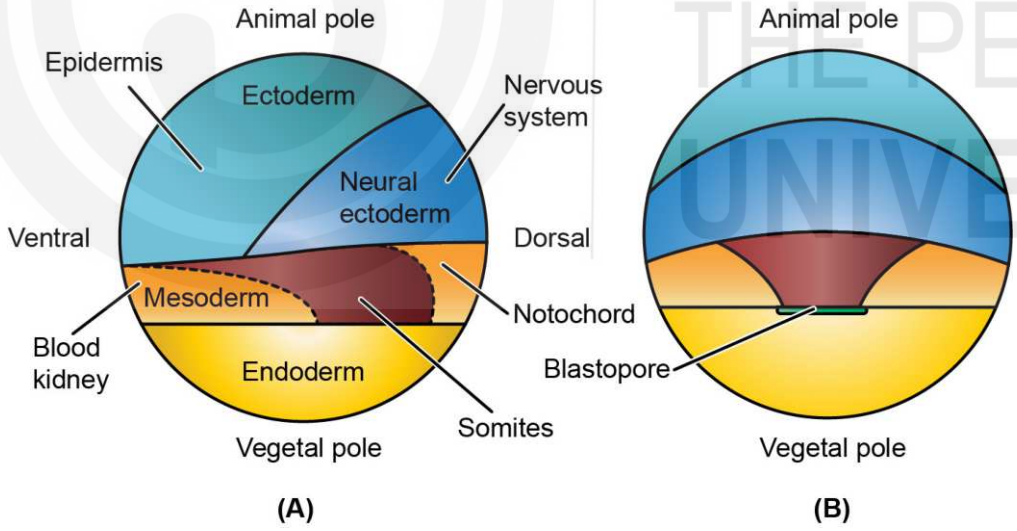


Fig.14.5: Fate map of late blastula just before gastrulation: a) lateral view in which blue denotes the animal zone with prospective ectodermal and neural ectodermal cells, yellow is the prospective endodermal cells in the vegetal zone and the mesoderm is derived from the superficial and deep cell layers in the marginal zone between the animal and vegetal zones; b) dorsal view showing the position of the blastopore where gastrulation will start.

1. **Presumptive ectoderm area:**

The pigmented area around the animal pole forms the presumptive ectoderm including the neural and epidermal ectoderm.

2. **Presumptive mesoderm area**:

Beneath the presumptive ectoderm area is present a belt like area the **marginal zone** or gray crescent. This region develops into presumptive mesodermal cells that are induced from the prospective ectoderm by a general signal arising from the ventral vegetal region that operates around the circumference and induces the ventral mesoderm (Fig.14.6). A dorsal signal that arises from the vegetal cells of Niewkoop center induces the axial mesoderm (Spemann's organizer). The patterning of mesoderm along the dorso-ventral axis produces regions from dorsal to ventral, that, give rise to **chordamesoderm** which forms the notochord above the region of the foregut. **Somatic mesoderm** gives rise to somites, which are present on both sides of the notochord area. The lateral and ventral part of the marginal zone constitutes the **ventrolateral mesoderm** which gives rise to the circulatory system, kidneys and gonads etc.

3. **Presumptive endoderm area:**

The yolky non pigmented area around the vegetal pole is the prospective endoderm. The yolk provides the nutrition and is used up during development. In *Xenopus* but not all amphibians there is a thin outer layer of presumptive endoderm overlying the presumptive mesoderm in the marginal zone.

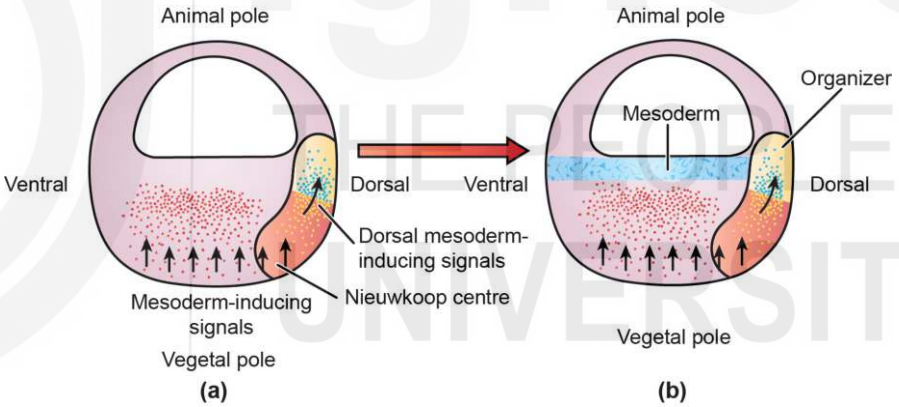


Fig.14.6: Signals involved in mesoderm induction in *Xenopus.* Signals from vegetal cells first induce mesoderm from prospective ectodermal cells of the animal zone. The organizer is specified on the dorsal most side where the inducing signal is strongest and of longest duration. Signals from the ventral zone of the embryo, pattern the rest of the mesoderm and the extent of their influence is counteracted by signals coming out of the Spemann's organizer.

SAQ 2

1) What is the dorsal most structure derived from the mesoderm?

b) What does the ectoderm give rise to?

c) What induces the formation of prospective mesoderm?

d) What is the prospective fate of the vegetal blastomeres?

14.3 GASTRULATION

The process of gastrulation in amphibians is quite complex. It involves various morphogenetic movements and rearrangement of cells. From the extensive studies done on amphibian gastrulation, it has emerged that the gastrulation movements are not uniform among the major amphibian groups. The process of gastrulation described below is based on studies chiefly done on *Xenopus laevis.* We know from Unit 13 that the main task of gastrulation is to bring cells from the surface of the blastula to the inside for forming the endodermal organs, to surround the embryo with cells capable of forming ectoderm and to place the mesoderm in its proper place between the external ectoderm and internal endoderm layers in order to give rise to organs derived from it. The main morphogenetic movements involved are chiefly – invagination, to initiate gastrulation which is followed by a variety of cell movements including convergence, divergence, involution and epiboly.

14.3.1 Initiation of Gastrulation

Gastrulation in frog is initiated on the future dorsal side just below the equator in the marginal zone i.e. the area of gray crescent where animal and vegetal hemispheres meet. Here the endodermal cells are not so large or yolky. The small endodermal cells in this region invaginate (sink in) to form a slit-like groove. The formation of the groove is the first external sign of gastrulation. The invaginating cells change their shape dramatically. They constrict at their apical surfaces and expand at their bases, but remain attached with the outside surface by their slender neck. These cells are called **bottle cells**. The change in shape of the cells into bottle shaped cells is associated with an inward pulling movement which results in the formation of a small groove. The initial groove is probably due to a coordinated contraction of the apical ends of "bottle cells" in a plane perpendicular to their long axis as occurs in the invaginating epithelia in general. The opening of this groove is called the **blastopore**.

14.3.2 Involution of Endoderm

Before the bottle cells are formed, there is an internal cell rearrangement that pushes the cells of the dorsal floor of the blastocoels made up of vegetal cells towards the animal cap. This rotation of the vegetal cells places the pharyngeal endoderm cells adjacent to the blastocoel immediately above the mesodermal cells that begin to move inside (Fig.14.7). Pharyngeal endoderm cells start migrating along the roof of the blastocoel towards the future anterior end of the embryo.

The initial shallow groove formed in the **dorsal blastopore lip** is the beginning of a groove or cavity known as **archenteron**. As the bottle cells which invaginate to form the groove leave the dorsal lip, their place is taken by new groups of cells derived from the more anterior regions of the marginal zone (gray crescent). They roll over the dorsal lip (involute) and continue to migrate inward deepening the groove. These endodermal cells that migrate from the superficial marginal zone form the roof of the deepening groove which becomes the tubular archenteron. This lining of cells is the future gut lining.

The superficial cells of the marginal zone that form the lining, migrate in passively because they are pulled along with the active migration force of the deep cells that are present under the superficial cells of the marginal zone and that also start to involute (you must remember that all the cells that are migrating inwards are doing so at the same time though we describe the movement step wise).

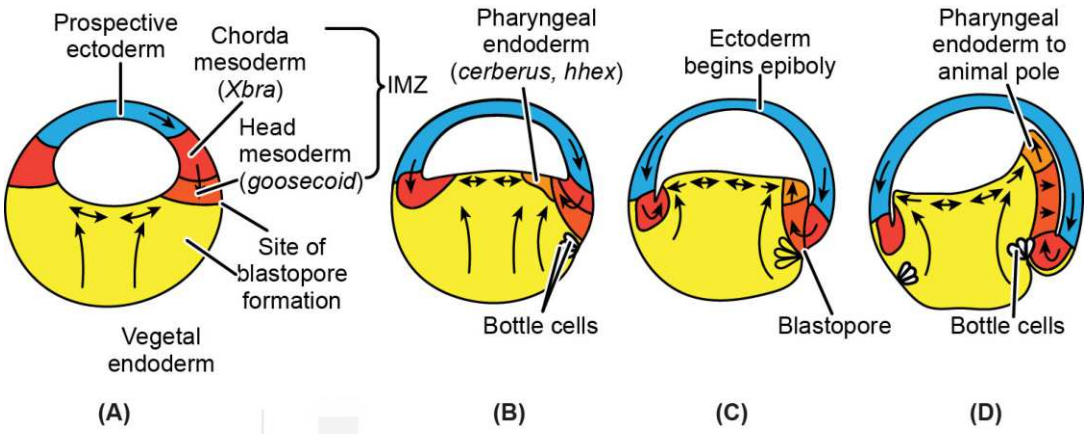


Fig.14.7: (A) At the beginning of gastrulation, the involuting marginal zone (IMZ) forms. Pink represents the prospective head mesoderm. Chordamesoderm is red; (B) Vegetal rotation (arrows) pushes the prospective pharyngeal endoderm (orange) to the side of the blastocoel. (C & D) The vegetal endoderm (yellow) movements push the pharyngeal endoderm forward, driving the mesoderm passively into the embryo and toward the animal pole. The ectoderm (blue) begins epiboly. (After Winklbauer and Schürfeld 1999)

Experiments show that endodermal lining along with the attached bottle cells is moved passively because it has been observed that removing the bottle cells that are attached to the tip of the archenteron, does not stop the migration. However it is seen that if the deeper involuting marginal cells are removed then the archenteron does not form. Continued inward migration of the involuted cells extends the archenteron into the blastocoel anteriorly until its tip reaches up to the inner surface of the animal pole region (Fig.14.7D).

As the archenteron expands the blastocoel cavity gets reduced and ultimately is obliterated. The large yolky cells of the vegetal zone also perform slow inward streaming movements so that the cells initially situated in the vegetal zone just below the initial groove reach the anterior part of the archenteron floor and those from the vegetal pole region come to be located in its posterior part.

Thus the first cells to undergo involution over the dorsal lip of the blastopore are the **pharyngeal endodermal cells**.

14.3.3 Involution of Mesoderm

The presumptive mesodermal cells lie in the deep marginal zone while a thin layer of endodermal cells lie in the superficial marginal zone. During early gastrulation, several layers of deep involuting marginal zone cells interdigitate

by a process known as radial intercalation to form a thin broad single layer. Further intercalation takes place as the cells involute into the embryo. This intercalation causes a convergent extension thereby, integrating several mesodermal streams to form a long narrow band (imagine on the road, several rows of cars are lined side by side and they all have to move forward to form one row of cars that will enter a single lane). The anterior part of this band migrates towards the animal cap followed by continued migration of mesodermal streams. The radial and mediolateral intercalations of the deep cells appear to be responsible for the continued movement of mesoderm into the embryo (see Fig.14.8 to understand this process).

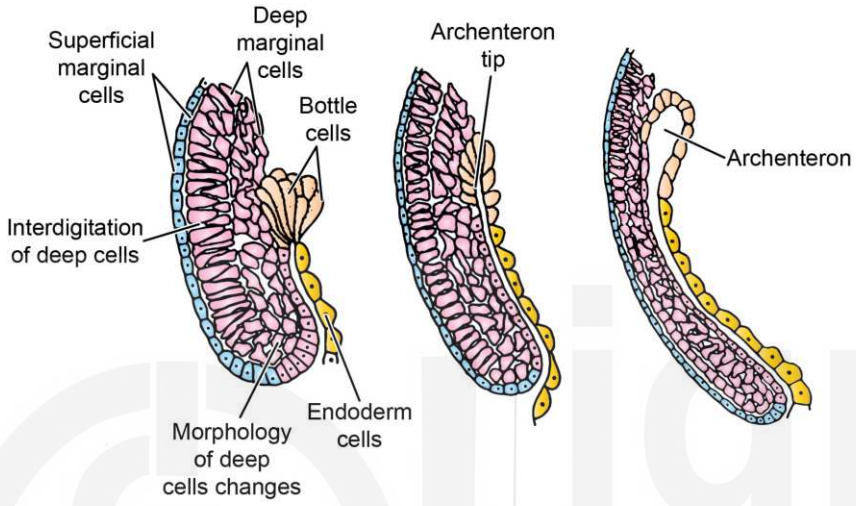


Fig.14.8: During early gastrulation the cells in the deep marginal zone show interdigitation to form a band of mesodermal cells and move in by involution. As they move in they drive the movement of surface endodermal cells with the bottle cells leading and the archenteron lengthening.

The extension of the dorsal mesoderm by convergent cell movement is due to several factors:

a. **Polarized cell cohesion:** in which the involuting mesodermal cells send out protrusions to contact one another which requires an extracellular fibronectin matrix under the ectodermal roof of the blastocoel.

b. **Differential cell adhesion:** genes encoding for axial protocadherin in notochordal cell and paraxial protocadherin in the somatic mesodermal cells ensure that proper cell sorting occurs so that only the trunk mesoderm undergoes convergent extension but head mesoderm does not

c. **Calcium flux:** also seems to regulate convergent extension by regulating the contraction of actin microfilaments. A surge of calcium ions across the dorsal tissue undergoing convergent extension causes contraction in the tissue. This was shown to be true because if calcium release from the intracellular store is blocked, no convergent extension occurs.

Involution of cells into the embryo follows a precise sequence. As explained earlier, the pharyngeal endoderm cells are the first to involute over the dorsal lip of the blastopore. As the cells move to the interior they are followed by

prechordal plate cells (precursor the of head mesoderm). The next cells involuting into the embryo through dorsal lip of blastopore are the **chordamesodermal cells** (precursor of notochord). Thus the cells that form the dorsal lip are constantly changing as one type of cells follows the previous type.

Meanwhile the blastopore lip also extends laterally forming the **blastopore crescent** with **lateral lips** (14.9 b &c). At the same time the ectodermal cells of the animal hemisphere begin to cover the embryo by epiboly to converge at the extending blastopore. As epiboly continues, a **ventral lip** of the blastopore forms and soon it becomes a complete circle (Fig.14.9 d).Invagination of cells is much more extensive around the dorsal lip of the blastopore than at the lateral and ventral lips. The yolky endodermal cells of vegetal hemisphere encircled by the blastopore form the yolk plug (Fig.14 d). By the end of gastrulation, blastopore is reduced to a narrow vertical slit. (Fig.14.9 e).

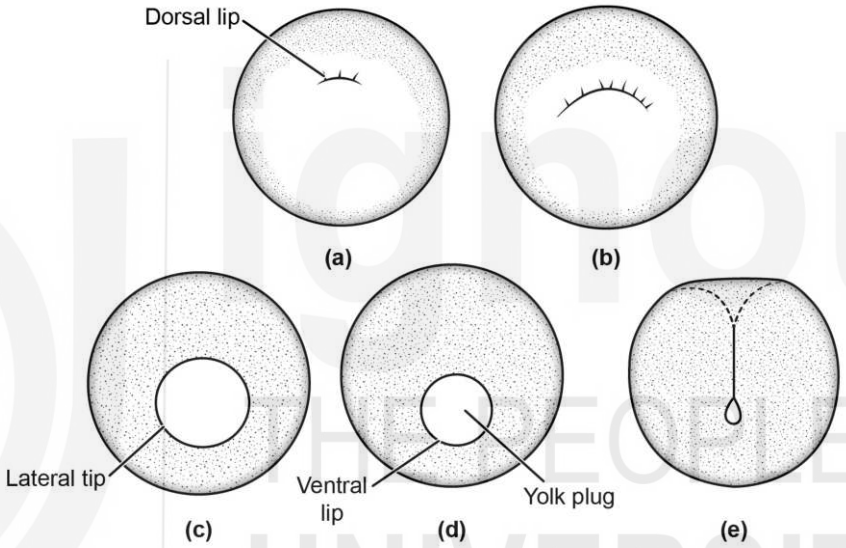


Fig.14.9: Formation and closure of the blastopore. a) formation of blastopore dorsal lip; b) elongation of blastopore slit laterally; c) and d) completion of blastopore with dorsal, lateral and ventral lips enclosing the yolk plug and; e) end of gastrulation in which the blastopore is a narrow vertical slit.

Later the prechordal plate becomes a part of archenteron roof in front of the anterior end of the notochord. The anterior part of the notochord extends along the dorsal side of the archenteron and occupies the mid-dorsal region of the archenteron roof. Meanwhile, the presumptive posterior notochordal cells involute over the dorsolateral lips of blastopore. Later they join the anterior notochordal cells. Thus those mesodermal cells that enter over the dorsal lip form the central dorsal mesoderm (notochord and somites) and majority of the mesoderm consisting of presumptive somitic mesoderm and ventrolateral mesoderm involute by rolling over the lateral and ventral lips of blastopore. Once the involution of the mesoderm is over, these cells move from the posterior (blastopore) side towards the anterior side as a single layer called **chordamesodermal mantle**. It occupies a space between the ectoderm and endoderm, except for a small part at the anterior end of the gastrula. This part does not contain mesoderm and later forms the mouth. The point where the endoderm and ectoderm meet at the blastopore becomes the anus.

14.3.4 Epiboly of Ectoderm

From the time of the first appearance of the blastopore slit on the dorsal side, the presumptive ectoderm, including epidermal and neural, expands to cover the whole embryo by a process known as epiboly. As gastrulation proceeds the cells of the animal cap and non involuting marginal zone cells (NIMZ) expand by epiboly to cover the entire embryo. This process helps in the formation of the circular blastopore and in the enclosing of all the yolky endodermal cells inside the embryo. These presumptive ectodermal cells form the surface ectoderm. The expansion of ectoderm is a very active process. There are various mechanisms to explain the process of epiboly. One such mechanism involves i) increase in cell numbers by cell divisions and cell flattening in the superficial layer ii) intercalation of several deep layers into one layer by radial intercalation. Second, mechanism of epiboly involves assembly of fibronectin fibrils by the blastocoel roof. This fibrillar fibronectin plays an important role in migration of vegetal cells and the animal cap cells and also in enclosure of the embryo.

As a result of epiboly the ectoderm covers the whole embryo after the mesoderm and the endoderm have disappeared into the interior of the embryo (Fig.14.10).

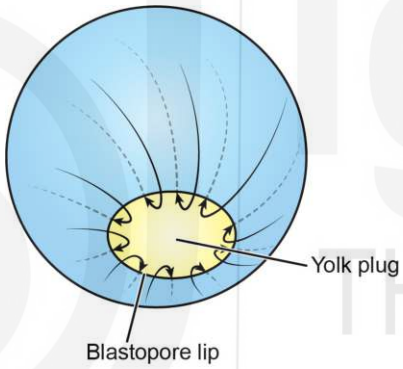


Fig 14.10: Epiboly of ectoderm to enclose all the prospective endodermal cells. The arrow indicate involution of vegetal cells through the narrowing blastopore which also shows the yolk plug.

See Fig 14.11 that summarizes the cell movements in gastrulation. At the end of gastrulation the following features are seen in the developing zygote:

1. Radially symmetrical monoblastic blastula is converted into triploblastic gastrula having anterior–posterior axis with the head in at one end and the future tail at the other.
2. Externally gastrula is covered by ectoderm. Mesoderm and endoderm occupy the internal position. Thus the three germ layers move into positions in which they will develop into structures of the larva/adult.
3. The ectoderm forming the mid dorsal band is the presumptive nervous system and the rest of the ectoderm forms epidermis.
4. The gastrocoel or archenteron is the presumptive lumen of the gut
5. The roof of archenteron is the chordamesodermal mantle and the floor and sides are formed of endodermal cells

The gastrula then undergoes a process of neurulation and becomes the neurula.

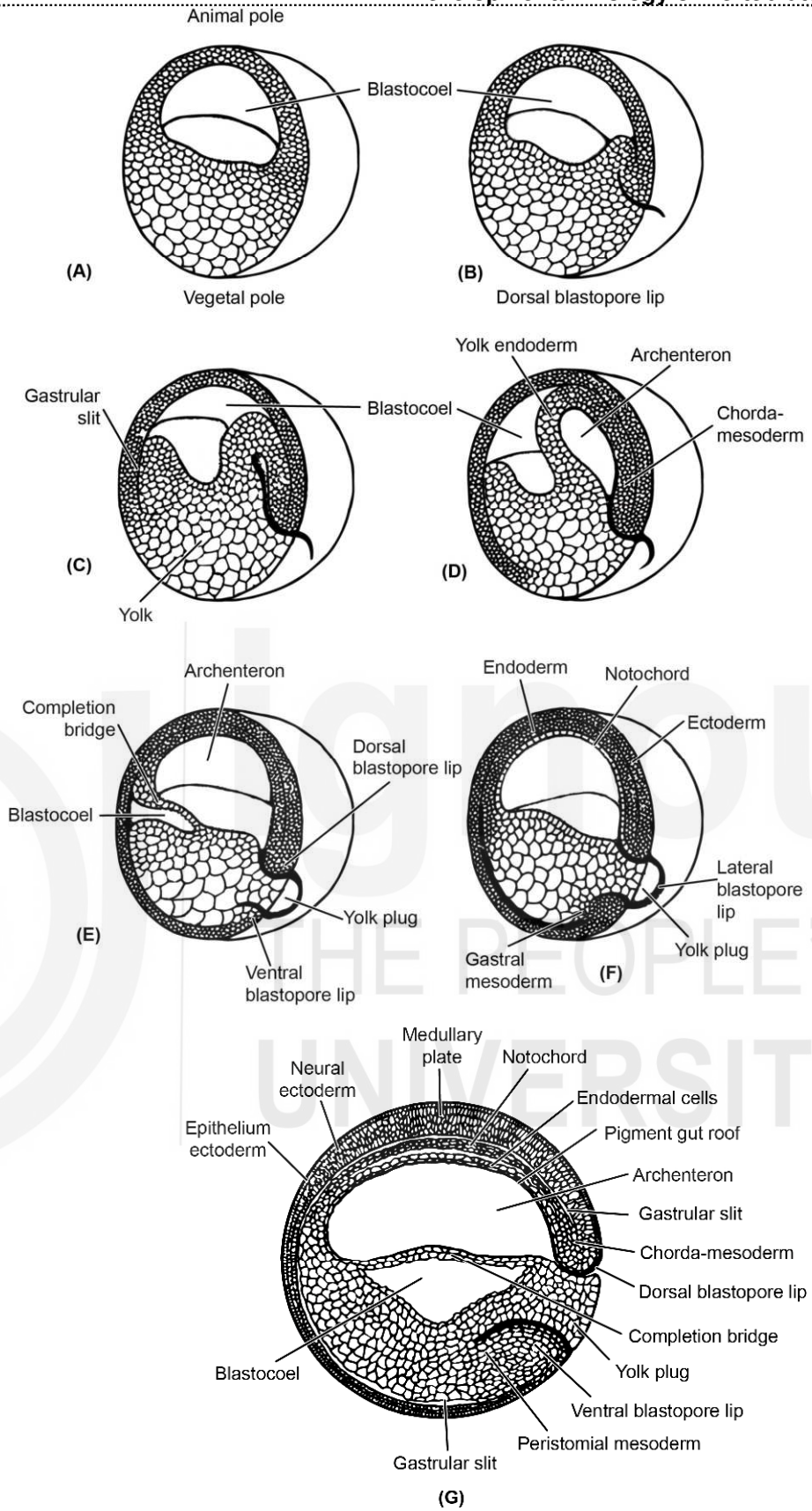


Fig.14.11: Cell movements in gastrulation: (A to G) drawings of section cut through the middle of the embryo. (B) shows the formation of blastopore lip on the dorsal side. (C&D) Archenteron forms by involution of endoderm and displaces the blastocoels, the cells migrate from the lateral and ventral lips of the blastopore. (E&F) the blastocoels is reduced and the embryo is surrounded by ectoderm while mesoderm is placed between the ectoderm and endoderm that is internalized. (G) Section of gastrula at the end of gastrulation.

SAQ 3

Match the following given in A with the terms given in B.

	A		B
a	The first cells to involute over the dorsal lip of blastopore	i	mouth
b	Cells carried passively at the tip of the archenteron	ii	chordamesoderm
c	Drives the involution of cells inside the embryo and carries other superficial cells with its force	iii	Lateral blastopore lip
d	The axial mesoderm that forms notochord	iv	Archenteron
e	Cells invaginating to form blastopore slit, located on dorsal side	v	Vegetal cells below grey crescent
f	Mesodermal cells that form part of archenteron roof, anterior to notochord	vi	Chordamesoderm mantle
g	Place for involution of presumptive somatic mesoderm	vii	Pharyngeal endoderm
h	Mesodermal cell sheet that moves from the blastopore side towards the anterior side	viii	Deep mesodermal cells
i	The anterior end of embryo where there is no mesoderm	ix	Prechordal plate
j	Presumptive lumen of gut	x	Bottle cells

14.4 NEURULATION

You have learnt that the dorsal mesoderm or the organizer region involutes to form the prechordal plate just ahead of the notochord. This will give rise to the ventral head mesoderm and the notochord that lies just behind it is formed from the chordamesoderm. When gastrulation is near completion the notochord becomes flanked on both sides by blocks of somatic mesoderm which start from the anterior to posterior direction. As this happens, the presumptive area of the nervous system becomes differentiated from rest of the ectoderm. This region of the embryo is called **neural plate**. The process by which this tissue forms a neural tube (rudiment of central nervous system) is called **neurulation.** An embryo undergoing such changes is called a **neurula.**

During neurulation, the original ectoderm is divided into following three sets of cells:

1. Outer ectodermal cells forming the epidermis of skin.
2. Neural ectodermal cells giving rise to central nervous system.
3. Neural crest cells that give rise to peripheral nervous system and a variety of other cell types which later migrate to distant regions as the embryo develops.

14.4.1 Neural Tube Formation

As mentioned earlier, neurulation begins with the formation of a **neural plate**. The presumptive area of the nervous system becomes differentiated from rest of the ectoderm and the cells become flat and thick in the form of neural plate. Cells of the neural plate change in shape, become elongated and arrange themselves into a columnar epithelium layer. During this process, the embryo lengthens along the anterio-posterior axis, narrowing itself so that subsequent bending will form a tube. In both amphibians and amniotes, the neural plate lengthens and narrows by convergent extension, intercalating several layers of cells into a few layers.

