Contents

Syllabus

PAPERII : DEVELOPMENTAL BIOLOGY
(SC-136)

UNIT1 :

Chapter 1 : Spermatogenesis and its significance.

Chapter 2 : Oogenesis and its significance.

Chapter 3 : Fertilization and post fertilization events.

Chapter 4 : Parthenogenesis.

Chapter 5 : Types of animals eggs and patterns of cleavage.

Chapter 6 : Fate maps and cell lineage.

Chapter 7 : Gastrulation

Chapter 8 : Organizer Concept and induction process.

Chapter 9 : Salient features of chick development.

Chapter 10 : Extra embryonic membranes of chick.

Chapter 11 : Types of placenta in mammals.

UNIT

1

INTRODUCTION

STRUCTURE

- Study of spermatogenesis
- Testis Structure
- Study of spermiogenesis
  - Summary
  - Test Yourself

LEARNING OBJECTIVES

After going through this unit you will learn :

- Gametogenesis : Spermatogenesis and Oogenesis.
- Spermatogenesis : Development of sperms in male human beings.
- Spermatogenesis includes formation of spermatids and spermiogenesis, i.e., formation of sperms.
- Formation of spermatids includes multiplication phase of the primary germinal cells or primordial germ cells; growth phase i.e., formation of primary spermatocytes, which are diploid; and third is maturation phase in which primary spermatocytes undergo meiosis to form secondary spermatocytes which are haploid spermatids.
- Significance : Primary germ cells in the testes are diploid which are transformed into haploid spermatids. A single germ cell produces four haploid spermatids (sperms).
- Spermatids by the process of spermeiogenesis form active motile sperms, each of which has a head and a vibratile tail.

• 1.1. SPERMATOGENESIS

Spermatogenesis and oogenesis collectively form the gametogenesis. Gametogenesis, i.e., formation and ripening of two dissimilar and specialized sex cells or gametes, namely a small and motile sex cell the spermatozoon or sperm in male and large, non-motile nutrient-filled sex cell the ovum or egg.

Spermatogenesis takes place in the testes of male animals including men. Thus, the process by which testes produce sperms (male gametes) is called spermatogenesis.

• 1.2. TESTES : STRUCTURE

Testes are paired structures in vertebrates and they perform two reproductive functions : as an exocrine organ in producing sperms and an endocrine gland in secreting male hormone. In the majority of mammals these are lodged in **scrotal sacs,** which are postero-verntral pouch-like extensions of abdominal wall and cavity. This is to keep the testes at a low temperature than the abdominal temperature, because the mature sperms survive best at a temperature few degrees lower than the abdominal temperature in monotremes, while elephant, seals and rhinoceros testes are abdominal (scrotal sacs are absent).

Each testis is covered by a thin layer of **peritoneal epithelium** below that lies a tough connective tissue, called the **tunica albuginea**, and numerous coiled seminiferous tubules which produce **sperms**. The seminiferous tubules) short or long) walls consist of outer connective tissues capsule, a thin basement

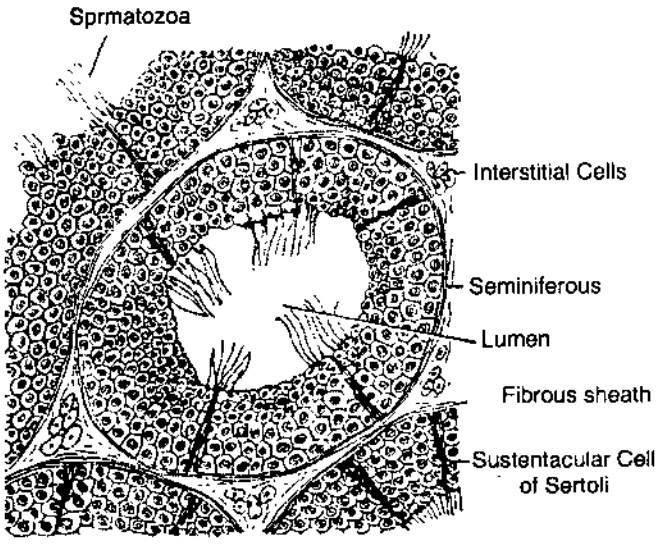


Fig. 1. T.S. of mammalian testis (a part)

membrane and a lining of stratified germinal epithelium. Spermatogenesis takes place in seminiferous tubules. By a series of cell divisions the sperm mother cells or **spermatogonia** located near the basement membrane transform into mature sperms and enter into the cavity of the seminiferous tubules. A transverse section through the tubule shows many stages of differentiating sperms, namely spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and mature sperms. All these cells of germinal epithelium are connected to one another by cytoplasmic bridges. These bridges break down when sperms are released into the lumen of the tubules. Among the spermatogonia are found interspersed **Sertoli cells (Enrico Sertoli)**, which are tall, columnar and phagocytic cells. These cells function as nurse cells for the sperm. During spermatogenesis Sertoli cells phgocytose excess cytoplasm cast off by spermatids.

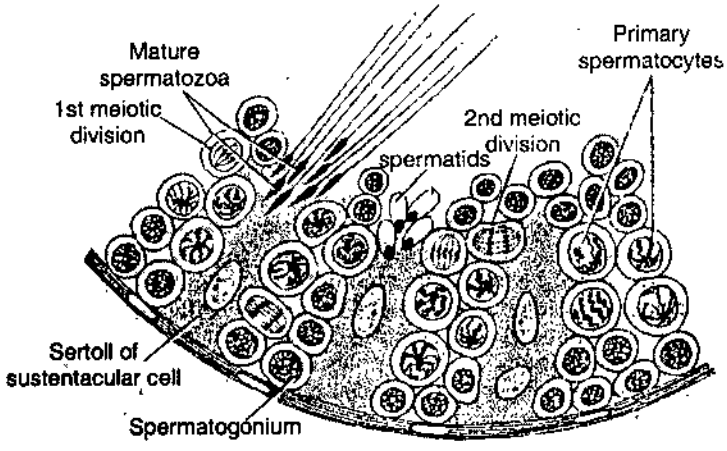


Fig. 2. T.S.of mammalian seminiferous tubule

In the testes are also found clusters of endocrine gland cells, called interstitial cells or **Leydig cells (Franz von Leydig)**, located between seminiferous tubules. These cells secrete **testosterone** (steroid hormone) responsible for the development of male secondary sexual characters at puberty. It is also essential for the continued function of seminiferous epithelium.

Process of Spermatogenesis

Spermatogenesis can be divided into two stages : Formation of spermatids and spermiogenesis.

1. Formation of Spermatids

Cells of germinal epithelium producing spermatozoa are called primary germinal or **primordial germ cells**. Each germinal cell undergoes following three phases for the formation of spermatids :

(i) Multiplication phase : Primordial germ cell repeatedly divides mitotically to produce the sperm mother cells or **spermatogonia**. Spermatogonia are diploid (2X) cells found below the basal membrane of the tubules. Spermatogenesis usually continues throughout the life of the adult animal. In man are found seven distinct classes of spermatogonia : A_1 spermatogonia divides twice to form four A2 spermatogonia – one of these four cells remain dormant for the further spermatogenesis cycle, while the remaining three A_2 spermatogonia divide mitotically into six intermediate cells, which further divide mitotically into 12 A_1 cells and then into 24 B_2 cells.

(ii) Growth phase : These B_2 spermatogonial cells do not further divide and grow by accumulating nourishing material obtained from germinal cells and become double in volume. These cells are called **primary spermatocytes**.

(iii) Maturation phase : These primary spermatocytes undergo first meiotic or maturation division of the nucleus followed by division of cytoplasm (cytokinesis). Thus, two haploid secondary spermatocytes are formed. Later secondary spermatocytes under go meiosis II which is mitotic producing four **haploid spermatids**. These four haploid spermatids undergo differentiation, called **spermiogenesis** or **spermioteliosis**.

• 1.3. SPERMIOGENESIS

During spermiogenesis spermatids discard their superfluous materials and undergo specialization to form the sperms, each sperm has a head and a long tail for active mobility.

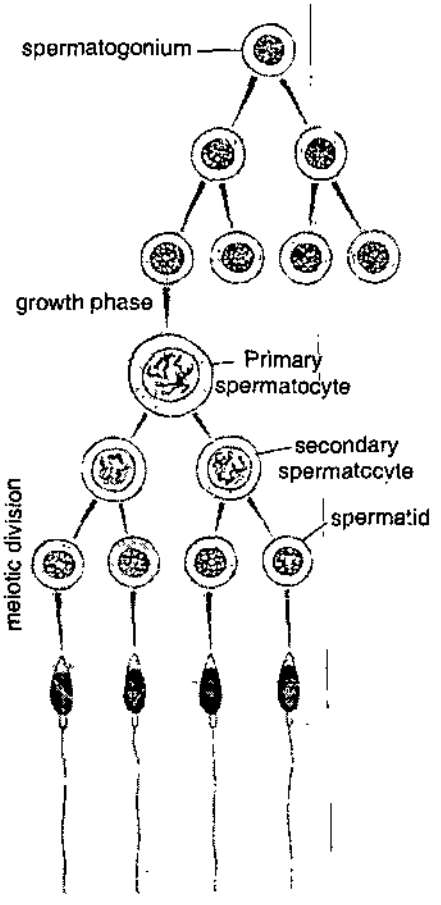


Fig. 3. Spermatogenesis.

(i) Formation of head of sperm : During transformation from the spermatid into spermatozoon, its nucleus loses its entire fluid content, nucleolus and most of its proteins. Its nucleus (haploid DNA) persists, and it becomes elongated and narrow. In different animals nucleus assumes different shapes.

Acrosome of the spermatozoon is formed by the Golgi apparatus of the spermatid. Golgi apparatus of spermatid consists of a series of cisternae which are arranged around of an aggregation of small vacuoles. During acrosome formation, one of the vacuole enlarges and inside it appears a small, **proacrosomal granule**. The vacuole or acroblast containing proacrosomal granule enlarges in size by fusing with other small vacuoles and become closely applied with the forwarding tip of the nucleus. Acrosomal granule increases further to form the acrosomal granule that forms the core of the acrosome. The vacuole now loses its liquid contents and its wall spreads over the acrosomal granule and anterior half of the nucleus, covering them with a double membranous sheath (plasma membrane, membrane of acrosomal vesicle). It is thus, a cap of sperm. The remaining parts of Golgi apparatus are reduced and finaly discarded from the sperm as Golgi rest, along with some cytoplasm.

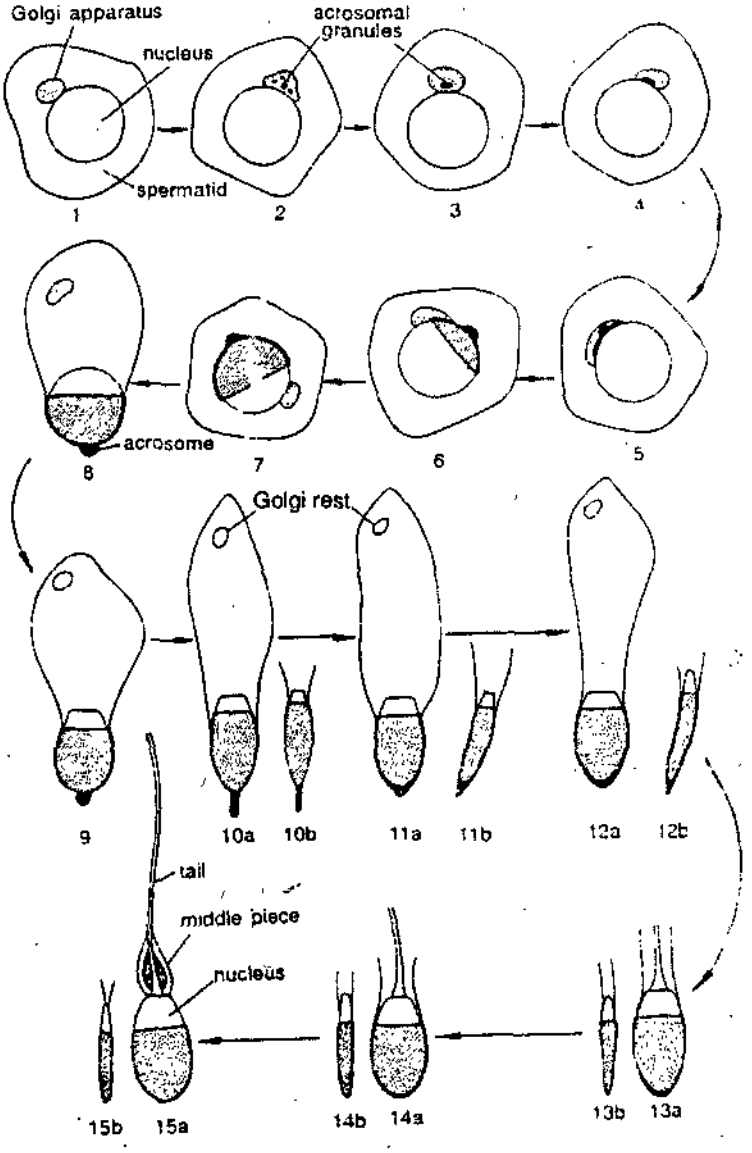


Fig. 4. Stages of spermiogesis (metamorphosis of spermatid into spermatozoon).

2. Formation of tail : Centrosome of the spermatid consists of two centrioles. During spermiogenesis both the centrioles move and take a position behind the sperm nucleus. A depression is formed in the posterior surface of the nucleus and one of the two centrioles is placed in this depression with its axis about at right angles to the main axis of the sperm. This is **proximal centriole** and the other centriole is the **distal centriole**, it takes a position behind the proximal centriole. Its axis conicides with the longitudinal axis of the sperm. Distal centriole forms the axial filament of the flagellum of the sperm and it acts as basal granule for the axial filament.

Most of the mitochondria of sperm concentrate around the distal centriole and proximal part of the axial filament and forms the neck and middle piece of the tail of sperm. In the middle piece of sperm, mitochondria lose their individuality by fusing with each other and are either arranged spirally or form a large mitochondrial body. Thus, the tail of sperm comprises of an axial filament which is differentiated into a principal piece and a tail piece which contains only the axial filament covered with cytoplasm and plasma memrane.

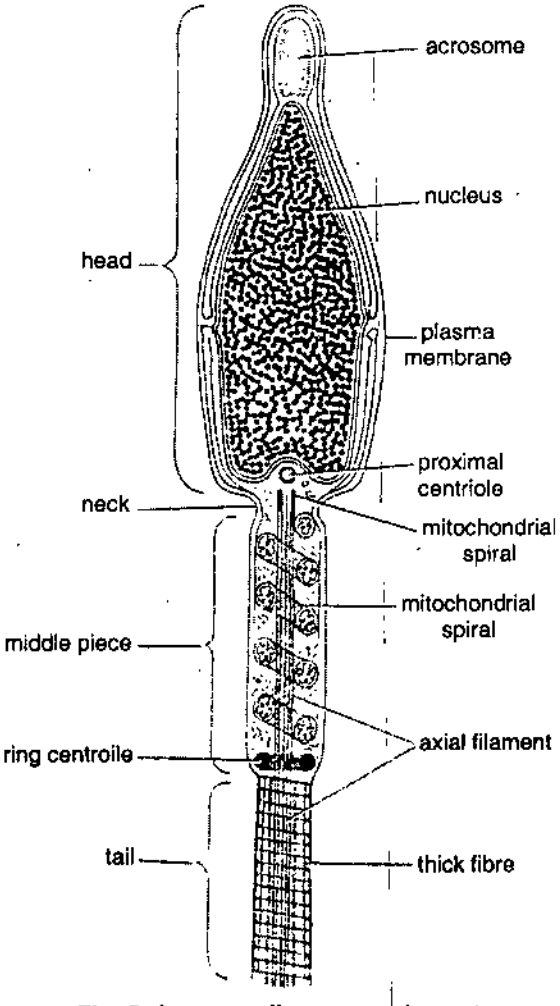


Fig. 5. A mammalian spermatozoon (semidiagrammatic)

Principal piece of tail consists of a central core formed of an axial filament with 9 + 2 microtubular arrangement and nine longitudinal fibres. Around this core is a microtubular fibrous tail sheath.

• SUMMARY

1. **Gametogenesis** is he collective name of spermatogenesis and oogenesis.

2. **Gametogenesis** is the formation and ripening of specialized gametes or sex cells, *i.e.*, spermatozoon and ovum or egg.

3. Testes in males are exocrine organs in producing sperms and an endoctine gland in secreting male sex hormones.

4. Testis has numerous coiled seminiferous tubules in which sperms are produced.

5. Sprms are produced from **spermatogonia** (sperm mother cells) located near the basement membrane. Among spermatogonia are found interspersed **Sertoli cells** which function as nurse cells for the sperm.

6. Testes also possess clusters of **interstitial cells** or **Leydig cells** in between seminiferous tubules. These cells secrete steroid hormone that is responsible for the development of male secondary sexual chrracters at puberty.

7. Cells of germinal epithelium which produce sperms are called **primordial germ cells.** Each germinal cells undergos multiplication phase, growth phase and maturation phase, finally producing **four haploid spermatids.**

8. Each spermatid undergoes differentiation called **spermiogenesis** to produce **sperm.**

9. **Each spermatid** undergoes specialization to form the **sperm** that has a head and a long vibratile tail.

10. During transformation from spermatid into sperm, its nucleus loses its fluid content, nucleolus and most of its proteins. Its nucleus becomes elongated and narrow.

11. **Acrosome** of sperm is formed by the **Golgi apparatus** of the spermatid. **Centrosome** of spermatid has two centrioles, during spermiogenesis both the centrioles move and come to lie behind the sperm nucleus, one forms the **prodimal centriole** and the other forms the **distal centriole.**

Distal centriole forms the **axial filament** of the flagellum of sperm and thus it acts as basal granule for the axial filament.

Mitochondria of sperm mostly concentrate around distal centriole and proximal part of axial filament and thus forms the neck and middle piece of the tail of sperm. **Mitochondrial body** is the fused mitochondria in the middle piece of sperm.

12. **Tail** of sperm has an axial filament differentiated into a **principal piece** and a **tail piece** that has only axial filament covered with cytoplasm and plasma membrane.

• TEST YOURSELF

Long Answer Type Questions

1. Write an essay on spermatogenesis.
2. Define spermiogenesis. Discuss the process of spermiogenesis.

Very Short Answer Type Questions

1. **Define spermatogenesis.**

   Ans. The process by which sperms are produced in the seminiferous tubules of the testes.

2. **In human male what is the position of testes ?**

   Ans. In human males the testes are lodged in the scrotal sacs out side the abdomen.

3. **Why in most of the vertebrates, testes are located outside the abdominal cavity.**

   Ans. Mature sperms need a lower temperature for their survival than the temperature of the abdominal cavity.

4. **Where sperms develop in the testes or Name the part of the testes where sperms are formed ?**

   Ans. Seminiferous tubules which are long coiled tubes present within each testis.

5. **Which cells of the seminiferous tubules give rise to sperms ?**

   Ans. Primary germinal or primordial germ cells lining the seminiferous tubules.

6. **What are the three phases of the formation of spermatids ?**

   Ans. Multiplication phase, growth phase and maturation phase.

7. **In which phase diploid nucleus of primary germ cells undergoes meiosis to reduce the number of chromosomes half of that of primary germ cells ?**

   Ans. In maturation phase, primary spermatocytes diploid nucleus undergoes reduction division to form the haploid nucleus of the secondary spermatocytes.

8. **Define spermiogenesis.**

   Ans. Transformation or differentiation of the spermatid into spermatozoon is called spermiogenesis.

UNIT

2

OOGENESIS AND ITS SIGNIFICANCE

STRUCTURE

- Study of oogenesis : Proliferative phase and meiotic phase
- Study of structure of ovary
  - Summary
  - Test Yourself

LEARNING OBJECTIVES

After going through this unit you will learn :

- 1. Oogenesis (Oon = egg; genesis = origin)
- Female gonads are ovaries, located in the abdomen.
- Ovaries like testes are exocrine and endocrine organs.
- Oogenesis is divided into three phases; Multiplication phase, growth phase and maturation phase.
- Growth phase is of long duration and varies in different animals : three years in case of Rana pipiens and in hen 6 to 14 days.
- Vittellogenesis is the synthesis and deposition of yolk in the egg.

• 2.1. OOGENESIS

Oogenesis takes place with in the ovaries of female vertebrates including human beings; growth and maturation of the egg or ovum in the ovary Oogenesis or manufacturing of ova (egg) includes two important phases : **Premeiotic or proliferative phase** and **meiotic phase**. Meiotic phase includes growth of oocyte, yolk synthesis (vitellogenesis) and maturation, and deposition of egg membrane etc.

1. Premeiotic phase or Proliferative phase

Like spermatogenesis, the primordial germ cells undergo division by mitosis forming egg mother cells or oogonia. Oogonia later give rise to primary oocytes. Thus, proliferative phase is a period of oogonial proliferation within the ovary. Proliferative phase is completed once in the life of an animal; long before sexual maturity. Oogonia do not proliferate in an adult ovary, of birds and mammals. But in fish and amphibians proliferative phase is seasonal throughout the adult life of an animal.

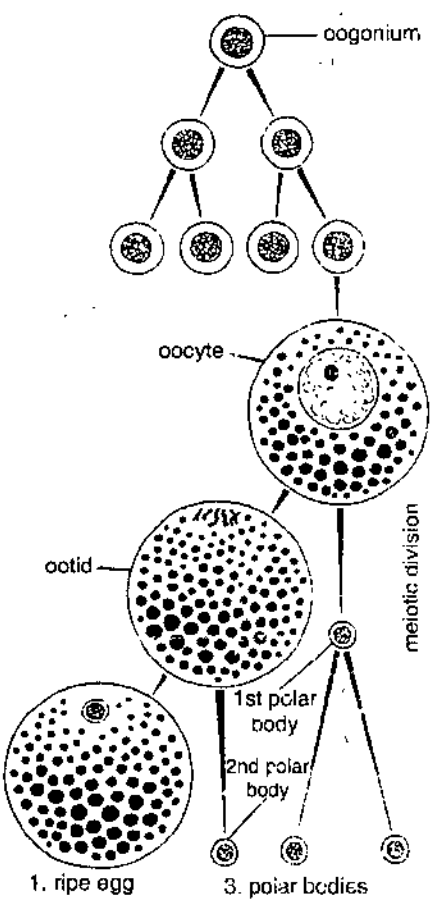


Fig. 1. Oogenesis in female vertebrates.

All oogonia do not mature to form the functional eggs. Survived oogonia begins meiosis in larval and foetal stages and form primary oocytes. In a five months foetus, seven million oogonia are present. At this stage mitosis usually stops and many oogonia enter the first meiotic prophase and become oocytes. Several oogonia degenerate and hence only two million oocytes remain at birth. After birth also oocytes undergo degeneration. Every month several oocytes are stimulated to mature, but only one usually completes meiosis and ovulates, remaining ones degenerate.

2. Meiotic phase :

During meiotic prophase of oogenesis, oocyte grows and acquires its developmental information. Primary oocytes grow in size, accumulates nutrients and other reserves necessary for embryonic development and genetic programming for development.

Oocytes grows in size enormously and thus becomes the largest cell of the animal body. It is due to accumulation of cytoplasm and yolk. During growth of primary oocyte qualitative and quantitative changes take place in the nucleus as well as in cytoplasm.