At the same time the edges of the neural plate become thickened and become raised above the general level as ridges called **neural folds**. Neural folds become higher and consequently there is formation of the neural groove, which extends throughout the length of the embryo. Neural folds meet each other in dorsal midline and fuse to form neural tube. At the time of rolling of the neural plate to form the neural tube, the neuroepithelial cells at the lateral folds of the neural plate develop constrictions at their apical edges. This changes the shape of columnar epithelial cells into pyramidal cones. As the surface area of apical end of neural epithelial cells becomes smaller relative to the basal surface, the rolling up of the neural plate and formation of neural tube takes place. The neural tube separates from the surface ectoderm and forms a closed cylinder. The epithelial cells at the surface form a continuous layer to cover the neural tube. The embryo at this stage is called **neurula**. Thus the change in the shape of cells in the neural tube results in the regionalisation of the neural tube. In the cephalic end, i.e. the end which will differentiate into brain, the wall of the tube is broad and thick and a series of swellings and constrictions develop that define the various divisions of brain. Caudally, towards the posterior end, the tube is simple and narrow, extending into the tail while the neural tube overlying the notochord will develop into spinal cord (Fig.14.12).

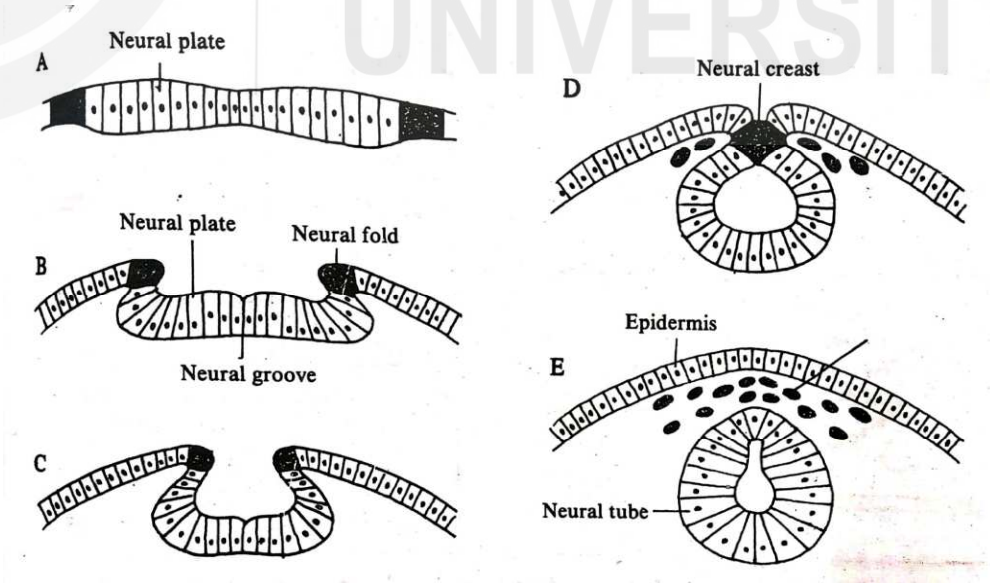


Fig.14.12: Stages in neurulation In frog: (A) thickening of dorsal ectoderm to form neural plate; (B) formation of neural folds and neural groove; (C) deepening of neural grooved) fusion of neural folds and formation of a neural tube; (D) neural tube has separated and is covered over by epidermis; (E) Neural crest cells have separated from neural folds.

The neural plate is induced both by early signals in the ectoderm and from signals from the mesoderm that comes to lie below the prospective neural plate ectoderm during gastrulation. Studies show that the notochord is necessary for neural plate formation. In *Xenopus* the organizer region is crucially important for inducing the anterio-posterior axis as well as the neural tissue from the ectoderm.

Separation of the neural tube from surface ectoderm is brought about by the expression of different cell adhesion molecules (recall cell adhesion and different cadherins from Unit 11). The cells of the surface ectoderm synthesize cell adhesion molecule, E – Cadherin. The ectodermal cells forming the neural tube which initially synthesize E-Cadherin, stop producing this protein and instead synthesize N – Cadherin and N – CAM. Because of the different cell adhesion molecules expressed, the neural tissue separates from the surface ectoderm. It has been observed that separation of neural tube from the surface ectoderm fails to occur if N – Cadherin m RNA is injected into the surface ectoderm of early *Xenopus* embryo.

To summarize, the process of neurulation can be divided into three distinct stages:

1. Formation and shaping of neural plate.
2. Bending of neural plate to form neural groove and neural folds.
3. Closure of the neural groove to form a neural tube.

14.4.2 Neural Crest Cells

Neural crest cells are induced at the borders of the neural plate. They rise up at neurulation to form the neural folds and form the dorsal most portion of the neural tube. These cells lie between the dorsal part of the neural tube and dorsal epidermis and run the full length on both sides of the neural tube(Fig. 14.12).Neural crest cells undergo extensive migration and form various types of cells. For example, neural crest cells give rise to glia, and neurons of the autonomic nervous system, the glial cells (Schwann cells) of the peripheral nervous system, pigment cells of the skin, adrenal producing cells of the adrenal glands. They also give rise to bone, cartilage, muscles of the facial region- cell types that would commonly be of mesodermal origin! The induction of neural crest cells in *Xenopus* is a multi-stage process that starts in the early gastrula stage and continues till the closure of the neural tube.

SAQ 4

Indicate whether the statements given below are **true or false** and correct the false statements.

i) The term neurulation refers to the partitioning of ectoderm, and the formation and inward displacement of neural tube. (T/F)

ii) During neural plate formation, the neuroeptithelial cells change in shape from columnar to pyramidal ones. (T/F)

iii) Neural cells are ectodermal in origin and migrate in the embryo to form various other tissue. (T/F)

iv) Cells of the notochord induce the formation of neural tube. (T/F)

14.5 METAMORPHOSIS

Metamorphosis is the post embryonic transformation of a larva of an organism into a juvenile adult. The changes during this period of development are dramatic and rather rapid. The phenomenon of metamorphosis is well established in amphibians, specially anurans (tail less amphibians) e.g. frogs and toads. They show dramatic metamorphic changes during their development, affecting nearly all organ systems and major functions. Amphibians are the first class of vertebrates to show transition from aquatic to terrestrial mode of life. Therefore, the metamorphic changes are associated with the change in their habitat. A number of features distinguish metamorphic changes from early embryogenesis and other post embryonic changes. The signals that bring about metamorphic changes are usually hormonal unlike the short term signals during embryogenesis that are typically protein growth factors. The synthesis of these hormones is directed by the central nervous system in response to environmental cues and there is a complex feedback mechanism that controls their production as well.

Figure 14.13 shows metamorphosis in frog. You can see that the egg develops into a larvae which is strikingly different from the adult in morphology. Apart from the appearance there are other significant changes in the physiology and biochemistry in the larva. Larva of frog is called tadpole. It is free swimming, and possesses a tail. It has three pairs of external gills covered by operculum for respiration. Locomotion is brought about by tail and chordal fin. The alimentary canal is long and coiled to aid herbivorous mode of digestion. Brain of tadpole is quite small and simple. Heart is two chambered consisting of auricle and ventricle.

14.5.1 Metamorphic Changes

The transformation of aquatic tailed tadpole larvae to a terrestrial adult frog involves major metamorphic changes. These can be classified as:

1. Ecological with respect to change in habitat.
2. Morphological with respect to change in morphology.
3. Physiological / Biochemical with respect to change in different bodily functions.

Ecological Changes:

The habitat of the tadpole larvae is fresh water whereas adult frog is land dwelling. Tadpole is herbivorous. Its horny beak and teeth help in rasping away the plant tissues. Frog is carnivorous feeding on insects and worms.

Morphological changes:

The morphological changes during metamorphosis are of three types; a) regressive changes, b) progressive changes and c) remodeling of existing structures in larvae.

a) Regressive changes: The organs or structures necessary during larval life, but redundant in adult are gradually reduced and ultimately

disappear in adults. Resorption of the long tail, chordal fin, external gills and ventral suckers take place. The gill clefts are closed. Peribranchial cavities are lost. Horny teeth and horny lining of jaw are shed. The cloacal tube gets reduced. Lateral line of the larva disappears. Aortic arches get modified due to reduced branchial arteries.

b) Progressive changes: Some organs develop and become functional only during and after metamorphosis. These involve the development and differentiation of fore limbs and hind limbs; the middle ear from the first pharyngeal pouch, tympanic cartilage and tympanic membrane; and hyoid apparatus from the pharyngeal arch to support the tongue which develops from floor of the mouth. The eyes protrude on the dorsal surface of the head developing eyelids and nictitating membranes.

c) Remodeling of existing larval structures: Some structures which function both in larval and adult form but undergo remodeling during metamorphosis. These involve basically skin, intestine and brain. The skin thickens and becomes multilayered with moucus and serous glands so that it remains moist. The skin acquires a characteristic color and pattern of pigmentation. The outer layer becomes keratinized. The long and coiled intestine shortens. Mesonephric kidney develops from pronephric kidney. Heart becomes three chambered from the earlier two chambered heart. Liver and pancreas become functional. Brain gets highly differentiated.

Biochemical / Physiological changes:

Along with morphological changes, certain biochemical / physiological changes take place during metamorphosis as explained below:

a) Tadpole larva is ammonotelic, excreting nitrogenous waste as ammonia which can be easily disposed off by diffusion in the aquatic medium. After metamorphosis, adult frog becomes ureotelic, excreting urea. This change occurs when the liver starts producing appropriate enzymes for the urea cycle.

b) The larval eye pigment porphyropsin is replaced by rhodopsin.

c) The respiratory pigment, larval haemoglobin is replaced by adult haemoglobin having more oxygen carrying capacity.

d) The site of erythropoiesis changes from liver to bone marrow and spleen.

e) Various digestive enzymes and hydrolytic enzymes are secreted.

f) Amoeboid macrophages by phagocytosis bring about autolysis of larval organs like gills and tails etc.

g) Shrinkage of body occurs because of suspended feeding and loss of some body parts like tails and gills. As a result degrowth (reduction in body mass) occurs. Head and trunk become small in adult.

Some important metamorphic changes seen in frog are summarized in Table 14.1

Table 14.1

System	Larva	Adult
Locomotory	Aquatic; tail fins	Terrestrial; tailless tetrapod
Respiratory	Gills, skin, lungs; larval hemoglobins	Skin, lungs; adult hemoglobins
Circulatory	Aortic arches; aorta; anterior, posterior and common jugular veins	Carotid arch; systemic arch; cardinal veins
Nutritional	Herbivorous; long spiral gut; intestinal symbionts; small mouth, horny jaws, labial teeth.	Carnivorous; short gut; proteases, large mouth with long tongue.
Nervous	Lack of nictitating membrane; porphyropsin, lateral line system, Mauthner neurons	Development of ocular muscles, nictitating membrane, tympanic membrane; rhodopsin; lateral line system lost, Mauthner neurons degenerate
Excretory	Largely ammonia, some urea (amoniotellic)	Largely urea; high activity of enzymes of ornithine-urea cycle (urotelic)
Integument	Thin, bilayered epidermis with thin dermis; no mucous or granular glands.	Stratified squamous epidermis with adult keratins; well-developed dermis contains mucous and granular glands secreting antimicrobial peptides

Three distinct stages of Metamorphosis:

Morphologically as seen in the Figure 14.13, the typical frog metamorphosis is divided into three stages.

1. Premetamorphosis – it is the first stage of metamorphosis when the tadpole has no limbs. This is the initial period of extensive growth but little developmental changes. Size of tadpole increases.

2. Prometamorphosis – during this stage developmental changes occur such as shortening of tail, gills becoming internal and growth of hind limbs take place.

Metamorphic climax – emergence of forelimbs marks the beginning of metamorphic climax. It is a brief period in which morphological changes occur very rapidly in quick succession including full limb growth and tail resorption.

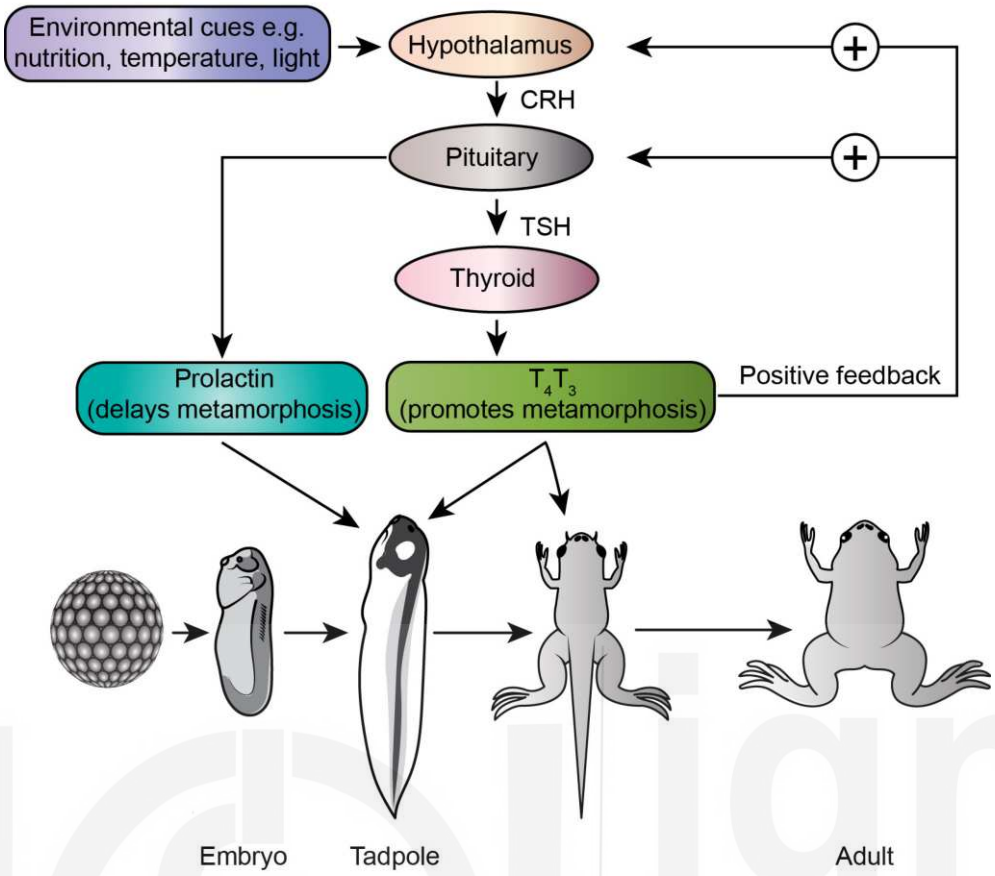


Fig.14.13: Environmental and nutrional cues cause the initiation of metamorphosis by secretion of corticotrophin stimulating hormone (CRH) from the larval hypothalamus which acts on the pituitary gland to release TSH. This in turn acts on the thyroid stimulating hormone (TSH) to release T4 and T3 that cause metamorphosis to happen. The thyroid hormones also act on the hypothalamus and pituitary to maintain the secretion of CRH and TSH.

These morphologically defined stages are influenced by different hormones which regulate the process of metamorphosis.

SAQ 5

i) Fill in the blanks with suitable words:

a) In anurans, metamorphosis is usually associated with a transition from …… to …… mode of life.

b) The three distinct stages of metamorphic changes in anurans are ……... and ……..

c) The three categories of morphological changes occurring during the metamorphosis of amphibians are ……, ……. and …… changes.

ii) Indicate the following changes that occur during metamorphosis in amphibians either as progressive or regressive or remodeling

a) The development of middle ear in connection with the pharyngeal pouch.

b) The change in the shape of the mouth and the shortening and reduction of the cloacal tube.

c) Disappearance of lateral line organs of skin and reduction of blood vessels.

d) The differentiation of brain.

e) The changes in the portal system and the changes in the vascular system to supply blood to the lungs.

f) The shortening and straightening of intestine.

g) The conversion of the heart into a three chambered one.

h) The development of fore and hind limbs.

i) Closing of gill clefts and loss of horny teeth.

j) Development of tongue from the floor of the mouth.

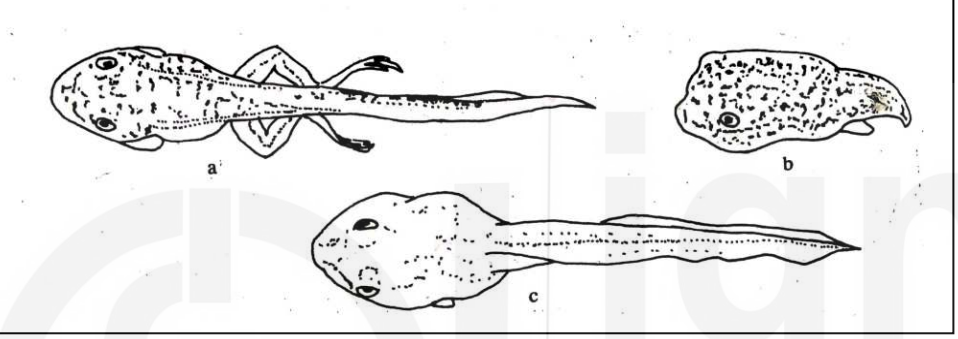
14.5.2 Hormonal Regulation

In amphibians, nutritional status and environmental cues such as, light and temperature, cause the neurosecretory cells of the hypothalamus to release **corticotrophin- releasing hormone (CRH)**. This hormone acts on the pituitary gland to release **thyroid stimulating hormone** (**TSH**) which in turn causes the release of thyroid hormones. This action of corticotrophin releasing hormone is peculiar to non-mammalian vertebrates and occurs in *Xenopus* only at the tadpole stage. In the adult frogs the release of thyroid stimulating hormone is due to **thyrotropin stimulating hormone**. The principal hormones which regulate metamorphosis in frog are **thyroid hormones** (TH) secreted by the thyroid gland (see Box 14.1). **Prolactin** and thyrotropic hormones released from the pituitary along with thyroxin from thyroid gland regulate metamorphic events in a sequential and coordinated manner (see Fig. 14.13).

The thyroid hormones exist as thyroglobulin in the thyroid follicles. These large proteins consists of tri-iodothyronine (T 3) containing three iodine atoms attached to tyrosine and tetra – iodothyronine (T 4) containing four iodine atoms attached to tyrosine. T 3 and T 4 are released during metamorphosis from the thyroid gland. T 4 is the precursor while T 3 is the active form of hormone. T 3 causes metamorphosis in thyroidectomized tadpoles in much lower concentration than T 4. Hypothalamus receives the exogenous signals such as temperature, light and nutrition etc. to release neurosecretory hormones. Pituitary also secretes another hormone, prolactin which is antagonistic to T 3. It inhibits metamorphosis and promotes growth in the larva. The action of prolactin is suppressed by dopamine produced by hypothalamus. Hypothalamus is the controlling centre for maintaining a balance between thyroid hormones and prolactin. During premetamorphosis, prometamorphosis and metamorphic climax the concentration of thyroid hormones circulating in blood vary, resulting in metamorphic changes.

Box 14.1

The role of thyroid hormone in metamorphosis was first demonstrated by Gudernatsch in 1912. He discovered that the tadpole when fed with powdered sheep thyroid glands, metamorphosed prematurely. Allen in 1916 showed that when thyroidectomy (removal of thyroid gland) was done on early tadpoles, the larvae failed to metamorphose and grew into giant tadpoles. If these tadpoles were fed dried powdered thyroid gland or immersed in iodine solution, they underwent metamorphosis. Subsequent studies by E.W.Etkin (1968) demonstrated the important role played by various hormones during metamorphosis. In the diagrams given below (a) shows normal metamorphic stage; (b) shows a tadpole exposed to thyroxin in the earlier stage undergoes premature metamorphosis and (c) shows the effect of removal of thyroid or pituitary gland which results in inhibition of metamorphosis



During premetamorphosis thyroid gland is still developing therefore, the **TH/Prolactin ratio is very low**. Prolactin secreted by pituitary gland stimulates growth of larval body. As hypothalamus develops in premetamorphosis, it produces CRH which causes rise in the level of TSH. As a response to this, T 3 and T 4 begin to rise causing premetamorphic changes. It also exerts positive feed back effect on the pituitary. As pituitary develops further, it causes increased flow of CRH between hypothalamus and pituitary. Hypothalamus releases prolactin inhibitor, causing increase in T 3 and T 4 and decrease in the level of prolactin. **In prometamorphosis, TH / prolactin ratio is intermediate.** When the level of T 3 and T 4 reaches the threshold, rapid metamorphic changes take place. *At this stage, TH/Prolactin ratio becomes very high*. High concentration of T 3 and T 4 exert a negative feed back on thyroid and hypothalamus causing decrease in CRH. Consequently thyroid gland partially degenerates.

Conversion of T 4 to its more active form T 3 takes place in the target tissue by the enzyme De – Iodinase II. Thyroid hormone acts on target cells by crossing the plasma membrane and interacting with thyroid receptors (TRs). T 3 binds to the nuclear thyroid receptors which has higher affinity than T 4. There are two types of thyroid hormone receptors; namely **TRα** and **TRβ**. Expression of thyroid receptors is under developmental control. Studies on *Xenopus* have shown the presence of TRα in all the tissues even before development of thyroid gland in the organism. The level of TRβ in the target tissue increased with high level of thyroid hormone as metamorphosis progresses. The thyroid receptors activate gene expression during

metamorphosis in the presence of TH. **In prometamorphic** tadpoles, the receptors are unliganed and so gene expression is repressed in the absence of TH to prevent metamorphosis, thus ensuring proper tadpole growth period. However, in the presence of TH, transcription is activated. At **metamorphic climax** the level of TR β reaches maximum due to which, rapid metamorphic changes take place.

In summary, metamorphosis in frog is regulated hormonally in a sequential manner as follows:

1. The thyroid gland secretes hormone thyroxin, T 4 into the blood.
2. T 4 reaches the target tissue and is converted to by enzyme Deiodinase II T 3 which is a more active form of the hormone.
3. T 3 binds to the nuclear thyroid hormone receptors. T 3 has a higher affinity to bind to thyroid receptors (TRs) than T4.
4. T 3-TR complex binds with retinoid receptor forming a complex which activates transcription of genes responsible for metamorphic changes.

Tissue reactivity and competence:

As described earlier, TH brings about diverse metamorphic changes. The hormone acts directly on the target tissue. This was demonstrated by implanting a pellet of inert material absorbed with T 4 on one side of the snout of frog. This caused widening of the mouth on that side only where the pellet was implanted. This clearly indicated that the hormone acts directly on the responding tissue.

Diversity of tissue response to T 4 is very striking i.e. different tissues respond to the hormone differently. The muscles of tails and fins etc. regress whereas, the tissues of limbs differentiate and grow as a response to the hormone. As you have learnt before in Unit 11, this difference in the reactive ability of different tissues to thyroid hormone is known as Competence and only cells that are competent to respond to TH do so.

Threshold and rate of response to thyroxin hormone:

Different tissues are sensitive to different doses of thyroxin level. This is known **as threshold value.** Experiments have shown that different tissue have different threshold values e.g. forelimbs require a high concentration of thyroid hormone to develop than hind limbs which are sensitive to low threshold value. For resorption of tail much higher concentration of the hormone is needed during metamorphic climax (that is why tail is reabsorbed only after the limbs grow). Thus each tissue type is responsive to a certain threshold for reactivity. Once this threshold is reached, the tissue responds.

When a very high dose of hormone is supplied to a young tadpole, all the metamorphic changes take place at once. This results in a chaotic situation leading to its death. Thus, developmental events during metamorphosis are sequential with progressively increasing threshold of the hormone.

SAQ 6

Indicate whether the following statements are true or false:

i) CRH secreted by the pituitary gland activates the thyroid gland to secrete thyroid hormone. (T/F)

ii) Tetraiodothyronine (T4) is a less potent hormone and is the precursor of triiodothyronine (T3). (T/F)

iii) Prolactin has an antagonistic action to thyroxine, and so promotes larval growth and inhibits metamorphosis. (T/F)

iv) The reactivity and responses of target tissues of the tadpoles to the thyroxine hormone are extrinsic and non-specific. (T/F)

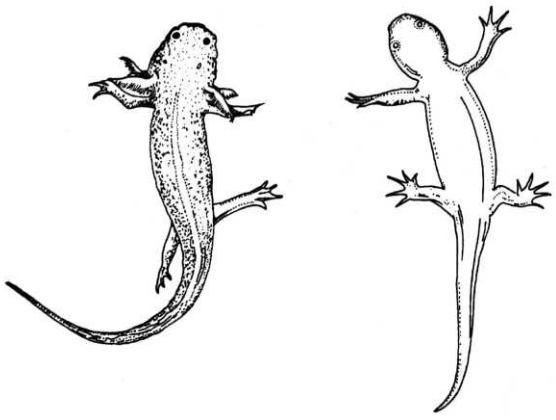
v) According to the threshold concept, different tissues of the tadpole larva are sensitive to different concentrations of the thyroxine. (T/F)

vi) Expression of thyroid hormone receptors in cells is under development control and not dependent on release of thyroid hormone. (T/F)

14.6 NEOTENY

Neoteny is referred to as retention of larval characters in sexually mature adult animals. Many urodeles (tailed amphibians) exhibit this interesting phenomenon of neoteny.

In some Salamanders, juvenile characters are retained by retarded body development while the gonads achieve maturity at the normal time. *Ambystoma mexicanum* (Mexican axolotl) does not undergo metamorphosis in nature. Sexually mature form has external gills and a long tail with dorsal and ventral fins and an reproduce (Fig.14.14 a). Failure of metamorphosis in the larva is because of absence of TSH from the pituitary gland. Axolotl can be made to metamorphose into an adult form when it is administered with either a thyroid hormone or thyrotropin (fig.14.14 b). This indicates that Axolotl larvae do have functional thyroid hormone receptors as they respond to the artificially administered thyroid hormone.



(a) (b)

Fig. 14.14: The sexually mature aquatic Mexican axolotl retains larval features like gills but reproduces. If the neotenic form is administered thyroxine which it does not produce, it metamorphoses to become the terrestrial salamander.

Some species of *Ambystoma* like *A. tigrinum* fail to metamorphose until environmental conditions change. The aquatic form *A.tigrinum* remain neotenic. However, if provided with terrestrial environment it metamorphosis into a land dwelling adult, tiger salamander. Some urodeles, e.g. *Necturus* and Siren exhibit permanent neoteny in which the tissue fail to respond to even artificially administered TH. Though the functional TH receptors are present in these, they do not activate those genes that initiate metamorphosis.

SAQ 7

Match the statement on the right side (A) with the species on the left (B).

A		B	
a)	No metamorphosis in nature, retention of all larval characters but sexually mature.	i)	*Ambystoma tigrinurn*
b)	Metamorphosis occurs only if environmental cues are provided. Otherwise neotenous larvae successfully reproduce	ii)	*Necturus*
c)	Administration of thyroxine or TSH results in the metamorphosis of the otherwise neotenous larva into an adult	iii)	*Ambystoma mexicanum*
d)	Administration of thyroid hormone has no effect on the larva as the larval tissues have lost their capacity to respond to thyroid hormone. These forms show permanent neoteny		

14.7 SUMMARY

In this unit you have learnt:

- Amphibian cleavage is holoblastic, but it is unequal because of the presence of yolk in the vegetal hemisphere. The mature unfertilized egg of frog already has a distinct animal-vegetal axis laid down by maternal genes in the egg during its development in the ovary. The dorsal-ventral axis is specified by the site of sperm entry and the resulting cortical rotation brings the maternal factors opposite to the SEP on the prospective dorsal side.
- The blastula is composed of thousand of cells with micromeres in the animal hemisphere and macromeres in the vegetal hemisphere with an eccentric cavity called blastocoel. The fate map of blastula shows presumptive ectoderm in the animal zone and endoderm in the vetal zone with a belt like marginal zone, induced by the underlying vegetal cells and the dorsal organizer region.
- Amphibian gastrulation begins with the formation of blastopore on the dorsal surface, just below the grey crescent by invagination of the bottle cells. This is followed by the coordinated involution of the mesoderm and the epiboly of the ectoderm. Vegetal rotation plays a significant role in

directing the involution. The driving forces for ectodermal epiboly and the convergent extension of the mesoderm are the intercalation events in which several tissue layers merge and migrate into the embryo.

- The result of gastrulation is the formation of ectoderm including epidermal and neural ectoderm, covering the surface of gastrula with the internalisation of endoderm with the placement of mesodermal cells between the two. The blastocoels get obliterated by the formation of a new cavity, the archenteron. The blastopore becomes the anus and the mouth develops as a new cavity on the ventral surface.
- Gastrulation is followed by the process of neurulation in which the neural plate is induced in the ectorderm followed by formation of neural tube and the neural crest cells. Differential cell adhension molecules are mainly responsible for separation of neural tissue from the surface ectoderm.
- In frogs the embryos develop and hatch as a larval stage tadpole which is free swimming and undergoes a process of metamorphosis before it is transformed to the young adult. Metamorphosis entails morphological, biochemical and physiological changes in the larva that can be catergorised into progressive, regressive or remodelling events regulated by thyroid hormones.
- Some amphibians (urodeles) retain their larval characters though they attain sexual maturity by a process called neotany. Some species showing neotany can be induced to undergo metamorphosis by exposure to thyroid hormones or environmental cues, while some may show permanent neoteny.

14.8 TERMINAL QUESTIONS

1. How are the body axes determined in frog?
2. Describe the process of internalisation of mesoderm in frog. What are the end results of the gastrulation process?
3. Make a flow chart to show the events in metamorphosis.
4. Describe the process of neurulation in frog.
5. Why is mid-blastula transition stage significant?

14.9 ANSWERS

Self Assessment Questions

1. i) organizer; dorso-ventral axis; ii) dorsal;
   iii) vegetal; left; right; iv) animal; vegetal;
   v) zygotic.
2. i) notochord;
   ii) epidermis and nervous tissue;

iii) paracrine signals from endodermal cells of vegetal pole and signals from organizer;

iv) endoderm.

3. a) vii; b) x; c) viii; d) ii; e) v;
f) ix; g) iii; h) vi; i) I; j) iv

4. i) T ii) F iii) T iv) T

5. i) a) aquatic, terrestrial;
b) premetamorphosis; prometamorphosis and metamorphic climax;
c) regressive, progressive and remodelling.

ii) a) progressive; b) regressive;
c) regressive; d) remodelling;
e) progressive; f) remodelling;
g) remodelling; h) progressive;
i) regressive; j) progressive

6. i) F ii) T iii) T iv) F v) T vi) ….

7. a) matches iii); b) matches i);
c) matches iii); d) matches ii).

Terminal Questions

1. Refer to section14.2
2. Refer to section 14.3.3
3. Refer to section14.5
4. Refer to section 14.4
5. Refer to section 14.2.3

UNIT 15

DEVELOPMENT OF CHICK

Structure

15.1 INTRODUCTION

Different animals have evolved a variety of strategies of development. However, since all animals are related, the basic mechanism of early development has been conserved in the course of evolution, and so there are some important similarities in early embryonic development of all metazoan animals as you have already learnt in Block 3.

This unit speaks about development of chick as an example of an amniote organism. Recall that amniotes are those vertebrates (reptiles, birds and mammals) that have a water sac or amnion surrounding the developing

organism protecting it from the external environment. Chick has been one of the first model organisms to be studied in detail as it is easy to maintain and large enough to be manipulated surgically and genetically during all stages of development.

You will study about strictly coordinated sequential changes that take place during the course of chick development viz. fertilisation, cleavage, formation of morula, blastula, gastrula and organogenesis that give rise to the new individual.

Chick egg is macrolecithal which undergoes discoidal and meroblastic cleavage. Cleavage results in morula which is converted into blastula. During gastrulation there is displacement of the parts of blastoderm so that the presumptive endodermal and mesodermal cells are removed and brought into the interior of embryo through morphogenetic movements. Neurulation, the next step, consists of the formation of first the neural plate and then the neural tube. Then due to future changes and development of extra embryonic membranes, the new organism emerges.

Objectives

After studying this Unit you should be able to:

- describe the structure and fertilization of chick egg;
- describe cleavage and formation of blastula in chick egg;
- discuss the process and mechanism of chick gastrulation;
- describe the morphogenetic processes that lead to the formation of neural tube and neural plate in chick;
- discuss the development of extra embryonic membranes in chick; and
- compare the development of chick with that of frog.

15.2 STRUCTURE OF EGG OF CHICK

The egg is about 3.0 cm in diameter and is macrolecithal. It is entirely filled up by the yolk and over it, i.e., in animal pole lies a small cytoplasmic disc with a nucleus. This disc is called blastodisc.

The yolk contains 48.7% water, 32.6% phospholipids and fats, 16% proteins, 1% carbohydrates and 1.1% other chemical molecules.

Yolk is covered by a layer of dense viscous albumen which forms a thin chalaziferous layer around the vitelline membrane of the yolk. This dense albumen forms two twisted cords or chalazae, one at each end of the egg. They are formed by rotation of the egg during its movement through the oviduct (Fig.15.1).

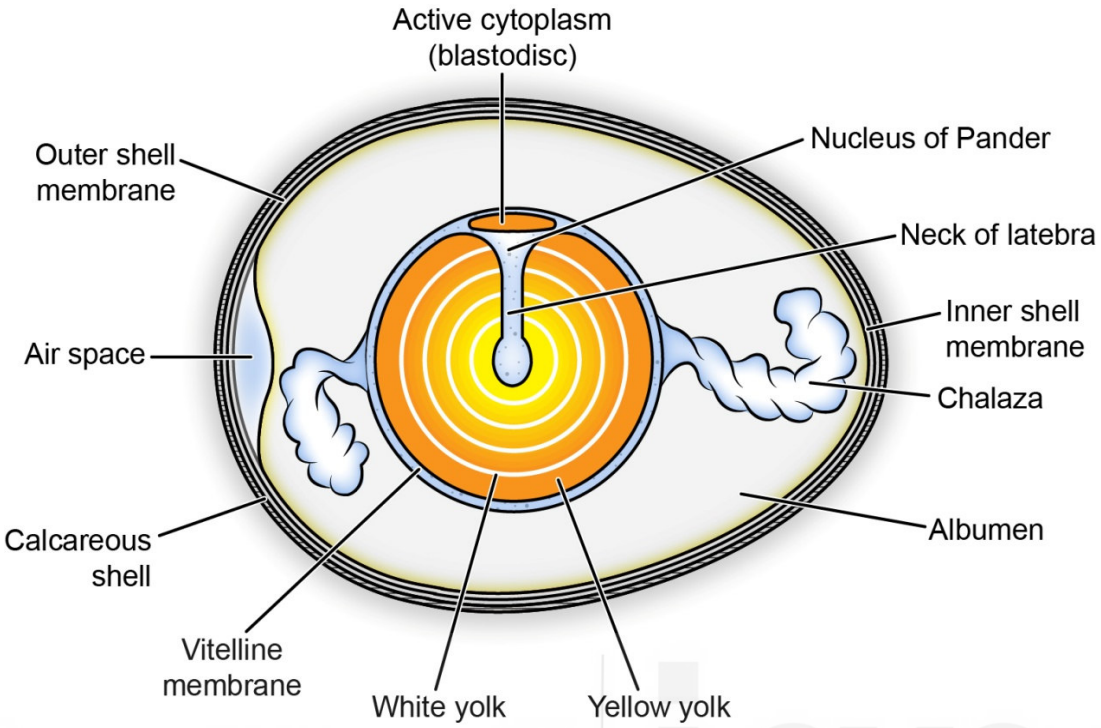


Fig.15.1: Diagrammatic longitudinal section of a hen's egg.

Around the chalaziferous layer is a thick layer of watery albumen. All albumen is secreted by the upper glandular walls or magnum of the oviduct. The functions of albumen are to provide nutrition to the embryo, serves as a water store and also acts as protective envelope for the embryo protecting it from mechanical and chemical injuries.

The albumen and yolk also contain a variety of enzymes, vitamins, pigments and phosphorus. The isthmus part of the oviduct secretes two shell membranes made of tough keratin fibres matted together.

The two shell membranes are closely applied except at the blunt end of the egg where they are separated by an air space formed after the egg is laid. The nidamental glands of the oviduct secrete a porous, calcareous shell over the two membranes which soon hardens after the egg is laid. The shell is pierced by a large number (about 7,000) of fine pores filled with a protein related to collagen. The diameter of the pores varies from 0.04 to 0.05 mm. These pores allow exchange of gases (oxygen and carbon dioxide) during respiration of the developing embryo. Once the egg is laid there is a cooling in the egg temperature and a contraction of the egg mass creating an air space between the two shell membranes at the broad end of the egg.

SAQ 1

Choose the correct word from the parentheses.

a) The egg of hen is (macrolecithal/microlecithal).

b) Cytoplasmic disc with a nucleus at animal pole is called (blastoderm/blastodisc).

15.3 FERTILISATION

The ova are released from the ovary in the form of primary oocytes. The second maturation division occurs after ovulation in the oviduct. The released primary oocyte in the body coelom is grasped and swallowed by the ostium of the oviduct.

The secondary oocyte or ovum after second maturation division is surrounded by several (5 or 6) sperms which enter in it (polyspermy), but only one sperm succeeds in the fertilization process. The nucleus of one sperm fuses with the female nucleus (amphimixis) and the nuclei of other sperms degenerate.

As the fertilised egg passes downward inside the oviduct, it rotates, undergoes cleavage and various accessory egg membranes are laid down over the developing egg. Thus, fertilisation is internal. Various egg membranes secreted around egg are albumen, shell membranes and shell. The egg is laid 24 hours after fertilisation, and its further development takes place, when the egg is incubated by the female. Incubation must continue steadily for 21 days at a temperature of 37.44℃.

15.4 CLEAVAGE AND BLASTULATION

As you know, cleavage, depends to a large extent upon the amount, distribution and orientation of yolk in the egg. As hen's egg is macrolecithal, so the cleavage in chick is discoidal meroblastic. That is, cleavage is confined only to the germinal disc or blastodisc situated at the animal pole (Fig.15.2). Cleavage starts about three hours after fertilization while the egg is still in the hen's oviduct. First two cleavages are at right angles to each other in the centre of blastodisc. These cleavage furrows do not cut the germinal disc completely through in the vertical plane.

The third set of cleavage furrows is vertical, cutting across the second set of vertical furrows. The fourth cleavage furrow is also vertical and circular cutting across all the cleavage furrows, forming eight central blastomeres which are surrounded by eight marginal blastomeres. Thus, these cleavage furrows separate the daughter central blastomeres from each other, but not from the yolk. The central blastomeres are continuous with the underlying yolk at their lower ends (Fig.15.3). At this time the maternal determinants are directing the cleavage, but by the 7thor 8th cleavge division (when there are 128 cells), the switch from maternal to zygotic gene expression occurs.

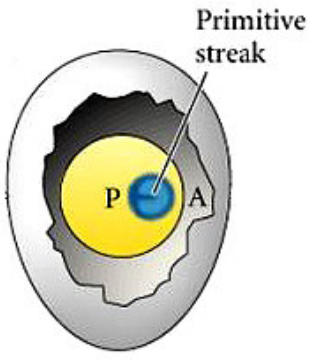


Fig.15.2: Surface view of egg showing the blastodisc with primitive streak. A denotes anterior and P denotes posterior part.

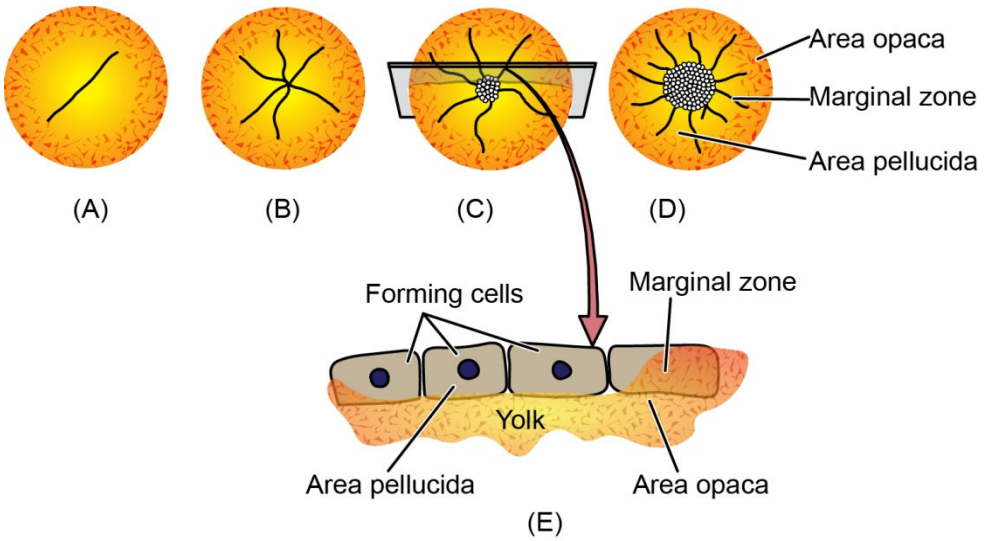


Fig. 15.3: Surface view of germinal disc of hen's egg showing early cleavage. A) Two-cell stage; B) Six-cell stage; C) Twenty-cell stage; D) Late cleavage stage E) Showing relationship between yolk and surface blastomeres.

The marginal blastomeres are continuous with the uncleaved cytoplasm at their outer edges. Further cleavages are irregular. The central cells divide more rapidly. The marginal cells also divide by the appearance of new horizontal and radial furrows. The newly formed inner cells of marginal blastomeres are added to the central cells, resulting in the increase of volume of this area. The radial furrows extend peripherally and these peripheral cells are still continuous with the uncleaved peripheral cytoplasm (Fig.15.3).

In later stage of cleavage, the blastomeres of the central area become separated from the underlying yolk due to the appearance of a horizontal cleavage in these cells. This cleavage extends peripherally cutting the inner ends of the blastomeres. Thus, a space also appears beneath the central cells which also extends peripherally as the horizontal cleavage extends outward.

The cavity beneath the central cells, i.e., in between central cells and yolk is called the **subgerminal cavity**, which is filled with a fluid diffused from the albumen through vitelline membrane. Thus, due to further cleavage the blastodisc becomes cellular, called the blastoderm- a round disc, 5 to 6 cells deep in the centre but only 1 to 2 cells deep at the periphery.

The appearance of subgerminal cavity separates the blastoderm from the underlying yolk. Some of the cell layers from the center of blastoderm are shed and die so that only a single or a few layer of cells in this area remains making the area translucent. Thus the central area is known as **area pellucida,** but the marginal cells remain overlapping the yolk and appear opaque and darker. Therefore, this area is termed **area opaca**. These cells in area opaca process the yolk but do not contribute in embryo formation (see Fig.15.4A). Between the area pellucida and area opaca is a thin layer of cells which is known as **marginal zone**. The embryo is now called the blastula.. At blastula stage the embryo reaches the uterus. It may be compared to the blastula of *Amphioxus* and frog, its sub-geminal cavity is equivalent to the blastocoel, the blastoderm is the animal pole, and the yolk is the vegetal pole.

The egg is laid by the female about the time the blastula is formed or even a little later. At the time of laying, the blastoderm is composed of 20,000 to 60,000cells. Most of the cells of the area pellucida remain at the surface, forming an “upper layer” called the **epiblast** (Fig.15.4A).

Shortly before the egg is laid some cells delaminate from the epiblast and ingress into the subgerminal cavity in clusters forming the **primary hypoblast** (Fig.15.4B). Shortly after the egg is laid, a small thickening of the epiblast, called **Koller’s sickle,** is formed at edge of the area pellucida which is now marked as the posterior end of the embryo. In between the area opaca and Koller’s sickle is a belt like region of cells called the **posterior marginal zone (PMZ)**. At the same time a sheet of cells begins to delaminate and migrates from the posterior edge of the area pellucida under the surface. These delaminated cells at the posterior edge of area pellucida gradually link up with each other and with the primary hypoblast, to form a continuous layer of flattened cells, which lie over the yolk on the floor of the subgerminal cavity. This layer is called as **secondary hypoblast or endoblast** and the upper layer of the blastoderm is the epiblast containing ectoderm and mesoderm cells. Hypoblast is exclusively composed of endoderm cells (Fig.15.4C).

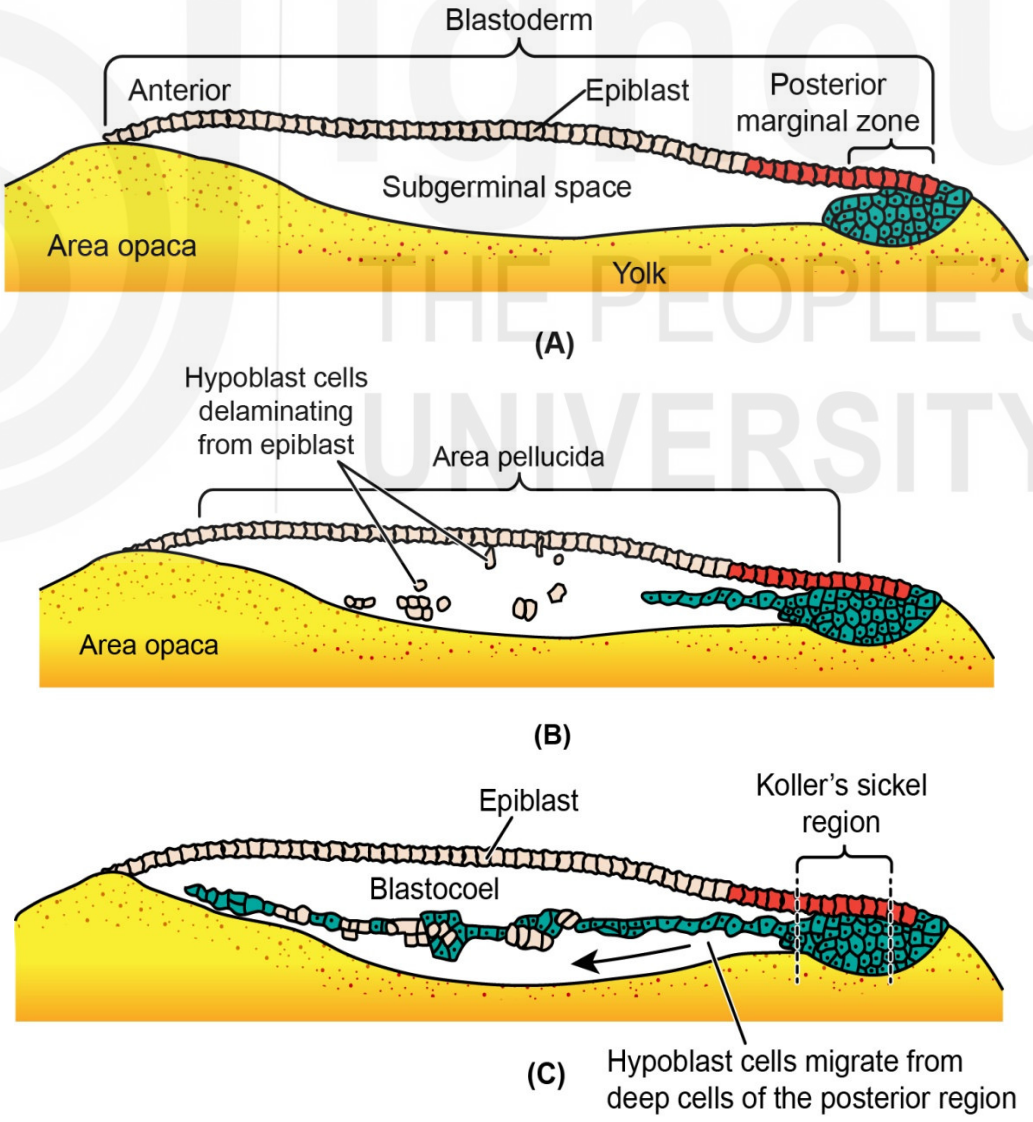


Fig.15.4: Formation of subgerminal cavity, epiblast and hypoblast and Koller’s sickle in the chick blastula.

SAQ 2

a) Which type of cleavage is observed in chick?

b) What is blastoderm?

15.5 GASTRULATION

In chick, gastrulation is a prolonged process. It is initiated soon after the beginning of incubation after laying of the egg and is completed in about 4 days. The actual process of gastrulation is preceded by some pre-gastrular movements of certain cells resulting in their separation from the blastoderm and formation of a lower layer called the **hypoblast**. Most of the cells, however, remain in the upper layers of the blastoderm which now constitute the **epiblast** (Fig. 15.6).

15.5.1 Role of Hypoblast

In the earlier section you have already learnt how the hypoblast forms from the blastoderm.The hypoblast thus formed expands and spreads peripherally to give rise to a complete thin layer below the remaining part of blastoderm, now called the epiblast. The hypoblast and epiblast are joined together at the margin of area opaca and the space between them is the blastocoel. The structure of the embryo is now somewhat similar to that of the **frog blastula** but the hypoblast is not the precursor of either ectoderm, mesoderm or endoderm. It is later displaced by endodermal cells derived from epiblast.

Hypoblast contributes cells for parts of some extra-embryonic membranes especially the yolk sac and the stalk linking the yolk mass to the endodermal digestive tube but none at all for the formation of the body of the embryo. However, the hypoblast has an important role in the development of the embryo. Its removal at an early stage stops all further development until a new hypoblast is regenerated from the epiblast. Hypoblast induces the formation of the primary embryonic axis (the primitive streak) in the epiblast and determines its orientation. Hypoblast cells also provide chemical signals that specify the migration of epiblast cells. However, the three germ layers of the embryo proper (plus the amnion, chorion, and allantois i.e. extraembryonic membranes) are formed solely from the epiblast.

15.5.2 Fate Maps

A chart or diagram showing the prospective fate of each part of blastula or embryo at any stage of development is called a fate map. Fate maps of the blastula of chick have been prepared artificially by using the vital stains such as carmine or carbon (charcoal) particles or radioactive thymidine. Fate map shows that blastomeres of area opaca do not form any part of the embryo proper, they form only extraembryonic membranes.

The epiblast and hypoblast of area pellucida have different fates in the course of embryonic development. The fate maps prepared by the use of tritiated thymidine have shown the following structures. In the centre of area pellucida

lies a small area destined to produce the notochord. Posterior to it, in the median plane is found an elongated oval area of presumptive endoderm which will form the gut (Fig. 15.5).

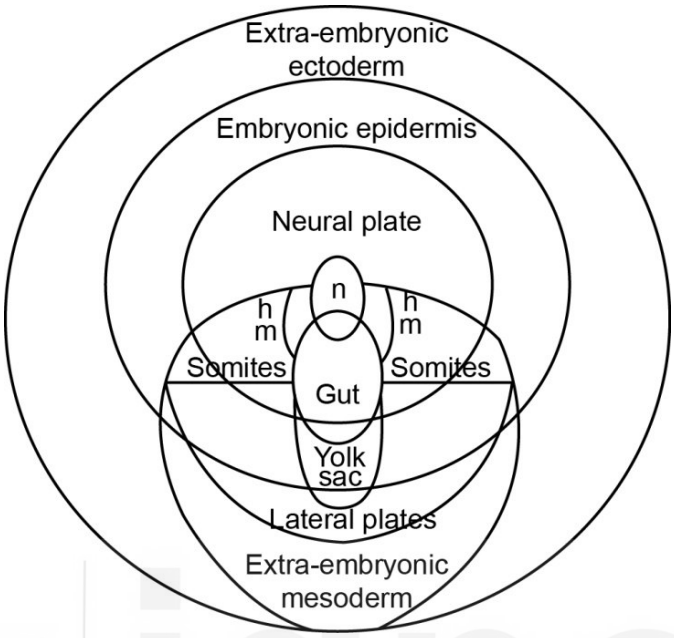


Fig. 15.5: Fate map of chick blastoderm immediately prior to gastrulation. The primitive streak is not yet formed but it will extend eventually to the region of the notochord. .

Further toward the posterior edge of area pellucida lies the extraembryonic endoderm which will form the lining of yolk sac. To the right and left of the presumptive notochord and endoderm, and posterior to extra-embryonic endoderm lie the various subdivisions of presumptive mesoderm, i.e., prechordal plate or head mesoderm, mesodermal somites, lateral plate mesoderm and extra-embryonic mesoderm.

As a result of morphogenetic movements during gastrulation the cells from the various areas reach their respective specific destinations forming the ectoderm on the surface, endoderm below and mesoderm between the two.

The anterior half of epiblast is the presumptive ectoderm containing central presumptive neural plate area, anterior to it is the presumptive embryonic epidermis and outer to it in the form of complete ring is the extraembryonic ectoderm.

15.5.3 The Gastrulation Process: Formation of Primitive Streak

Gastrulation in all amniotes including eutherian mammals is related to a characteristic structure called the **primitive streak** formed on the epiblast surface during the first 10-18 hours of incubation at 37.5-385°C. The primitive streak can be considered as the equivalent of an elongated blastopore lip of amphibian embryos. It forms as a result of convergence of epiblast cells to the dorsal midline of the blastoderm. Dyemarking experiments and time-lapse photography indicate that the primitive streak first arises from Koller's sickle and the epiblast above it the beginning of primitive streak formation is first indicated by a thickening in the posterior marginal zone region of area

pellucida immediately after the formation of endoblast. The thickening narrows and elongates growing anteriorly in the centre of area pellucida. When fully formed the primitive streak is a narrow structure with a groove (primitive groove) in its floor along its length flanked by a fold (or ridge) on either side. If we consider the primitive streak is homologous to the blastopore lip then the primitive groove is homologous to the amphibian blastopore, The primitive streak extends anteriorly upon about three fourth the length of area pellucida where it ends in a deep pit called **Hensen's Node** with thick borders (Fig. 15.6).The center of Hensen's node contains a funnel-shaped depression through which cells can enter the embryo to form the notochord and prechordal plate. Hensen's node is the functional equivalent of the dorsal lip of the amphibian blastopore or the organizer.