The nucleus of primary oocyte enlarges due to accumulation of a large amount of nuclear sap. The chromosomes incease in length, in amphibian oocytes chromosomes acquire a very characteristic shape. Numerous paired threads or loops project transversely from the main chromosomal axes, and look like **lamp brush**, thus they are called **lamp brush chromosomes**. Loops of these chromosomes represent loci of gene activity, that is, at these sections messenger RNA is synthesized which later contols protein synthesis in the cell. Amount of DNA in the chromosomes does not increase in proportion to the enlargement of the nucleus.

Nucleolus in the nucleus is for the synthesis of ribosomal RNA. Nucleolus also increases in size in the growing oocyte.

Cytoplasmic substances. Amount of cytoplasm increases during growth of the oocytes. Within cytoplasm various cell inclusions like mitochondria, Golgi bodies, endoplasmic reticulum, cortical granules, etc., also increase in number.

Mitochondria are few in young oocytes but they increase in number during growth of oocytes. In amphibia, and birds they are aggregated in the form of large mitochondrial clouds. They are required for respiratory metabolism (oxidative phosphorylation).

Golgi bodies present in oocytes perform secretory, storage and transport functions. Stacked lamellae vesicles around centrosome synthesize yolk. They also develop membranes of endoplasmic reticulum. Golgi bodies also manufacture the cortical granules which are membrane bound. These contain acid mucopolysaccharides for the synthesis of fertilization membrane during fertilization. Cortical granules are found in rabbits, man, etc., but absent in birds and rat guinea pig (mammals). Cortical granules are found close to the plasma membrane arranged in a layer.

Smooth endoplasmic reticulum are in the form of small vesicles and are found dispersed evenly in the cytoplasm. **Rough endoplasmic reticulum** is often not found in young or mature primary oocyte. Growing oocytes also contain annulated lamellae in the cytoplasm. These are membranous structures appearing as stacks of cisternae or filattened sacs with an intracellular space of 100 to 200 Å wide. They are formed by the budding of the nuclear envelope having pore complexes like those of nuclear envelope.

Vitellogenesis : Vitellogenesis is the synthesis and deposition of yolk in the oocytes. Yolk is the major nutritional reserve of the oocyte and it accumulates during growth phase. Yolk is composed of proteins, phospholipids and neutral fats. Yolk is of two types; protein yolk and fatty yolk.

Protein yolk is found in the form of fine granules found evenly distributed in the cytoplasm of the oocyte.

Yolk platelets are large granules found in the oocyte of cyclostomes, fishes, amphibians, reptiles and birds. Yolk platelets of amphibian oocytes contain phosvitin and lipo-vitellin (proteinaceous substances).

Yolk proteins are synthesized in amphibian liver or vertebrate liver and transported to the oocyte via circulation in the overies and from here the yolk protein precursors are taken by oocyte membrane by micropinocytosis. The yolk platelets begin at the cell periphery as a small droplets which gradually increase in size as they migrate into the interior. Here they crystallized into definite pattern, i.e., **crystalline yolk platelets.**

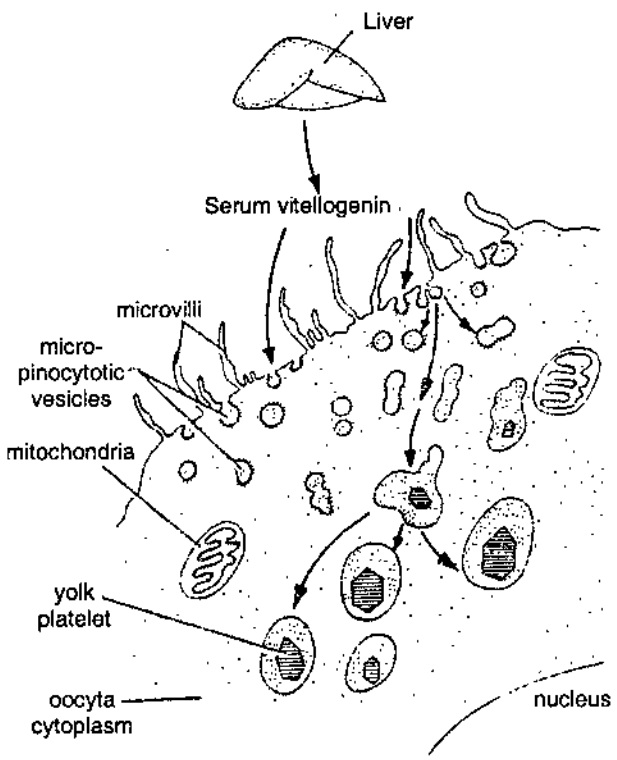


Fig. 2. Formation of yolk platelets in amphibian oocyte.

Animals with microlecithal eggs (coelenterates, echinoderms and molluscs), the oocyte takes up raw materials (amino acids etc.) and synthesize the yolk (autosynthesis of yolk by membrane system of Golgi apparatus and endoplasmic reticulum.

• 2.2. OVARIES

Vertebrate ovaries are exocrine as well as endocrine in function. They produce eggs (ova) and sex hormones **estrogen** and **progesteron** (steroid hormones). These hormones regulate the reproductive tract, develop secondary sex characters and mating behaviour of the females. In human females estrogen broaden the pelvis, develop the breast including mammary glands, uterus and vagina, change in voice, growth of pubic hairs and onset of menstrual cycle.

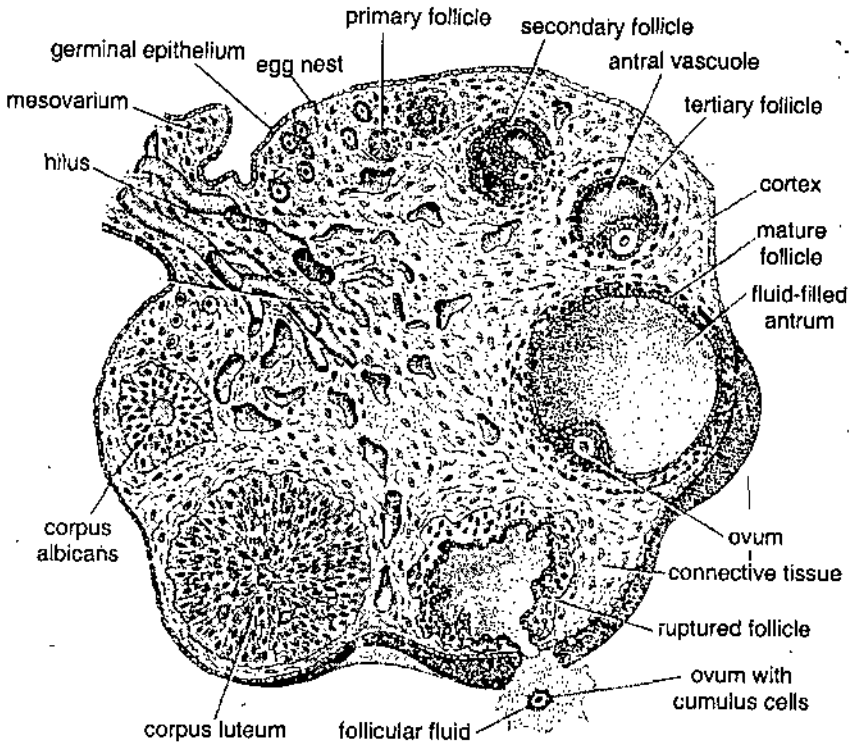


Fig. 3. T.S. of mammalian ovary (three dimensional view) showing its morphology and cyclic changes, formation of ovum, its liberation and formation of corpus luteum and corpus albicans in case of non-fertilization of ovum.

A pair of ovaries are abdominal found suspended from dorsal body wall just behind the kidneys by peritoneum, **mesovarium**. Ovaries in human females are small, walnut-shaped, measuring 4 × 2 × 1 cm. A single egg is commonly matured in a month in human females.

Each ovary is a compact (solid) structure and has two regions :

1. Outer region **cortex** containing several ova in various stages of development. Germ cells are found in this region and clusters of epithelial cells surround each ovum forming a **follicle**.

2. Inner region **medulla** having irregularly arranged connective tissue, blood and lymph vessels.

Cortex is covered by a dense connective tissue layer, called **tunica albuginea,** and it is externally covered by the peritoneal or coelomic epithelium.

• 2.3. SIGNIFICANCE OF OOGENESIS

In the process of oogenesis, in human females a single ovum is developed in a month, i.e., each month several primary oocytes are stimulated to mature, but only one completes meiosis (number of chromosomes becomes half in comparison to that of oocytes) and then ovulation (liberation of ovum from the ovary) takes place.

Each primary oocyte after going meiosis. only one egg develops, while other three are polar bodies having very little cytoplasm and no yolk and later they die off.

Eggs possess reserve food material in the form of yolk that is used up during development. Where as in mammals including human eggs contain no yolk and

during development after fertilization they are nourished by the mother through placenta (a chord-like structure which connects the uterine tissues with the developing foetus).

• 2.4. SUMMARY

Oogenesis (Gr. Oon = egg; genesis = origin) is the formation and maturation of ova in the ovary. Ova are derived from the germinal epithelium lining the ovary.

Germinal cells are diploid. Ovary is solid attached by its anterolateral border to the broad ligament.

Hilus is the region of attachment in which smooth muscle fibres are found. Tissue of broad ligament extends into the ovary called **stroma**.

Beneath the surface epithelium is found tunica abuginea that is condensed stroma. Stroma consists of outer cortex region and innr medulla. Cortex has developing Graafian follicles, discharged follicles, corpus luteum (pleura corpora lutea), degenerating atretic follicles and corpus albicans (fibrous hyaline scar), shrinked corpus luteum when fertilization has not occurred.

Oogenesis is divided into three phases :

A. Multiplication phase : Primordial germ cells proliferate mitotically to form **oogonia** or egg mother cells. These further multiply to give **primary oocyte,** which enter a period of growth.

B. Growth phase : Primary oocytes undergo first meiotic division and end *in a suspended state. Nucleus and cytoplasm carry synthetic activities.* Oocyte increases in size and volume. Growth phase is prolonged. Nucleus enlarges in size due to production of large amount of nuclear sap. Chromosomes increase in size. Lampbrush chromosomes are formed. mRNA is synthesized at loci of lampbrush chromosomes. rRNA is synthesized by nucleolus. Amount of *cytoplasm also increases quantitatively.*

C. Maturation phase : Primary oocyte is diploid. After the growth of oocyte, meiosis takes place due to which chromosomes number becomes half. Thus, ovum or egg becomes haploid. This is called **maturation.**

In oogenesis one ovum is produced out of four unequal cells derived from the primary oocyte.

• QUESTIONS

Long Answer Type Questions

1. **Describe the process of oogenesis in detail.**
2. **Describe the process of oogenesis and its significance in a mammal.**

Very Short Answer Type Questions

1. **Define oogenesis.**

   Ans. It is the process of formation and maturation of ova from the primordial germ cells of the ovary.

2. **Name the two phases of oogenesis.**

   Ans. Multiplication phase, growth phase and maturation phase.

3. **In which phase primary oocytes undergo meiotic or reductional division.**

   Ans. Meiosis takes place in growth phase. Each primary oocyte divides by meiosis due to which number of chromosomes becomes half to that of primary oocyte.

4. **Which phase of oogenesis is of long duration ?**

   Ans. Growth phase is very prolonged and developing egg increases in size appreciably. It is 200 microns in diameter in mammals.

5. **What are polar bodies ?**

Ans. In maturation phase, primary oocyte divides into a large and a very small cell, each with a haploid number of chromosomes. The smaller cell is called the **polar body** or **polocyte** and it has a very small quantity of yolk. The larger cell having other half number of chromosomes is called the **secondary oocyte** and it contains a large amount of cytoplasm.

6. **Define vitellogenesis.**

Ans. Synthesis and deposition of yolk in the oocytes is called the vitellogenesis.

7. **What are yolk platelets ?**

Ans. In amphibian eggs the yolk is found in the form of large granules called yolk platelets. They contain phosvitin and lipovitellin (proteinous substances).

8. **What are the functions of yolk ?**

Ans. It is a nutritive food substance that is used up during development. Yolk is found in eggs of Amphibia, fishes, reptiles, birds and egg laying mammals (Prototherian mammals).

UNIT

3

FERTILIZATION AND POST-FERTILIZATION EVENT

STRUCTURE

LEARNING OBJECTIVES

After going through this unit you will learn :

- Study of fertilization process.
- Study of process of fertilization.
- Study of mechanism of fertilization :
  1. Encounter of sperms and ova.
  2. Approach of the sperm to the egg.
- Study of Activation of ovum
  Monospermic and polyspermic fertilization.
  Metabolic activation of egg.
- Amphimixis : Union of both pronuclei of sperm and ovum.

• 3.1. INTRODUCTION TO FERTILIZATION

1. Fertilization is the process of fusion of sperm head with the egg or ovum.

2. Tail of flagellum of sperm propel the sperm towards the egg surface and thus it makes contact and fuses with the egg membrane and finally enters the egg cytoplasm.

3. Later the nucleus of sperm head fuses with the nucleus of egg in a process called **amphimixis** (syngamy). Both the nuclei of sperm and egg are haploid.

4. After fusion of both the nuclei in fertilization process, the resulting nucleus becomes **diploid**.

5. Fertilization is a species-specific, i.e., sperm of one species can not fertilize the egg of another species.

6. In fertilization process genes of two gametes (sperm and egg) mix due to which genetic variation appears in the resulting species.

7. Fertilization triggers the developmental process, increased metabolic activity.

8. Fertilization process survives the egg. In the absence of fertilization egg or ovum degenerates.

9. Fertilization in most animals needs a fluid medium, that may be sea water in marine animals, freshwater in freshwater animals and body-fluid in viviparous animals.

10. Sperms are short-lived, life time of human sperm in the female tract is of 24 hours.

11. Number of sperms must exceed the number of eggs to increase the probability of fertilization. In man per ejaculation averages 3 ml. Its 20 percent by volume is sperm.

Fertilization Process

Fertilization of ova either occurs outside the body of the maternal parent, called **external fertilization** or inside the oviduct of the female, called **internal fertilization.** Internal fertilization needs insertion of sexual appendage in the body of female. Fertilization triggers the developmental process, i.e. rapid burst of metabolic activity. Fertilization saves the life of the egg. Unfertilized eggs have a limited life after ovulation, often of a few hours. If they are not fertilized, their nuclei and cytoplasm become disorganized. Mature unfertilized eggs ripen or age and if such eggs are fertilized they develop abnormalities, including chromosomal aberrations. Many known congenital abnormalities in human like Down syndrome, are due to the development of overripe eggs.

• 3.2. REQUIREMENTS OF FERTILIZATION

1. Fertilization requires a fluid medium, like sea water in marine forms, freshwater in freshwater animals and some body-fluid in viviparous animals, such as mammals.

2. Life-spans of gametes (sperms and ova) are limited. Mature eggs become overripe if not activated promptly. Eggs led into water, like most invertebrates, fish and amphibians, must be fertilized within a few minutes. Eggs that are fertilized within the body or female, like human egg, can be fertilized within 24 hours after ovulation.

3. For the probability of fertilization the number of sperms must be more to the number of eggs. In mammals, in a single ejaculation, i.e., fluid medium in bat is 2 ml, rat have 500 ml contain 6 million and 2 to 5 million. In man the single ejaculate has the average about 3 ml of which about 20 percent by volume is sperm i.e., about 100,000 sperms per microliter.

• 3.3. MECHANISM OF FERTILIZATION

1. Encounter of sperms and ova : In external fertilization, that is in marine animals, sexually mature adults shed eggs and sperms into the surrounding water. The oviducal fluid liberated at the of eggs sheding stimulate other ripe females and also ripe males in the vicinity. Thus, clouds of eggs and sperms are formed in the seawater at the same time and thus, fertilization occurs. Among freshwater animals, like fish, amphibians and freshwater invertebrates, sperms are delivered directly to the eggs of an individual female at the time of laying, because their sperms remain active in freshwater for only minutes.

In internal fertilization, the sperms are delivered internally in the body of female by intromittent organs of male. Fertilization occurs either in lower portion of the oviduct, or in the upper portion of the oviduct, or in the ovarian rollicles in viviparous fishes and certain eutherian mammals (*Ericulus*). In

terrestrian animals, there is no problem of timing of spawning of eggs and shedding of sperms, because mature sperms are commonly stored in a physiological medium which maintain their life and potential activity for days, weeks or months, in moisture conserving capsules or in compartments of male or female body.

2. Approach of the spermatozoon to the egg : The sperm moves near the ovum by one of the following two methods :

(i) Chemotaxis : This is operative in coelenterates and fishes. In them sperm appears to be guided to the egg by chemical substances. For example, in *Campanularia,* the eggs are produced by reduced female gonagia, which are enclosed in theca with an opening at the distal end. At the time of fertilization the sperm converge toward the opening of theca. The attractant chemical substance extracted from female gonangia was found to be a small molecule, whose molecular weight is less than 500 daltons.

In fish eggs (sturgeon) and lamprey, each egg is enclosed in a hard chorion. The chorion is perforated by narrow canal, the **micropyle** through which sperm enters to reach the egg cytoplasm.

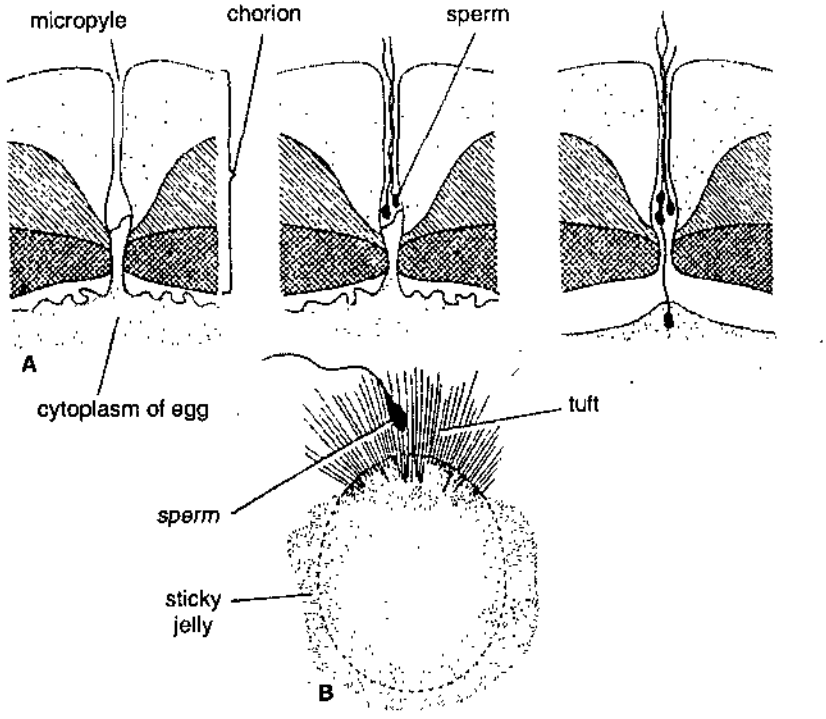


Fig. 1. A - Micropyles in the eggs of some teleost and sturgeon fishes through which sperms pass through the outer covering of eggs.
B - A sperm penetrates the chorion of lamprey egg at the region of tufts.

(ii) Fertilizin-antifertilizin interaction : Eggs and sperms of some animals such as sea urchin (echinoderms), polychaetes, molluscs, tunicates, cyclostomes, fishes, amphibians and some mammals are chemically attracted towards one another. These eggs are released into the surrounding medium substances which cause agglutination of sperm and adherence of sperm to the egg.

Fertilization theory proposed by **F.R. Lillie** (1914) proposed that egg water (sea water around unfertilized sea urchin eggs) agglutinated the sperms and activated their motility. This reaction was specific because some sperms

from related species were unaffected. The factor was called **fertilizin** and it came from the egg jelly coat which slowly dissolved as eggs remain in sea water.

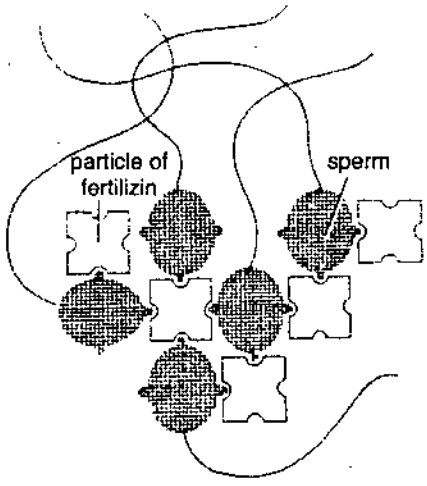


Fig. 2. Binding of sea urchin sperm by particles of fertilizin.

Grant (1978) expressed that fertilizin is the constituent of both jelly coats and egg membranes like vitelline membrane and plasma membrane. Plasma membrane of sperms have receptor sites called **antifertilizins** which interact with fertilizin molecules.

Chemically a fertilizin is a glycoprotein or mucopolysaccharide, now called **proteoglycan** (**Trinkaus,** 1984). Monosaccharides and amino acids of fertilizin vary from one species to another. Thus, each species have specific type of fertilizin. Each molecule of fertilizin has more than one **active group,** thus, one fertilizin particle may attach to two or more sperms, binding them together. **Antifertilizins** are acid proteins with a fairly small molecule in comparison to fertilizin.

In sea urchin, sperms remain immotile in undiluted semen but become active immediately as they become suspended in sea water. This is due to spontaneous influx of Na+ in the sperm.

(iii) Capacitation : In mammals, the ejaculated sperms do not show acrosome reaction unless they remain for some time in the female vagina and Fallopian tube. This time period varies with species and physiological condition of the female. It is more than one hour in mouse, 2 to 4 hours in rat and 6 hours in rabbit, and man. During this time the receptor sites on the acrosome of sperm are gradually removed. Thus, exposed sites of the sperm enable the sperm to recognize signals emanating from the ovulated egg. This is called **capacitation.**

(iv) Acrosome reaction and contact of sperm and ovum : Acrosome reaction takes place when there are optimal physiological conditions like optimum pH, Ca++, Mg++ ions concentration and temperature. Ca++ is essential for acrosome reaction. During acrosome reaction breakdown of

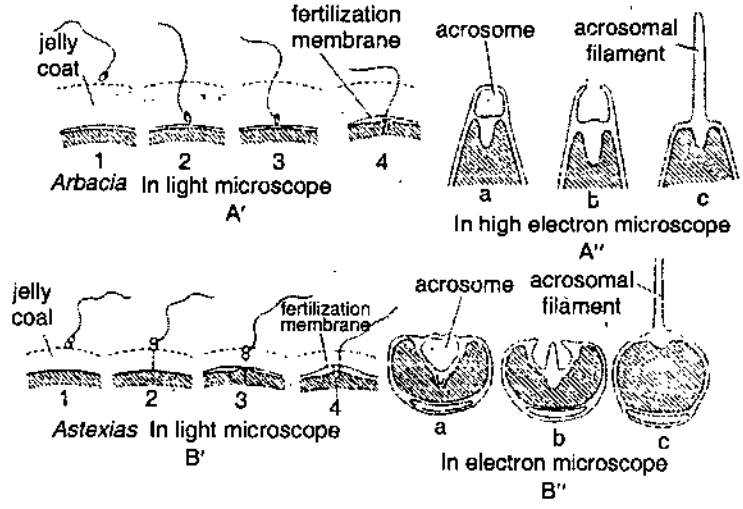


Fig. 3. Acrosome reaction in echinoderms. A'- Acrosome reaction in sea urchin Arbacia in light microscope A''. In electron microscope. B'-Acrosome reaction in light microscope. B''-In electron microscope.

membrane of acrosome vesicle, release of acrosomal enzymes, formation of acrosomal tubule and fusion of plasma membranes of sperm and egg takes place.