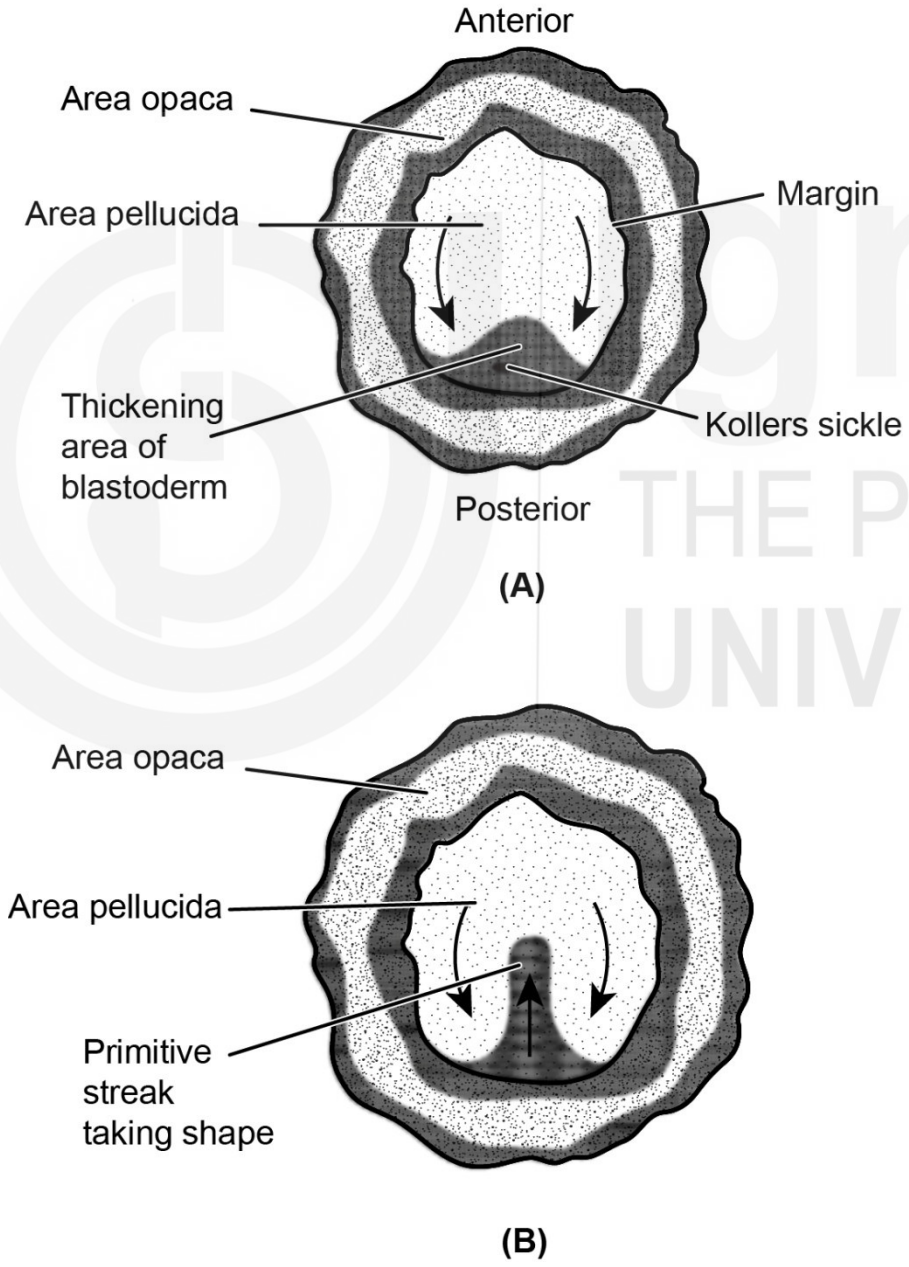


Fig. 15.6: Initiation of primitive streak formation from the thickening known as Koller's sickle. The streak corresponds to the blastopore lip of the amphibians.

During the formation of primitive streak the shape of area pellucida gradually changes from circular to pea shaped with the broad side anterior and narrow side posterior. This change is due to convergence of cells toward dorsal midline beginning at posterior end and progressing anteriorly but stopping where the Hensen's Node is formed. Primitive streak marks the median anterior-posterior axis of the embryo and establishes bilateral symmetry (Fig.15.7 and. see Box 15.1). It also establishes the dorso- ventral axis of the embryo.

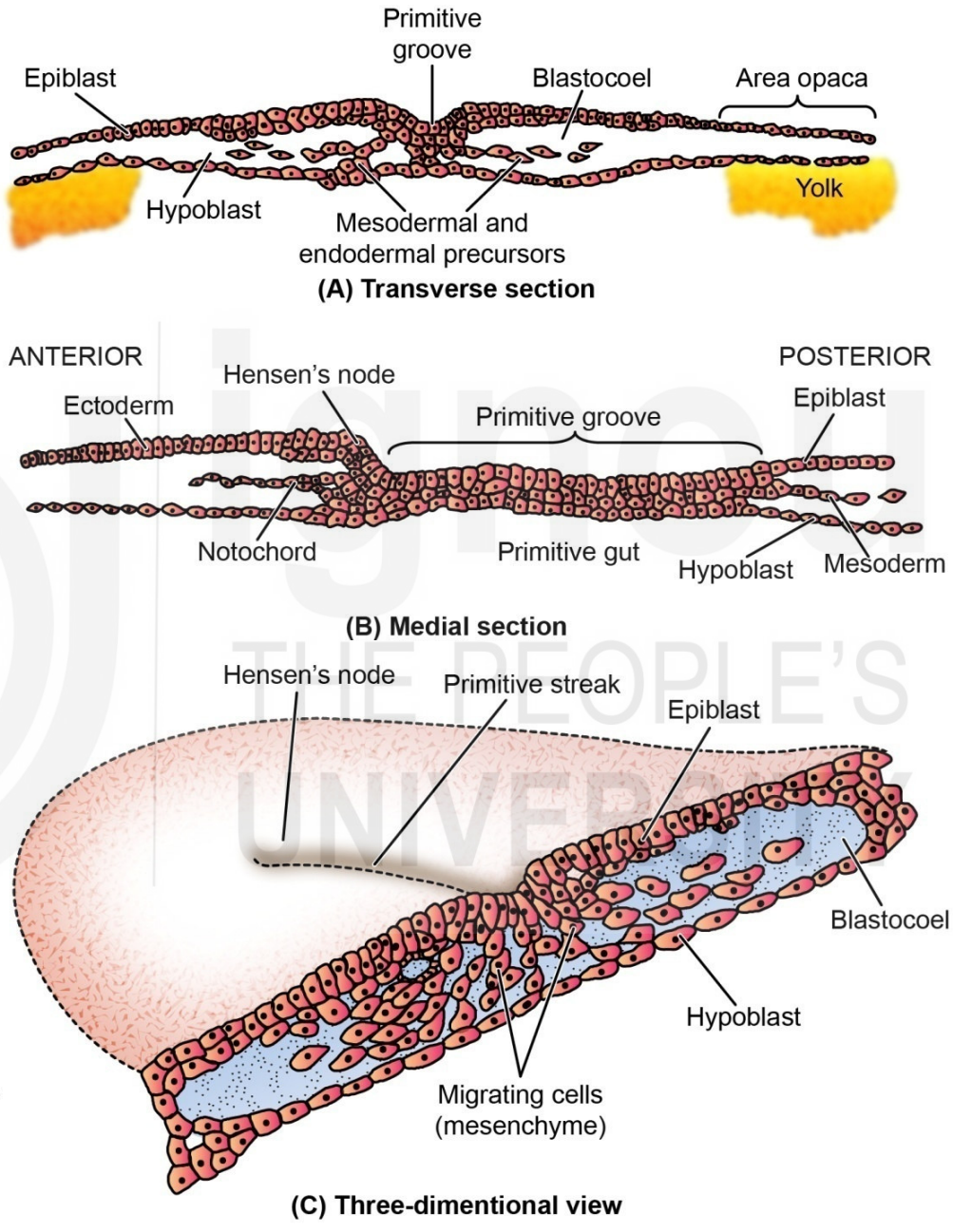


Fig. 15.7: Migration of endodermal and mesodermal cells through the primitive streak. A) Diagram of transverse section through a 17 hours embryo, illustrating the blastocoel; B) Diagram of medial section through the same embryo, showing that those cells migrating through Hensen's Node condense to form the notochord (head process); C) Stereogram of gastrulating chick embryo, showing the relationship of the primitive streak, the migrating cells, and the two original layers (epiblast and hypoblast) of the blastoderm.

Box 15.1: Setting up the antero-posterior axis in chick embryo.

How do we know what will be the posterior side in the blastoderm where the primitive streak will start to develop? As early as 1828, von Baer gave a general rule that enabled the antero- posterior axis to be predicted in the majority of eggs. The rule states that 'if the egg is horizontal with the pointed end to the right then the tail of the embryo should be towards the observer'. This happens because the egg undergoes a continuous rotation when it is in the uterus and this is usually in the same direction relative to the sharp and blunt ends of the egg. The embryo and yolk do not rotate along with the outer surface of the egg but are nevertheless tilted in the direction of rotation .It is thought that the rotation of the egg causes the lighter components of the yolk to accumulate under one side of the blastoderm. These yolk components are probably maternal determinants for development. This tilted end of the blastoderm becomes the posterior marginal zone where the primitive streak is initiated. And the opposite end becomes the anterior side towards which the primitive streak will extend. The cells of PMZ initiate gastrulation and once PMZ is formed it controls the surrounding regions of the margin between area pellucida and area opaca and prevents the formation of any other primitive streak. Thus we see that a radially symmetrical cleavage pattern gives rise to a bilaterally symmetrical organism and all because of gravity and rotation of the egg!

Movements of Epiblast Cells

The cells for the presumptive mesodermal and endodermal organ areas pass from the epiblast into the blastocoel below through the Hensen's Node or primitive groove of the primitive streak. This occurs by the cells sheets converging toward the Hensen's Node or the streak. On reaching there the cells change shape becoming "bottle cells", the sheet breaks up into separate cells which pass into the blastocoel individually by involution through the Hensen's Node or the primitive streak. Once inside the blastocoel, the cells flatten and continue to migrate as streams of loosely connected mesenchyme vertically down or laterally and anteriorly.

As cells enter the primitive streak, they undergo an epithelial-to-mesenchymal transformation and the basal lamina beneath them breaks down. In contrast to the amphibian gastrulation where sheets of cells involute during gastrulation, avian grastrulation is an **ingression** of epiblast cell which form a loose **mesenchyme** within the blastocoel. The first cells to enter blastocoel are those of the presumptive foregut endoderm located immediately around the Hensen's Node through which they move in. On entering the blastocoel they move anteriorly and ventrally and displace the cells of the anterior part of hypoblast. Later, infolding of this endodermal layer forms the foregut.

Next, the cells of presumptive chorda-mesoderm migrate into the blastocoel also through the node and then move anteriorly in the mid line just below the overlying epiblast to form the head mesoderm and anterior part of notochord called the head process. Later, the remaining endodermal and mesodermal cells of the epiblast migrate through the anterior and posterior regions of the streak, respectively. Once inside the blastocoel they form two streams of

migrating cells. One stream contains the endodermal cells which move down along the midline pushing the hypoblast to the side and forming a continuous sheet with that of the foregut endoderm. The other stream contains cells of presumptive somite and lateral plate mesoderm. They remain within the blastocoel, move laterally and anteriorly to take up positions on either side of the forming notochord as a loose sheet of mesoderm between the epiblast and hypoblast. The movement of cells within the blastocoel is facilitated by hyaluronic acid secreted by the ectodermal cells of epiblast. This polysaccharide accumulates in the blastocoel and coats the surface of the incoming cells which keeps them separate allowing their migration as individual cells.

SAQ 3

Choose the appropriate word given in the parenthesis.

i) Most of the cells remain in the upper layers of the blastoderm which constitute the (epiblast/hypoblast).

ii) As a result of morphogenetic movements during gastrulation the cells from various areas reach their respective specific destinations forming the (ectoderm/endoderm) on the surface.

iii) During the formation of primitive streak, the shape of area (opaca/pellucida) gradually changes from circular to pea shaped with broad side anterior and narrow side posterior.

iv) The cells for the presumptive mesodermal and endodermal organ areas pass from the epiblast into the (blastocoel/gastrocoel) through the Hensen's Node or primitive groove of the primitive streak.

v) The (polysaccharide/disaccharide) accumulates in the blastocoel and coats the surface of incoming cells.

15.5.4 Completion of Endoderm

The first cells that migrate through the anterior part of streak form the endoderm. By 22 hours of incubation most endodermal cells have migrated internally while the mesodermal cells continue to migrate till later. As the mesodermal ingression continues, the primitive streak begins to regress moving the Hensen's node from near the center of area pellucida towards a posterior position. As the Hensen's node recedes backward, and the notochordal process elongates, the presumptive endoderm of the middle and posterior part of the gut, located just behind the node, migrate inside as an endodermal strip beneath the notochord. These will give rise to the endodermal organs, major part of the gut of the embryo and most of the extraembryonic membranes. The remaining part of extraembryonic membranes will be formed by the hypoblast and cells of the area opaca.

In chick no archenteron is formed during gastrulation (Figs.15.8 and 15.9).

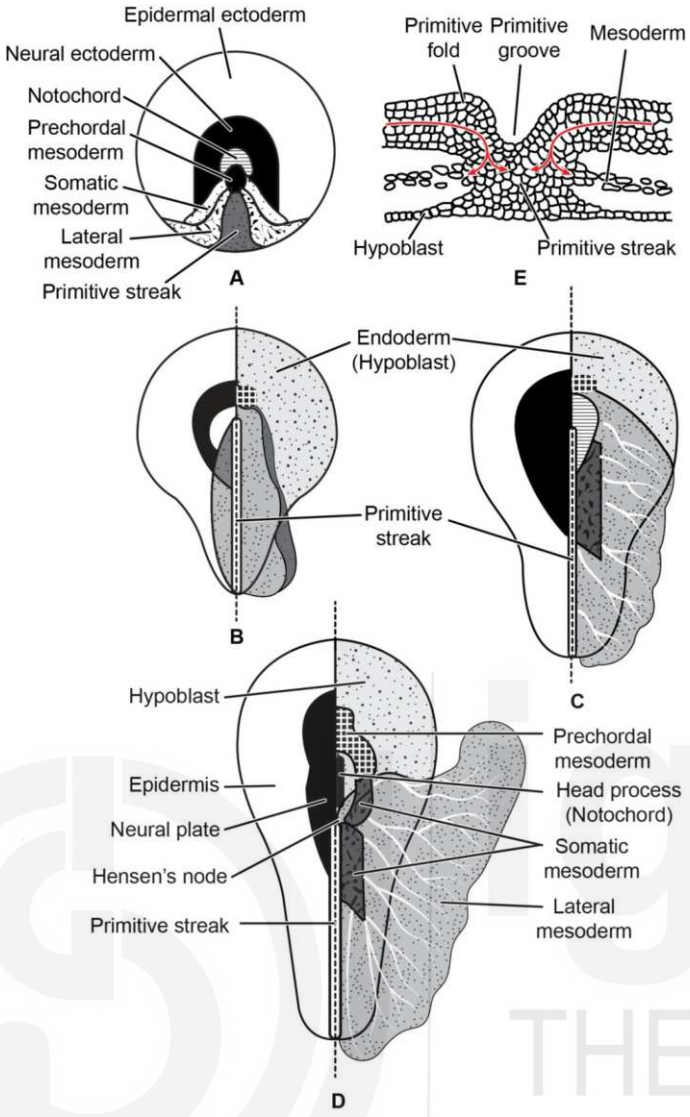


Fig.15.8: Primitive streak and germ layer formation in the chick embryo. A- Surface view of the epiblast; In B, C and D the left side pictures show the surface and the right side depict the pattern of the mesodermal areas as they spread over the hypoblast below; E- is a diagrammatic cross section showing the involution of mesoderm through the primitive streak.

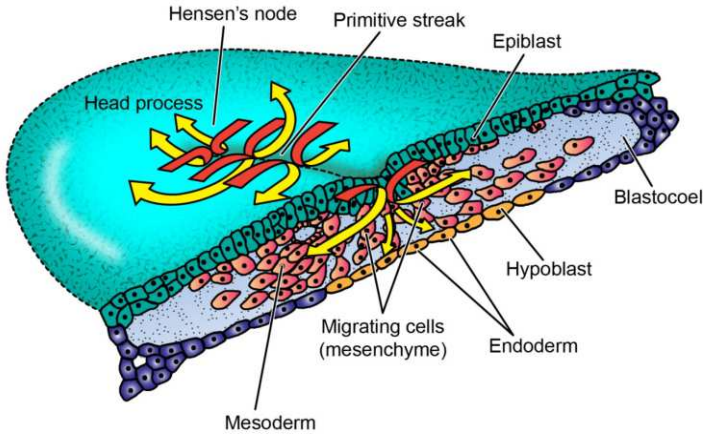


Fig.15.9: Ingression of endodermal and mesodermal cells through the Hensen's node and the primitive groove. The migrating cells of the endoderm from the Hensen's node move anteriorly to form the head process and the migrating endoderm cells move deep laterally over the hypoblast. The mesodermal cells form a loosesheet of mesenchyme which will form the mesodermal organs.

15.5.5 Regression of Primitive Streak

As the inward migration of cells through the primitive streak ends progressively along its anterior to posterior regions, the streak gradually regresses in the same direction. As the streak shortens, the Hensen's node, which forms its anterior end, also moves in a posterior direction (Fig.15.9). Simultaneously, the presumptive notochordal cells still remaining in the epiblast migrate inward through the posteriorly moving node adding to the length of the notochord. The regressing streak thus leaves behind in its path the dorsal axis of the embryo represented by the posteriorly lengthening notochord (Fig.15.9). By the time the streak disappears and the Hensen's node arrives at the final position at the posterior border of area pellucida all the presumptive endodermal and mesodermal cells have left the epiblast which now consists of only the ectodermal cells. Regression of streak begins after about 22 hours of incubation and is completed in the next about 20 hours. It should be noted that unlike amphibian gastrulation, differentiation of axial structures and some organs such as neural tube, somites, foregut, heart, occurs cephalo-caudally along with the progress of later stages of gastrulation in chick embryos (Fig.15.10). This means that head structures are already forming while migration of cells is still going on in the regressing primitive streak (See Fig.15.11A and B).

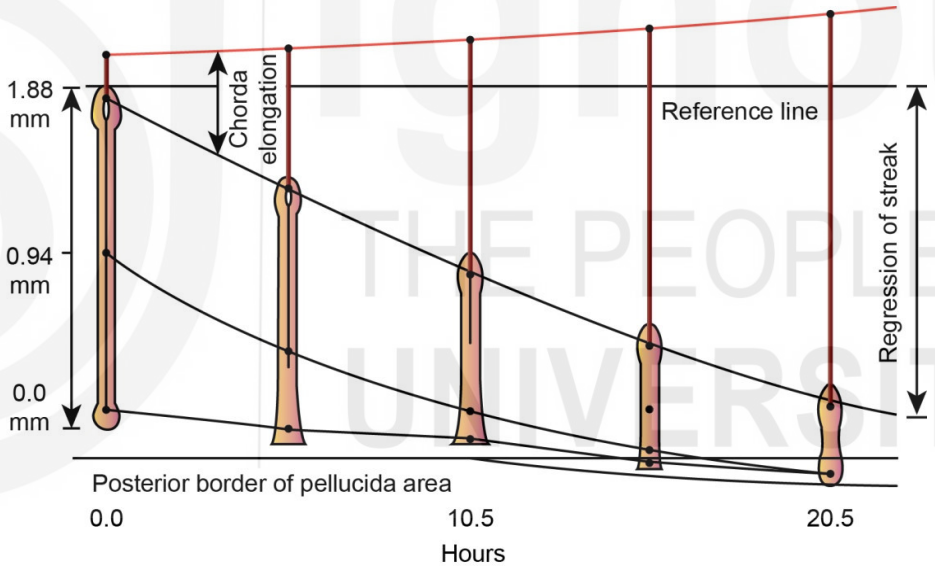


Fig. 15.10: Regression of the primitive streak, leaving notochord in its wake. Time represents hours after achieving maximum length by the primitive streak.

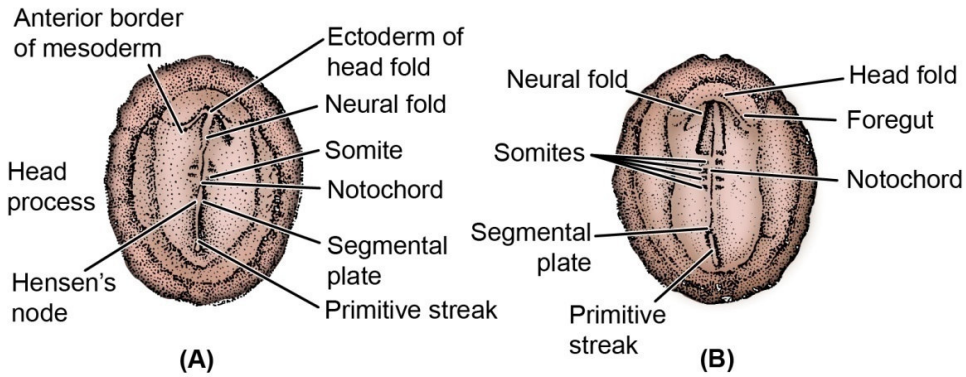


Fig. 15.11: Dorsal view of regression of primitive streak. Note that while the streak is regressing from anterior to posterior region the organs are already forming in the anterior region.

15.5.6 Epiboly of Ectoderm

As the presumptive endodermal, notochordal and mesodermal cells migrate inward the epiblast is made entirely of ectodermal cells (of the embryonic epidermal and neural ectoderm). Simultaneously the ectodermal precursors proliferate, expand as a sheet beneath the vitelline membrane outward from the area opaca ultimately encircling the yolk through epiboly (recall morphogenetic movents from Unit 12). The marginal cells of area opaca are different from other blastodermal cells, they can extend enormous cytoplasmic extensions or fillopodia to form a firm contact with the inner surface of vitelline membrane.. It appears that these marginal cells of area opaca migrate outward along the inner surface of the vitelline membrane with the help of these pseudopodia and at the same time drag along the expanding sheet of ectodermal cells of which they are the leading members. It is believed that the fillopodia connect to protein fibronectin in the vitelline membrane and if the fibronectin is broken experimentally, ectoderm migration stops. Similarly removal of the vitelline membrane inhibits epibolic movements and expansion of ectoderm.

15.5.7 Characteristic Features of Avian Gastrulation

i) Formation of hypoblast and its important role in formation of the axis and orientation of embryo.

ii) Presence of the cells of all the three germinal areas in the epiblast.

iii) Formation of the primitive streak and its regression in later stages of gastrulation.

iv) Absence of archenteron formation. Gastrular cell movements include ingression, involution, convergence, divergence and epiboly.

v) Cephalo-caudal differentiation of axial structures and growth of the embryo during gastrulation.

15.5.8 Comparison with Amphibian Gastrulation

On comparison of chick gastrulation with amphibian gastrulation you will find many similarities along with important differences. Primitive streak is analogous to the blastopore although there is no actual pore. The Hensen's node represents the dorsal lip of amphibians and the folds on the lateral sides of primitive groove represent the lateral lips. However, there is no ventral lip. As in amphibians, the prechordal endoderm and chorda mesoderm of chick migrate inward through the Hensen's node (dorsal lip) and the remaining endoderm and mesoderm do so by involution over the lateral folds of the streak (lateral lips). The topographic locations of the presumptive ectodermal chordamesodermal; mesodermal and endodermal area relative to each other in the epiblast are nearly the same as on the surface of amphibian blastula. As in amphibians, the involuting mesodermal and endodermal cells change shape to become bottle cells in the gastrulating chick embryos as well.

15.5.9 Fully Formed Gastrula

Gastrula is fully formed when primitive streak completely disappears. The fully formed gastrula consists of three germ layers-ectoderm, chordamesoderm and endoderm. The ectoderm and chorda-mesoderm remain in continuity along the axis of primitive streak. The endoderm is also united with the mesoderm and ectoderm at the anterior and posterior end of streak (Fig.15.12).

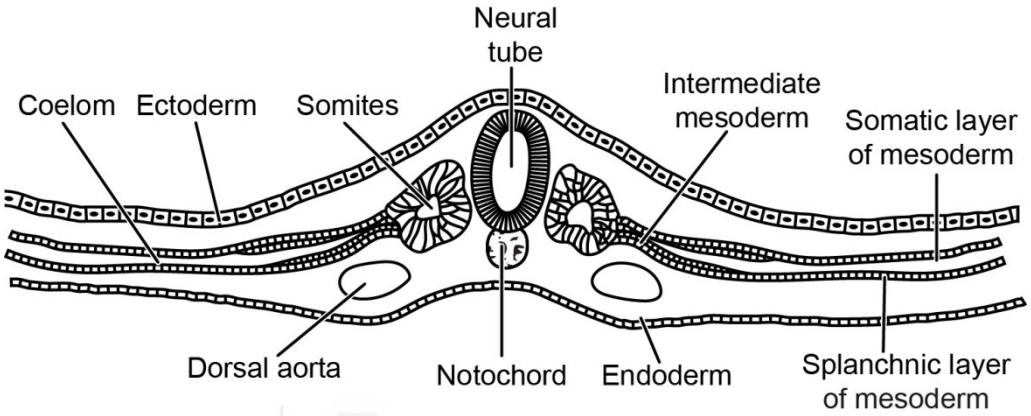


Fig.15.12: Cross section of an early chick embryo, illustrating the relationship of the germ layers.

SAQ 4

i) List the types of morphogenetic movements of cells that occur during gastrulation in chick embryos.

ii) What is the role of hypoblast in chick embryos?

iii) Cells of which presumptive germinal layers are constituents of the epiblast in chick embryo?

iv) How do mesodermal and endodermal cells migrate inward?

v) Which structures are formed from cells of area opaca?

vi) List similarities between gastrulation in chick and amphibians.