Breakdown of acrosome : In some animals like *Hydroides* (annelids), *Mytilus* (molluscs), *Arbacea* and *Asterias* (echinoderms), *Procambarus* (decapod crustacean) and *Saccoglossus* (hemichordate), apical part of sperm plasma membrane and outer acrosomal membrane dehisce. The severed edges of plasma membrane and outer acrosomal membrane fuse to form an opening through which contents of acrosomal vesicle are released. Inner acrosomal membrane grows into one or many **acrosomal tubules** which in some cases as long as the entire sperm. The membrane surrounding the acrosomal tubule is derived from the inner membrane of acrosomal vesicle.

In mammals, the plasma membrane and outer acrosomal membrane break and fuse to form extensive vesiculation. The inner acrosomal membrane joins with the plasma membrane at the level of acrosomal collar. In mammals inner acrosomal membrane does not participate in gametic fusion. The sperm is probably phagoytosed by the egg and the sperm plasma membrane does not form part of the zygote plasma membrane.

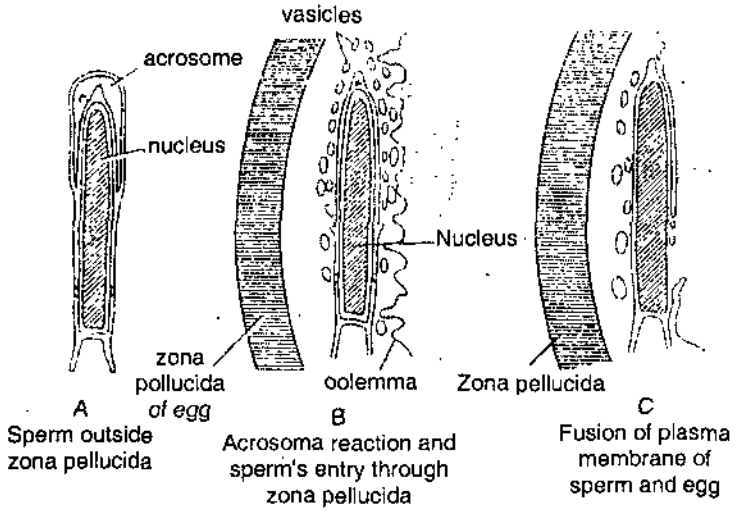


Fig. 4. Acrosome reacton and sperm entry in rat ovum.

(b) Release of acrosomal contents : Lytic enzymes or lysins found in the acrosome are released. The lysins help the sperm to penetrate the egg envelopes by liquifying them locally without affecting the egg plasma membrane or oolemma. In sea urchins like *Strongylocentrotus purpuratus* and *S. franciscanus*, acrosomal reaction liberates a species-specific egg-binding protein, **bindin** and a protease or lysin called **acrosomin. Bindin** causes adhesion of sperms to eggs of the same species. **Acrosomin** digests the vitelline membrane covering the unfertilized egg.

In mammals including human females, fertilization of ovum takes place soon after the ovum enters the Fallopian tube. Sperm first penetrate the **granulosa cells** (follicular cells) attached to the outer side of ovum, forming the **corona radiata**. The follicular cells of corna radiata are held together by an adhesive substance called **hyaluronic acid** (a glycosaminoglycan). The sperm entry through corona radiata and zona pellucida require hyaluronidase and proteolytic enzymes released by the acrosome of the sperm. Hyaluronidase dissolves the cement substance between the cells of corona radiata. Only one sperm is required to fertilize the ovum. The proteolytic acrosomal enzymes are responsible for the passage of sperm through the zona pellucida.

(c) Formation of acrosomal tubule : Apical part of plasma membrane of sperm (inner acrosomal membrane) extends forward to form the **acrosomal tubule**. It penetrates the egg envelopes (outer and inner egg envelopes) to reach the egg plasma memorane (oolemma). The shape and size of the acrosomal tubule varies with species- long in *Asterias, Saccoglossus,* and *Mytilus;* short in *Pseudocentrotus,* and filamentous in *Limulus.* It is completely absent in mammals.

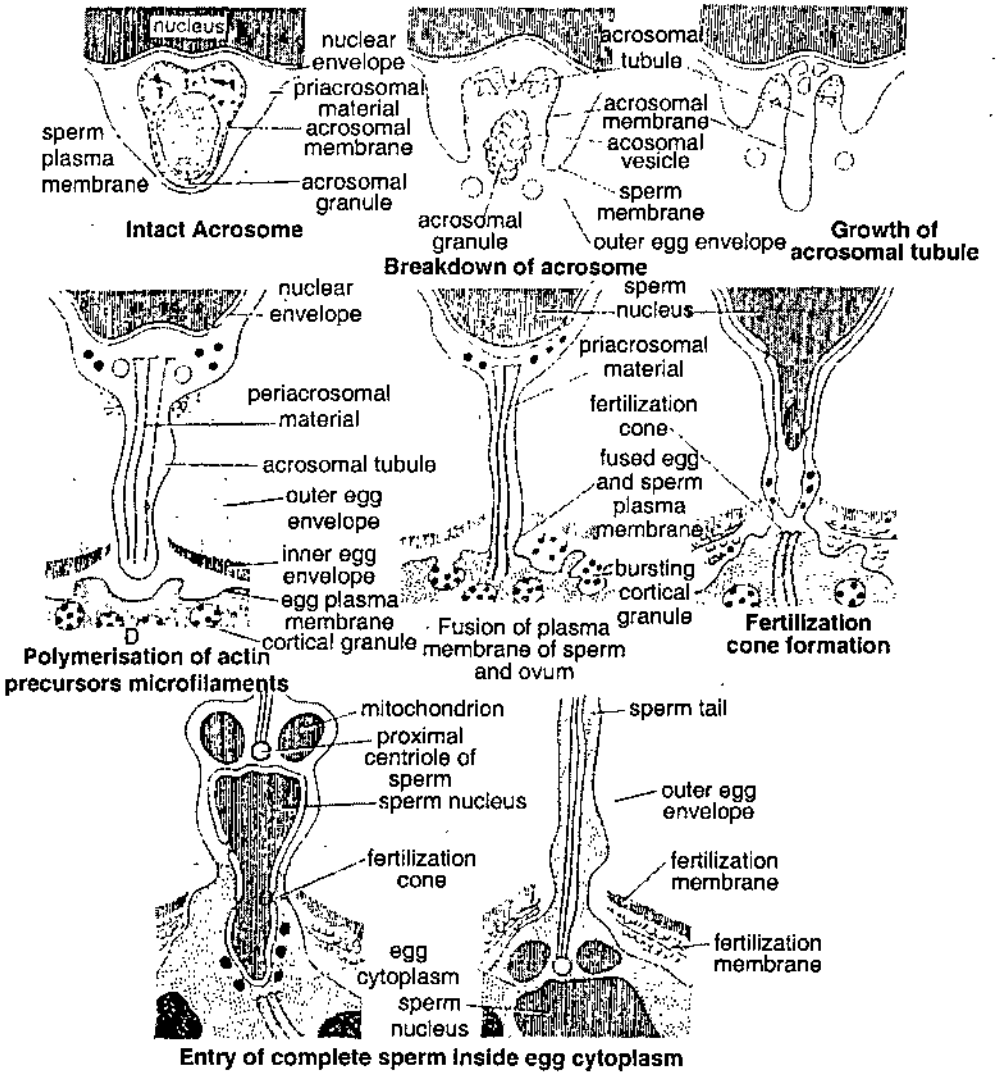


Fig. 5 Fertilization in *Saccoglossus*.

(d) Fusion of sperm and egg plasma membrane : Tip of the acrosomal tubule fuses with the plasma membrane of the egg that develops continuity between cytoplasms of the egg and sperm. In mammals, due to the absence of acrosomal tubule, sperm makes contact with the egg surface by its lateral aspects. Later the plasma membranes of the egg and sperm dissolve at the point of contact and the nucleus and cytoplasmic components of the sperm are drawn into the interior of the egg, forming a single cell **zygote**.

In teleost fishes, acrosome is not found in their sperms, hence acrosome reaction is not found in them. In such cases, sperm plasma membrane fuses with the plasma membrane of the ovum.

Activation of Ovum :

Egg activation causes : change in ionic permeability of egg plasma membrane, transient increase in intracellular Ca++ and pH, cortical reaction and elevation of fertilization membrane, formation of fertilization cone, cytoplasmic rearrangement, changes in physical properties and metabolism of egg, restart of meiosis and preparation of mitotic cell division for cleavage.

1. Change in ionic permeability of egg's plasma membrane : As the sperm makes contact with the egg plasma membrane, the egg surface undergoes a change in ionic permeability and electric potential of the membrane also changes. Electrical potential change at the time of fertilization resemble changes taking place in a nerve when an impulse passes over it, although it is slow in comparison to nerve impulse. Response of sodium and potassium ions suggests that primary activation event of the egg is a change in ion permeability at the site of sperm contact. Sodium, potassium and calcium ions diffuse rapidly into frog's egg, reaching a peak within a minute after sperm attachment. This permeability change is propagated as a wave over the membrane within seconds and involves various transport proteins (pumps) of membrane.

2. Transient intracellular rise in calcium ions : First intracellular change is the transient rise in Ca++, released in the egg cortex from intracellular calcium store. Calcium store is recharged after 40 minutes. Transient Ca++ increase is enough to cause cortical reaction is sea urchins and fish eggs. In the absence of calcium, fertilization does not take place. In calcium free medium, sperm viability, motility and fertilizing capacity also decreases. Acrosome reaction is also calcium dependent- without calcium acrosome filament does not explode. Egg stability also depends upon calcium.

Calcium is found bound to the plasma membrane and cortex. After membrane fusion, calcium is released and diffused within cytoplasm. Calcium activation may change protein configuration in membrane and cortex. with consequent changes in functional properties. Conformational changes of membrane proteins cause increased permeability for sodium, potassium ions and other substances. Finally calcium exchange across membranes in excitatory event is coupled to cyclic AMP, a regulatory molecule that activates many enzymes including **protein kinases.** Protein kinases phosphorylate proteins such as **histones** and also phosphorylate microtubular proteins that function in cell division.

3. Transient intracellular increase in pH : Along with the increase of *calcium there is intracellular increase in pH* by 0.3 to 0.4 units. This increase is due to inflow of Na^+ and outflow of H^+ ions. H^+ ions causes the formation of **fertilization acid** in the surrounding sea water. Thus, pH of the cytoplasm increases from 6.84 to 7.27 in sea urchin eggs.

4. Cortical reaction : Activation of egg membrane is intimately connected to changes in egg cortex. After the membranes of sperm and ovum contact, changes take place in the egg cortex. The fine microvilli of egg membrane are withdrawn and egg surface becomes smooth. The egg slightly shrinks (decreases in volume). The dense granular layer of cortex becomes homogeneous region of oriented microfilaments adjacent to plasma membrane.

The cortical reactions in most groups fundamentally ends into the formation of a **fertilization membrane** outside the egg plasma membrane. Fertilization membrane blocks the entrance of late arriving sperms. The process of

fertilization membrane and cortical reactions have been studied more extensively in sea urchins and starfishes.

(i) Fertilization Membrane Formation in Sea Urchins

In sea urchins, as soon as the apical end of acrosomal tubule touches the egg surface, from the site of contact a wave like colour change from yellow to white travels rapidly around the egg cortex and soon elevation of fertilization cone from the egg surface and formation of fertilization membrane around the egg plasma membrane take place.

In its unfertilized egg, the egg cortex is bounded by two membrane : (i) an *outer 30 Å thick vitelline membrane and (ii) an inner 60° thick* plasma membrane. Beneath the plasma membrane is found a layer of cortical granules (about 15,000 granules). A fertilization membrane is formed as follows :

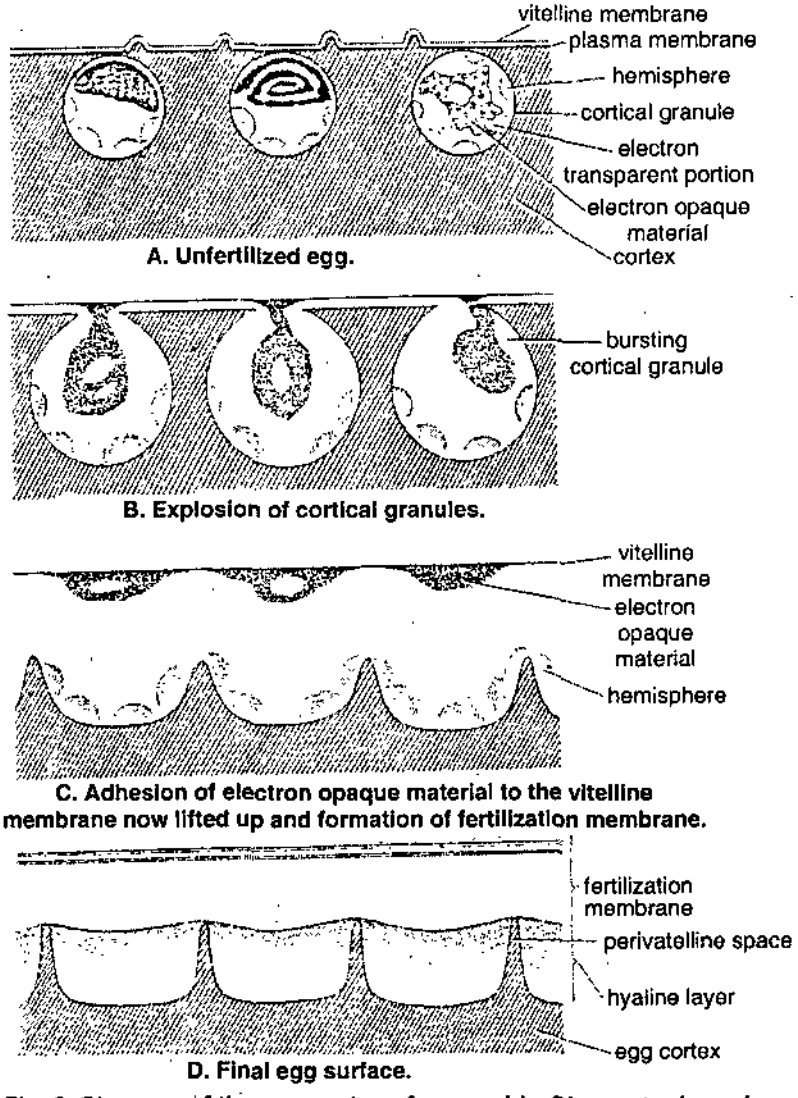


Fig. 6. Changes of the egg cortex of sea urchin *Clypeaster japonicus* after fertilization.

1. Outer vitelline membrane separated from the plasma membrane undergoes expansion and becomes outer layer of the fertilization membrane.

2. Cortical granules explode and release three components :

(i) Lamellar and folded parts of granules unfold and fuse with the inner side of the already elevating vitelline membrane.

(ii) Globules fuse together and form a new surface of viscous hyaline layer outside the egg plasma membrane. This hyaline layer adheres closely to the surface of the egg during cleavage, it keeps the blastomeres together.

(iii) Liquified component of the cortical granules fills the perivitelline space. It contains mucopolysaccharides and abundant water. Cortical granules contents also contain a trypsin-like protease enzyme.

Thus, vitelline membrane and contents of cortical granules form a fertilization membrane.

Finally, membrane of cortical granules fuses with the egg plasma membrane to form a mosaic. This membrane contribute to the change in permeability.

Minutes after fertilization, lipases and proteases become active and they digest cortical granules membranes to initiate breakdown. Calcium-dependent adenosine triphosphatase activated at fertilization. Splitting of ATP furnish energy for explosive release of granules or for contractions of the egg surface.

In barnacle *Barnea,* cortical granules explode independently of fertilization. In *Chaetopterus* (annelid worm), cortical granules do not breakdown at fertilization but are retained during cleavage.

(ii) Fertilization Membrane Formation in Vertebrates

In **bony fishes** and **frogs,** cortical granules contain mucopolysaccharides. In these animals, cortical granules are broken down after sperms penetration into the egg cytoplasm and their contents become liquified and extruded on the surface of the plasma membrane of the egg. Thy gradually fill up perivitelline space, which is present in between chorion and egg plasma membrane in bony fishes and in frogs space lies in between the vitelline membrane and egg plasma membrane. In both (fish and frog) vitelline membrane or chorion itself does not change into the fertilization membrane like sea urchins.

The unfertilized eggs of some mammals like rabbit, hamster and man, have cortical granules in their cortical regions. In them, sperm penetration is not followed by formation of fertilization membrane, but the cortical granules burst and release their contents into the perivitelline space (space between egg plasma membrane and zona pellucida). They finally disappear.

Many vertebrates like urodele amphibians, mammals etc., lack cortical granules. In these animals, fertilization membrane formation do not take place. In caudate amphibians a hyaline perivitelline space is formed in fertilized egg.

Monospermic and Polyspermic Fertilization

When only one sperm enters the egg, this is called **monospermic fertilization.** Such fertilization is found in many species, e.g., sea urchins, *Xenopus laevis* (hamster), fish, rabbit, man etc.

When many spermatozoa penetrate the single ovum, this is called **polyspermic fertilization.** If in some mammals and sea urchins immature or aging or overripe egg is kept in a dense sperm suspension, bispermy or trispermy occurs. This is called **pathological polyspermy.** In polylecithal eggs like some insects, elasmobranch fishes, amphibian urodeles, reptiles and birds, and also in microlecithal egg of bryozoans, several sperms enter the egg. This is called **physiological polyspermy.** In these cases, genetic material from only one sperm unites with the nucleus of ovum to form the **zygote nucleus.** All other sperm nuclei are degenerated.

Metabolic Activation

In animals in which the egg remains metabolically inactive before fertilization, fertilization spurt the metabolic activity. Following metabolic changes take place after fertilization :

1. Respiratory changes : In fertilized egg, oxygen consumption is generally increased and it is related with the oxidation of glycogen and other foodstuffs of the egg and synthesis of numerous ATP molecules.

2. Changes in coenzymes : In fertilized egg, NAD due to phosphorylation converted into NADP and also NADPH in the presence of enzyme **NAD kinase**.

$$NAD + ATP \xrightarrow{NAD\ kinase} NADP + ADP$$

NAD kinase enzyme is present in unfertilized egg in an inactivated state. At the time of fertilization it is made activated (**Epel** and **Iverson**, 1965). Increased NADP and NADPH contents may initiate many synthetic pathways of fertilized egg.

Change in Rate of Protein Synthesis

Cytoplasm of mature unfertilized egg contains DNA molecule, tRNA, mRNA, ribosomes and enzymes required during protein synthesis, no or very little protein synthesis occurs in it. During later phase of oogenesis, some inhibitor or repressor proteins are manufactured in sea urchin's eggs and these proteins inactivate chromosomal genes, mRNA, ribosomes, etc., (**Metafora**, *et. al.*, 1971). During fertilization some proteolytic enzymes remove these inhibitor proteins from them and activate protein synthesis.

After five minutes of fertilization, rate of protein synthesis increases three to twelve folds. After about 20 minutes of fertilization, DNA synthesis in the pronuclei begins. Protein and DNA synthesis herald cleavage and development.

Resumption of Meiosis : After fertilization, when polar bodies are extruded, meiosis started. It has been found that sperm enters the egg at different stages of maturation in different animals.

Initiation of Mitosis : After fertilization, DNA synthesis increases with greatest pace. The unfertilized egg cytoplasm has its own centriole, but this centriole is incapable to divide and to form a mitotic spindle of microtubules. The spermatozoon introduce its own centriole, for forming mitotic spindle by the egg to divide in normal way.

Amphimixis :

At the time of penetration of sperm inside the egg cytoplasm, sperm nucleus remains compact and is mitochondria and centriole remain situated behind it. As the sperm nucleus moves inward from the site of fertilization cone, it soon rotates through the angle of 180°, its mitochondria and centriole take the leading position. The sperm nucleus swells and its chromatin becomes finely granular. It finally becomes vesicular and becomes like the interphase nucleus, which is called **male ponucleus.** At the same time, sperm aster forms in the egg cytoplasm around *the proximal centriole of the sperm. As the male* pronucleus develops and migrates toward the side of amphimixis, sperm aster lead it. As the sperm pronucleus and centriole move inward, it is accompanied with some cortical and subcortical cytoplasm, forming the **penetration path** as visible in amphibian eggs.

Before, amphimixis, haploid egg nucleus found near the surface of the egg in the form of several vesicles, called **karyomeres.** In fertilized egg, these

karyomeres fuse together to form **female pronucleus.** It swells, increase in volume and becomes vesicular. It also migrates towards the site of amphimixis.

Amphimixis : In animals, two pronuclei fuse together, i.e., nuclear membrane of both pronuclei are broken at the point of their contact and contents of both pronuclei unite into one mass and finally bounded by a common nuclear envelope.

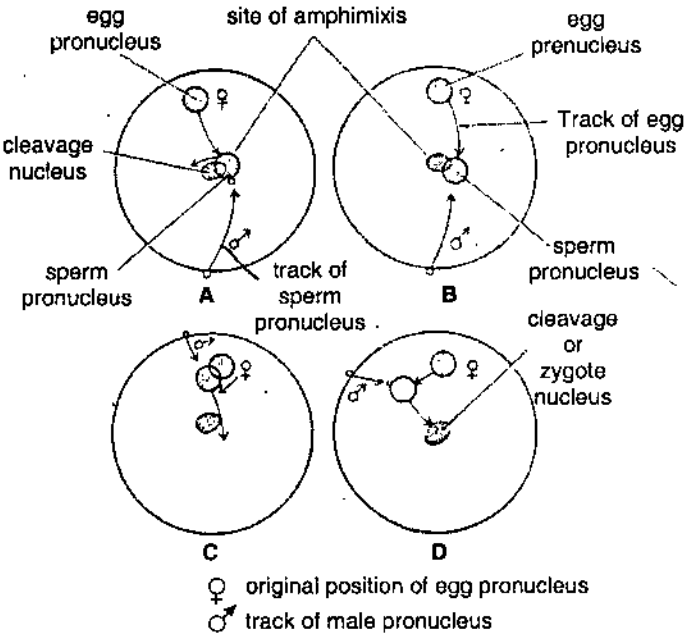


Fig. 7. Amphimixis in sea urchin.

In other cases like *Ascaris,* some annelids and molluscs, male and female pronuclei do not fuse, nuclear membranes in both dissolve and chromosomes are released. In the mean time centrosome of the sperm has divided into two and achromatic spindle is formed and to this spindle, chromosomes of sperm and egg pronuclei become attached. After the completion of first division of the fertilized egg, paternal and maternal chromosomes become enclosed by common nuclear membranes forming the nuclei of two blastomeres.

• SUMMARY

1. Fertilization is of two types : external fertilization occurs outside the body of maternal parent. Internal fertilization occurs inside the oviduct of the female.

2. Life spans of gametes (sperms and ova) are limited. Mature eggs become overripe if not activated promptly. Human egg can be fertilized within 24 hours after ovulation.

3. Approach of the sperm towards the eggs is **chemotaxic** in coelentrates and fishes. In sturgeon and lamprey (fishes), each egg is enclosed in a hard chorion perforated by a narrow canal, **micropyle**.

4. **Fertilizin-antifertilizin interaction :** Eggs (egg membranes) release a chemical attractant, called **fertilizin**. Whereas sperms release antifertilizin. which interact with fertilizin.

5. **Capacitation :** Ejaculated sperms in the oviduct lose the receptor sites over the acrosome and thus sperm recognize the signals emanating from the ovulated egg. This is called capacitation.

6. **Acrosome reaction :** During acrosome reaction membrane of acrosome vesicle breaks down, acrosomal enzymes are released, acrosomal tubule or tubules are formed, and finally plasma membrane of sperm and egg fuse. Calcium is essential for acrosome reaction.

7. Sperm plasma membrane and outer acrosomal membrane are lost. Severed membranes of plasma membrane and outer acrosomal membrane fuse

to form an opening, through which contents of acrosomal vesicle are released. Acrosomal tubule or tubules are formed by the growing inner acrosomal membrane.

8. In mammals, plasma membrane and outer acrosomal membrane break and fuse to form many vesiculations. Inner acrosomal membrane joins with the plasma membrane at the level of acrosomal collar. Sprm is phagocytosed by the egg and inner acrosomal membrane does not participate in gametic fusion.

9. Lytic enzymes released from the acrosome help the sperm to penetrate the egg envelopes.

Acrosomal reaction in sea urchins liberate species-specific egg-binding protein, called **bindin** and **acrosomin.** Bindin causes adhesion of sperms to eggs of the same species. Acrosomin digests the vitelline membrane of the unfertilized egg.

10. In mammls, hyaluronic acid helds together the follicular cells or corona radiata. Hyaluronidase and proteolytic enzymes released by the acrosome of sperm dissolve the cement substance between the cells of corona radiata.

11. Acrosomal tubule is formed by the inner acrosomal membrane. Acrosomal tubule is not found in the mammals.

12. In teleost fishes, acrosome is not found in sperms, and hence acrosome reaction is not found.

13. As sperm makes contact with the egg plasma membrane, egg surface undergoes change in ionic permeability and electric potential. Calcium is released in the egg cortex from intracellular calcium store. In the absence of calcium fertilization does not take place. Acrosome reaction is also calcium dependent. Acrosome filament does not explode without calcium.

14. Changes in the egg cortex take place after the membranes of sperm and ovum make contact. Microvilli of egg membrane are withdrawn, egg decreases in volume, dense granular layer of cortex becomes homogeneous region. Fertilization membrane is formed at the end of cortical reaction. Vitelline membrane and contents of cortical granules form the fertilization membrane.

• QUESTIONS

Long Answer Type Questions

1. Describe the process of fertilization in animals.
2. Usually, interspecific fertilization is not possible. Explain the histochemical basis for this, found in zona pellucida.
3. Describe the mechanism of fertilization in detail.

Short Answer Type Questions

1. Write short notes on :
   (i) Fertilization
   (ii) Fertilizin- antifertilizin interactions
   (iii) Capacitation
   (iv) Acrosome reaction
   (v) Cortical reaction.

Very Short Answer Type Questions

1. **Fertilizin**

   Fertilizin is secreted from jelly coats and egg membranes of eggs. **Lillie** postulated that fertilizin was continuously secreted by the egg before fertilization. Fertilizin is a glycoprotein, now called proteoglycan.

2. Acrosome

Acrosome is found at the anterior end of sperm in between anterior half of nucleus and plasma membrane of the sperm tip. It is bounded by a unit membrane and is formed by the Golgi apparatus. It contains acid hydrolases such as acid phosphatase, cathepsin and hyaluronidase.

3. Antifertilizin

Antifertilizin is found on the surface of sperms plasma membrane. Antifertilizins interact with fertilizin molecules.

4. Acrosomal tubules

After the dehiscence of sperm plasma membrane and outer acrosomal membrane, both the membranes edges fuse to form an opening. Inner acrosomal membrane grows into one or many acrosomal tubules.

5. *Bindin and acrosomin*

In sea urchins *Strongylocentrous purpuratus* the acrosomal reaction results in the liberation of a species-specific egg binding protein, **bindin** and a protease called **acrosomin.** Bindin causes adhesion of sperms to eggs of the same species. Acrosomin digests the vitelline membrane of the unfertilized egg.

6. Amphimixis

Fusion of male and female pronuclei is called amphimixis.

Objective Type Questions

1. Entry of a single sperm into the ovum is called ..... fertilization.

Ans. Monospermic.

UNIT

4

PARTHENOGENESIS

STRUCTURE

- Study of parthenogenesis
- Type of parthenogenesis
- Natural parthenogenesis. Complete and incomplete or cyclic parthenogenesis
- Natural parthenogenesis based on the behaviour of chromosomes and based on the sex of chromosomes
- Arrhenotoky and thelytoky (diploid parthenogenesis).
- Study of artificial parthenogenesis.
  - Summary
  - Test Yourself

LEARNING OBJECTIVES

After going through this unit you will learn :

- Parthenogenesis : Natural and Artificial.
- Natural parthenogenesis : Complete and Cyclic parthenogenesis Haplo-diploid parthenogenesis.
- Natural parthenogenesis based on sex of offsprings.
- Artificial parthenogenesis.
- Signification of parthenogenesis.

• 4.1. PARTHENOGENESIS

Parthenogenesis is a special type of sexual reproduction. In parthenogenesis (virginal development) egg or ovum develops without fertilization. A mature ovum contains all the material potentiality to form a new being.

Parthenogenesis is the development of a female gamete into the embryo without any genetic contribution from the male gamete, with or without the attainment of adulthood (**Beatty**, 1967). Parthenogenesis (Gr. *Parthenos* = virgin; *genesis* =origin) literally means the birth of an individual from a virtin. The individuals reproducing by parthenogenesis are called **parthenotes.** Parthenogenesis is found in nature, in certain orders of insects such as Hymenoptera (bees and wasps), Homoptera (aphids), Coleoptera (beetles); rotifers and lower crustaceans (*Apus* or tadpole fish, *Artemia* or brine shrimp, *Cypris* and *Daphnia*) and certain desert lizards. It has also been successfully induced artificially in echinoderms, molluscs, annelids, amphibians and even mammals. Parthenogenesis is of two main kinds : 1. Natural (spontaneous) parthenogenesis 2. Artificial (induced) parthenogenesis.

• 4.2. NATURAL PARTHENOGENESIS

Parthenogenesis occurs as a natural reproductive phenomenon in bees, and other social insects, rotifers, water fleas and other crustacea. In some organisms it is the usual **(facultative)** or exclusive **(obligatory)** mode of reproduction. Natural parthenogenesis is of two kinds :

1. Complete Parthenogenesis. In some platyhelminthes, rotifers and certain wasps, parthenogenesis is the only form of reproduction. This is called **complete parthenogenesis.** In complete parthenogenesis, individuals arise from unfertilized eggs and all are entirely females, males **rarely appears.** Sexuality is lost in these groups. The entire adult life is devoted to feeding and reproduction.

2. Cyclic Parthenogenesis : In cyclic parthenogenesis, found in aphids and some rotifers. In this several generations of parthenogenetic reproduction alternate with biparental reproduction in which the egg is fertilized. It retains the advantages of sexuality. **Aphids** increase rapidly during the parthenogenetic portion of the life cycle. **In spring,** eggs hatch as females, which invade plants and reproduce many parthenogenetic offspring throughout the summer months. In summers, eggs from some winged females become sexual individuals which mate. From these fertilized eggs, female nymphs emerge in the spring in the following year to repeat the cycle.

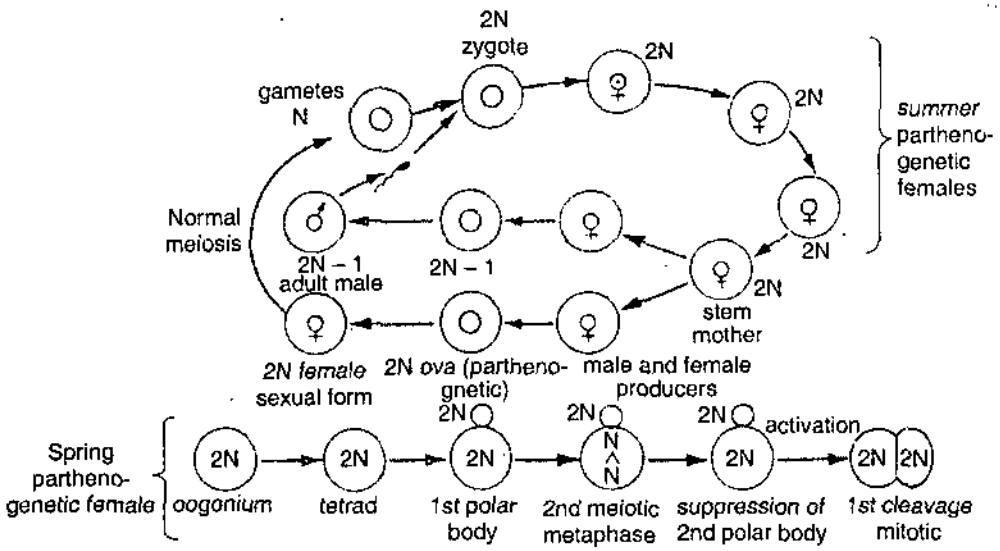


Fig. 1. Cyclic (diploid) parthenogenesis in aphids. Females emerging in the spring produce many generations of females by diploid parthenogenesis resulting from *suppression of first or second polar body (shown below).* At summer's end some females produce sexual males and females by diploid parthenogenesis, males differing from females in lacking one sex chromosome. Both of these sexes, in turn, produce haploid gametes through normal meiosis which fuse to form diploid zygotes that emerge again in the spring as parthenogenetic females.

Haploid parthenogenesis in Rotifers : There are generally separate sexes in rotifers. Females are of two types, **amictic** and **mictic,** each produces separate types of eggs : **Mictic eggs** are haploid and can be fertilized by males. A fertilized mictic egg becomes dormant and eventually develops into a **diploid female.** If mictc eggs are not fertilized, they develop into haploid males. **Amictic females** produce diploid eggs which develop directly into diploid females without fertilization, *i.e.,* by the process of parthenogenesis.

Natural parthenogenesis has also been further classified into two main types :

1. Natural parthenogenesis based on the behaviour of chromosomes

Three types of natural parthenogenesis have been recognized on the basis of chromosomes during meiosis :

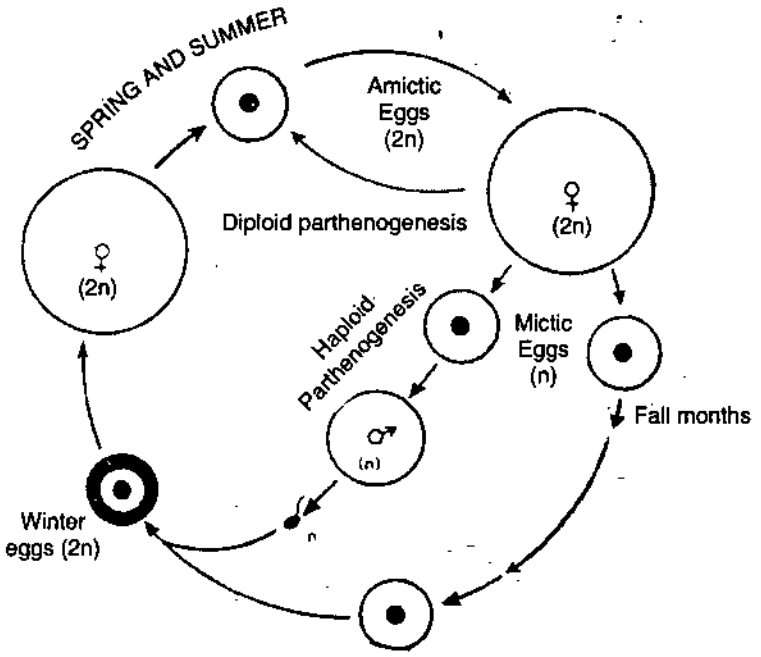
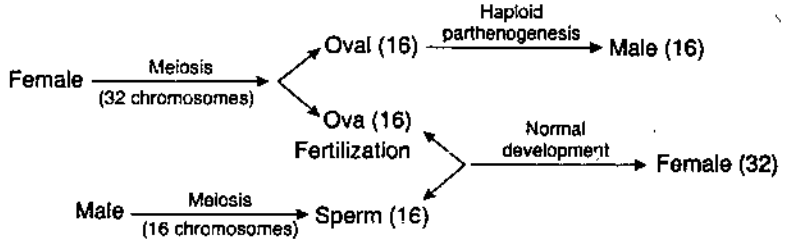


Fig. 2. haploid and diploid parthenogenesis in the life cycle of rotifers.

(i) Haplo-diploid parthenogenesis (Haploid parthenogenetic males, diploid females) : In this case normal meiosis occurs and parthenogenetic haploids develop into **males.** Queen honeybee is fertilized only once by a male (**drone**) or sometimes by more than one drone. She stores the sperms in her seminal receptacles. As she lays her eggs, they are either fertilized or passed out unfertilized. **Fertilized eggs** (diploid with 32 chromosomes) develop into **females** (queens or workers) and unfertilized eggs (haploid with 16 chromosomes) become males (drone).



(ii) Automictic, meiotic or diploid parthenogenesis : In this case, meiosis occurs, but the chromosome number is doubled (**diplosis**) either before (*Moraba,* Orthoptera) or after (*Solenobia,* Lepidoptera) meiosis. Diplosis may take place by the following two methods :

(A) Autofertilization : During oogenesis, the first meiotic division is normal and produces a first polar body (polocyte). However, the second part of meiotic division is suppressed and the second polar body's nucleus fuses with the egg nucleus to produce the **diploid cell.** It gives rise to a parthenogenetic female. Autofertilization takes place in *Artemia salina* (crustacea) found in saline water.

(B) Prestitution : In insects order Hymenoptera (*Nemertis consesceus*) and Lepidoptera, nuclear division or karyokinesis (meiosis I) of primary oocyte forms a nucleus of secondary oocyte and a nucleus of the first polocyte. This karyokinesis is not followed by the cytokinesis. The chromosomes of both daughter nuclei get arranged on the equator and proceed for second meiotic division to form a **diploid ovum** and a **diploid polocyte**. The diploid ovum develops into a parthenogenetic **diploid individual.**

(iii) Apomeiotic (ameiotic) parthenogenesis : In this case no meiosis occurs. For example, in brine shrimp (*Artemia salina*) meiosis is entirely suppessed in the female, the maturation divisions of the egg being entirely mitotic or equational in characters. Similarly in *Trichoniscus* (Isopoda), *Daphnia pulex* (Crustacea), *Campelona rufum* (Mollusca), weevils and long-horned grasshoppers during oogenesis, first meiotic division is completely suppressed and primary oocyte directly undergoes second maturation division (**Meiosis** II). Consequently such ova (eggs) contain diploid number of chromosomes and develop into new individuals without fertilization. Due to absence of meiosis, this type of parthenogenesis is called apomictic or ameiotic parthenogenesis.

2. Natural parthenogenesis based on the sex of offspring :

Based on the sex of the offspring, parthenogenesis is of three types :

(i) Arrhenotoky (Haploid parthenogenesis) : Here only parthenogenetic males are produced, *e.g.,* honeybees. Other examples of arrhenotoky are the following :

(a) Hymenopteran insects (bees, ants, wasps) show arrhenotoky. In parasitic wasp (*Habrobracon*), males are haploid having 10 chromosomes, while females have 20 chromosomes since they are diploids. In male, during spermatogenesis, reduction division is omitted, hence sperms contain haploid (n) number of chromosomes in the adult. Female honeybee contains 32 chromosomes (2n-diploid number). Oogenesis results in the formation of haploid (n) ova, each has 16 chromosomes. Males develop parthenogenetically from unfertilized eggs having 16 chromosomes only. Union of these sperms with ova results in the formation of a zygote with 32 chromosomes. It develops into a female.

(b) In Coleoptera (*Micromalthus debilis*), males are haploid and develop from unfertilized eggs.

(c) In Thysanoptera (*Anthothrips verbasi* and *Frankliniella insulasis*) virgin females produce all haploid males.

(d) Rotifer *Asplanchna amphora* is an example of arrhenotoky, where the males are dwarf and develop from haploid eggs. In another rotifer, *Bolelloida*, males are unknown as arrhenotoky is the only mode of reproduction.

(e) In Arachnida, arrhenotoky (haploid parthenogenesis) found in **ticks** and **mites.** In them, males are haploid and develop from the unfertilized eggs, whereas, the females are diploid arising from fertilized eggs. **Schrader** (1923) has reported male haploidy in the red spider *Tetranynchus* and **Patau** (1936) has described male haploidy in *Pediculoides ventriconus.*

(ii) Thelytoky (diploid parthenogenesis) : In this case only parthenogenetic females are produced. In thelytoky, reduction division is abortive and the young individuals develop from the unfertilized diploid eggs. It includes the already discussed categories of parthenogenesis such as meiotic and ameiotic parthenogenesis, *e.g. Solenobia, Ptinus, Lacerta saxicola armeniaca* (No males exist in nature in *Lacerta saxicola armeniaca*.. Its population is propagated exclusively by parthenogenetic females.

(c) Amphitoky : In this case parthenogenetic eggs may produce individuals of either sex, *e.g., Aphits.*

2. ARTIFICIAL PARTHENOGENESIS

The development of a ripe egg can be incited to start development by certain treatments. This type of fertilization is called **artificial parthenogenesis.**

Eggs of many animals, such as sea urchin, starfish, moth, fish, frog, turkey, hen, rabbit, etc., have been activated by the following ways and it has been found that the eggs which have been induced by artificial means seldom reach maturity and seldom develop into the functinonal adults.

(1) Chemical treatment of eggs with various substances : Hypo- and hypertonic sea water; weak organic acids (butyric, lactic, oleic acids); alkalis (0.01 N NH_3); chloride salts of lithium, sodium, potassium, calcium and magnesium; strychnine (an alkaloid obtained primarily from the plant ***Nux vomica*** or *Strychnos nuxvomica*); fat solvents (toulene, ether, alcohol, benzene and acetone); and other chemicals such as chloroform, urea, sucrose, corrosive sublimate, etc.

(2) Treatment with physical agents such as heat or cold shock (*e.g.*, when the egg is transferred from 30°C to –10°C), electric shock, shaking, pricking, etc.

(3) Treatment with radiations such as fertilization by genetically inactivated (ultra-violet or UV-treated) sperm.

Frog eggs can be activated by pricking them with a clean fine glass needle. If needle is not clean and is smeared with freshly drawn frog blood, so that a nucleate cell is introduced into the egg at the time of pricking, a second aster will form around the injected cell. Mitosis will then be normal and development will proceed until the tadpole stage. Constituent parts of cells may also be introduced into the egg with a micropipette.

Certain compounds, called **calcium ionophores** can activate parthenogenetic development of the eggs of a wide group of animals including sea urchins, tunicates, molluscs, amphibians and mammals. Ionophores make cellular membranes permeable to certain ions and not only the outer cell of plasma membrane but internal membranes as well.

SIGNIFICANCE OF PARTHENOGENESIS

1. Means of reproduction : Parthenogenesis is a well established means of reproduction. It is even simpler and easier than sexual reproduction.

2. Rapid multiplication : Parthenogenesis represents a device of high multiplication. For example, aphids multiply very fast during their parthenogenetic phase of the life cycle.

3. Polyploidy : Pathenogenesis permits the establishment of **triploid** (3n) and **aneuploid** races having three or more sets of chromosomes. In triploid forms, three sets of chromosomes fail to pair during meiosis at the time of gamete formation. This leads to sexual sterility. The parthenogenesis is, thus, a means for aneuploid individuals to propagate their own kind.

4. Persistence of advantageous characters : Parthenogenesis encourages advantageous combination of characters or genes originated by mutation to remain as such, because these are not liable to segregate by crossing over as there is no meiosis.

5. Parthenogenesis is a means of sex determination in honeybee and other animals. It supports the chromosome theory of sex determination.

6. Parthenogenesis eliminates the need for mating and allows the whole energy of the adult life to be devoted to feeding and reproduction.

• SUMMARY

Parthenogenesis or virginal development is a special type of sexual reproduction.

In parthenogenesis, egg develops without fertilization. Parthenogenesis is found in certain orders of Insecta, such as Hymenoptera, Homoptera, Coleoptera, rotifers, certain lower crustaceans and certain desert lizards.

Parthenogenesis is of two types. Natural and Artificial.

Natural parthenogenesis is of two types :

1. Complete parthenogenesis : It is found in some platyhelminthes, rotifers and certain wasps. In this type individuals arise from unfertilized eggs, and all are entirely females. Male rarely appear.

2. Cyclic parthenogenesis : It is found in aphids and some rotifers. In it several generations of parthenogenetic reproduction alternate with biparental reproduction in which egg is fertilized.

Haploid parthenogenesis in rotifers. In rotifers, sexes are separate.

Females are of two types : **Amictic** and **mictic.** Each produces separate types of eggs. **Mictic eggs** are haploid and can be fertilized by males. Fertilized mictic egg eventually develops into a diploid female.

Amictic females produce diploid eggs which develop into diploid females without fertilization.

Haplo-diploid parthenogenesis (Haploid males and diploid females). Here normal meiosis occurs and parthenogenetic haploids develop into males. Fertilized eggs (diploid) develop into females and unfertilized eggs (haploid) develop into males. This is found in honey bees. **Automictic** or **meiotic** or **diploid parthenogenesis.** In this case meiosis occurs but the chromosome number is doubled. This diplosis takes place by the following two methods :

1. Autofertilization. In this process during oogenesis first meiotic division is normal produces first polar body, but the second part of division is suppressed, and second polar body nucleus fuses with egg nucleus to produce the diploid cell. It develops into females, e.g., ***Artemia salina*** (Crustacea).

2. Restitution. In this process, nuclear division of primary oocyte forms a nucleus of secondary oocyte and a nucleus of first polar body. This nuclear division is not followed by cytokinesis. Chromosomes of both daughter nuclei are arranged on equator and proceed for second meiotic division to form a diploid ovum and a diploid polar body. Diploid ovum develops into a diploid individual.

Artificial parthenogenesis : A ripe egg is incited by certain chemicals to start development.

• QUESTIONS

Long Answer Type Questions

1. Write a short essay on parthenogenesis.
2. What is parthenogenesis ? Explain how various methods of induced parthenogenesis help us in understanding the mechanism of fertilization.

Short Answer Type Questions :

1. Differentiate between the following :
   (i) Arrhenotoky and thelytoky

(ii) Natural and induced parthenogenesis

(iii) Complete parthenogenesis and cyclic parthenogenesis.

2. Artificial parthenogenesis.
3. Parthenogenesis in brief.

Very Short Answer Questions :

1. **Define parthenogenesis.**

   Ans. It is a special type of sexual reproduction. Eggs develop without fertilization.

2. **What are parthenotes ?**

   Ans. Individuals reproducing by parthenogenesis are called parthenotes.

3. **In which animals parthenogenesis is a natural phenomenon.**

   Ans. It is found in bees, wasps, aphids, beetles, rotifers, *Apus, Artemia, Cypris, Daphia,* Lizards etc.