15.6 NEURULATION IN CHICK

You already know from Unit 13 when studying about the development of frog that the vertebrate brain and spinal cord start their development as a flat plate of neuroepithelial cells that folds up along most of its length to form a tube. The process of forming this neural tube is called neurulation. Neurulation in chick is similar to that of amphibians but there are certain differences. In amphibians, the formation of neural tube occurs simultaneously along the entire length of the embryo. In birds, reptiles and mammals even as the neurulation has begun in the anterior part of the embryo, the posterior region is still in the process of gastrulation. At a time when the neural folds are just about to form in the posterior region, in the anterior region the neural folds have already started fusing forming the neural tube (Fig.15.13).

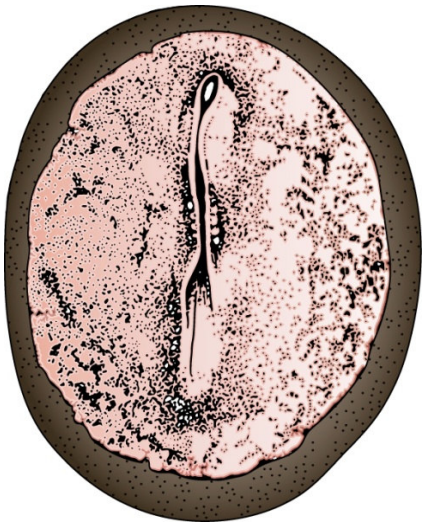


Fig.15.13: Dorsal view of chick embryo of 25-26 hours with 5 pairs of somites. Neural folds approaching each other fuse and form neural tube in the cephalic region; but in the posterior region gastrulation is still occurring and primitive streak and neural folds are yet to form.

We described two important changes in cell shape while discussing neurulation in amphibians. Such changes occur in chick embryo as well. One is related to changes in neural ectoderm cells from cuboidal to columnar shape that results in narrowing and thickening of neural plate. The second is related to narrowing of the apical ends of some neural epithelial cells when the neural plate rolls up to form two parallel neural ridges or folds that eventually meet to form the neural tube.

15.6.1 Mechanisms of Neural Plate Formation

You are now familiar with the concept that the neurulation comprises of two processes i) the neural plate formation and ii) the neural tube formation. All the mechanisms responsible for these two processes are not very clear.

Following induction by the underlying mesoderm during gastrulation, the ectodermal cells that will give rise to the neural tube appears as a thick plate known as neural plate. The shaping of neural plate from an oval to a long, narrow plate is believed to occur by the elongation of neural epithelial cells and the accompanying apical shrinkage. Although it was earlier believed that microtubules may be involved in the elongation process, evidence for this is inconclusive. The role of microtubules appears to be important in stabilizing the elongated state of the cells rather than in the elongation process itself.

The bending of neural plate to form neural tube is associated with changes in cell shape as well. The cells at the edge of the plate are constricted at the apical end and they become wedge shaped. This change in shape pulls the edges on both sides up so that the neural plate folds. Cells in the midline of the neural furrow become wedge shaped that is, the apical edge is constricted because of the contraction of actin microfilaments and the base becomes broad. This forms the **medial hinge point (MHP)**. The cells of the MHP are firmly attached to the notochord which enables the formation of the neural furrow (Fig.15.14B). Two additional **dorsolateral hinge points** from where the

ridge folds curve in to meet (Fig.15.14C). These are anchored to the surface ectoderm cells. The neural tube is formed as the lateral folds meet in the midline (Fig.15.14D). When the tube forms, the cells on the apex of the folds delaminate and fall off as neural crest cells that migrate to their respective spaces in the embryo.

The neural tube seperates from the presumptive epidermal layer due to change in cell adhesiveness because neural cells now begin to express N-cadherin and N-CAM instead of the E-cadherin which is expressed by the nearby epithelial cells (recall mechanism of cell adhesion from Unit-10). This allows the neural tube to sink below the surface and the ectodermal cells then form a continuous layer over it.

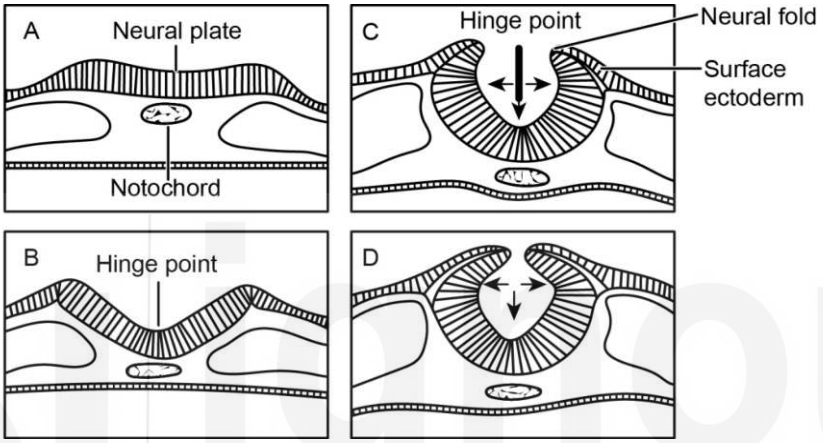


Fig.15.14: Stages in the neurulation of chick. A) Neural plate formation; B), C), and D) The bending of plate in three locations (indicated by arrows), just above the notochord (N) and on each side of the neural plate, just below the tips of neural folds

Before we proceed to discuss the mesodermal derivatives, attempt the following SAQ.

SAQ 5

State whether the following statements are True or False.

i) During post-gastrulation development, different germ layers of the gastrula become segregated from each other to form tissues and organs.

ii) The term neurulation refers to the partitioning of ectoderm, and the formation and inward displacement of neural tube.

iii) Neurualtion results in the separation of ectoderm into epidermal ectoderm, neural ectoderm and neural crest cells.

iv) Neurulation process in chick is entirely different from that of amphibians.

v) During neural plate formation, the neuroepithelial cells change in shape from columnar to pyramidal ones.

vi) Microtubules play significant role during the rolling up of the neural plate into neural tube.

vii) In chick the neurulation process occurs simultaneously through out the length of the embryo.

15.6.2 Morphogenesis of Mesodermal Derivatives

Recall from the section on gastrulation that the presumptive mesodermal cells from the epiblast ingress from the Hensen's node and from the different regions of the primitive streak along the dorso-ventral axis into the embryo.to form the loose sheets of mesodermal cells. All the organs that lie between ectoderm and endoderm tissues arise from mesoderm. In the neurula stage of the embryo, the mesoderm cells are arranged in the following distinct regions:

- Chordamesoderm or axial mesoderm: After the primitive streak is fully extended in length some cells migrate from the Hensen's node to move forwards in the middorsal line. This mesodermal tissue is chordamesoderm that separates as a middorsal strip from the rest of the mesodermal tissue. Chordamesoderm gives rise to the notochord which establishes the anterior-posterior body axis of the embryo. The notochord gives support to the developing embryo and later is cleared due to apoptosis remaining only as the intervertebral discs (Fig.15.15B).

- Dorsal mesoderm or paraxial mesoderm: As the notochord is laid down, the mesoderm on each side forms blocks of tissues called somites present on each side of the neural tube (Fig.15.15C). These will form the muscles and connective tissue in the back of the embryo, such as dermis, muscle and skeletal elements (vertebrae and ribs) surrounding the spinal cord and some muscles in the limb and ventral regions mainly the abdominal walls. The anterior most paraxial mesoderm does not form somites and is called **head mesoderm.** Located in the head region, it will give rise to head muscles.

- **Intermediate mesoderm:** Intermediate mesoderm is located as a thin stalk connecting paraxial mesoderm with the rest of the mesodermal sheet. The urinogentital system arises from intermediate mesoderm (Fig.15.15).

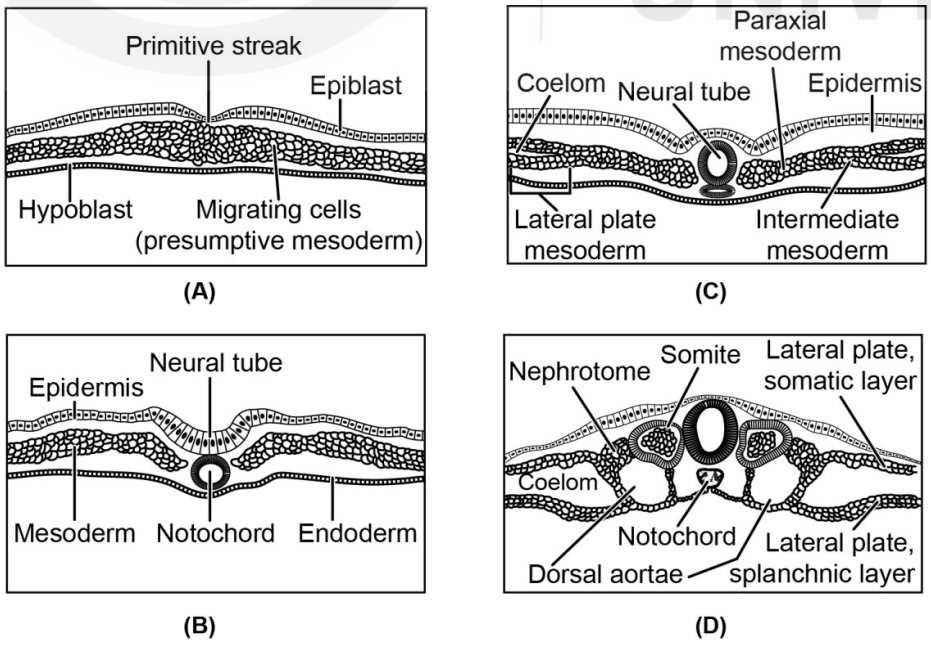


Fig. 15.15: Stages in the development of mesoderm shown in transverse sections of chick embryo at trunk level.

- **Lateral plate mesoderm:** Lateral plate mesoderm is a sheet of loosely connected cells on either side of gut (Fig.15.15 C and D). This loose sheet of epithelial cells splits into two layers, the somatic mesoderm which becomes closely associated with ectoderm and the splanchnic mesoderm which becomes closely associated with endoderm. The space between the two regions of mesoderm is the future coelomic cavity (Fig. 15.15D). Lateral plate mesoderm gives rise to heart, blood vessels and blood cells and the lining of the body cavities. All the components of pelvic limb skeleton except muscles are derived from lateral plate mesoderm. Lateral plate mesoderm also helps to form some of the extraembryonic membranes that are used for transporting nutrients to the embryo.

SAQ 6

Name the organs derived from

i) Dorsal mesoderm

ii) Lateral plate mesoderm

15.7 FOLDING OF EMBRYO

You have learnt at the beginning of the unit that the entire blastoderm does not form the embryo. Its central portion, called the area pellucida, only forms the embryo, and its outer portion, the area opaca forms the extra-embryonic membranes, such as yolk sac, amnion, serosa (chorion) and allantois.

The body of the embryo becomes separated from the yolk sac. This is achieved by the formation of folds which appear all around the body of embryo. The folds involve all three germ layers and are directed downward and inward, undercutting the body of the embryo proper. They are known as body folds. The various folds do not appear simultaneously.

The first to appear is the head fold just in front of the head. It undercuts the head and anterior part of the trunk of the embryo, so that these parts project freely over the surface of yolk sac. The lateral and posterior parts of the body folds develop soon after.

The posterior fold undercuts the tail end and the posterior part of trunk which also projects freely over the surface of yolk sac. These body folds gradually contract underneath the embryo and eventually the body of embryo is connected with the yolk sac and other extra-embryonic membranes by a narrow stalk, the yolk stalk. Figure 15.16 shows the various body infoldings that give it shape and make it constricted off from the yolk.

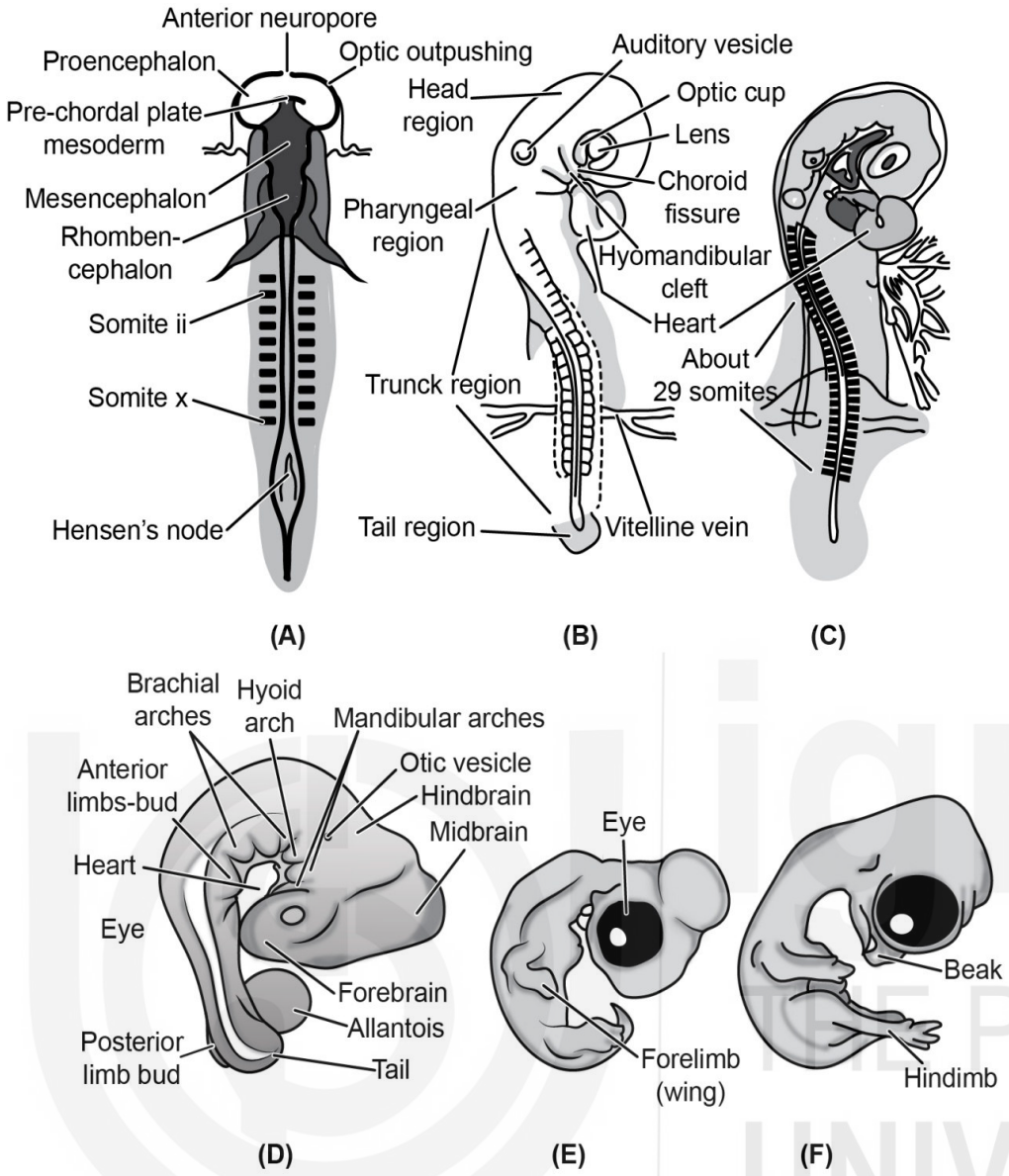


Fig.15.16: Post-neurular development. A) Stained preparation of the stage 10; B) External view of embryo of about 27-28 pair of somites (51 to 56 hours of incubation, stage 16); C) Internal anatomy of stage 16 (of B); D) Embryo of about 72 to 75 hours of incubation (Stage20); E) embryo of stage 27, F) Embryo of stage 23.

Flexure and Torsion

When the head is formed, it gets bent down ventrally with respect to the main axis of the embryo, this bending is called cranial flexure. Then first the head, and gradually the entire body turns sideways so that the embryo comes to lie on its left side over the yolk, this dextral twist is knows as torsion. The anterior end in its cervical region becomes still more bent over towards its ventral surface.

Various organs are formed on the third and fourth days of incubation, such as the nervous system, sense organs, four pairs of pharyngeal pouches out of which only the first three pairs meet the ectoderm to open as gill clefts, five pairs of visceral arches, heart and, blood vessels, and paired segmental somites.

15.8 DEVELOPMENT OF EXTRA-EMBRYONIC MEMBRANES

As you had seen in Figure 15.15, during neurulation, the lateral plate mesoderm splits into an outer somatic layer lying beneath the ectoderm and an inner layer lying outer to the endoderm layer. In between these two layers of mesoderm lies the coelomic space.

The ectoderm and somatic layer of mesoderm are collectively called somatopleure, while the splanchnic layer of mesoderm and endoderm form the splanchnopleure. During later developmental stages, the somatopleure and splanchnopleure gradually spread outward beyond the developing embryo.

As the body of embryo grows, it becomes separated from the foetal membranes by the appearance of body folds - cephalic, caudal and lateral folds. These folds limit the body of embryo and separate the embryonic region from the extraembryonic region.

The blastoderm besides forming the embryo, gives rise to certain other structures which do not take part in the formation of embryo proper, but are external to the developing embryo. These structure are collectively called extra-embryonic membranes.

These membranes are essential for complete development of the embryo. These extraembryonic membranes are concerned with nutrition, excretion, respiration and protection from desiccation and concussions. These membranes are amnion, chorion or serosa, allantois and yolk sac.

15.8.1 Development of Amnion and Chorion

The origin of amnion and chorion is considered together since they develop simultaneously from the extra-embryonic somatopleure. Around 30 hours of incubation, the extra-embryonic somatopleure of the blastoderm rises up in front of the embryo as a fold, the fold grows and forms an arch over the embryo.

It forms a double somatopleuric hood, called the cephalic amniotic fold. As this fold gradually extends backward, its caudally extending side limbs called lateral amniotic folds arch over the embryo from either lateral side. A similar fold or elevation over the tail appears, called the caudal amniotic fold. All these amniotic folds converge and fuse over the embryo, enclosing it within two sheets of somatopleure from all sides except the region of yolk stalk (Fig.15.17).

Functions of Amnion and Chorion

The amnion, chorion and amniotic cavity serve the following functions for the embryo:

a) The amnion protects the embryo from desiccation and sudden temperature changes.

b) The amniotic fluid is an efficient shock absorber and protects the embryo from the mechanical shocks.

c) The shell and shell membranes are protective and prevent desiccation of the embryo.

d) The mesoderm of amnion during later development forms muscle cells which contract rhythmically, rocking the embryo within the amniotic fluid so as to prevent it from adhesion to the embryonic membranes.

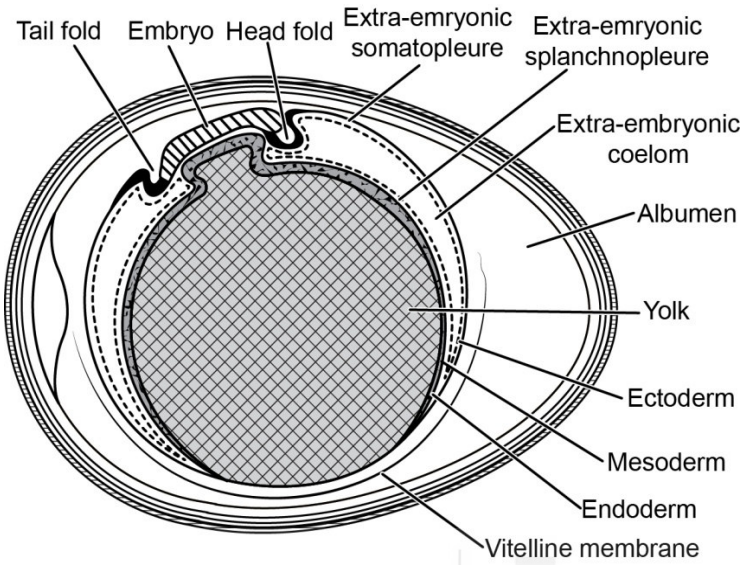


Fig. 15.17: An early chick embryo showing body folds delimiting from extra-embryonic areas.

15.8.2 Development of Allantois

About the third day of incubation the floor of the endodermal hindgut begins to bulge as a bladder, called the allantois. The allantoic evagination is formed from the splanchnopleure, that is, inner layer of endoderm and an outer layer of splanchnic mesoderm. It grows rapidly and spreads into the extra-embryonic coelom, the space between the yolk sac, the amnion and the chorion.

The distal part of the allantois expands and remains connected with the hindgut of the embryo by means of a narrow allantoic stalk. When the body folds contract, embryo is separated from the extra-embryonic parts, the allantoic stalk is enclosed together with the stalk of yolk sac, or an umbilical cord is formed (Fig. 15.18).

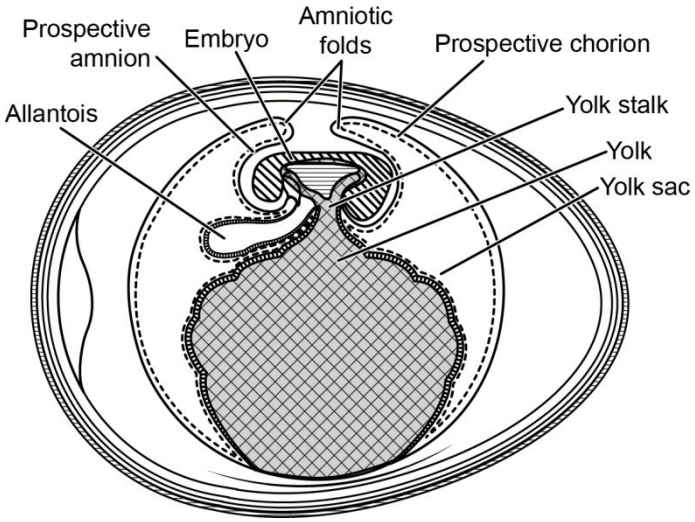


Fig.15.18: Early stage in the development of the extra-embryonic membranes of chick.

As the allantois vesicle enlarges and spreads outward, its distal part becomes flattened and expands between the amnion and yolk sac on one side and the chorion on the other side. Due to this, the splanchnic mesoderm of the allantois fuses with the inner somatic mesoderm of the chorion to form an allanto-chorion.

The allanto-chorion grows around the albumen of the egg to enclose it in an albumen sac. It aids in absorption of water and albumen. The allantois becomes highly vascular due to the appearance of blood vessels in the splanchnopleure. It also receives a pair of allantoic or umbilical arteries, and its blood is returned into a pair of allantoic or umbilical veins.

The chorio-allantoic circulation continues until the young chick breaks the egg-shell and begins to breathe the surrounding air. Thus, the umbilical vessels close, the circulation ceases and the allantois dries up and separates from the body of the young chick. At the time of hatching, the allantoic vesicle with excretory wastes is detached from allantoic stalk and is left attached to the broken shell.

Functions of Allantois

The allantois serves following functions for the embryo:

i) The allantois serves as an embryonic urinary bladder collecting uric acid from the kidneys which is, thus, prevented from escaping into other parts of the embryo where it might be harmful.

ii) The vascular allanto-chorion is in contact with shell membranes and it brings about the respiration of the embryo by an exchange of oxygen and carbon dioxide through the porous shell.

iii) **Development of Yolk-Sac:** During neurulation, the gut region of embryo has a roof and side but no floor. The ventrally open gut rests on the yolk mass. The formation of yolk sac first among extraembryonic membranes is essential, since nutrition is supplied to the developing embryo with the help of yolk sac. The extraembryonic splanchnopleure grows over the yolk and eventually surrounds the entire yolk to form the yolk sac.

It has an inner layer of endoderm and an outer layer of splanchnic mesoderm. The yolk sac in the beginning is joined by a wide yolk stalk to the midgut of the embryo. But later on, as the body folds move toward one another beneath the embryo, a floor of the gut is formed except in a small region of the midgut through which it communicates with the yolk sac.

Thus, the yolk sac cavity remains in continuity with the gut cavity through yolk duct of yolk sac stalk. The endodermal surface of the yolk sac is thrown into folds called the yolk sac septa that penetrate the yolk mass. The rich blood circulation develops within the splanchnic mesoderm layer of the yolk sac. These are the paired vitelline arteries and veins, now called the omphalomesenteric blood vessels (Fig.15.19).

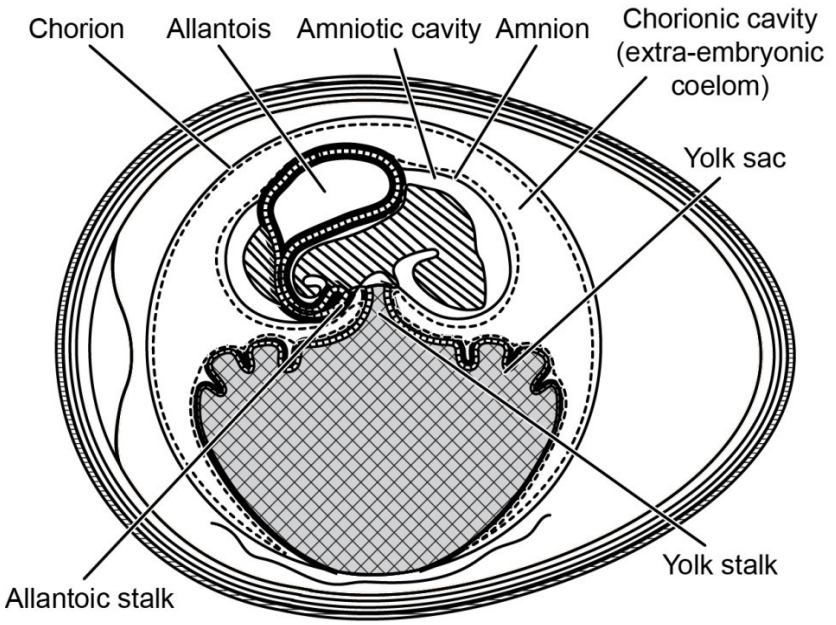


Fig.15.19: Later stage in the development of the extra-embryonic membranes of the chick.

The endodermal cells of the yolk sac secrete digestive enzymes which digest the yolk. The digested yolk is collected by left and right vitelline veins, both of which open into an unpaired ductus venosus which opens into sinus venosus of the heart.

Thus, the yolk sac serves as a digestive and absorptive surface by which yolk is made available to the embryo. Shortly before hatching the shrivelled up remains of the yolk sac are retracted into the abdominal cavity of the embryo, and the walls of the abdominal cavity close behind it (Fig.15.20).

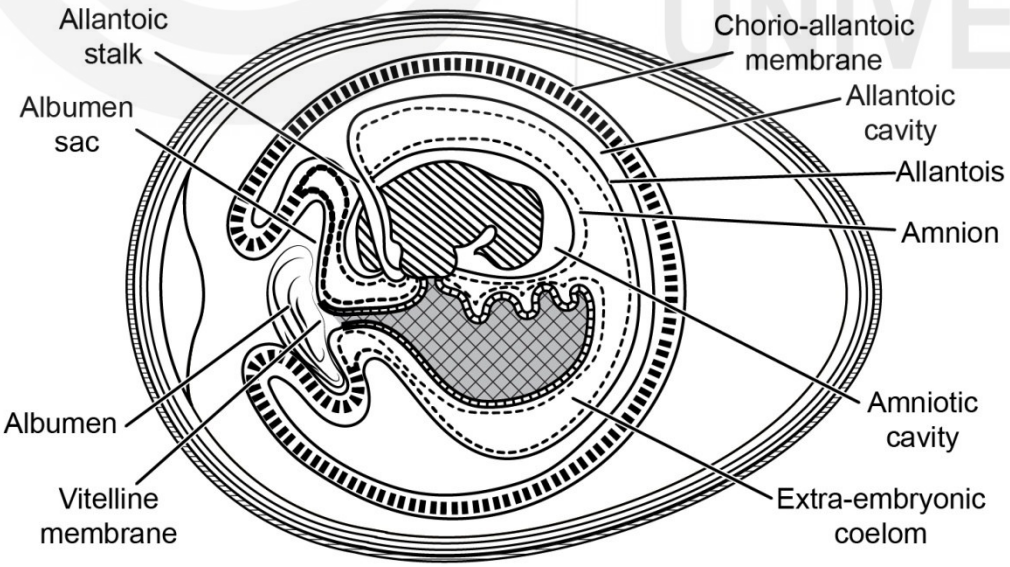


Fig.15.20: The fully matured extra-embryonic membranes of the chick.

The amnion, chorion, and allantois are devices by which the embryo can develop on dry land (as also in reptiles and mammals). The yolk sac (also found in fishes, reptiles and mammals) develops for the absorption of yolk.

SAQ 7

Write one important function of each of the following:

i) Amnion

ii) Chorion

iii) Allantois

15.9 HATCHING

The early embryo was at right angles to the long axis of the egg, later it comes to lie along the long axis with its head near the blunt end. The beak of the chick is covered by a horny caruncle by which it first pierces the inner shell membrane to take air into the lungs from the air space. Soon after the caruncle breaks the egg shell and the chick emerges after 21 days of incubation at 103°F.

Since the inside surface of shell is concave, it is easier for the chick to break open the shell and shell membrane as all the force is concentrated at one point. The outer surface of the shell is convex, hence, the force exerted on the outside of the shell is distributed on all sides and the shell does not break easily.

The chick breaks open the shell by repeated blows of the egg tooth and hatches out leaving behind the allanto-chorion and amnion in the shell. The newly hatched chick is precocious or nidifugous, i.e., it is fully clad with wet feathers which soon dry up on the exposure to air, and is able to walk and feed.

15.10 SUMMARY

Let us summarise what you have learnt so far:

- Chick egg is macrolecithal. It is entirely filled by yolk and yolk is covered by albumen. Cleavage in chick is discoidal and meroblastic.
- Cleavage produces morula which is converted into blastula or a blastula-like structure the blastodisc. The organization of blastula is dependent on the amount of yolk originally deposited in the egg. The amount of yolk influences the whole course of development and structure of embryonic stages. Such an influence continues even during subsequent stages of gastrulation.
- During the gastrulation of aves (also in the reptiles, egg laying mammals), the primitive streak provides the passage for the movement of presumptive notochordal, mesodermal as well as endodermal cells. Hence the primitive streak may be called the closed blastopore of Amphibia.
- Neurulation consists of, first the neural plate formation and then the neural tube formation. The neural plate is formed by changes in the

shape of the cells by the cell elongation and accompanying apical shrinkage. The rolling of neural plate into neural tube due to changes in cell shape is brought about by the contraction of actin filaments in the apical end of the cells.

- Post neural development processes include, folding, flexure and torsion of embryo.
- The blastoderm besides forming embryo gives rise to extra embryonic membranes i.e. amnion, chorion and allantois. All these perform specific functions.

15.11 TERMINAL QUESTIONS

1. Discuss the process of gastrulation in chick.
2. Define the following terms:
   a) Blastoderm
   b) Morphogenetic movements
   c) Meroblastic cleavage
   d) Fate maps
   e) Coelobastula
   f) Epiblast
   g) Inner cell mass
3. What is the fate of presumptive notochordal area of blastula of the chick in the adult stage?
4. Describe the process of neurulation in chick.
5. Discuss the process of development of extra embryonic membranes in chick.

15.12 ANSWERS

Self Assessment Questions

1. a) Macrolecithal b) Blastodisc
2. a) Meroblastic, discoidal
   b) When due to cleavage, the blastodisc becomes cellular, round disc like structure, it is called blastoderm.
3. i) epiblast
   ii) ectoderm
   iii) area pellucida

iv) blastocoel

v) polysaccharide

4. i) Convergence, divergence, involution, poly-invagination, epiboly.

ii) Influences formation of antero-posterior medial axis and orientation of embryo.

iii) Ectoderm, endoderm, mesoderm.

iiii) Individually through primitive streak.

iiv) Extra embryonic membranes (yolk sac, amnion, allantois, chorion).

iv) Refer to Subsection 15.5.7.

5. i) F ii) T iii) T iv) F v) T vi) F vii) F.

6. i) Muscles, cartilage, dermis.

ii) Heart, blood vessels and blood cells.

7. i) The amnion projects the embryo from desiccation and sudden temperature changes.

ii) The function of chorion is to contribute to the development of the placenta in placental animals.

iii) The allantois serves as an embryonic urinary bladder collecting uric acid from the kidneys which is, thus, prevented from escaping into other parts of the embryo where it might be harmful.

Terminal Questions

1. Refer to Section 15.5.
2. Refer to the relevant parts of the text.
3. The presumptive notochordal area of zygote of a chick forms notochord in the early embryonic stages but it is destined to be obliterated completely by the adult stage except in the cyclostomes.
4. Refer to Section 15.6.
5. Refer to Section 15.9.

UNIT 16

EARLY DEVELOPMENT IN HUMANS

Structure

16.1 INTRODUCTION

Human development is an area of immense interest largely to learn about our beginning from a single fertilized cell and how eventually it develops into a fully functional adult. The other interest is to learn about the various genetic and environmental factors that influence this process of development to improve the quality of human life.

The process of human development is a continuous cascade of miraculous events. A single cell is transformed into a multicellular human being with the help of cell division, growth, differentiation, and even cell death. An ovum is fertilized by a sperm which is followed by the formation of the organ systems in the prenatal period (between fertilization and birth). Prenatal period refers to the period before birth. During this period for the first eight weeks the developing human being is called an **embryo** because the organ systems are

forming. From the 9th week onwards the term **foetus** is used. The foetal period (9 weeks to birth) is characterised by growth and elaboration of structures. But, the important maturational changes continue to occur during the postnatal period (infancy, childhood, adolescence and even adulthood)

Humans reproduce and bear live offspring by the human reproductive system provided all the organs present have normal structure and are capable of functioning properly. The important events of human reproduction are:

i) an ovum, or egg, is released at a particular time in the female reproductive cycle,

ii) spermatozoa, or sperm cells fertilize the ovum,

iii) the fertilized ovum is then transported to the womb or uterus,

iv) blastocyst implantation, the development of an early embryo from the fertilized ovum,

v) formation of a placenta and maintenance of the embryo,

vi) child birth and placenta expulsion. You will study about these events in the present unit.

The 266 days between conception and birth are traditionally divided into about three month periods, each called a **trimester**. We deal with each trimester but more emphasis is given to the first trimester as more dramatic changes occur during this period. But before we discuss the development of the human embryo it is important to recapitulate the process of gametogenesis and the general structure of the female reproductive tract as the entire prenatal period is spent inside the mother's womb.

You should remember that all the general principles involved in animal development that you had studied in Block 3, with the help of examples taken from invertebrates, and vertebrates, hold good for human beings as well. Revising those concepts will help you to appreciate and understand the complexities that occur during the period of prenatal development.

Objectives

After studying this unit, you should be able to:

- explain the key biological events in early human development;
- explain the structure of female reproductive tract and steps in fertilization;
- summarize the events that occur during blastocyst formation, implantation an prenatal period;
- describe the role of extra-embryonic membranes and process of formation of placenta, and;
- analyse some of the causes of flaws in early human development.

16.2 FEMALE REPRODUCTIVE TRACT

The female reproductive tract includes the fallopian tubes, uterus and vagina (Fig.16.1). You would notice that the ovaries lie one on each side of the pelvic cavity. The fallopian tubes extend from the uterus and are not attached to the ovaries but have fingerlike projections called **fimbriae** that sweep over the ovaries. During the time of ovulation, when the oocyte is released from the ovary, it is usually swept into the fallopian tubes by the action of the fimbriae and beating of the cilia that line the tubes.

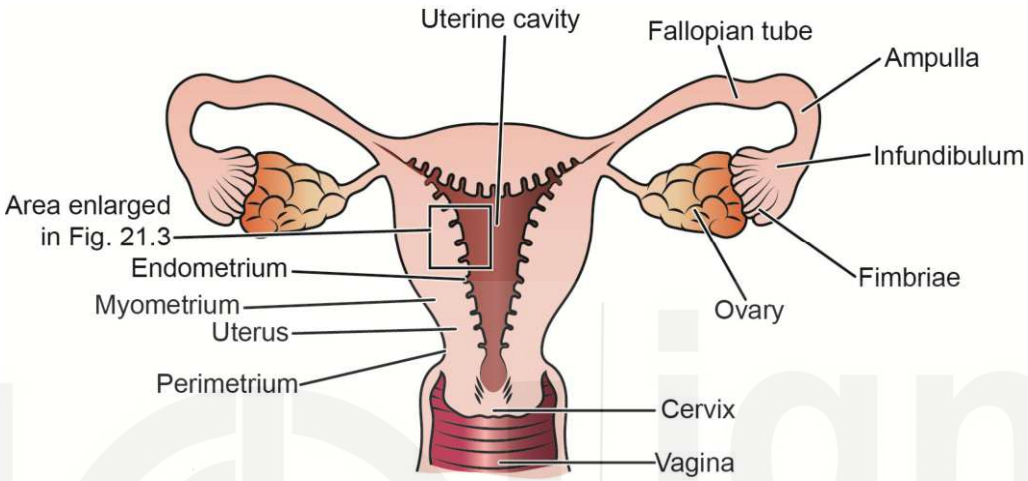


Fig.16.1: Female reproductive tract along with the ovaries.

The wall of the uterus is relatively thick and consists of three layers-the endometrium, myometrium and perimetrium (Figs.16.1 and 16.2). The **endometrium** forms the inner mucosal layer lining the uterine cavity. This lining is covered with columnar epithelium and contains numerous glands. The **myometrium** is thick and muscular. The **perimetrium** consists of the outermost thin layer covering the body of the uterus.

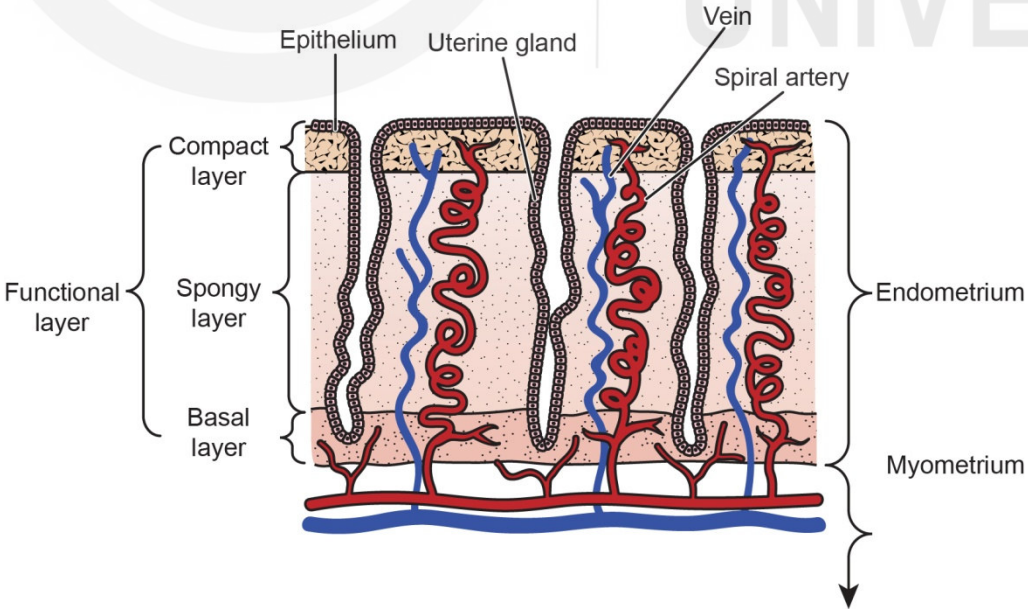


Fig.16.2: Structure of uterine wall.

Out of the three layers of the uterus only the endometrium participates in the formation of the placenta. In a non-pregnant female the endometrium varies in

its thickness according to the monthly reproductive cycle or the menstrual cycle. The term menstrual cycle as you know refers specifically to changes in the uterus. During the monthly reproductive cycle in non-pregnant females only the functional layer of the endometrium (See Fig.16.2) undergoes cyclic changes and varies in thickness.

The entire reproductive cycle consists of an ovarian cycle and an uterine cycle. Fig.16.3 illustrates one complete reproductive cycle. You can see in the figure the interrelationship among the hypothalamus, pituitary, ovaries and uterus. The average reproductive cycle is spread over 28 days. It commences at puberty and normally continues throughout the reproductive years. The cycle prepares the reproductive system for pregnancy.

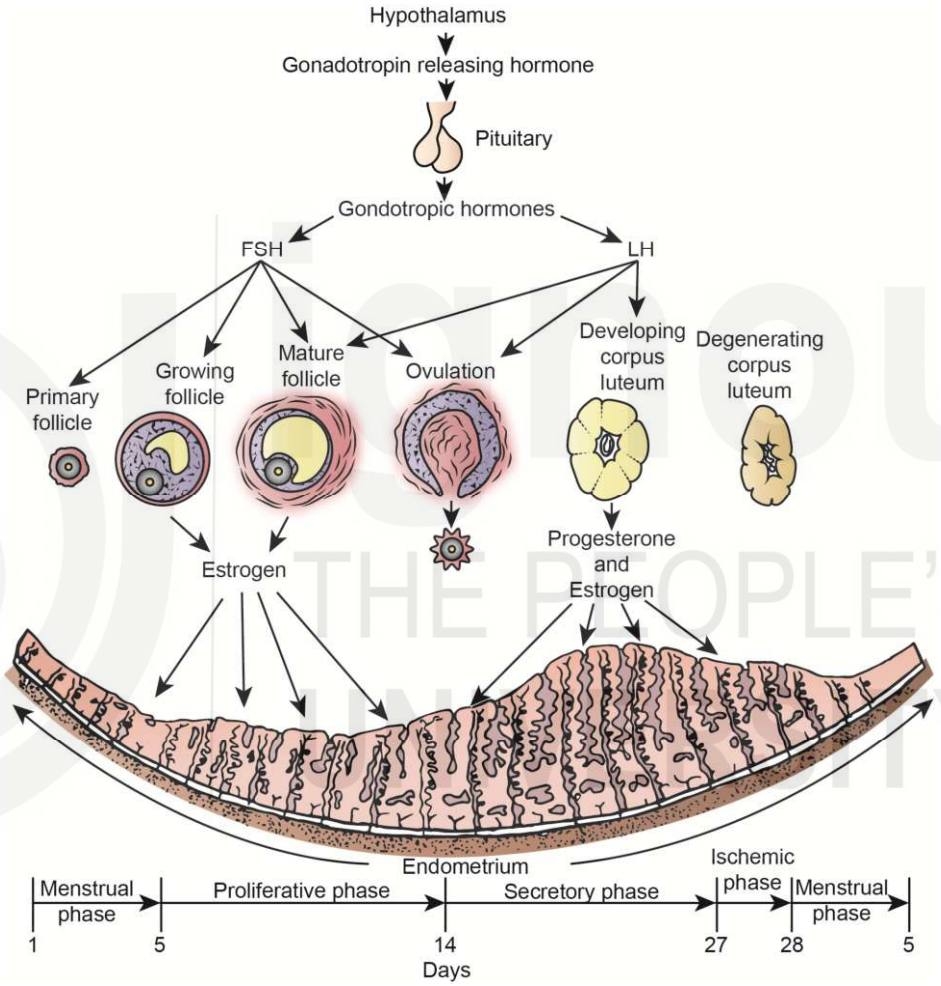


Fig.16.3: Interrelationship of the hypothalamus, pituitary, ovaries and endometrium. One complete menstrual cycle shown. Note that the cyclic activity of the ovary is intimately linked with the changes in the endometrium.

The reproductive cycle in human females is known as the menstrual cycle which has a duration of 28 days. It is characterised by regular recurring changes in uterine lining which prepare the uterus for pregnancy. In case pregnancy does not occur then the uterine endometrium is shed from the uterus through the cervix and vagina in a bleeding known as menstruation. The menstrual cycle is accompanied by a follicular growth phase in the ovary or the ovarian cycle.

The hypothalamus synthesises the gonadotropin releasing hormone (GnRH) that stimulates the anterior pituitary to secrete follicle stimulating hormone (FSH) and luteinizing hormone (LH). The ovarian cycle begins with the phase of follicular growth in the ovary after the end of preceding menstrual cycle and lasts for about 14 days. In the first half of this period FSH stimulates the growth of 5-12 primary follicles out of which usually only one matures at a time. The maturing follicle secretes estrogen which exerts a feedback control on anterior pituitary inhibiting further FSH production hence preventing maturation or more follicles. The follicular phase comes to an end with the

maturation of one follicle and its release from the ovary leading to ovulation, on day 14. At the time of ovulation the ovum is in the secondary oocyte stage surrounded by the non-cellular zona pellucida and follicle cells that form the zona radiata (or corona radiata). Following ovulation, from day 15 to 28 production of LH from anterior pituitary stimulates the formation of corpus luteum from the follicle cells left behind in the ovary. The corpus luteum secretes the hormone progesterone which prepares the endometrium for implantation of embryo and later maintenance of pregnancy during early stages. If the ovum is not fertilized, the corpus luteum degenerates and menstruation begins again.

The cyclic changes in the ovary are matched by cyclic changes in the uterine wall as you can see from Fig.16.3. From day 1 to 5 the level of sex hormones in the body is low, hence uterine wall breaks down and menstruation begins. This is externally manifested by bleeding. This is the menstrual phase. As the level of estrogen produced by the growing follicle increases from day 5-14 the endometrium gradually thickens and becomes glandular. This is the proliferative phase. From day 15 to 28 the endometrium doubles in thickness and is richly vascularized preparing to receive the embryo for implantation, this is the **secretory phase**. If pregnancy does not occur, the uterine wall breaks down leading to bleeding into the uterine cavity and the menstrual cycle begins all over again.

Now that you know how the uterus prepares itself for pregnancy we proceed to study the events in early development after fertilization takes place.

SAQ 1

Match the following stages with their characteristic features:

i)	Ovulation	a)	Corpus luteum and uterine wall break down, sex hormones low
ii)	Menstrual phase	b)	Under influence of FSH estrogen secreted, uterine wall becomes thick and vascularised
iii)	Proliferative Phase	c)	Post ovulation estrogen and progesterone secretion, corpus luteum enlarges, functional layer of uterus thickens
iv)	Secretory phase	d)	Ovum released under the influence of LH and FSH

16.3 FERTILISATION AND IMPLANTATION

All the events of sexual reproduction that take place before the fusion of gametes (syngamy) are included under pre-fertilization events.

Gametogenesis and gamete transfer are two main pre-fertilisation events. Before studying about actual process of fertilization, let us discuss about these two events:

16.3.1 Gametogenesis

You have learnt in Unit 11 of this course that the process of formation of male and female gametes is known as gametogenesis. Gametes are haploid cells. In humans, the parent body is diploid thus, the gametes are formed by the process of meiosis. In humans, the male and female gametes are morphologically dissimilar hence, they are called heterogametes. The male gamete is called sperm and female reproductive gamete is known as egg or ovum. Though all our somatic cells are diploid, only the gonads i.e. the ovaries in the females and the testes in males have some specialised cells which take part in the production of gametes i.e. gametogenesis. These cells are called as meiocytes. During gametogenesis, meiocytes undergo meiotic division due to which, the number of chromosomes in the daughter cells get reduced to half in number and thus from the diploid meiocytes, four haploid gametes are formed. Gametogenesis in the males is known as spermatogenesis and in females it is called oogenesis. The spermatogenesis in males gets started at puberty and oogenesis in females gets started at the embryonic stage before birth.

Spermatogenesis takes place in the testes of male reproductive system. The process of spermatogenesis takes place at a temperature less than that of body temperature that's why the testes are present in the scrotal pouch which is located outside the body. In the testis, the process takes place in seminiferous tubules, where the spermatogonial cells undergo division which leads to the formation of primary spermatocytes (immature) which then undergo spermiation in the Sertoli cells to develop into spermatids as the Sertoli cells are the nurse cells which provide nutrition to primary spermatocytes to form the functional spermatids. These spermatids then reach epididymis and get temporarily stored in it till the ejaculation. Spermatids undergo spermiogenesis to produce sperms.

Oogenesis takes place in the ovary and starts with the development of primary oocytes, from oogonia. Oogenesis is complete either before or shortly after birth. Primary oocytes reach their maximum development at 20th week of gestational age, at that time approximately seven million primary oocytes have been created; however, at the birth of a female, this number gets reduced to approximately 1-2 million. Thus at birh the human female has all its future ova arrested at primary oocyte stage. After menarche (starting of menses at puberty) under the influence of pituitary hormones, these primary oocytes begin to develop again and, as a result of meiosis I, the primary oocyte develops into the secondary oocyte and the first polar body. This completes meiosis I, and the secondary oocyte undergoes meiosis II immediately. This process is also halted at the metaphase II stage until fertilization. Every month, the secondary oocyte is released by the ovary and waits for one day i.e. 24 hours for the sperm for fertilization, if the egg doesn't get sperm for fertilization then it gets released during menses and the secondary oocyte does not complete meiosis II. But if fertilization takes place then meiosis II

gets completed. As a result the mature ovum with all the cytoplasmic contents and the maternal complement of chromososmes and another polar body is formed (at this stage both secondary oocyte and polar body are haploid). Both the polar bodies disintegrate at the end of Meiosis II, leaving only the fertilised ovum with the full complement of 46 chromosomes from the sperm and egg.

Gamete Transfer

In humans, the male gametes are motile and the female gametes are stationary. After gamete formation, male and female gametes should come in physical association for fusion of gametes. During gamete transfer a large number of male gametes fail to reach the female gametes so, to fulfill this loss, the number of male gametes produced is several thousand times the number of female gametes. The process of transfer of male gamete into female genital tract is known as insemination.

16.3.2 Fertilisation

The process of fusion of male gametes (sperm) with the female gametes (ovum) is called fertilization or syngamy. It usually takes place in the ampullary-isthmus junction of oviduct in the uterus. It results in the formation of zygote. Now let us discuss about the mechanism of fertilisation.

After insemination process, the sperm undergoes a series of changes, as freshly ejaculated sperm is unable or poorly able to fertilize the egg. The sperm undergoes **capacitation** in the female reproductive tract over several hours, which increases its motility and destabilizes its membrane, preparing it for the **acrosomal action** which helps it in the penetration of the egg's tough membrane, the **zona pellucida**, which surrounds the oocyte. When the sperm reaches the ovum (secondary oocyte), it invades the follicular cells (Fig.16.4). An enzyme **hyaluronidase** is released from the acrosome of the sperm head. This enzyme removes the extracellular matrix and disperses the corona radiata cells. Another enzyme **acrosin** digests a path for the sperm through the zona pellucida. In the acrosome of the sperm this trypsin like enzyme is inactive and needs to be activated by a glycoprotein in the female reproductive tract.

Once a sperm passes through the zona pellucida, this covering becomes impenetrable by other sperms. This is known as the **zona reaction**. The structure of the membrane changes and lysosomal enzymes are released from the secondary oocyte that prevent other sperms from attaching to the membrane.

Usually only one sperm enters the ovum. Two sperms may participate in fertilization under an abnormal process known as **dispermy**. The resulting embryo contains 69 chromosomes and may appear normal, but it is always aborted. Sometimes a triploid infant may be born but it dies shortly. Thus polyspermy does not produce viable embryos.

After penetration of sperm in the cytoplasm of the oocyte, the tail and the outer covering of the sperm disintegrate and the cortical reaction starts to prevent polyspermy. The oocyte undergoes second meiotic division and results in the

formation of the haploid ovum and releases a polar body. The sperm nucleus then fuses with the ovum which results in fertilization.

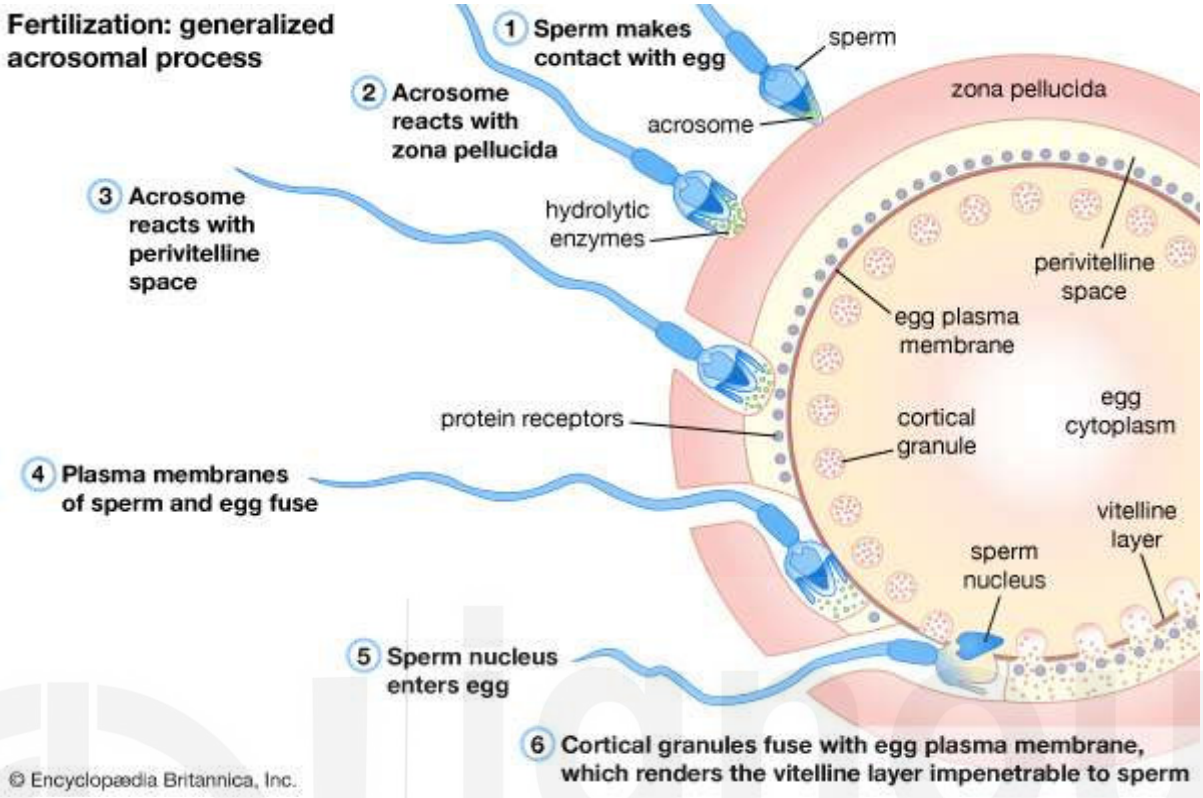


Fig.16.4: Mechanism of Fertilization.