4. **Define complete parthenogenesis.**

   Ans. Individuals develop from unfertilized eggs, this phenomenon is called complete parthenogenesis.

5. **Write about cyclical parthenogenesis.**

   Ans. Parthenogenesis alternate with sexual reproduction is called cyclical or incomplete parthenogenesis.

6. **What is arrhenotoky ?**

   Ans. In certain animals such as bees, wasps and ants, plantlice and scale insects, beetles and weevils and thrips, males arise from unfertilized eggs (haploid) and females develop from fertilized eggs (2n). This is called arrhenotoky (haploid parthenogenesis).

7. **Write about thelytoky.**

   Ans. Plantlice (aphids) young individuals develop from unfertilized diploid eggs. This is called diploid parthenogenesis. Diploid condition is maintained by omitting reduction division or by fusion of one of the polar bodies with haploid ovum.

8. **What is ameiotic or apomictic thelytoky ?**

   Ans. It occurs due to omission of first reduction division and primary oocyte directly undergoes second meiotic division in eggs, thus such eggs remain diploid and they develop into new individuals without fertilization. It is found in *Trichonisus* (Isopoda), *Daphnia pulex* (Crustacea) etc.

9. **What about meiotic thelytoky.**

   Ans. Certain eggs of insects of order hymenoptera and Lepidoptera develop by usual process of oogenesis, but at some stage of reduction division some abnormality occurs resulting in diplosis (doubling of chromosomes). This is called meiotic thelytoky.

UNIT

5

TYPES OF ANIMALS EGGS AND PATTERNS OF CLEAVAGE

STRUCTURE

- Study of types of eggs :
  1. Based on amount of yolk
  2. Based on distribution of yolk
  3. Based on presence or absence of shell
  4. Based on type of development
- Study of types of Cleavage
- Study of patterns of cleavage
  - Summary
  - Test Yourself

LEARNING OBJECTIVES

After going through this unit you will learn :

- Various types of eggs :
  Based on amount of yolk
  Based on distribution of yolk
  Based on type of development
- Types of Cleavage
- Patterns of Cleavage

5.1. TYPES OF ANIMALS EGGS

Amount of yolk (reserve food) in a fully developed egg varies greatly in different groups of animals. Its distribution also varies in different groups of animals. Thus, based on the amount and distribution of yolk (vitellin), animal eggs have been classified as follows :

A. Types of Eggs Based on Amount of Yolk

1. Microlecithal or Oligolecithal eggs : These eggs are of very small size and contain a very small amount of yolk. **Kent** (1969) described them as **alecithal eggs**. Such eggs are found in certain marine invertebrates, like *Hydra* (Coelenterata), and sea urchin and in various chordates, like *Amphioxus* (Cephalochordata), tunicates, and eutherian mammals.

2. Mesolecithal eggs : Such eggs contain moderate amount of yolk. These are found in annelid worms, molluscs, *Petromyzon,* lung fishes (Dipnoi) and amphibians (frog etc.).

3. Megalecithal, macrolecithal or polylecithal eggs : These eggs contain enormous amount of yolk. These are found in insects, *Myxine,* elasmobranch fishes (Chondrichthyes), reptiles, birds and prototherian mammals.

B. Types of Eggs Based on Distribution of Yolk

1. Homolecithal or isolecithal eggs : In these eggs amount of yolk is very little and evenly distributed throughout the cytoplasm. Its examples are sponges, *Amphioxus,* tunicates and eutherian mammals.

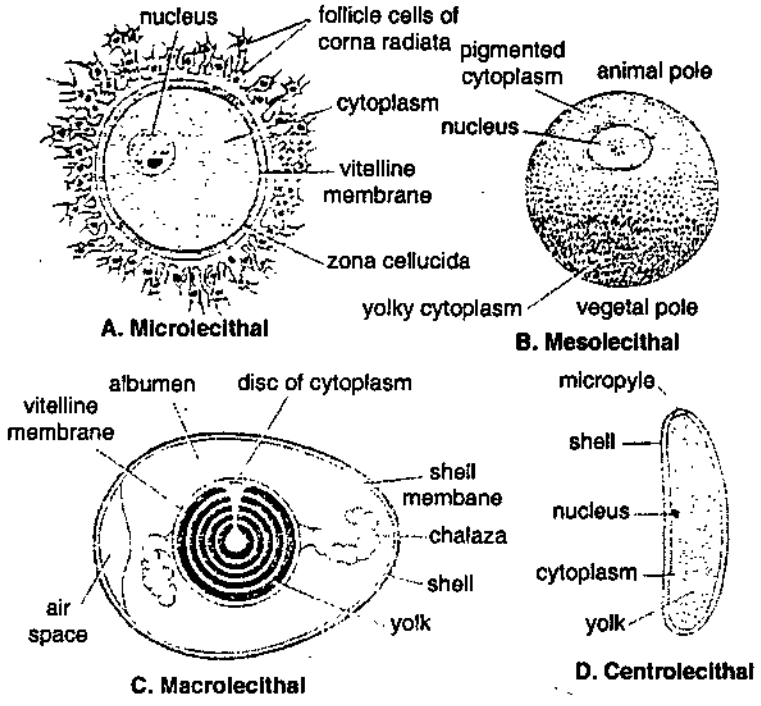


Fig. 1. Representative types of ova.

Types of Animals Eggs and Patterns of Cleavage

2. Telolecithal eggs : In such eggs, the distribution of yolk is more in one hemisphere (lower part) of the egg than in the other half (upper part). Due to uneven distribution of yolk in the egg, two poles are established : lower vegetal pole or half where concentration of yolk is greatest and upper animal pole where concentration of yolk is lesser. Such distribution of yolk is found in mesolecithal eggs. Its examples are many annelids, and molluscs and most amphibians.

3. Centrolecithal eggs : The amount of yolk is large and concentrated in the centre of the egg. Cytoplasm is distributed outside around the yolk. A tiny mass of cytoplasm containing the nucleus is also present in the centre of the egg. Its examples are some coelentrates and most arthropods.

4. Discoidal eggs : In such eggs, amount of yolk is enormous and occupies most of the part of the egg except a very small disc-shaped area of the cytoplasm over the yolk, called **blastodisc.** Such eggs are found in squids and octopuses, fishes, reptiles, birds and prototherian mammals.

C. Based on the Presence or Absence of Shell over the Eggs

1. Cleidoic eggs : Eggs of reptiles and birds laid down on dry land are fully laden with yolk and are surrounded by albumen and a water poof shell. Such self-sufficient eggs are called **cleidoic eggs.**

2. Non-Cleidoic eggs : Such eggs are devoid of outer calcareous shell. Such eggs are found in those animals in which development is internal, i.e., within the body (uterus).

D. Types of Eggs Based on the Types of Development

1. Determinate or mosaic eggs : During the development of eggs, the fate of every part of the egg becomes fixed before or at the time of fertilization. The head-tail, left-right axes and dorsal-ventral parts of the future embryo are pre-determined in the zygote. Removal of a particular portion of the egg, developing embryo will be devoid of a particular organ. Such eggs are called **determinate** or **mosaic,** in these fate of the egg parts can not be changed after

fertilization. Its examples are Protozoa, Porifera and Platyhelminthes (acoelomates). Aschelminthes (pseudocelomates), Annelida, Mollusca and Arthropoda (schizocoelomates) and tunicates.

2. Indeterminate or Regulative Eggs : In such eggs, predetermination and fate of various egg portions is not fixed upto three cleavages (eight celled stage). If blastomeres are separated before eight-celled stage, each develops as a whole embryo. Its examples are coelentrates and most enterocoelomates (echinoderms and chordates).

• 5.2. TYPES OF CLEAVAGE

Major types of cleavage, on the basis of amount and distribution of folk in the egg are:

1. Holoblastic and (2) meroblastic.

1. Holoblastic or total cleavage :

In it the entire egg divides by each cleavage furrow. It is of two types :

(i) Holoblastic Equal Cleavage. In this type the daughter blastomeres are roughly of the same size. It is found in microlecithal and isolecithal eggs. Examples are tunicates, *Amphioxus, marsupials* and entherian mammals.

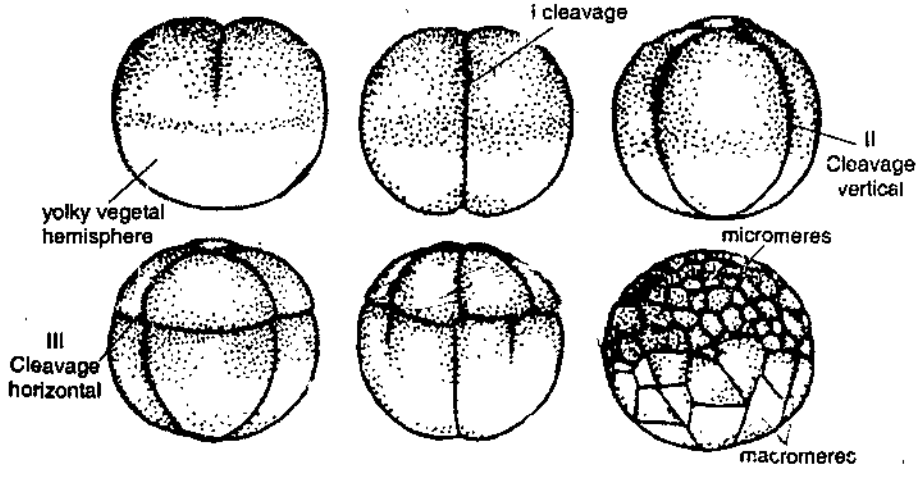


Fig. 2. Effect of yolk on cleavage in frog egg.

(ii) Holoblastic Unequal Cleavage : In this type, daughter blastomeres are of unequal size. This is found in mesolecithal and telolecithal eggs. Examples are cyclostomes, elasmobranch fishes, dipnoi and amphibians etc.

2. Meroblastic or Partial Cleavage :

In this type, cleavage furrows divide only a small amount of active cytoplasm found at the animal pole or on the periphery around the central yolk. Yolky portion of egg remains undivided. This is found in polylecithal eggs. This is of two types :

(i) Superficial Cleavage : This type of partial cleavage is found in centrolecithal eggs of insects and other arthropods. In the beginning of cleavage, the nucleus located in the centre divides repeatedly and then daughter nuclei migrate towards the periphery. Each nucleus is surrounded by a small portion of original central cytoplasm. When nuclei reach at the periphery of egg, their cytoplasm fuses with the superficial layer of cytoplasm. Later cytoplasm divides by furrows moving inward from the surface. Thus, a superficial (peripheral) cells surrounds central yolk mass.

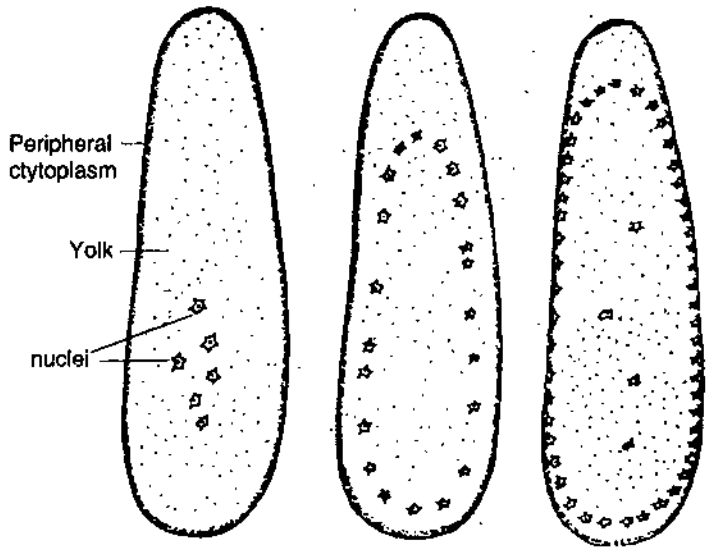


Fig. 3. Superficial meroblastic cleavage in insects (beetle)

(ii) Discoidal Cleavage : This is found in macrolecithal eggs of elasmobranchs, bony fishes, reptiles, birds and egg-laying mammals. Cleavage remains restricted to the blastodisc at the animal pole.

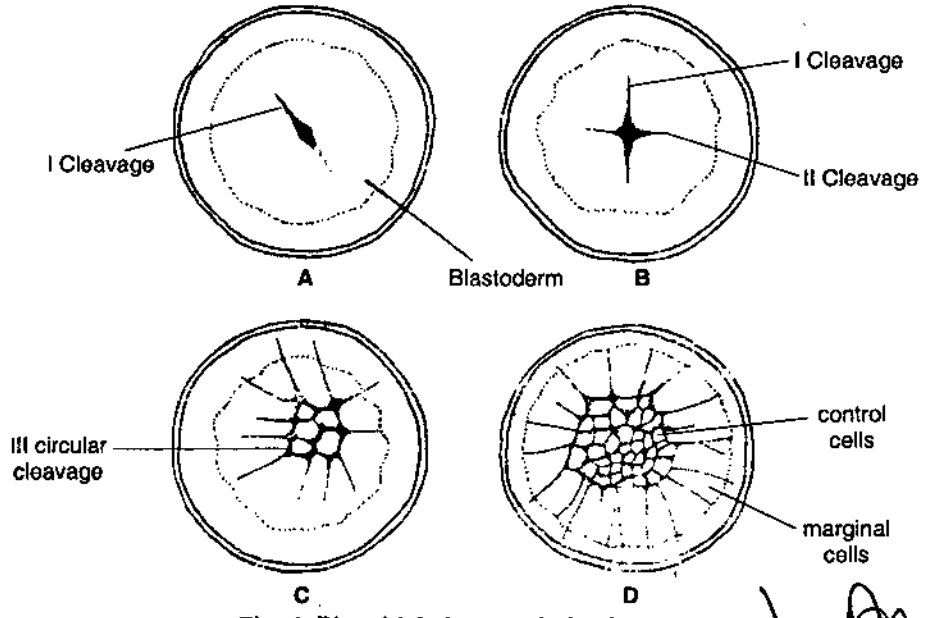


Fig. 4. Discoidal cleavage in hen's egg.

5.3. PATTERNS OF CLEAVAGE

Cleavage or segmentation is the division of an activated egg by a series of mitotic cell divisions, transforming the fertilized egg into a multitude of cells. These cells are called **blastomeres.** Cleavage prepares the ground for differentiation and morphogenesis.

During segmentation, cleavage furrows are not formed at random, but are oriented in a particular manner with reference to the animal-vegetal axis of the egg. Based on the organization of egg, cleavage pattern is of following types :

1. Radial Cleavage : In this type of cleavage, divisions take place in such a way that all blastomeres are placed in a radially symmetrical fashion around the polar axis. If such egg is seen from the poles, blastomeres seem to be

arranged in a radially symmetrical fashion. Examples are sponges, coelentrates, sea urchin and sea cucumber (echinoderms) and *Amphioxus*.

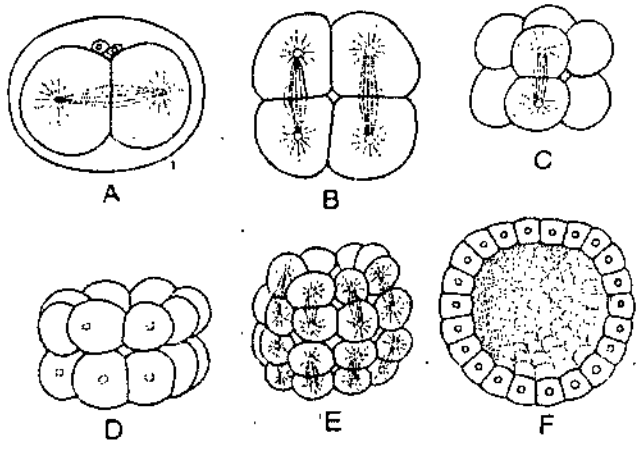


Fig. 5. Radial cleavage of echinoderms (*Synapta, Asterias*).

2. Biradial Cleavage : In such cleavage pattern, first three division planes do not stand at right angles to each other. Its example is Acoela like Ctenophora.

3. Spiral Cleavage : It is diagonal to the polar axis. In this case, spindles of third cleavage are oriented diagonally due to which the resulting upper tier of cells is displaced sideways. Upper four cells are placed over the junctions between four lower cells. Upper four cells are called **micromeres** and lower larger cells are called **megameres**. Spiral cleavage results due to oblique positions of the mitotic spindles. Here four spindles during third cleavage are arranged in a sort of spiral.

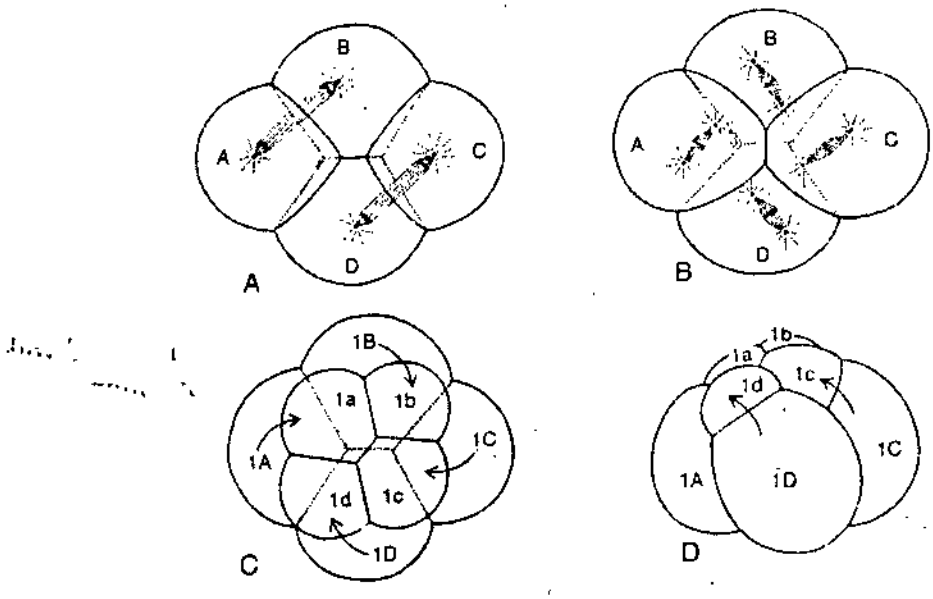


Fig. 6. Spiral cleavage in mollusc (*Trochus*).

Turn of spiral as seen from the animal pole may be in clockwise direction, called **dextral cleavage** or in anti-clockwise direcion, called **sinistral cleavage**. Its examples are annelids, molluscs, nemerteans and some planarians.

4. Bilateral Cleavage : In this type of cleavage, blastomeres are so arranged that right and left sides become disrinct. In this case two of the first

four blastomeres may be larger than the other too, establishing a plane of bilateral symmetry in the developing embryo. Examples are nematodes, cephalopod molluscs, some echinoderms and tunicates.

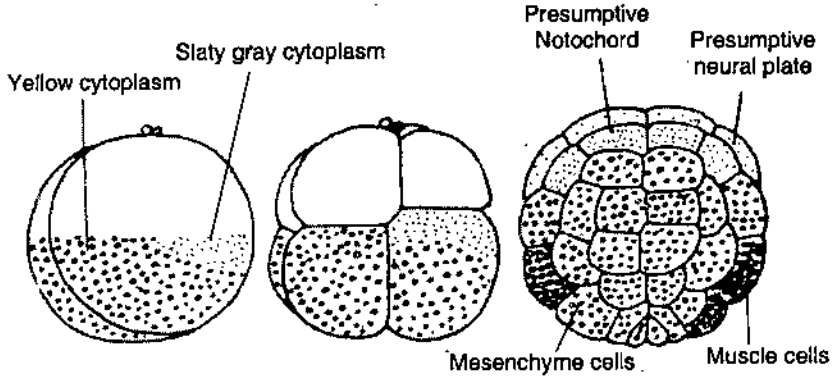


Fig. 7. Bilateral cleavage.

Determinate and Indeterminate Cleavages

Determinate Cleavages : In this type cleavage follows a precise pattern and each blastomere has its characteristic position and unchangeable fate. It converts a fertilized egg into a blastula which is mosaic, because its blastomeres have received assignments according to an inflexible plan which is already completed at time of fertilization. Each blastomere at the same period becomes precursor of a definite part of the embryo. Its examples are tunicates, molluscs and annelids etc.

Indeterminate Cleavage : In this type, cleavage pattern have no definite relation to the embryo. Its examples are echinoderms, *Balanoglossus*, coelentrates and amphibians. If a first cleavage blastomere from the embryo is isolated, it can change its usual destiny and could develop into a perfect, but small embryo.

• SUMMARY

1. Types of eggs based on amount of yolk are :

Microlecithal : Very small in size and contain very small amount of yolk. Examples are *Hydra*, sea urchin, *Amphioxus* and eutherian mammals.

Mesolecithal : These contain moderate amount of yolk and are found in Petromyzon, lung fishes and amphibians.

Megalecithal (Polylecithal) : These contain enormous amount of yolk and are found in elasmobranch fishes, reptiles, birds and egg laying mammals.

2. Types of eggs based on distribution of yolk :

Homolecithal : Amount of yolk is very little and evenly distributed. Examples are *Amphioxus*, tunicates and eutherian mammals.

Telolecithal : Distribution of yolk in such eggs is more in one half (lower) of the egg than in the other upper half. Examples are most amphibians. In these eggs, yolk is concentrated in the lower half of the egg.

Centrolecithal : Amount of yolk is more and concentrated in the center of the egg. Cytoplasm is arranged around the yolk. Examples are insects.

Discoidal : Amount of yolk is enormous occupying most part of the egg except a very small disc-shaped area of cytoplasm over the yolk, called blastodisc. Examples are fishes, reptiles, birds and egg-laying mammals.

Cleidoic eggs : Reptiles and birds lay their eggs on dry land. Their eggs are fully laden with yolk that is surrounded by albumen and a water proof shell.

None-cleidoic eggs : These eggs are devoid of calcareous shell. Determinate or mosaic eggs. During development of egg, fate of every part of the egg becomes fixed before or at time of fertilization.

Indeterminate eggs : In such eggs fate of various egg portions is not fixed.

Patterns of cleavage : Cleavage is the division of an activated egg by a series of mitotic cell divisions transforming the fertilized egg into multitude of cells. These cells are called **blastomeres.**

Cleavage furrows are oriented in a particular manner with reference to the animal-vegetal axis of the egg.

'Cleavage pattern is of the following types :

Radial cleavage : Blastomeres are arranged in a radially symmetrical fashion around the polar axis. Examples are *Amphioxus*, **Biradial cleavage**. First three division planes do not stand at right angles to each other. Example *Ctenophora.*

Spiral cleavage : Spindles of third cleavage are oriented diagonally due to which resulting upper tier of cells is displaced sideways. Upper four cells are placed over the junctions between four lower cells. Upper four smaller cells are called **micromeres** and lower larger cells are called **macromeres.** Examples are nemerteans and some planarians.

Bilateral cleavage : Blastomeres are so arranged that right and left sides become distinct. Two of the first four blastomeres may be larger than the other two, establishing a plane of bilateral symmetry. Examples are some echinoderms and tunicates etc.

Determinate cleavage : Here cleavage follows a precise pattern and each blastomere has its characteristic position and unchangeable fate. Blastula is mosaic. Examples tunicates.

Indeterminate cleavage : Cleavage pattern have no definite relation to the embryo.

• TEST YOURSELF

Long Answer Type Questions

1. Describe the various cleavage patterns of eggs in the chordates.
2. Write the various patterns of cleavage.
3. What is cleavage ? Describe various types of cleavages and also mention difference between mitosis and cleavage.