After fertilization, the cytoplasm of sperm and ovum fuse. Then fusion of their nucleus takes place that results in the formation of zygote. After the formation of zygote, it starts moving towards the uterus and ultimately results in the formation of blastocyst which undergoes implantation. Then, embryogenesis takes place which ultimately leads to formation of fully developed baby.

SAQ 2

a) What is the site of fertilization in humans?

b) At what stage the spermatogenesis begins?

c) Mark the statements given below true or false:

i) The sperm cell penetrates the zona pellucida of the ovum using an enzyme hyaluronidase.

ii) The sperm swims to reach the ovum only due to the lashing of its whip like tail.

iii) After penetration of the ovum by the sperm head polyspermy is prevented due to structural changes in the cell membrane of the ovum.

iv) The second polar body and the sperm nucleus fuse to form the zygote.

16.3.3 Blastocyst Formation

In humans, the blastocyst is formed after five days of fertilization. There are various stages which are involved in the process of blastocyst formation.

About thirty hours after fertilization the zygote undergoes mitosis giving rise to 2 blastomeres (Fig.16.5). These blastomeres undergo further cleavages and by the 3rd day after fertilization, the tiny mass of cells reaches the uterus. By this time it is a solid ball of cells called **morula**. In the next three days the morula is transformed into a hollow ball of cells called **blastocyst** with layers of cells and fluid filled cavity called blastocoel.

The cavity of blastocyst is filled with fluid secreted by the cells. One portion of the blastocyst contains a concentrated mass of cells that will give rise to the entire body of the new individual. This is known as the **inner cell mass** (ICM). Interestingly, each cell of this mass has the ability to develop into a complete individual. Identical twins are formed if the ICM splits (See Box 16.1). The outer ring of cells of the blastocyst surrounding its cavity is known as **trophoblast**. It helps in providing the nourishment to the developing embryo and gives rise to all the extra-embryonic membranes including much of the placenta. It also consists of several enzymes which help in implantation process by dissolving the endometrium and implanting the blastocyst in the lining. The side of the blastocyst where the inner cellular mass is located is known as the animal pole, and the opposite side is called as the vegetal pole.

The development in trophoblast results in formation of structures which helps in implantation of embryo to the mother's womb (Fig.16.5). Simultaneously, the inner cell mass continues to differentiate which leads to formation of various parts or organs of the embryo and ultimately fully developed embryo.

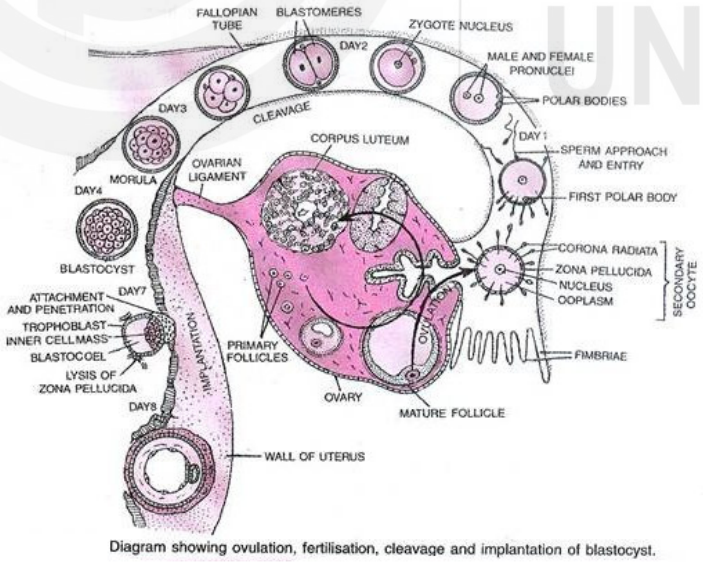


Diagram showing ovulation, fertilisation, cleavage and implantation of blastocyst.

Fig.16.5: Structure showing ovulation, fertilization, cleavage and implantation of blastocyst.

16.3.4 Implantation

After entering the uterus and formation of ICM, the blastocyst begins to embed in the endometrium of the uterine wall. By one week after fertilization the

HCG secretion which starts shortly after fertilization is excreted in urine. Its presence in urine is used to detect pregnancy. A positive pregnancy test can be detected within about 8 to 10 days of fertilization.

trophoblast secretes enzymes that digest the tissues and blood vessels of the uterine wall. The invading trophoblast differentiates into two layers, the outer **syncytiotrophoblast** and the inner **cellular layer.** As the syncytiotrophoblast swallows more blood vessels in the uterine wall, lacunae develop in the syncytiotrophoblast which get filled up with blood from the mother and exchange of gases takes place here. Thus a primitive uteroplacental circulation is established (Fig.16.6). This nourishes the embryo till the placenta is formed.

By the 10th day the blastocyst is completely embedded in the uterine wall. This type of implantation in which the embryo gets fully embedded is known as **interstitial implantation.** The trophoblast begins to secrete human chorionic gonadotropin (HCG). HCG causes the corpus luteum to be maintained and to continue to secrete estrogen and progesterone.

Sometimes implantation may occur outside the uterus at some other location. In that case it is an ectopic pregnancy. The implantation site may be the fallopian tube or even the abdominal cavity. In ectopic pregnancy the embryo has to be surgically removed because if it is not done, it can lead to tubal rupture, internal bleeding, shock and possible death.

At the start of the second week a small cavity appears between the trophoblast and ICM. This is the amniotic cavity which will grow around the embryo and later the foetus. It is a fluid filled cavity which acts as an insulator against shocks, cold and heat. At the same time the ICM also differentiates into two layers, the upper **epiblast** which gives rise to the embryo and the lower **hypoblast** which gives rise to the extraembryonic membranes. You can see the two primary germ layers and the beginning of third in Fig.16.6.

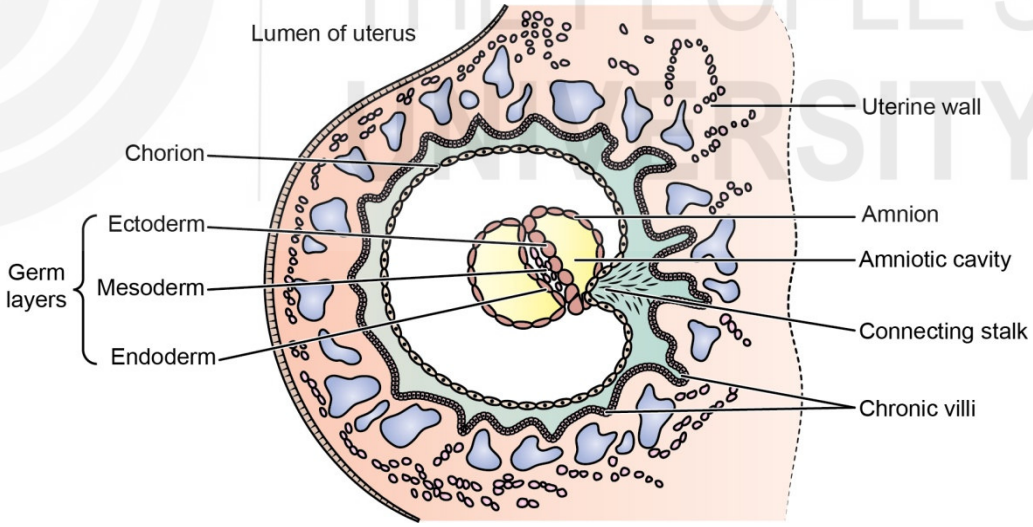


Fig. 16.6: Fully implanted embryo and formation of three germ layers.

Box 16.1

Identical Twins

Human twins may be identical, when they are formed from one egg (monozygotic) or fraternal when they are the result of fertilization of two different ova released at the same time and fertilized by different sperms (dizygotic).

From an embryological point of view identical twins are of greater interest. 0.25% of

all births may be monozygotic twins. Current evidence indicates that twinning occurs at the blastocyst stage by the splitting of the inner cells mass. The cells of the ICM are considered totipotent *i.e.,* they have the potential of forming a single, dual or even a multiple embryonic disc. If by chance, the separation of inner cell mass occurs before day 5, even before the formation of trophoblast, which occurs in 33 percent of identical twins, then they have two separate chorions and amniotic cavities. (Fig.16.7). Identical twins that share a common chorion, but two separate amniotic cavities indicate that the split in ICM came between the formation of chorion on day 5 and formation of amnion on day 9 as shown in Fig.16.7. This is the case in two thirds of identical twins. A small percentage of identical twins are born within a single chorion sharing a single amniotic cavity as shown in Fig.16.7. This indicates that the division occurred after day 9 after the implantation of the blastocyst.

As seen in Fig.16.7, the embryonic disc may divide incompletely *i.e.,* the two new embryonic axes fail to separate completely and the outcome is conjoined twins. The degree of union may be slight or quite extensive and the twins may be joined at any part of their body.

It should be remembered, however, that conjoined twins represent an aberration in development that would normally lead to formation of identical twins. Most conjoined twins do not survive after birth but nowadays it is possible to separate some of them surgically and then one or both may survive to lead fairly normal lives.

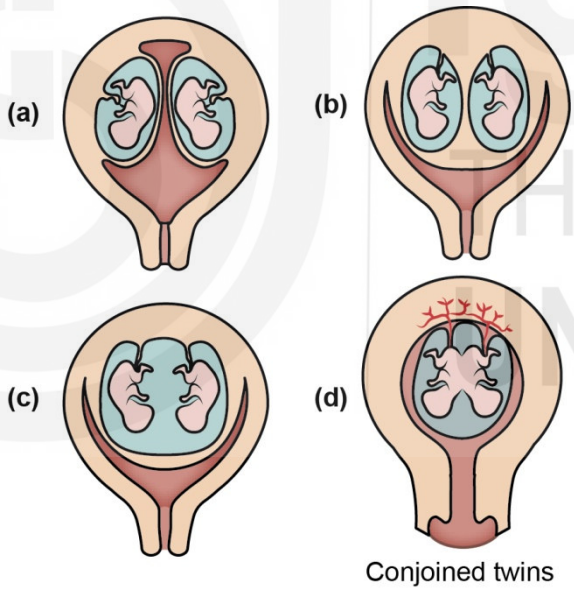


Fig.16.7: Conjoined twins

SAQ 3

Choose the correct term:

i) The morula/blastocyst implants in the uterine endometrium.

ii) The ICM/trophoblast gives rise to the embryo.

iii) Ectopic pregnancy is the result of implantation inside/outside the uterus.

iv) HCG maintains/degenerates the corpus leuteum.

v) Uteroplacental circulation occurs due to development of blood filled space in syncytiotrophoblast/inner cellular layer of trophoblast.

16.4 EMBRYONIC DEVELOPMENT

The embryonic stage extends from the second week through the eighth week and is characterised by formation of placenta, the development of internal organs and appearance of the major external body structures. During this period the embryo takes on a human shape by morphogenetic processes. The rudiments of various organs get established during the 3rd week but further development will take place from then on to 8th week by organogenesis, a process you are already familiar with. Because of the simultaneous developmental changes taking place during this period the developing embryo is particularly sensitive to teratogens, that is, certain agents like alcohol or drugs etc., can induce malformations in the rapidly forming tissues and organs.

16.4.1 Third Week

During the third week of development the ICM separates from the trophoblast and forms the flattened embryonic disc, which at first contains cells of all three germ layers, ectoderm, endoderm and mesoderm and is called the epiblast. From this a lower layer separates to form the endoderm. The second layer, mesoderm, is formed by migration of cells through the primitive streak which forms as the embryonic disc elongates. The cells remaining in the upper layer form the ectoderm.

At 8 to 10 weeks of development doctors can take samples of tissue from chorionic villi to detect genetic defects in the embryo because chorionic cells and foetal cells contain identical genetical information.

These three germ layers will give rise to all the organs of the body as indicated in Fig.16.8.

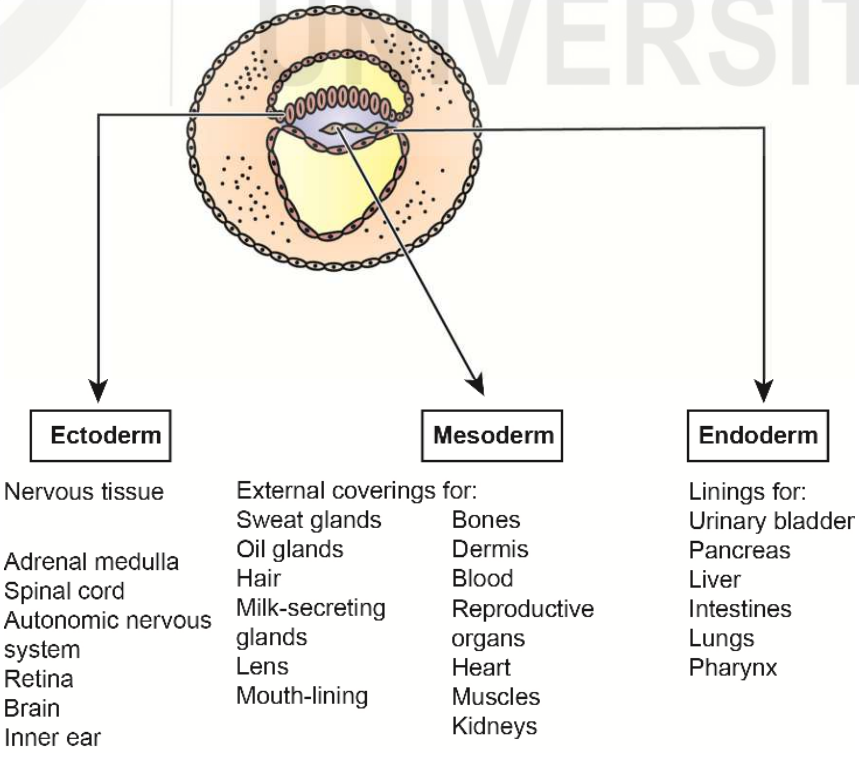


Fig.16.8: The organs of the body formed from the three germ layers.

The embryo's heart also begins its development as a pair of microscopic tubes. The cardiovascular system is the first system to become functional in the embryo. At the end of the third week the heart tubes fuse and become linked to the blood vessels in the embryo, body stalk, chorion and yolk sac, to form a primitive blood circulatory system. Chorionic villi (which will form the placenta later) also begin to form during this period.

16.4.2 Fourth Week to Eighth Week

The general changes in body shape and plan of the embryo from fourth week to eighth week are shown in Fig.16.9.

The fourth week embryo is cylindrical and has a blunt head with a very primitive brain (Fig.16.9). Vague rounded elevations on the lateral surfaces of the brain indicate the eye rudiments. The simple tube like heart which arose in the third week is functional, pumping blood through the umblical arteries to the placenta. Heartbeats, however, cannot be recorded yet. Oral and anal openings appear but are nonfunctional. At the end of fourth week the embryo is 1/4 of an inch long. A characteristic feature of the fourth week is the alternating series of elevated ridges and depressions, the pharyngeal pouches and grooves respectively. These 4 pharyngeal pouches correspond to the gill arches and grooves to the gill slits of fishes. However, these grooves in humans never become functional or perforated. The pharyngeal pouches in humans form the eustachian tubes (1st pair of pouches), walls of tonsils (2nd pair of pouches), thymus and parathyroids (3rd and 4th pair of pouch).

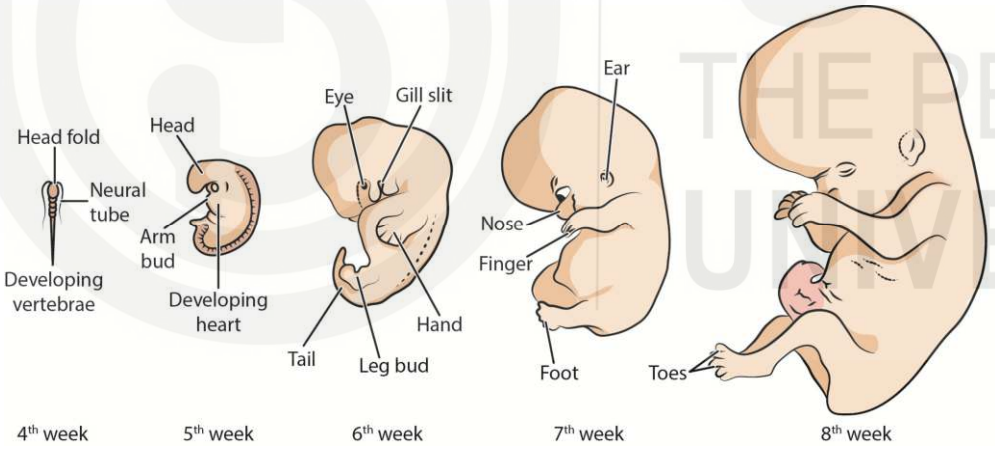


Fig.16.9: Human embryonic development from fourth to eight week.

During the **fifth** week the embryo becomes curled so that the head almost touches the tail (Fig.16.9). The rudimentary arms and legs (limb buds) appear about the middle of the fifth week. The head is now much larger than the trunk region. The brain is the most prominent feature of the embryo. Nerves spread out in the body, paired gonads form, .though not recognizable yet, as testes or ovaries.

In the **sixth week** the head is disproportionately large and the stomach bulges out because of the large liver. Future fingers are seen as indentations on the paddle shaped hands and feet. The hands develop faster than the feet. This is an expression of the general rule that anterior structures grow more rapidly than posterior structures (Fig.16.8). Rapid growth occurs in the facial region.

By the end of the sixth week, the main systems, nervous, muscular, circulatory, excretory, reproductive, digestive and skeletal have been initiated.

After the sixth week the embryo starts looking more human and all the internal organs are formed by the **seventh week** (Fig.16.8). Along with the development of brain, the head achieves its normal relationship with the body as the neck appears. The nervous system is developed enough to permit reflex actions such as the 'startle response' to touch. By the end of **eight week** of development, the embryo is usually 30 mm in length and weighs less than 5 mg (Fig.16.8).

16.5 FOETAL DEVELOPMENT

The foetal stage of development begins in the ninth week and lasts till birth. During this period the existing body structures continue to grow and mature and only a few new parts appear. Figure 16.10 shows that the growth rate is rapid during the foetal period and the body proportions also change greatly. You can also see from Figure 16.10 that at the beginning of the foetal period the size of the head is disproportionately large and legs are short but later the growth of the head slows down.

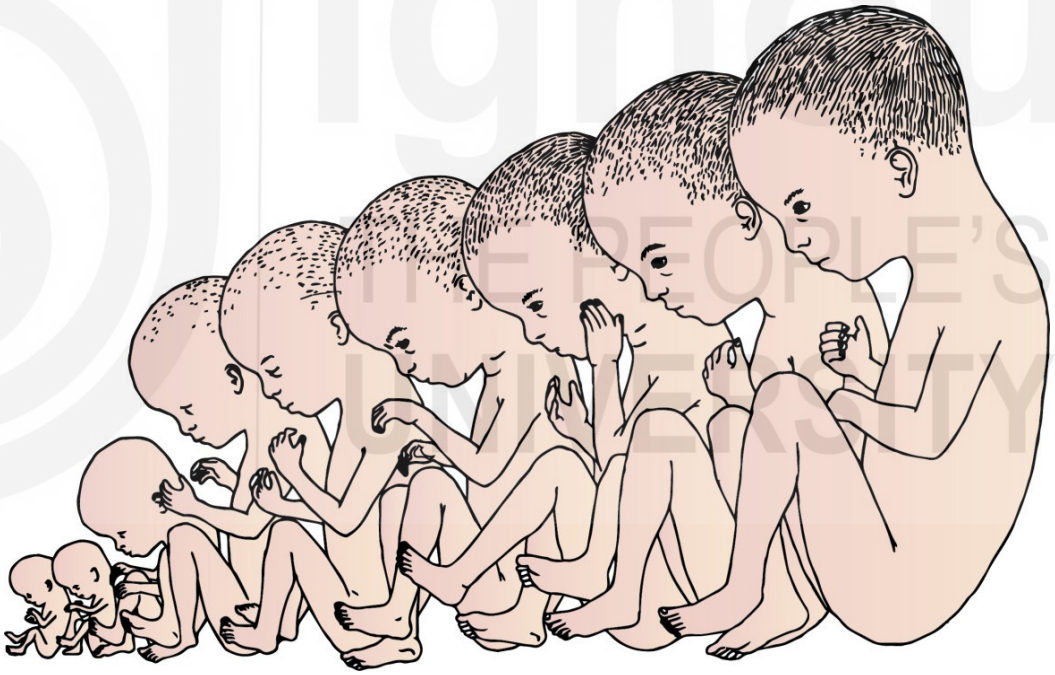


Fig.16.10: Growth during foetal period. The above 9 – 38 week fetuses are less than half actual size.

In the third month, the arms attain the relative length they will maintain during development. Cartilage is replaced by bone as ossification centres appear in most bones. By the end of third month, it is possible to distinguish male foetus from the female. The Y chromosome triggers the formation of a protein called the H-Y antigen that causes the differentiation of testes from the indifferent gonads. The testis secretes testosterone that stimulates the growth of external genitalia. In the absence of testosterone, female genitalia form. Estrogen need not be produced by foetal ovaries as there is enough maternal estrogen circulating in the blood.

In the **fourth month**, the body grows rapidly to reach a length of 13-17 cm, the legs lengthen and the heart beat is loud enough to be heard by the physician's stethescope.

In the **fifth month** the rate of growth decreases slightly. The legs achieve their final relative proportion, the skeletal muscles become active and the mother may feel the foetal movements. Some hair appear on the head and the skin is covered by downy hair. The skin is also covered by a cheesy coating made up of dead epidermal cells and secretions of sebaceous glands.

During the **sixth month**, the body gains substantial amount of weight. Eyebrows and eyelashes appear, skin is wrinkled and translucent.

In the **seventh month** fat gets deposited in the subcutaneous tissues. The eyelids that were fused together in the third month reopen. At the end of the seventh month a foetus is about 37 cm in length. If a baby is born in the seventh month, it is possible that it may survive.

In the **eighth month**, the testis of the male descends into the scrotal sac from regions near the kidney. During the **ninth month** the foetus reaches a length of about 47 cm. The skin becomes smooth and the body appears chubby due to accumulation of subcutaneous fat. An average full term foetus is about 50 cm long and weighs 2.5 to 3.6 kg. Fingers and toes have well developed nails. Fig.16.11 shows the full term foetus with its head positioned towards the cervix.

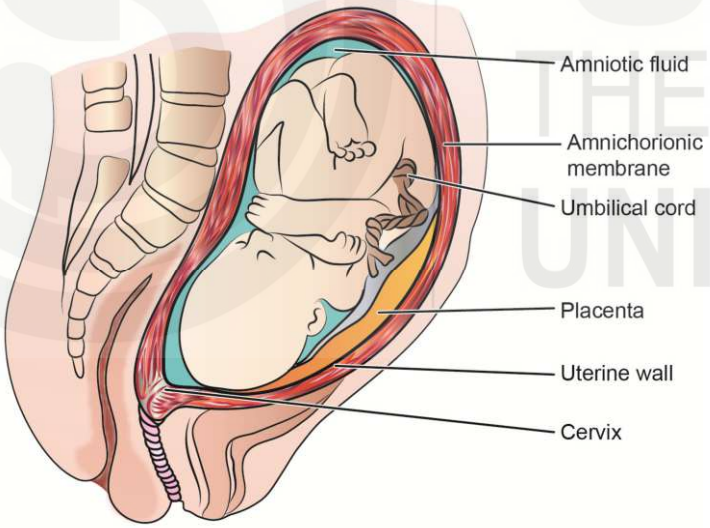


Fig.16.11: Full Term Foetus.

Birth takes place at the end of the third trimester, 38 weeks after conception. The uterus begins a series of powerful contractions. This is due to hormonal as well as mechanical changes in the uterus.

During the last two months of pregnancy estrogen secretion begins to increase while progesterone starts to level off. Since estrogen stimulates and progesterone inhibits contractions, stimulation or contraction dominates. Another hormone oxytocin also stimulates contraction and its levels increase sharply at the beginning of labour. In addition, simple stretching of the uterus also increases the contradictions. At the same time placental tissue especially

the trophoblast exhibits degenerative changes which lead to separation of the foetal and maternal tissues at the time of birth. Now investigations have revealed that at parturition the placenta contains very high concentration of prostaglandins which are powerful stimulators of uterine contractions. These placental prostaglandins are secreted under the influence of foetal hormones. It appears, therefore, that the foetus controls its own birth! Foetal hypothalamus activates the pituitary to release adrenocorticotropin which stimulates the adrenal gland in the foetus to release cortisol. There is evidence that cortisol directs the production of prostaglandins in the placenta and these in turn may influence uterine muscles to contract or cause increased secretion of oxytocin from maternal pituitary gland.

SAQ 4

a) Choose the correct alternative.

i) Growth rate is rapid during the foetal/embryonic period.

ii) It is possible to distinguish male foetus from female foetus at the end of second/third month.

b) Fill in the blanks

i) Foetal heart beat can be heard in the ..... month.

ii) Foetal movements can be felt by the mother by ..... month.

iii) Eyelids that are fused in the third month reopen in the ..... month.

iv) Accumulation of subcutaneous fat to give a chubby appearance takes place in the ..... month.

16.6 DEVELOPMENT CHANGES AFTER BIRTH

The first independent breath may take as much as 10 times the inhalation force required of an adult. As much as 40 percent of the air inhaled remains inside the lungs so less force is required to expand them in subsequent breaths.

During the birth process, some important developmental changes occur in the infant's respiratory and circulatory systems. These changes occur in response to the change in its environment from aquous to gaseous. The most immediate need at birth is to obtain oxygen and excrete out carbon dioxide. Therefore, the first breath is critical. Let us examine the changes that take place in the respiratory and circulatory systems.