Short Answer Type Questions

1. Write about types of cleavage.
2. Write about the various types of eggs.
3. Write a short description of cleavage.

Very Short Answer Type Questions

1. What is centrolecithal ?

   Ans. Centrolecithal are the eggs of some coelentrates and most arthropods. In such eggs amount of yolk is large and concentrated in the centre of the egg. Cytoplasm is a clear layer at the periphery around the yolk.
2. Write about equatorial cleavage.

Ans. Equatorial cleavage bisects the egg at right angles to the main axis and half way between the animal and vegetal poles, e.g. first cleavage plane of eggs of higher mammals.

3. What are the various types of eggs ?

   Ans. According to the amount of yolk, eggs are microlecithal, mesolecithal and megalecithal. According to the distribution of yolk, eggs are isolecithal or homolecithal, telolecithal, centrolecithal and discoidal.

4. Cleavages in various types of eggs are of which type ?

   Ans. Mitosis type.

Fill up the Blanks

1. When the cleavage furrows do not divide the entire egg, the cleavage is known as .....

   Ans. Meroblastic.

2. During cleavage the amount of DNA .....

   Ans. Increases (Great increase in the snthesis of DNA takes place because it is needed for duplication of chromosomes.).

3. During cleavage ..... consumption is greatly increased.

   Ans. Oxygen.

4. Cleavage in *Ascaris* is of ..... type.

   Ans. Determinate.

5. Yolk-laden cytoplasm is also called .....

   Ans. Deutoplasm.

True or False Questions :

1. Egg of frog is polylecithal.

   Ans. False

2. Large quantity of yolk in egg interferes in cleavage.

   Ans. True (Yolk retards and even inhibits the movement of cleavages).

UNIT

6

FATE MAPS AND CELL LINEAGE

STRUCTURE

- Blastula
- Fate maps
- *Methods of preparation of Fate maps*
- Examples of Fate maps : *Amphioxus*, Frog blastula and chick embryo
- Study of cell lineage (Cytogeny) : Method of study of cell lineage.
  - Summary
  - Test Yourself

LEARNING OBJECTIVES

After going through this unit you will learn :

- To study formation of blastula and its various types.
- Study of fate maps and methods of Fate maps preparation.
- Study of fate maps of various animals.
- Study of cell lineage.
- Method of study of cell lineage.

• 6.1. BLASTULA

Isolecithal eggs undergo cleavage to form numerous equal–sized cells, forming a tight cluster of cells, called **morula.** This is the most early cleavage stage in which cluster of blastomeres are tightly packed within fertilization membrane. Cells are not organized into a sheet. Morula is not found in centrolecithal eggs of insects.

A true morula is a solid ball of cells, e.g., coelentrates. In most morula, cells are loosely arranged and have narrow crevices between their round ends. Morula in macrolecithal and telolecithal eggs is formed of a small flattened disc of cells found at the animal pole.

Blastula : A morula undergoes further cleavage, its blastomeres increases and undergo rearrangement. Blastomeres adhere with each other to form an epithelium, that may be single cell thick (coelentrates, echinoderms, *Amphioxus,* etc.), or many cells thick as in most vertebrates. This forming epithelium is called **blastoderm.** Due to rearrangement of blastomeres to form the blastoderm, a fluid-filled cavity or space appears in the center of blastoderm, called the **blastocoel.** Such hollow, spherical and single cell thick embryonic stage is called **blastula** and its process of the formation is called **blastulation.**

Types of Blastulae

1. Coeloblastula : It is a hollow sphere formed of a single layer of cells surrounding the blastocoel. It is found in echinoderms and *Amphioxus.*

2. Stereoblastula : In this blastocoel is not found and micromeres accumulate as a cluster of cells over vegetally placed macromeres. This is a solid blastula found in annelids, molluscs, neme teans and some planarians.

3. Periblastula or superficial blastula : In insects, superficially cleaving egg produce periblastula in which blastocoelic cavity is lacking.

4. Discoblastula : It is found at the animal pole over the yolk that is separated by a narrow subgerminal cavity. It is a small multilayered flat disc found in fishes, reptiles and birds whose eggs are large and yolky.

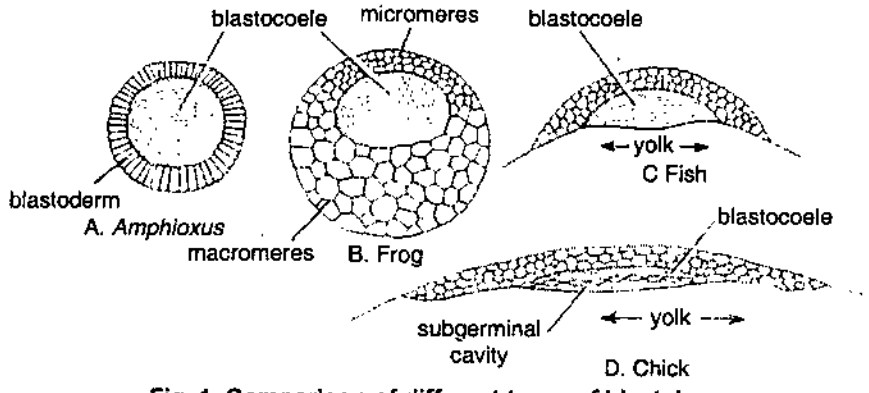


Fig. 1. Comparison of different types of blastulae.

5. Amphiblastula : Such blastula is formed of two types of cells, called micromeres and macromeres. In amphibians, blastula is formed of smaller micromeres and lower larger yolky macromeres. In *Sycon* anterior half of blastula is formed of flagellated cells, while posterior half is formed of large rounded granular cells.

6. Blastocyst : It is found in mammals. Cleavage is regular, a small cavity appears inside the dividing cells, called the **blastocoel** which gradually increases in size. Cluster of cells differentiate into two- an epithelium-like layer of **trophoblast or nutritive cells** surrounding the expanding cavity and an inner cell mass or **formative cells** of embryo that are displaced to one pole of the sphere. Inner cells mass spreads inside the cavity as a flat disc. This stage of development in mammals is called as **blastocyst.** At this stage embryo implants into the uterine wall. Trophoblast layer surrounding the blastocoele becomes the part of the placenta or extra-embryonic membranes.

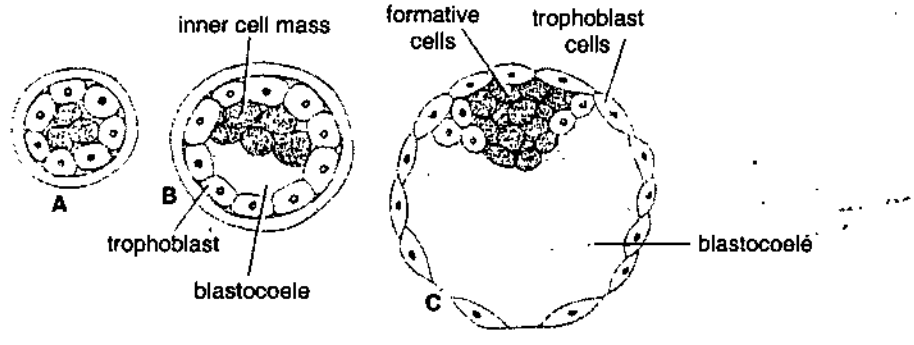


Fig. 2. Cleavage of mammalian egg forming a blastocyst having formative cells which form embryo proper and trophoblast cells.

• 6.2. FATE MAPS

In blastula cells are present on or close to the surface of the embryo and do not present in layers. Hence the area of future germ layers in blastula is called presumptive layer. Fate maps of blastula are constructed on the basis of path taken by the tagged or marked cells.

Preparation of Fate Maps :

Fate maps of different types of animal blastula have been prepared as given below :

1. Construction of Fate Maps by Natural Marking : Cytoplasm of the fertilized eggs of ascidians such as *Ciona, Styela* or *Cynthia partita* has natural colour in its various regions. In *Styela* egg cytoplasm, upper hemisphere is of light colour, postero-ventral crecent of yellow colour, antero-dorsal gray crescent, and dark gray yolky vegetative area. Due to natural differences in pigmentation, developmental fate of these regions has been prepared. Thus, upper clear protoplasm represents presumptive epidermal ectoderm, postero-ventral yellow crescent is presumptive mesoderm, dark gray yolky area is presumptive endoderm, and antero-dorsal gray crescent is presumptive neural ectoderm and notochord.

2. Construction of Fate maps by Artificial Markings : Wather Vogt in 1925 discovered artificial marking methods for constructing the fate maps of amphibians. These methods are as follows :

(i) Vital staining methods : Vital stains are retained by the cells for long time and they do not interfere with normal processes. For the construction of fate map of an amphibia, a small piece of **agar** or **cellophane** is stained with a vital stain like Nile blue sulphate, Neutral red, Janus green or Bismark brown. It is then pressed against a selected area of the blastula for a short period. Stain diffuses from the piece of agar into the blastomeres, which retain their distinctive colouration for several days. Stain usually does not diffuse into neighbouring cells. Hence, vital dye (stain) acts as an effective marker for the morphogenetic movement of such cell groups. Thus, by marking several areas simultaneously, movements of various areas may be observed during gastrulation. Persistence of the stains also makes the ultimate locations of the marked materials in later embryo. In this way one can construct a map of future organ regions in the blastula.

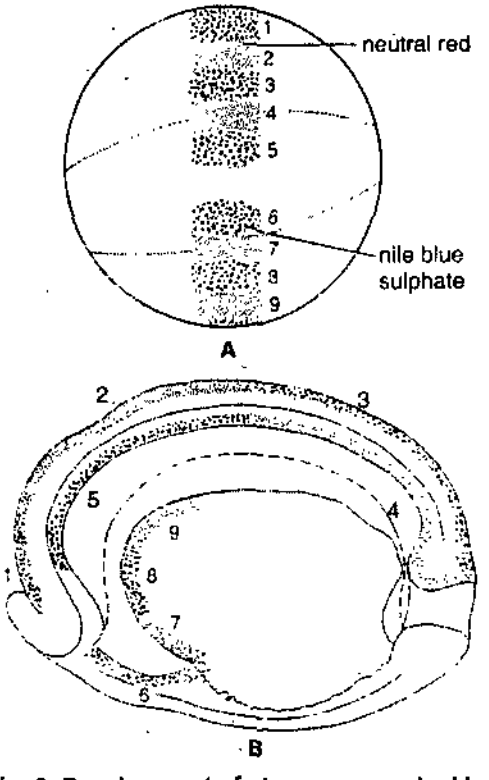


Fig. 3. Development of nine areas marked by vital stains during gastrulation of a urodele.

Agar and cellophane both are stain carriers. By vital staining methods, the future organ region maps (fate maps) have been prepared for a number of vertebrate species.

(ii) Carbon particles marking. Spratt (1946) devised another marking method for staining cells of a developing embryo. Tiny particles of carbon are applied to the surface of the embryo. Carbon particles stick to the surface of cells. Thus, one can follow the movements of cells and draw up fate maps. Recently carbon or chalk particles are injected inside a particular region of an embryo (blastula) and than their fate is followed in a subsequent dissections of the developing embryo. **Ballard**

(1981) adopted this technique in tracking the movement of cells within teleost blastoderm during gastrulation.

(iii) Radioactive labelling method : In this method the cells of an embryo are labelled by giving the embryo with a suitable radioactive metabolite. A part of this embryo (called donor) is then excised and quickly grafted over a normal unlabelled embryo (called host). The fate of the labelled primordium is easily found out by cutting sections of the host embryos at different time intervals and by autoradiography. **Rosenquist** labelled epiblast cells of chick with radioactive thymidine, tritiated thymidine, and grafted them to unlabelled blastoderms. Thus, he proved that some ectoderm as well as mesoderm originate from epiblast, invaginating at the primitive streak.

Tritiated thymidine gives cell to cell labelling where DNA synthesis occurs, without regard of tissue stage of organism. It labels an dividing cell in any species and the descendants of that cell. But it works only for dividing cells and if these cells are dividing rapidly, it soon be diluted to undetectable levels.

• 6.3. EXAMPLES OF FATE MAPS

1. Fate Map of Protochordate Blastula :

In *Amphioxus* blastula, the blastoderm forms a single layer around the blastocoele. Cells from the vegetal pole of the egg will become the floor of blastocoel or hypoblast and or future **endoderm** of embryo. Cells from the region of animal pole will form the epiblast and will give rise to eczoderm (epidermal and neural ectoderm) and chodamesoderm (notochordal and mesodermal) cells.

Most of the cells of epiblast give rise to epidermal ectoderm, and some small epiblast cells located at the margins of the prospective endoderm layer form a ring around the blastula, this ring of cells consists of two areas, **dorsal crescent** and **ventral crescent**. Cells of dorsal crescent give rise to presumptive notochord and neural plate cells. Ventral crescent contains the cells that form the presumptive mesoderm.

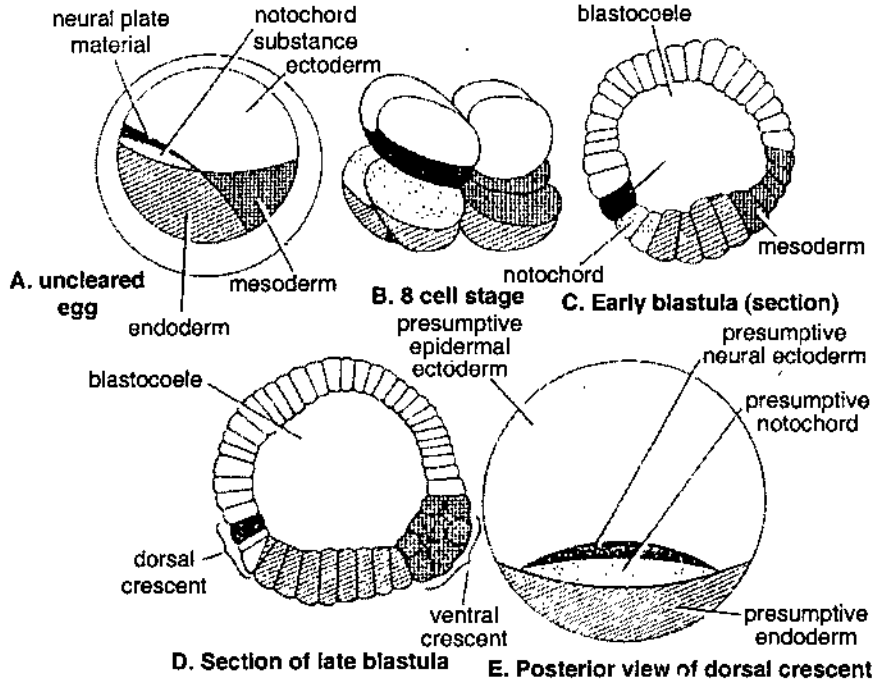


Fig. 4. Distribution of presumptive organ forming areas during cleavage and blastulation in *Amphioxus*.

2. Fate Map of Amphibian Blastula :

Amphbian blastula is a rounded structure and it can be divided into three regions :

1. Vegetal region : It includes hypoblast comprised of yolk-filled, non-pigmented **macromeres.** It represents **presumptive endoderm**, which contains materials for the formation of midgut and hindgut of embryo.

2. Animal region : It represents epiblast comprises of micromeres. Amphibian epiblast unlike *Amphioxus* is several cells thick. In frog, darkly pigmented micromeres, deriven from the animal pole of the egg give rise to future ectoderm of the animal. It includes two main regions : (1) Region of prospective epidermal ectoderm that develops into epidermis of the skin and (2) region of prospective central nervous system which contains material for brain, spinal cord and sub-areas for sense organs like eyes, ears and nose.

3. Intermediate or marginal region : This is grey-crescent area that in blastula represents a ring of cells around the equator. Most of these cells are presumptive mesodermal cells. It contains the following subregions : (i) Notochordal region present on the dorsal side of blastula and gives rise to notochord, (2) Area for anterior part of alimentary canal situated below the notochordal area and give rise to endodermal lining of mouth, gill region and pharynx. (3) Region of somites or segmental muscles present on both sides of notochordal area and develops into trunk muscles or somites. (4) Ventro-lateral mesodermal area lying on lateral and ventral parts of marginal zone and gives rise to mesodermal lining of body cavity, kidney and reproductive organs.

3. Fate Map of Avian Blastula :

Area pellucida consisting of yolk-less central cells forms the embryo proper. It is part of the chick blastoderm that is included in the fate map. Area pellucida consists of two layers of cells : upper layer **epiblast** and lower layer of cells below the cleft is **hypoblast**. Blastocoele is a slit-like cavity below the hypoblast. Blastula is flattened rests on a bed of undivided yolk. Cells of epiblast are organized around the notochord. Cells in the anterior part of area

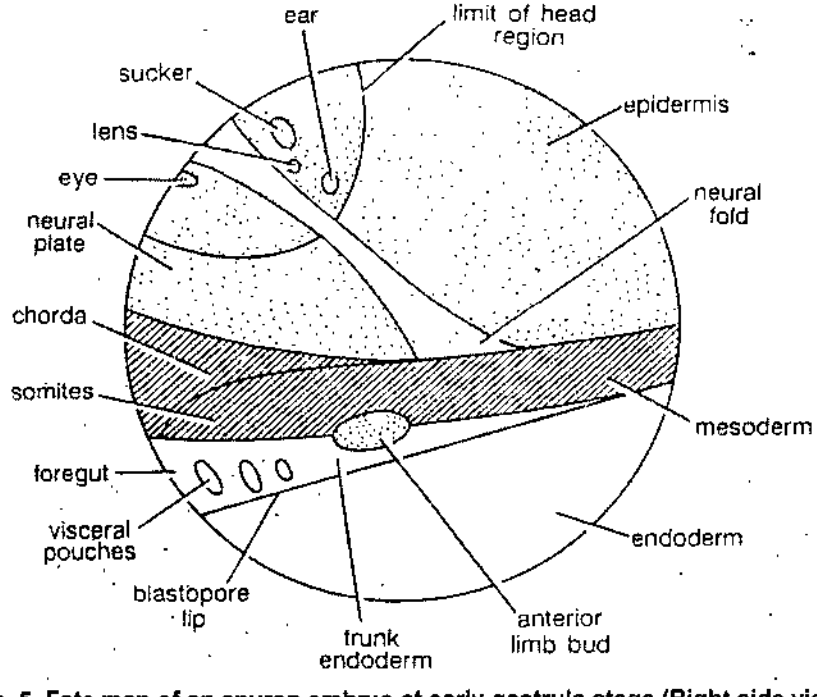


Fig. 5. Fate map of an anuran embryo at early gastrula stage (Right side view).

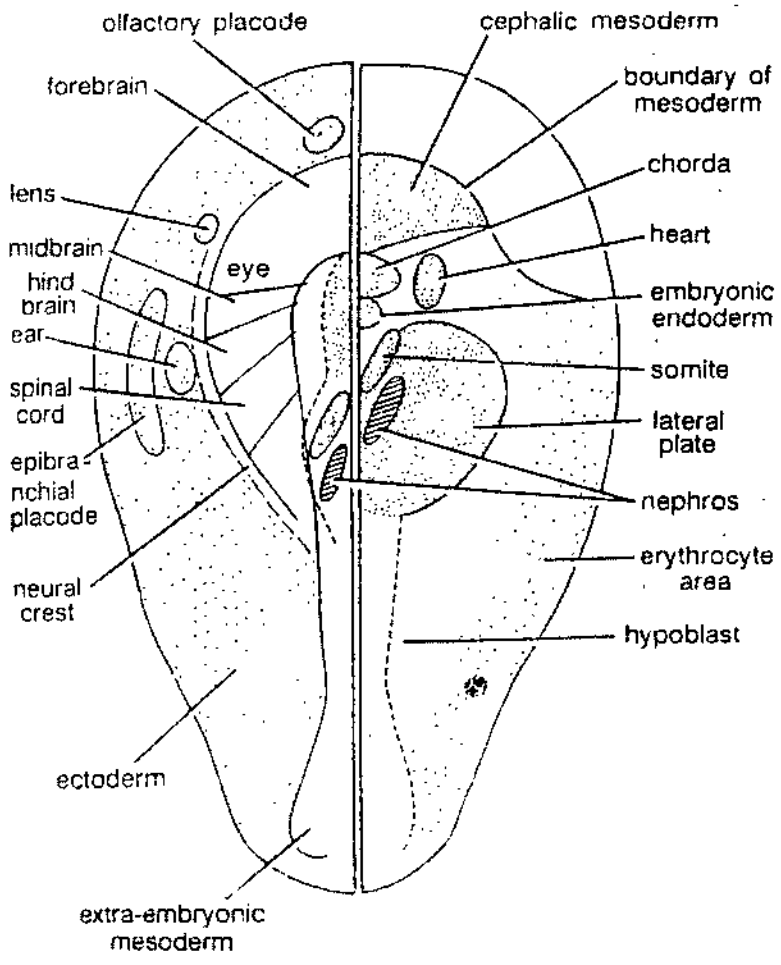


Fig. 6. Fate map of chick embryo at definitive primitive streak stage.

pellucida form the ectoderm (future epidermal ectoderm and future neural plate ectoderm) and embryonic membranes. Posterior half of area pellucida forms the mesoderm of the body proper and extra-embryonic mesoderm of the membranes. In the middle of area pellucida, presumptive neural plate cells take the form of a transverse crescent-shaped mass. Posterior to presumptive neural plate cell, near the middle of area pellucida, lies presumptive notochordal material. Cells of hypoblast area forms the endoderm. Some of the endoderm cells are incorporated into the wall of the gut but most of them form the lining of yolk sac.

• 6.4. CELL LINEAGE

The study of fate of each blastomere after first and second cleavage is called **cell lineage study** (cytogeny). Its study is easier in spirally cleaving mosaic eggs of flatworms, annelids and molluscs in comparison to radially cleaving regulative (in determinate) eggs. This is due to determinate cleavage found in spirally cleaving eggs.

Method of Study of Cell Lineage :

For the study of cell lineage, one has to trace the fate of particular blastomeres. Or find out which part of the embryo or larva is formed by which cell of the blastula. Thus, in the study of cell lineage identity, the early blastomeres and their progeny by giving them certain names. These names are given by letters of English alphabet and by numbers above the letters which indicates the power.

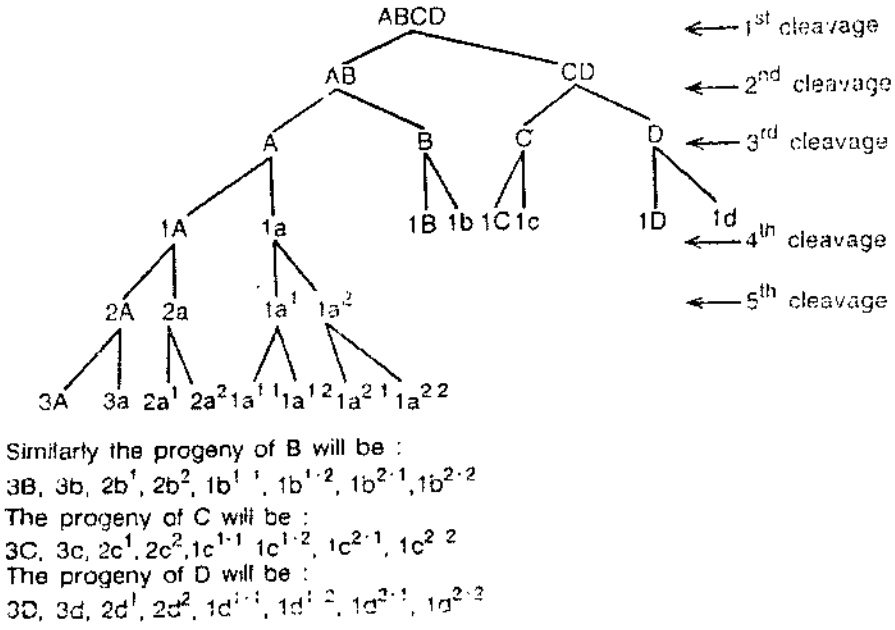


Fig. 7. Method of nomenclature of blastomeres in cell lineage studies.

Examples of Cell Lineage :

Study of cell lineage in annelid, *Nereis* by **E.B. Wilson** (1892). In *Nereis*, first four cleavages occur at the same time. First cleavage is unequal forming a small AB blastomere and a large CD. Second cleavage (after 60 minutes) produces two equal sized A, B blastomeres, while larger CD cell divides into a smaller C and a larger D cell. Cleavage is spiral, alternating dexiotropic to leiotropic (right to left) with each successive division. At third cleavage, first quarter is designated 1a – 1d, marking the micromere derivative of each A – D micromeres. With each succeeding macromere division, second through fourth micromeres quarters are formed, identified as 2a–2d through 4a – 4d respectively. Third cleavage division segregates yolk and oil droplets into macromeres, which remain there until the end of cleavage.

At fifth cleavage, divisions become a synchronous (occur at the different times) and 32. cell stage is reached in steps as cell groups divide at different times. First micromere quartet at the animal pole gives rise to the most apical structures in the larva. It divides into **4 central cells** at the animal pole, e.g. 1a′–1d′, and four **trochoblast cells.**

These trochoblast cells later (after 2 more divisions) develop into a band of powerful cilia encircling the larva, called as **prototroch.** Apical tuft of larva formed of large cilia extending from the former animal pole, is derived from four central cells 1a′–1d′. These divide unequally into four small cells at the animal pole, designated 1a″–1d″ and four larger cells $1a^{12}$ – $1d^{12}$. $1a^{11}$ –$1d^{11}$ differentiate into apical tuft cells with long cilia, while $1a^{12}$ – $1d^{12}$ contribute to larval epidermis. In this way each cell can be followed into the larval organs like gut, eye spots, ciliary bands and coelomic sacs.

Cleavage patterns and cell lineages in flat worms, annelids and molluscs are similar. Annelids and molluscs, both develop a **trochophore larva** having a common body plan. In each larval **ectoderm** develops from the first three micromere quartets, while remaining quartets give rise to **mesodermal** derivatives. **Endoderm** develops from the **macromeres.** Ciliated **trochophore bands** in these larvae come from $1a^2$ –$1d^2$ cells as described earlier. 2d cell of second quartet and 4d cell of fourth quartet (both derivative of

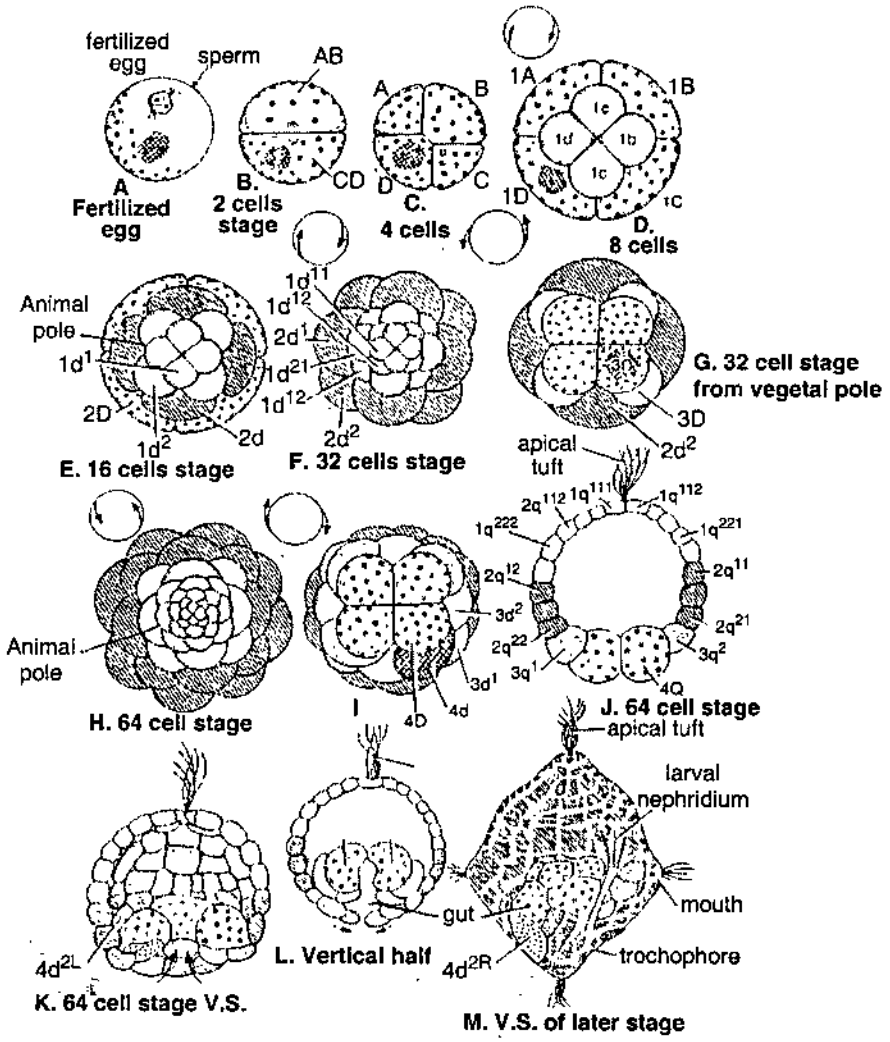


Fig. 8. Cell lineage pattern and spiral cleavage in *Nereis*.

D macromere) are called first and second **somatoblasts** respectively. Both these cells give rise to mosodermal derivatives such as heart, **muscle bands, ventral nerve cord** and other main tissues. The development of each phylum diverges from this common plan later on, as unique structural adaptations appear, like **shell glands** in mollusc larvae and **metameric** body plan in annelids. Studies of cell lineages also show that each blastomere carries out a prescribed number of divisions before differentiation into a larval structure. Position and division programmes seem to be set at the beginning of development i.e., at the first two cleavages. If any of the four macromeres is separated after second cleavage, each divides exactly as it would have in the intact embryo (both in number of divisions and position of progeny).

• SUMMARY

Isolecithal eggs due to cleavage develop into numerous equal sized cells forming a tight cluster of cells called **morula.** These cells are tightly packed within fertilization membrane.

Morula is not found in controlecithal eggs.

Morula in macrolecithal and telolecithal eggs is formed of small flattened disc of cells at the animal pole.

Blastula : Blastomeres of morula undergo further cleavage, undergo rearrangement, and adhere with each other to form an epithelium, called

blastoderm. A fluid-filled cavity called blastocoele also appears. Such hollow, spherical embryonic stage is called **blastula** and process of its formation is, called **blastulation.**

Blastula are of the following type :

Coeloblastula of echinoderms and *Amphioxus.*

Stereobastula of annelids, molluscs, and some planarians.

Periblastula of insects.

Discoblastula is found in fishes, reptiles and birds.

Amphiblastula is found in amphibians.

Blastocyst is found in mammals and a cavity appears inside the dividing cells, called blastocoele. Cluster of cells differentiates into two **trophoblast** and **inner cell mass.**

Fate maps : Fate maps are prepared in blastulae stage. Fate maps are constructed on the basis of path taken by the marked cells.

Fate maps of different types of animal blastulae have been prepared by natural marking, by the use of vital stains, carbon particles, Radioactive labelling, etc.

Natural marking method is used in ascidians (*Ciona, Styela* etc.). In these animals blastula has various colours in its various regions. In *Styela* egg cytoplasm, upper hemisphere is of light colour, posteroventral crescent is of yellow colour, antero-dorsal crescent and dark gray yolky vegetative area. Due to natural differences in pigmentation, developmental fate of these regions has been prepared. **Construction of fate maps by artificial markings :**

Use of vital stains (dyes). These are retained by the cells for long time and do not interfere with normal processes. For the construction of fate map of an amphibia, small piece of agar or cellophane stained with vital stains like Nile blue sulphate, Neutral red, Janus green or Bismark brown are used. These stains diffuse from the piece of agar into the blastimeres.

Agar and cellophane are stain carriers.

Carbon particles are applied to the surface of the embryo which stick to the surface of cells.

Radioactive thymidine, tritiated thymidine is used for labelling the cells of blastoderm.

Cell Lineage : Study of fate of each blastomere after first and second cleavage is called cell lineage study or cytogeny.

For the study of cell lineage, one has to trace the fate of particular blastomeres.

For the study of cell lineage, we find out which part of the embryo or larva is formed by which cell of the blastula.

• QUESTIONS

Long Answer Type Questions

1. What is the fate map ? Describe different methods of fate map construction.
2. Describe Fate map of amphibian embryo.
3. What is Fate map ? Describe the Fate map of amphibian embryo.
4. What is Fate map ? Describe the Fate map of bird in detail.

5. Define the term blastulation. Describe the process, types and importance of blastula in chordates.

Short Answer Type Questions

1. Describe blastulation in brief.
2. Write down about construction of fate map by vital stains.
3. Draw a well labelled diagram of fate map of chick embryo at definitive primitive streak stage.
4. Write about cell lineage.

Very Short Answer Type Questions

1. **Why Neutral Red and Bismark brown are used in embryological studies ?**

   Ans. Small piece of Agar or Cellophane is stained with each of these separately and then each stained piece is pressed aginast a chosen area of the blastula for a short period. Each stain diffuses from the piece of agar or cellophane into the blastomeres, which retain their distinctive colouration for a number of days. Only those cells receiving the stain retain the dye, and do not diffuse into neighbouring cells. These stain serves as an effective marker for the morphogenetic behaviour of small cell groups.

2. **For what purpose vital dye is used ?**

   Ans. Vital dye is used to prepare the fate maps of blastomeres of blastula or morphogenetic movements of blastomeres during gastrulation.

3. **Name fluid-filled cavity of the blastula.**

   Ans. Fluid filled cavity of blastula is called blastocoele.

4. **Write about blastula.**

   Ans. At the end of cleavage, embryo consists of a hollow of cells in holoblastic types and a layer of cells over the yolk in meroblastic types. This developmental stage is called blastula.

5. **Why agar or cellophane is used in vital staining method of preparing fate maps of any animal blastula or for viewing the morphogenetic movements of cells of blastula during gastrulation.**

   Ans. Agar or cellophane abosorb the vital stains like Nile blue sulphate, neutral red, Janus green, etc., and when such piece are kept over the blastomeres of blastula, they diffuse into the particular cells which are migrating during gastrulation. Thus, their future movement can be seen.

6. **Who discovered the artificial marking method of construction of fate maps ?**

   Ans. Wather Vogt (1925) discovered artificial marking methods for construction of fate maps of amphibians.

7. **Write about the discovery of Spratt.**

   Ans. Spartt in 1946 devised staining of cells of developing embryo by tiny carbon particles which stick to the surface of cells.

8. **Define cell lineage.**

   Ans. Study of fate of each blastomere after first and second cleavage is called cell lineage. This is easier to study those types of eggs which show determinate cleavage.

UNIT

7

GASTRULATION

STRUCTURE

- Gastrulation-Definition
- Prominent features of gastrulation.
- Morphogenetic movements during gastrulation.
- Chemical changes during gastrulation.
- Nuclear activation during gastrulation.
- Significance of gastrulation.
  - Summary
  - Test Yourself

LEARNING OBJECTIVES

After going through this unit you will learn :

- Study of gastrulation.
- Study of major events occurring during gastrulation.
- Study of morphogenetic movements in gastrulation*-Epiboly, Emboly-invagination, involution, ingression or polyinvagination, delamination, etc.
- A few examples of gastrulation.
- Physiology of invagination.
- Chemical changes during gastrulation.
- Significance of gastrulation.

• 7.1. GASTRULATION

During gastrulation a simple one-layered blastula is converted into a two-layered (**didermic,** e.g., *Amphioxus*) or a three-layered (**tridermic**) embryo, called **gastrula**. Single layer of blastula is called **blastoderm** (**ectoblast** or **protoderm**) and three layers of gastrula are called **ectoderm** (outer), **endoderm** (inner) and in between these two is the **mesoderm**. These are commonly known as germ layers. During gastrulation, blastocoele of the blastula is obliterated and a new cavity, the **archenteron** or **gastrocoel** is formed that is surrounded by endoderm layer. Gastrula also acquires its antero-posterior polarity and bilateral symmetry. Gastrulation is the embryo's way of laying down its body plan (**Trinquas,** 1984).

• 7.2. PROMINENT FEATURES OF GASTRULATION

During gastrulation, blastomeres of blastula are rearranged by the process of morphogenetic movements.

Cellular division is slowed down or inhibited. Growth of gastrula does not take place. Metabolism changes and rate of oxidation increases. Synthesis of new and different kinds of proteins takes place.

• 7.3. MORPHOGENETIC MOVEMENTS DURING GASTRULATION

Gastrulation is an integrated, dynamic process controlled largely by intrinsic forces bound up in the various presumptive organ forming areas of the

blastula and early gastrula. During gastrulation different kinds of morphogenetic movements take place. Movement of blastomeres from one place of the embryo (blastula) to another place to establish a particular structure is called **morphogenetic** or **formative movement.** Morphogenetic movements take place in the embryo during gastrulation, tubulation and organogenesis. Each part brought by morphogenetic movement remains in the same position, into which it has been brought earlier. Two types of morphogenetic movements are involved in gastrulation :

1. Epiboly
2. Emboly

1. Epiboly :

Epiboly means "throwing on" or "extending upon". Epibolic layer is usually outside (ectodermal) and envelopes and surrounds inner layers. Epiboly is characterized by a rapid proliferation of cells in the animal half and they spread over the cells of vegetal half. It involves the growth of blastoporal lip over the yolk or yolk-filled vegetal cells. This movement takes place due to active cell division in the overgrowing layers. This cell division is most intense in the region of blastoporal lip. Epiboly of ectodermal blastomeres has been observed in the development of eggs possessing abundant yolk, e.g. amphibian eggs. In rounded blastulae of amphibians, future ectodermal cells spread over the inwardly moving presumptive notochordal, mesodermal and endodermal blastomeres. In flasttened blastulae of teleost fishes, reptiles and birds, epibolic movements take place in the form of antero-posterior extension, along with migration and expansion of ectodermal blastomeres towards the periphery.

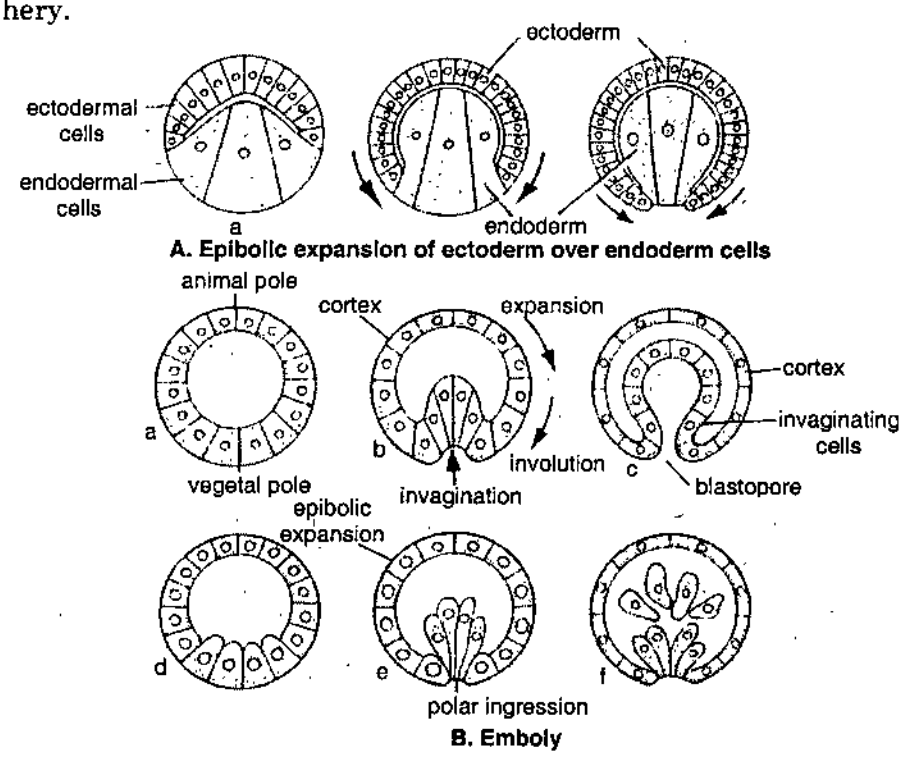


Fig. 1. Morphogenetic movements during gastrulation.

2. Emboly :

This term is also derived from Greek meaning 'throw in' or 'thrust in'. It involves growth of the endoderm forming regions beneath the ectoderm forming region. In emboly, chorda-mesodermal and endodermal blastomeres

move from the external surface of the blastula to the interior of the developing embryo. Embolic movements include invagination, involution, infiltration, ingression, concrescence, cell proliferation, divergence and extension or elongation.

1. Invagination : Infolding or inward bending of endoderm and mesoderm is called invagination. In invagination, vegetal half of blastula is pushed in or invaginates and extends beneath the animal half or ectoderm. Thus, spherical blastula is converted into a double-walled cup. The original blastocoele is obliterated and a new cavity develops that is called **archenteron** or **primary gut**. The opening of archenteron to the exterior is called the **blastopore**. Archenteron is lined by the vegetal cells which form the endoderm and mesoderm. The outer layer of the cells forms the ectoderm.

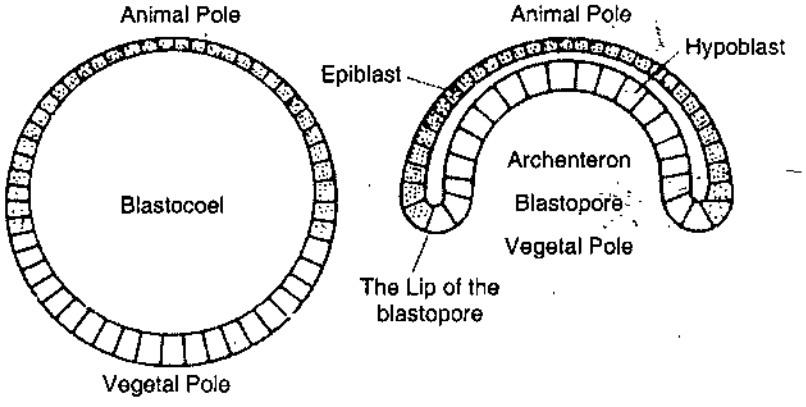


Fig. 2. Gastrulation by invagination (Diagrammatic).

2. Involution : Involution means "turning in" or "rolling under" of chorda-mesodermal cells through the blastoporal lip. It is often accompanied with invagination. Thus, cells which are at some distance from the site of invagination, move to the site of invagination, i.e., at the lip of blastopore (amphibia), and then migrate over the blastoporal lip and then move inside beneath the forming ectoderm.

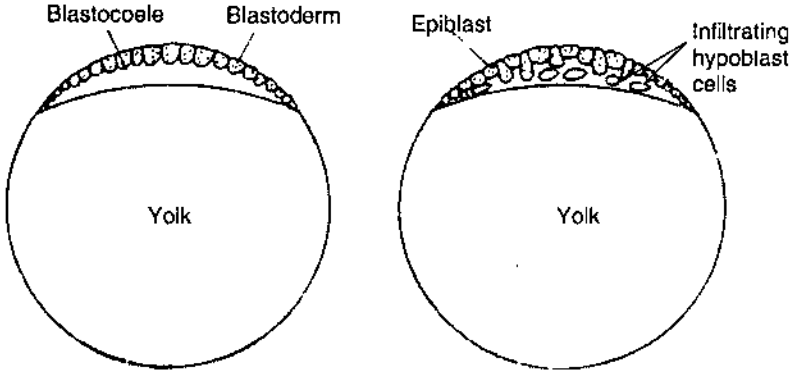


Fig. 3. Gastrulation by involution.

3. Infiltration : It involves infiltration of cells from the blastoderm into the blastocoele lying beneath. These infiltrated cells soon spread out to form a continuous layer, called **hypoblast.** The blastocoele now becomes the **archenteron.**

4. Ingression or polyinvagination : In this process, small groups of independent cells separate from the upper (epithelial) layer and migrate into the blastocoele and then unite with each other to form a layer of cells as found in the development of chick.

Delamination is a type of ingression, in which mass separation of a layer of cells froms an epithelium (outer layer of cells), which later unites to form an inside sheet.

5. Concrescence : Gradual drawing together of the blastoporal lips during epiboly resulting into a diminished blastopore in size, e.g., gastrulation in telolecithal eggs.

6. Cell proliferation : In the process of gastrulation, cell proliferation is essential, since without cell prolifaration gastrulation is not possible.

7. Divergence : It is opposite of convergence. After involution of cells over the blastoporal lips during gastrulation, they migrate and diverge to their future positions within developing embryo. This occurs in lateral plate and ventral mesoderm in frog's development and in lateral plate and extra-embryonic mesoderm in birds and mammals.

8. Extension : During gastrulation, gastrula begins to elongate in anteo-posterior axis. The elongation of presumptive neural and epidermal areas externally and notochordal, mesodermal and endodermal materials after they have migrated inward beneath the neural plate and epidermal material are examples of extension. It is essential for the development of the embryo.

Process of Emboly in Amphiblan (Frog) Development :

In frog's development, regions of blastula are clearly distinguished by pigment differences. In frog's blastula, black pigmented animal region of micromeres is the presumptive ectoderm, unpigmented vegetal region of macromeres is the presumptive endoderm and a roughly equatorial band between them is grey (grey crescent of fertilized egg) the presumptive mesoderm. During epiboly, epidermal sheet spread ventrally and in involution, a layer of cells rolls in at the dorsal lip.

In the fate map of frog, presumptive mesoderm is inside the embryo as an inner layer beneath the ectoderm. The future notochordal region is in the outer layer in the marginal region. This along with endoderm is displaced into the interior by gastrulation. Various events involved in gastrulation of frog take place in the following order :

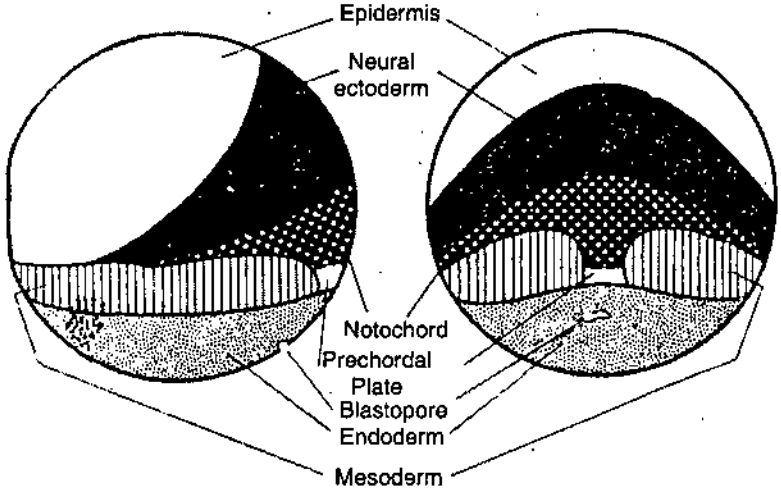


Fig. 4. Fore map of frog at the begning of gastrulation.