Respiratory System

The new-born's lungs are collapsed and require powerful breaths to inflate them; airways too are small, offering considerable resistance to flow of air. The surface tension tends to hold the moist membranes together. The lungs of a full term baby secrete surfactant which has the unique property of exerting high surface tension when the lungs are expanded but a low surface tension when the lungs are collapsed. Thus after the first powerful breath which expands the lungs, breathing becomes easier, because of the reduced surface tension of collapsed lungs.

Circulatory system

You have learnt that during foetal life, gas exchange takes places, only through the placenta and not through lungs. Therefore, the foetus has several features in its circulatory system that are not present in an adult.

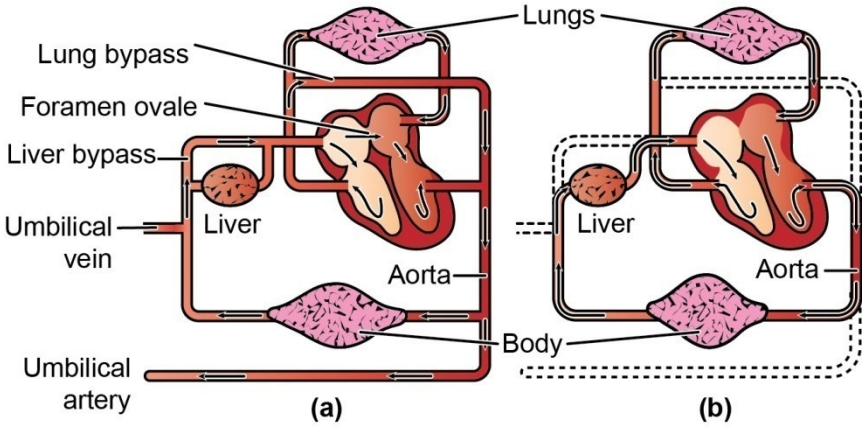


Fig.16.12: Changes that occur in the newborn circulatory system a) Foetal circulation; b) Infant circulation. In the foetus liver and lungs are bypassed by blood vessels. The foramen ovale permits blood to flow from right to left atria. Infant circulation mirrors adult circulation.

Fig.16.12 is a diagrammatic representation of foetal and infant circulation. In the foetus the oxygenated blood flowing back through the umblical vein largely bypasses the liver and goes to the right atrium. The blood then passes to the left atrium directly through an opening called **foramen ovale**. From the left atrium the blood flows to the left ventricle which sends the blood to the head and rest of the body. Blood returning from the head moves through the right atrium to the right ventricle which then does not pump the blood to the lungs but to a shunting vessel the **ductus arteriosis** that connects with the descending aorta. A pair of umblical arteries branch off from the aorta and carry deoxygenated blood to the placenta.

In some new born babies the foramen ovale fails to close leading to the blue baby syndrome due to mixing of oxygenated blood with deoxygenated blood. Earlier such babies did not survive more than a few years but now the condition can be corrected by open heart surgery.

At birth the umblical artery and vein collapse when the cord is tied or the placenta separates. As a result there is negative pressure in right atrium and blood flows back from the left atriurn to the right. The flow causes the one way valve to close in the **foramen ovale**. Thus the left and right atria are separated. The shunting vessels that bypassed the liver and the lungs, also collapse and adult pattern of circulation starts. The whole process takes place within a few hours of birth, though the permanent closure of foramen ovale may take upto a year.

Later Changes

We had said in the beginning of the unit that development does not stop once birth has taken place. It continues through the stages of life i.e., infancy, childhood, adolescence and adulthood.

Infancy lasts until about two years of age. The newborn has certain innate reflexes that help it to establish-a relationship with its caretakers. During this period sensory and motor development takes place which can be related to his or her ability to respond to stimuli or perform simple function.

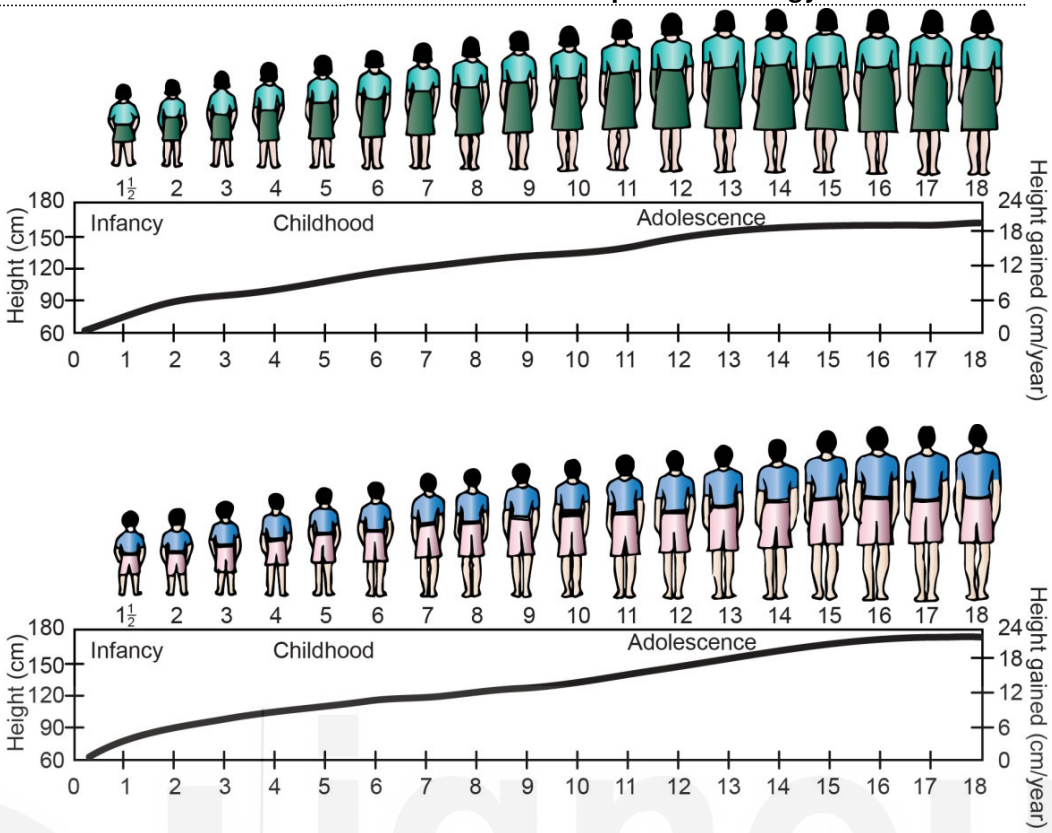


Fig.16.13: Comparative body growth pattern in girls (above) and boys (below) from infancy through adolescence. Note that the male's growth spurt occurs about two years after the females (adapted from 'Growing Up' by J.M. Tanner 1975).

Childhood lasts until puberty which is around 10-12 years in girls and 12-14 years in boys after which adolescence continues till adulthood. The general body growth of boys and girls is given in Fig.16.13. At the time of puberty the sex organs mature and secondary sexual characters begin to appear. This is related to the level of sex hormones in the body.

Actually the hypothalamus pituitary-gonad system functions long before puberty but it is very sensitive to feedback control. When puberty starts, the hypothalamus becomes less sensitive to feedback control and begins to secrete increasing quantities of releasing hormones causing the gonads to develop and start increasing their production of hormones. This sensitivity of the hypothalamus continues to decrease until gonadotropin and sex hormones secretion reaches adult levels.

The sex hormones in combination with other hormones have a profound effect on the body during puberty. There is an acceleration in growth causing changes in weight height, distribution of fat, and body proportions leading to adult appearance.

SAQ 5

Why does the newborn need to take a powerful first breath?

16.7 EXTRA-EMBRYONIC MEMBRANES AND PLACENTA

In this section we will have a look at the extra embryonic membranes in humans and how they make up the placenta which is the life line of the developing foetus inside the mother.

16.7.1 Extra-Embryonic Membranes

All amniotes including humans produce 4 extra embryonic membranes- amnion, yolk sac, chorion and allentois. These as you already know provide nourishment and protection to the developing young one. In Fig.16.14 you can see how the extra embryonic membranes begin to form. These membranes are essentially similar to the extra-embryonic membranes of reptiles and birds, though the method of origin differs in humans. During the third and fourth week, the **amnion** grows around the embryo enclosing it in a membraneous fluid filled sac within which the growing embryo and later the foetus floats and can move freely. This sac as mentioned in the earlier section is a shock absorber and encloses a fluid, the amniotic fluid, that helps to keep the temperature of foetal environment stable.

The other membrane, the **chorion** develops from the trophoblast cells. The chorion is a highly specialized extraembryonic tissue. It facilitates the transfer of gases, nutrients and wastes between the embryo and the mother. It is the primary part of the placenta which we will study a little later.

Recent studies have indicated yet another function of chorion. It protects the embryo from the immune response of the mother. The human body rejects any kind of foreign tissue implanted in its body, yet it tolerates the embryo which has major histocompatiblity antigens from both mother and father. One hypothesis is that the immune system of mother does not reject the embryo or foetus because the chorion produces substances that block the immune response.

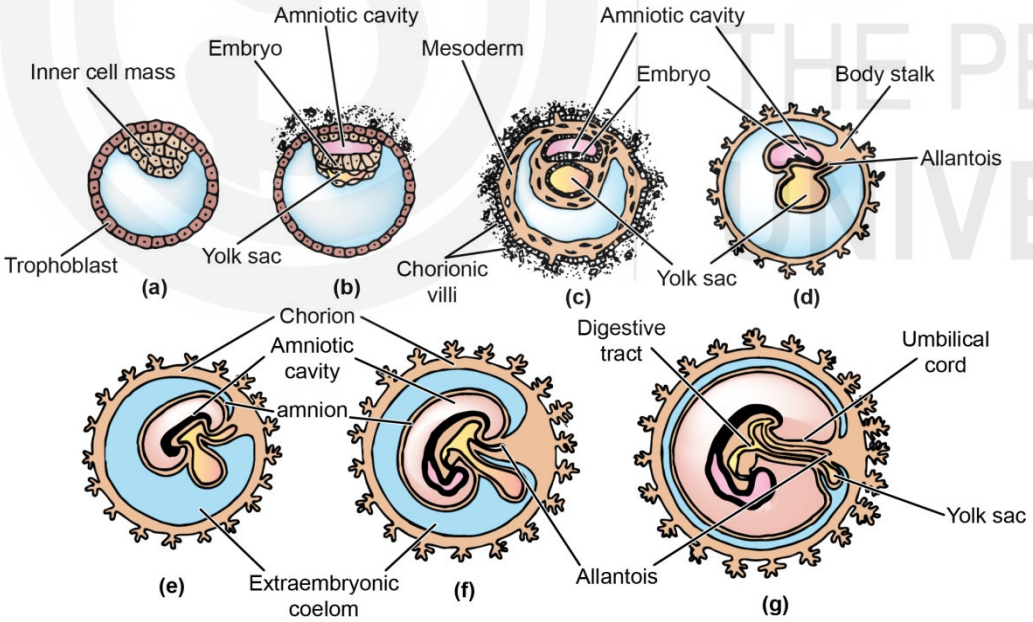


Fig.16.14: Development of extra embryonic membranes and umbilical cord, a) blastocyst and surrounding trophoblast b) amniotic cavity and yolk sac appear c) formation of first chorionic villi d) embryo connected to chorion by body stalk e-g) formation of umbilical cord.

Yolk sac develops during the second week of development although there is no yolk in the human egg. It does not provide nourishment to the embryo but it becomes surrounded by mesoderm which forms the blood cells till the liver of the embryo becomes functional in the sixth week. The yolk sac along with the

allantois form the umblical cord. Part of the yolk sac also forms the lining of the gut. The **allantois** which forms during the third week of development is a tiny sausage shaped pouch on the yolk sac. It too is responsible for producing blood cells and later develops into the umblical blood vessels.

SAQ 6

Compare the functions of human extra embryonic membranes with those of other vertebrates.

16.7.2 Placenta

We had said earlier in the unit that in the second week of development a primitive uteroplacental circulation is established. Early in this stage slender projections grow out from the trophoblast into the surrounding endometrium. These are the chorionic villi and they become branched (Fig.16.14). By the end of fourth week they are well formed.

As the chorionic villi develop embryonic blood vessels begin to form in them and these vessels are continuous with the vessels in the connecting stalk. Matching the chorionic villi, uterine crypts develop. Until about the end of the eighth week, the chorionic villi cover the entire surface of the former blastocyst. As the embryo and chorion enlarge only those villi that are in contact with the endometrium remain. The others degenerate. The region still in contact with the endometrium forms the disc shaped placenta. The manner in which the trophoblast interacts with the maternal tissue largely determines the morphology of the placenta. Very intimate contact between the mother and embryo is achieved in the placenta found in the humans and rodents. Here the chorionic villi grow deep into uterine tissue and break-down the maternal blood vessels until they are bathed in maternal blood. This kind is called the **haemochorial** placenta, in which a thin membrane separates the embryonic blood within the capillary of the chorionic villus from the maternal blood in the crypts of the endometrium. This membrane is the placental membrane and is composed of epithelial membrane of the capillary and the membrane of the villus. It is through this membrane that exchange of gases takes place. Oxygen and nutrients pass from the maternal blood to the embryo and carbon dioxide and other wastes from the foetal blood move across the membrane to the mother's blood.

By the **tenth week** of development, the placenta is fully formed. It begins to secrete estrogen and progesterone. These hormones by their negative feedback on the pituitary and hypothalamus prevent any new follicle from maturing and also maintain the lining of the uterus.

Various substances can move across the membrane by active transport and pinocytosis. The blood of the embryo normally never mixes with that of the mother, however, small molecules and various toxins and viruses do pass through across the placenta and this is the reason that many drugs taken by the mother or certain infections contacted by the mother are passed on to the embryo.

SAQ 7

i) What is the function of amniotic fluid?

ii) How are substances exchanged between the embryonic and maternal blood?

16.8 FLAWS IN DEVELOPMENT

The human growth and development despite its complexity works perfectly most of the time. But sometimes defects can be seen during development. All birth defects are loosely grouped as 'congenital defects'.. These are due to defective genes, abnormal arrangements of chromosomes and sometimes environmental factors and unfavourable factors occurring in the uterine environment during pregnancy. The list of hereditary birth defects is long and in some cases the specific chemical defects due to defective genes is known, for example, haemophilia, sickle cell, thalassemia, and phenylketonuria. But not all genetic defects are inherited. In some embryos the genetic instructions that govern growth are initially correct but somewhere during cell divisions the instructions become garbled and mishaps of this type usually involve not a single defective gene but a whole rearrangement, of the cell's genetic material.

Though foetal and maternal blood never mix during pregnancy, at the time of birth when the placenta separates (or if a miscarriage occurs) the placental membrane may be broken and some foetal blood may enter the maternal blood stream. This condition can lead to serious consequences in the next pregnancy if the mother is Rh negative and the foetus is Rh positive. The Rh positive cells of the foetus stimulate the maternal tissue to produce anti-Rh agglutins. In case the mother is pregnant with another Rh positive foetus then these anti-Rh agglutins can pass through the placental membrane and react with the foetal red cells causing them to agglutinate. The foetus then develops a condition known as **erythroblastosis foetails** which is often fatal or if the infant survives it may have severe motor and sensory losses and mental deficiencies.

a) **Problems with Embryo Development**

After implantation, there are several changes which takes place during embryo development such as cell differentiation which leads to formation of two layers in the blastula i.e. trophoblast and inner cell mass. The trophoblast layer is responsible for formation of chorionic villi which form various layers to cover the implanted blastula. The inability of trophoblast to form the chorionic villi due to lack of hormones results in poor embryo development.

b) **Problems with fetal development**

There are generally two types of defects in fetal development that are genetic and environmental which are discussed as follows:

i) **Genetic Problems**

Genetics play a vital role in development. However, sometimes the genetic problems can emerge that may affect both current and future development. Some of them are described below:

- **Down's Syndrome:** It is also known as trisomy 21 and is the most common genetic anomaly during prenatal development. It is caused due to an extra copy of the 21 chromosomes which show its impacts in approximately 1 out of every 1,000 infants. Flattened facial features, heart defects, and intellectual impairment are the typical features of Down's syndrome. The risk of having a child with Down's syndrome increases with increase in maternal age.

- **Inherited diseases:** If one or both parents carry a gene for the disease**,** a number of illnesses can be inherited. Sickle-cell anemia, thalisemia, Cystic fibrosis, and Tay-Sachs disease are some of the examples of the inherited diseases.

- **Sex-Chromosome Problems:** The third type of genetic problems involves sex-chromosomes. These include conditions such as Klinefelter's syndrome (in males), in which an extra X-chromosome is formed and Turner's syndrome (in females) in which a single X-chromosome is formed. Abnormal cell division is the major cause for the sex-chromosomal diseases.

ii) **Environmental Problems**

You have learnt in the last section that the embryo is very vulnerable to noxious influences. An infection or a toxic substance that can cross the placental barrier can cause serious defect in development. Thus environmental factors can also play a major role in prenatal development.Harmful environmental elements or teratogens affect the foetus. The highest sensitivity to such teratogenic agents is during 18-55 days after conception. The peak period being day 30. Pregnant women must, therefore, never use any drug without the advice of their attending physicians.

- **Maternal Drug Use:** During gestation period the use of some substances by the mother can have devastating consequences to the foetus. Smoking leads to low birth weight, which can result in a weakened immune system, poor respiration, and neurological impairment of the foetus. Alcohol use results in fetal alcohol syndrome (FAS), which is linked to heart defects, body malformations, and mental retardation in foetus. As little as 8.5 ml of alcohol per day or engaging in a single drinking binge by a pregnant woman can result in FAS. The use of illicit psychoactive drugs such as cocaine and methamphetamine are responsible for low birth weight and neurological impairment. There are many drugs that are apparently harmless to adults but may be injurious to developing embryos. They are not toxins but teratogens as they lead to malformations in the embryo. Perhaps the most unfortunate instance of the effect of a teratogen is the drug thalidomide which was used as a tranquilizer. Within a couple of years of its introduction over 8000 crippled children without limbs were born in Europe and England.

- **Maternal Diseases:** There are a number of maternal diseases that can cause negative impact on the foetus, including herpes, rubella, and AIDS. Herpes virus is one of the most common maternal diseases and can be transmitted to the foetus and results in deafness, brain swelling, or mental retardation. Infection by German measles (caused by Rubella virus) in a pregnant woman can cause congenital malformations in the embryo. If the rubella infection is contacted during the first four or five weeks of pregnancy, the embryo's eyes, ears and heart may be malformed

while exposure to this virus during later stages of development may result in defects in the central nervous system.

Exposure to radiations also poses a grave threat to normal development. Large doses of X-rays during the first trimester may cause mental retardation, skeletal malformation, small head size and predispose the person for later development of leukemia.

SAQ 8

Which common maternal diseases can lead to flaws in development?

16.9 SUMMARY

In this Unit you have studied that:

- Human development is a continuous process and can be divided into prenatal and postnatal periods of development.
- Gametogenesis in males starts at puberty and continues throughout life while in females, gametogenesis begins before birth but ova mature only after puberty. Oogenesis continues normally only until the age of 45-50 years beyond which menopause sets in.
- Cyclic changes in the ovary and uterus release the ovum for fertilisation and prepare the uterus for pregnancy.
- Fertilization takes place in the fallopian tube and the period from fertilization to about 2 weeks of development is known as pre-embryonic developmental period in which the implantation of the embryo in the uterine wall takes place.
- Embryonic period of development lasts from two weeks to eight weeks in the first trimester of pregnancy. During this period all the major organs and tissues differentiate from the three germinal layers namely ectoderm, endoderm and mesoderm.
- The foetal period begins at ninth week. During this period the existing body structures grow and mature rapidly. Body proportions change greatly. Birth takes place at the end of the third trimester, 39 weeks after conception.
- At birth important developmental changes occur in the respiratory and circulatory systems of the newborn that enable it to breath air and separate oxygenated blood from deoxygenated blood so that an adult pattern of circulation starts.
- Development does not stop at birth but continues throughout infancy, childhood, adolescence and adulthood.
- The placenta in humans is haemochorial and forms fully by the tenth week of development. It secretes estrogen and progesterone which maintain the pregnancy. Passage of all nutrients from the maternal blood

to foetal blood and exchange of gases takes place across the placental membrane. Various substances and teratogens can cross the placental membrane. Therefore, the embryonic period is especially crucial and sensitive to the effect of drugs and teratogens which can affect the normal development of the embryo.

- Some flaws in development can be the result of defects in fertilization, blastocyst development, implantation, embryo development due to genetic defects and environmental effects.

16.10 TERMINAL QUESTIONS

1. List the sequence of events that occur during fertilization.
2. How is the placenta formed? What are its functions?
3. Define the term embryo and foetus.
4. Why is the embryonic period particularly sensitive to teratogens?
5. Draw a flow chart to show how the three germinal layers are derived from the zygote.
6. Fill in the blanks with appropriate terms from the text and arrange the developmental events sequentially.
   i) The foetus increases its body weight maximum in the …….. trimester.
   ii) By the end of …….. trimester, most organ systems are atleast partially functional.
   iii) After the …… month the face starts looking more human and the embryo becomes a ……. .
   iv) The embryo forms its first organ system in the ……. week.
   v) Cartilage is replaced by bone during the …….. month.
   vi) A few days after implantation the ICM becomes a ……. layered embryonic disk separated from the chorion by the …… cavity.
   vii) The three germ layers form by …….. week of development.
   viii) Following fertilization cleavage, blastulation and implantation occupy the ……. week of development.
7. How do genetic and environmental defects cause problems in development?

16.11 ANSWERS

Self Assessment Questions

1) i) d, ii) a, iii) b, iv) c.

2) a) Ampullary-isthmus region in oviducts.

b) At puberty stage.

c) i) T ii) F iii) T iv) F.

3) i) blastocyst, ii) ICM, iii) outside the uterus, iv) maintains, v) syncytiotrophoblast

4) a) i) foetal, ii) third.

b) i) fourth, ii) fifth, iii) seventh, iv) eighth.

5) To open the collapsed lungs because in the foetus the exchange of gases takes place through the placenta and lungs remain nonfunctional.

6)

Humans	**Birds and reptiles**
Amnion – fluid filled sac around the embryo. Encloses amniotic cavity. Acts as a shock absorber	Fluid filled cavity which protects the embryo and prevents dehydration.
Chorion – forms foetal part of placenta; protects embryo against rejection by maternal antibodies. Secretes HCG which maintains the corpus luteum till placenta is fully formed.	Controls the overall permeability of the egg. Surrounds amnion, allentois and yolk sac.
Allantois – sac like connected to gut of embryo makes up most of the blood vessels of the embryonic side of placenta.	Stores wastes until egg hatches; also has respiratory functions.
Yolk sac – No nutrients but bears a clear fluid, manufactures blood cells for early embryo till liver takes up the task; forms part of umblical cord.	Contains large amount of yolk to nourish the developing embryo. The sac is highly vascularised to carry dissolved nutrients from yolk to embryo.

7) a) To protect the embryo from shocks and provides a stable environment. The embryo is also known to drink the amniotic fluid.

b) Through the selectively permeable membrane of the chorion and capillaries.

8) Herpes, Rubella, AIDS.

Terminal Questions

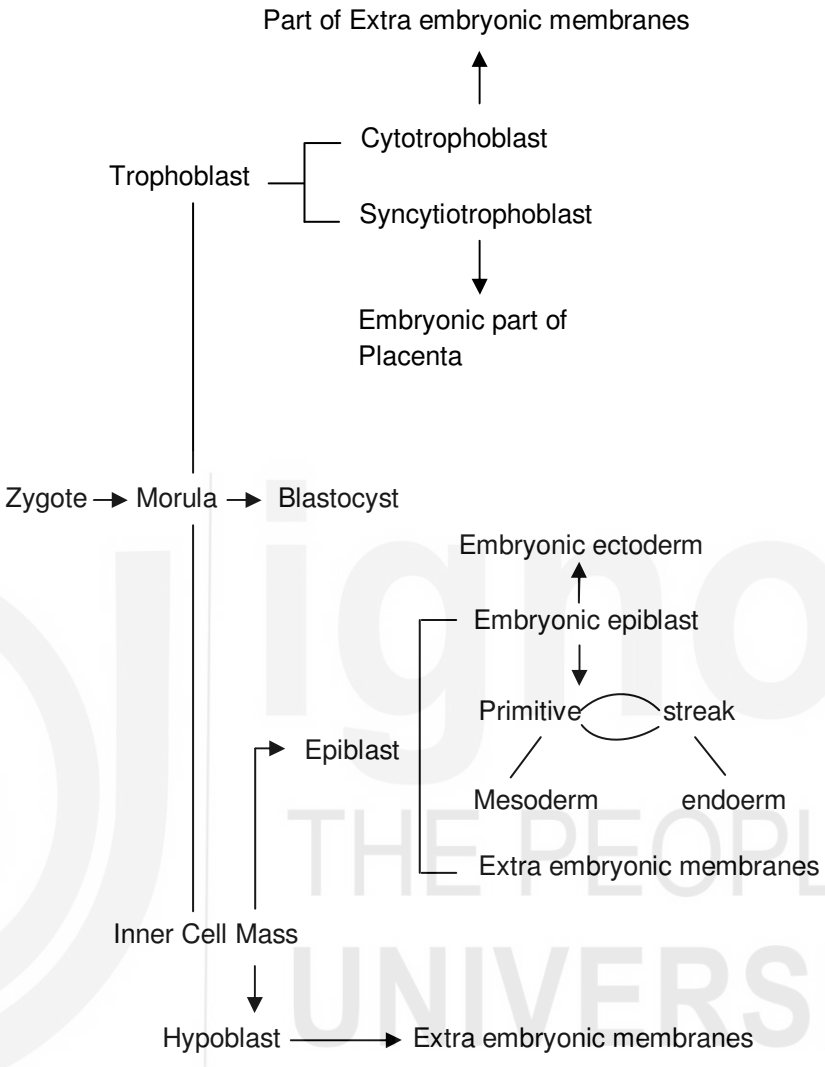
1. Refer to sub section 16.3.2.

2. Refer to sub section 16.7.2.

3. The developing individual from two weeks to eight weeks is termed embryo while from eight weeks to birth is referred to as foetus.

4. Because maximum tissue differentiation and organogenesis takes place during the first three months of pregnancy and any agent that will disturb the normal process will result in malformations in the developing individual.

5.



6. i) third

ii) first

iii) second, foetus

iv) third

v) third

vi) double; amniotic

vii) second

viii) first

Sequence for development events

viii); vi); vii); iv); iii); v); ii); i)

7. Refer to Section 16.9.

Acknowledgment of Figures

Fig. 16.1: Source: https://www.britannica.com/science/fertilization-reproduction/images-videos/media/205305/229000

Fig. 16.4: Source: http://cdn.yourarticlelibrary.com/wp-content/uploads/2013/10/image51.png

Fig. 16.6: Source: https://slideplayer.com/slide/2617594/9/images/4/Implantation+of +the+Blastocyst.jpg