1. In late blastula, cortical tension in the vegetal pole increases and it contracts, more on future dorsal side, i.e., in the region of grey crescent. During epibolic expansion, smaller pigmented animal half cells or micromeres in the

marginal zone are present well below the equator. Cells in the dorsal marginal zone are smaller than ventral marginal cells, it reduces cortical tension in this region.

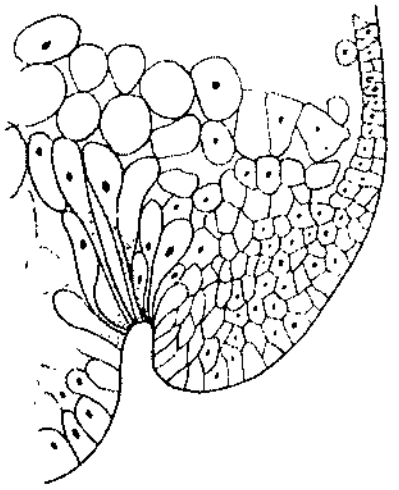


Fig. 5. Invagination of bottle cells (Median section through blastopore region of an early gastrula)

2. First visible sign of gastrulation is a sinking in (invagination) of a few presumptive endodermal cells at the original grey crescent or dorsal side of the embryo. These cells become motile and less adhesive, stretching into elongate bottle shapes, wider at inner surfaces than at the periphery. The distal tip of bottle cells remains attached to the outer surfaces by microvilli. Ultimately these cells sink into the interior and a groove called the dorsal lip of blastopore appears. Adjacent endodermal and chorda-mesodermal cells attached to bottle cells by tight junctions, are drawn in the interior. As a result, the depression lengthens into a crescent-shaped (horse-shoe-shaped) groove.

3. The entire animal half of blastula undergoes epibolic expansion. Dorsal lip enlarges and becomes crescent-shaped because animal half cells stretch, converge towards the blastopore and involute over the lip, spreading beneath the superficial layer.

Epiboly is most clear in dorsal region but gradually extends laterally and ventrally as dorsal lip enlarges. More cells invaginate at a ventral blastopore lip and the expanding blastopore becomes a circular opening. Blastopore is slowly reduced to yolk plug stage, while epibolic movements sweep superficial layers of cells into the interior. Inside, invading chorda-mesoderm pushes and pulls yolky endoderm forward to line the cavity of archenteron. Roof of archenteron comes from endoderm pushed in by chorda-mesoderm plus any superficial ectoderm that has invaginated over the dorsal lip. Floor of the archenteron consists of yolk-laden cells pulled forward from the blastocoele floor. Thus, ectoderm has a greater spreading tendency than endoderm.

4. Mesodermal sheet has a complicated origin. Presumptive mesoderm in frog's gastrula occupies an inner position just beneath the most superficial ectoderm layer. Thus, all mesoderm is derived from internal cells that do not invaginate. The combined endo-mesodermal sheet advances as a unit under the ectoderm, and the mesodermal sheet separates probably by delamination at the end of gastrulation.

Process of Emboly in Chick :

Primitive streak is a short, broad and longitudinal band, that develops in the epiblast after about eight hours of incubation in chick. Primitive streak initiates the embolic movements of gastrulation. Primitive strak indicates the future antero-posterior axis of the embryo. Formation of primitive streak is due to migration of epiblast cells from the sides to the central area of the area pellucida. Primitive streak elongates and occupies the posterior half of the area pellucida. Area pellucida soon begins to change its shape— first to an oval shape and then to pear shape. The fully formed streak (definitive primitive streak) has a characteristic morphology : Its anterior end forms **Hensen's node** in which is present **primitive pit**. Primitive streak length is formed by primitive groove bordered by primitive ridges. This longitudinal groove appears in the middle of streak as epiblast cells become flask-shaped and

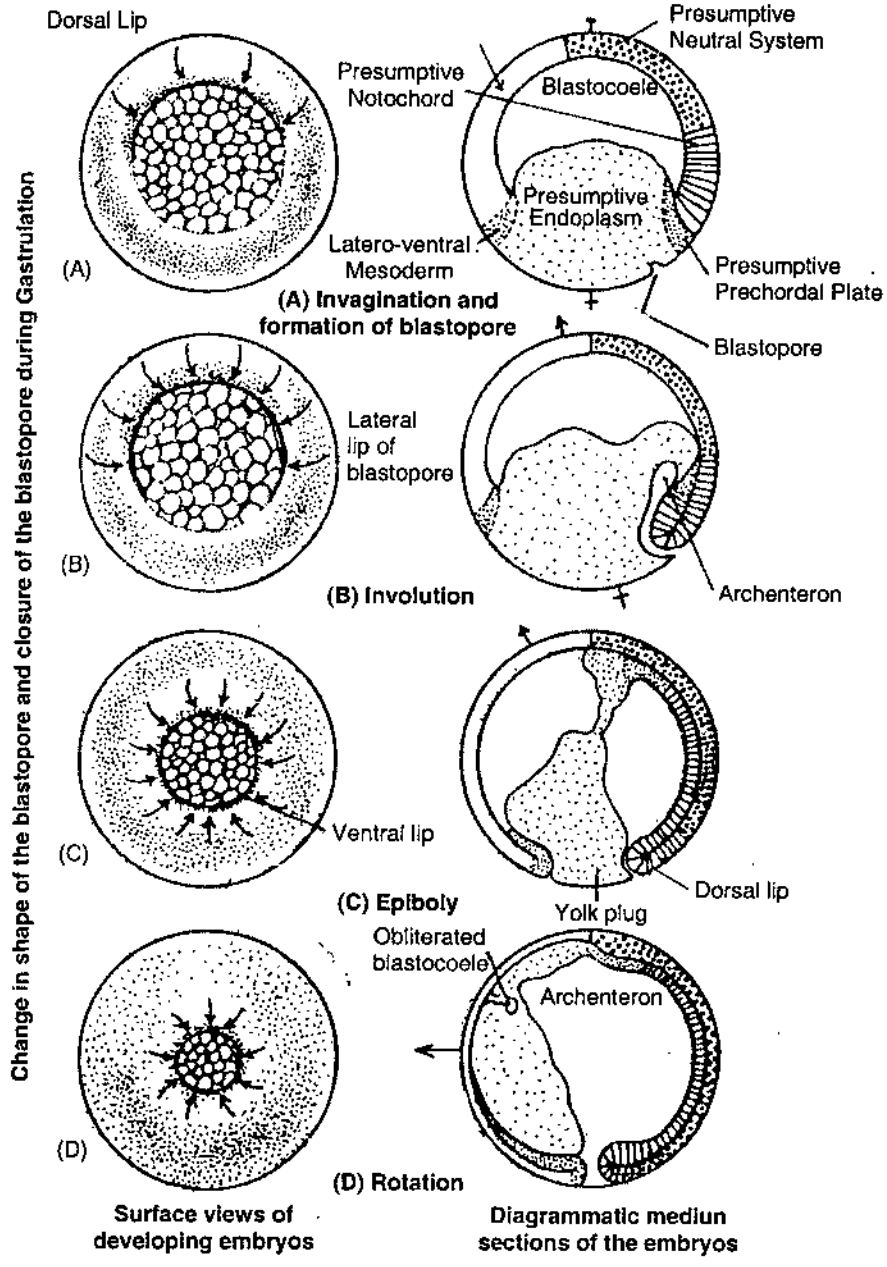


Fig. 6. Germ layers formation in frog (Gastrulation).

invaginate into the blastocoele. Basal ends of flask-shaped cells are highly motile, while their apical ends remain fixed in the surface by tight junctions. They pull neighbouring cells into the groove so that they migrate as a cohesive sheet.

Formation of streak marks the end of gastrulation. Two major types of cell movements take place during chick gastrulation :

1. Immigration of endoderm, mesoderm and notochordal cells from the epiblast, which then becomes the ectoderm.

2. Expansion of ectoderm, endoderm and mesoderm.

The first cells that move into the interior through anterior part of the streak form the endoderm, head mesoderm including prechordal plate and notochord.

Presumptive notochord cells located in the Hensen's node move forward beneath the epiblast as a **notochordal** or **head process.** Presumptive body mesoderm cells also move inside through anterior half of the streak. These then

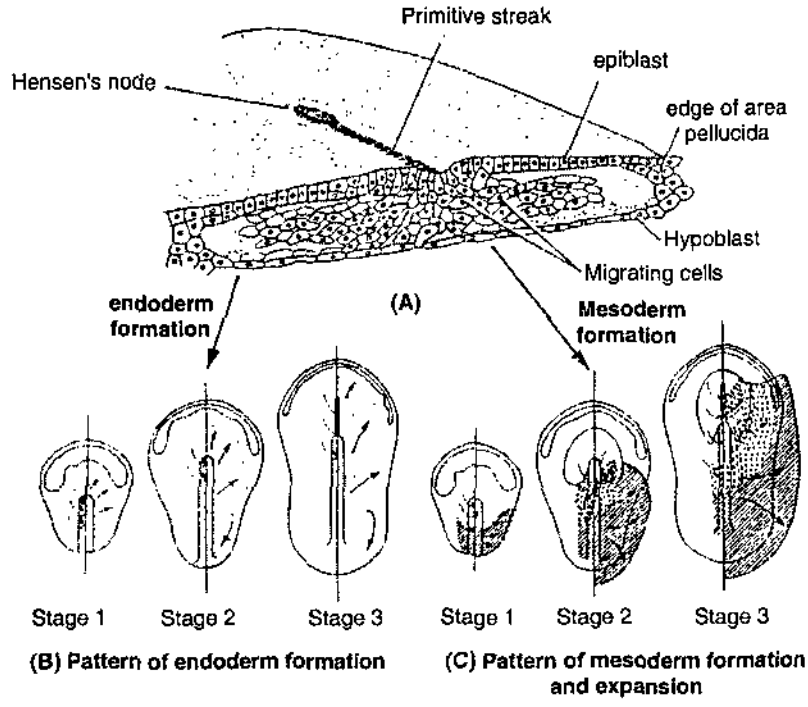


Fig. 7. Gastrulation in chick. A-Hemisected view of primitive streak showing invaginating and ingressing cells from epiblast entering segmentation cavity and spreading out to form endoderm and mesoderm.

migrate forward and come to lie lateral to the notochordal process. Later on this mesoderm forms the somites, nephrogenic tissue and lateral plate.

Cells that move through posterior half of the streak contribute only to extra-embryonic mesoderm formation.

During migration, epiblast cells abondon their epithelial character after entering the primitive groove. These cells form a loosely connected network and are in touch with the cells of epiblast, endoderm and mesoderm at the same time. From the groove these cells move singly, but in a synchronized and connected manner (ingression).

Regression of the primitive streak occurs as the embryonic area that is located in front of Hensen's node increases. It involves the shortening of streak and posterior shifting of Hensen's node. As the node shifts backwards and *notochordal process elongates the presumptive endoderm of middle and* posterior part of the gut, located just behind Hensen's node moves inside as an endodermal strip beneath the notochord. Finally Hensen's node and remnant anterior part of primitive streak are included into the **tail bud** of the embryo.

After streak disappears, continuity between three germ layers is also lost and they become independent expanding sheets. Original hypoblast at the floor of blastocoele forms the floor of archenteron,

Epiblast cells that invaginate through the streak form the roof and walls of developing archenteron.

Unlike *Amphioxus* and frog, notochord or mesoderm never lines the archenteron, because gut cavity develops between yolk and endoderm by lifting up of yolk and endoderm from the yolk.

• 7.4. CHEMICAL CHANGES DURING GASTRULATION

Gastrulation is the most active phase of embryonic development. During gastrulation morphogenetic activities and rate of metabolism increases.

During gastrulation, due to morphogenetic activities, expenditure of ATP molecules increases, resulting an increase in oxidation. During gastrulation glycogen and yolk (food reserves) are oxidized for the manufacture of ATP molecules.

During gastrulation, DNA-dependent RNA synthesis (transcription), small amount of DNA replication and biosynthesis of certain proteins also take place.

In frog gastrula, rate of DNA synthesis decreases. It is due to exhaustion of nucleotide reserves at the end of blastula stage. All phases of cell cycle lengthen, DNA synthesis is very much reduced. During gastrulation transcription of DNA and embryonic genome becomes active.

Nucleoli first make their appearance during late blastula or early gastrula stage, thus marking the onset of stable ribosomal RNA accumulation.

Genes remain dormant throughout the period of cleavage, but during gastrulation they become evident and control the process of development from this stage onward. Before gastrulation effect of genes is indirect, *i.e.*, their action is through mRNA molecules. If a nucleus containing maternal and paternal genes is removed from the egg, non-nucleated egg continues to divide upto morula stage and does not reach blastula or gastrula stage. Thus, nucleus is necessary for development beyond cleavage stage.

• 7.5. SIGNIFICANCE OF GASTRULATION

Gastrulation brings the presumptive structures from the external surface of the blastula into their normal positions in the gastrula. Gastrulation is necessary for the formation of nervous system.

• 7.6. SUMMARY

During gastrulation, a simpl one-layered blastula is converted into a two layered or a three layered gastrula or embryo. Three layers of gastrula are outer ectoderm, inner endoderm and in between these two is the mesoderm.

During gastrulation, blastocoele is obliterated and archenteron or gastrocoel is formed surrounded by endoderm.

During gastrulation, blastomeres get rearranged by the process of morphogenetic movements. Cellular divisions are slowed or inhibited. During gastrulation, growth does not occur, rate of oxidation is increased resulting into production of ATP molecules. Synthesis of new types of proteins takes place.

Morphogenetic or formative movement is movement of blastomeres from one place of the embryo to another place to form a particular structure.

Morphogenetic movements are of two types : epiboly and emboly.

Epiboly : Rapid divisions of cells in the animal half which spread over the vegetal half, forming blastoporal lips over the yolk-filled vegetal cells. Thus, an ectodermal layer is formed over the inwardly moving presumptive mesodermal and endodermal cells.

Emboly : In emboly, chorda-mesodermal and endodermal blastomeres move from the external surface of the blastula to the interior of the developing embryo.

Embolic movements are invagination, involution, infiltration, ingression or poly- invagination, concrescence, cell proliferation, divergence and extension or elongation.

In invagination, endoderm and mesoderm cells invaginate or turn inside and extend beneath the outer ectoderm. A new cavity develops obliterating the blstocoele, that is archenteron opening to the exterior by blastopore.

In involution, chorda-mesodermal cells involute through blastoporal lip.

In infiltration, cells from the blastoderm infiltrate into the blastocoele, forming a continuous layer called the hypoblast (endoderm).

In ingression, small groups of cells separate from the upper layer and migrate into the blastocoele, which then unite to form a complete sheet, e.g., chick.

Concrescence. In this process blastoporal lips gradually come closer to each other and fuse, resulting into a small blastopore.

Cell proliferation is the division of cells which is essential for the formation of embryo.

Divergence. It is opposite to convergence. Cells involute though blastoporal lips and diverge to their future positions in the gastrula.

Extension or elongation is the elongation of embryo in antero-postreior axis along with presumptive structures.

During gastrulation, morphogenetic activities and rate of metabolism *increases. Oxidation of glycogen and yolk is increased* producing more ATP molecules

Rate of DNA synthesis decreases during gastrulation.

• TEST YOURSELF

Long Answer Type Questions

1. Define the term gastrulation. *Describe briefly different types of morphogenetic* movements taking place during gastrulation.
2. What are the morphogenetic movements that occur during gastrulation? Describe them in brief.

Short Answer Type Questions

1. What is epiboly ? Discuss the type of cell movements in epiboly.
2. What is emboly : How does it differ from epiboly ?
3. Differentiate between convergence and concrescence.
4. What is the significance of gastrulation?

Multiple Choice Questions

1. The cells located along the external margin of the blastoporal lip move over the lip to inside edge of the lip. This is called :
   (a) invagination (b) involution (c) convergence (d) concrescence
   Ans. (b)
2. The process by which group of cells in the external layer of blastula migrate into the segmentation cavity is called as :
   (a) invagination (b) involution (c) polyinvagination (d) delamination
   Ans. (c)
3. Epibolic morphogenetic movements occur only in the presumptive :
   (a) mesodermal blastomeres (b) endodermal blastomeres
   (c) ectodermal blastomeres (d) all these
   Ans. (c)

4. The embolic morphogenetic movements are concerned with inward migration of presumptive :
   (a) endodermal cells (b) chorda-mesodermal cells
   (c) ectodermal cells (d) (a) and (b)
   Ans. (d)

Fill in the blanks with suitable words

1. ..... Morphogenetic movements occur in the presumptive ectodermal blastomeres.
   Ans. Epibolic
2. The actual migration of cells from the outer surface of the blastula to the external margin of the blastoporal lip is .....
   Ans. convergence
3. The movement of cell masses towards each other from two areas and their fusion into one cell mass is called .....
   Ans. Concrescence.
4. The movement of cells in chick towards the primitive streak and down into it is termed .....
   Ans. immigration
5. The morphogenetic movements during gastrulation cause ..... in expenditure of energy rich ATP and increase in .....
   Ans. Increase, oxidation.
6. During gastrulation, simple one-layered blastula is converted into didermic .....
   Ans. Gastrula in *Amphioxus*.
7. Single layer of blastula is called .....
   Ans. Blastoderm or ectoblast or proctoderm
8. Three layers of fully formed gastrula are ....., ... and .....
   Ans. Ectoderm, endoderm and mesoderm.

Very Short Answer Type Questions

1. **Write the names of cavities found in blastula and gastrula.**
   Ans. Blastocoele and gastrocoel or archenteron
2. **What are the two types of morphogenetic movements that occur during gastrulation?**
   Ans. Epiboly and emboly
3. **What is epiboly?**
   Ans. Epiboly occurs in presumptive ectodermal blastomeres which multiply, rearrange and expand and extend in antero-posteror direction and eventually envelopes the inwardly moving presumptive mesodermal and endodermal cells.
4. **Write about embolic morphogenetic movements**
   Ans. Embolic morphogenetic movements are inward migration of future chordamesodermal and endodermal blastomeres from the external surface of the blastula and their extension along anter-posterior axis of the developing embryo.
5. **What is invagination?**
   Ans. The process of infolding of a layer of endodermal cells to form a cavity, the archenteron surrounded by infolded cells and opens to the exterior by blastopore.
6. **Define involution.**
   Ans. Involution denotes a turning in or rolling under of cells which migrate to the blastoporal margin due to the process of convergence. The cells located along the external margin of blastoporal lip, move over the lip to the inside edge of the lip.
7. **Define migration.**
   Ans. The movement of cells toward the primitive streak in chick and then down into it is called immigration.

8. How is primitive streak formed in the chick blastoderm?

Ans. Primitive streak is formed as a result of convergence of epiblast cells to the middle line of the blastoderm.

UNIT

8

ORGANIZER : CONCEPT AND INDUCTOR PROCESS

STRUCTURE

- Organizer or Inductor
- Morphogenetic effect of inductor is induction.
- Embryonic induction.
- Types of embryonic induction.
- Examples of embryonic induction.
  - Summary
  - Test Yourself

LEARNING OBJECTIVES

After going through this unit you will learn :

- Meaning of organizer.
- Morphogenetic effect of organizer or inductor.
- To know the embryonic induction.
- Types of embryonic induction : Endogenous induction and exogenous induction with examples.
- Examples of embryonic induction : Either by blastomeres or by undifferentiated germinal layers or by mesenchyme or by spinal cord

• 8.1. ORGANIZER : CONCEPT AND INDUCTION PROCESS

When one embryonic tissue transmits a stimulus that influences another tissue to produce a structure that otherwise would not come into existence, then the former tissue is called **organizer** or **inductor** and its morphogenetic effect is called **induction. Spemann** thus, recognized a primary organizer in the form of dorsal lip or roof of archenteron in amphibian gastrula and got Nobel prize of 1935 for such an important discovery in experimental embryology.

Induction in modern terms can be defined as a type of intercellular communication which is required of differentiation, morphogenesis and maintenance (**Graham** and **Wareing**, 1976). It is also found that during induction some chemical is transmitted from one tissue to the other and this chemical acts on the genes of the cells which are being induced to develop into a particular manner. The embryonic tissue which exerts such an inductive influence (evocative action) is called an **inductor** and the chemical substance that is emitted by an inductor is called **morphogen.** The tissue on which a morphogen of inductor acts is called **responsive tissue**.

Induction is an interaction between two types of cells in which one, the inductor influences the fate of the other. i.e., **competent** or **responsive cell.**

• 8.2. EMBRYONIC INDUCTION

In ambhibian embryos, dorsal ectoderm cells in a mid-longitudinal region differentiate to form neural plate. It takes place only when chorda-mesoderm

lies beneath it. Chorda-mesoderm layer is formed by the invaginating cells from the region of dorsal blastoporal lips, that form the roof of archenteron.

Mangold (1927) had taken a small part of dorsal blastoporal lip from an early gastrula of *Triturus cristatus* and grafted it near the lateral lip of blastopore of the host gastrula of *Triturus taeniatus*. The grafted cells grew in number and spread inside host gastrula to form an additional chorda-mesoderm at this place. This chorda-mesoderm induced the ectoderm of the host gastrula to form an additional neural tube. The grafted cells also formed an additional notochord. As the host gastrula developed further, it developed into a double embryo united together. One of these embryos was the regular one, while the other embryo was the induced one, which could not develop a complete head.

This experiment clearly showed that dorsal lip of blastopore of the developing gastrula had the ability to induce the formation of neural plate in the ectoderm of the host. This is called **neural induction**. Similarly other parts of an embryo can induce the formation of other structures. Thus, **embryonic induction** is the influence of one structure in the formation of another structure.

• 8.3. TYPES OF EMBRYONIC INDUCTION

Embryonic induction is of two types : Endogenous and exogenous.

1. Endogenous Induction :

Certain embryonic cells gradually assume new diversification pattern through the inductors that are produced by them endogenously. Due to such inductors, these cells undergo either self-transformation or self-differentiation. Examples of such endogenous induction has been reported in mesenchymal cells of ventral pole of Echinoidea (echinoderms) and in small sized yolk laden cells of dorsal lip of amphibian blastopore.

2. Exogenous Induction :

When some external agent or a cell or tissue is introduced into an embryo, they exert their influence upon neighbouring cells by a process of contact induction. This is called **exogenous induction.** It may be **homotypic** or **heterotypic,** depending upon the fact that whether inductor provokes the formation of the same kind of tissue or different kind of tissue. In case of **homotypic exogenous induction**, differentiated cell produces the inductor. This inductor maintains the state of the cell proper and also induces adjacent cells to differentiate according to it, *e.g.,* neural ectoderm. Example of **heterotypic exogenous induction** is the formation of a secondary axis by an implanted presumptive notochord *i.e.,* primary inductor in amphibians.

Examples of Embryonic Induction :

The embryonic induction in different animals was found either by **blastomeres,** *e.g.,* sea urchin, Ascidia, etc., or by **undifferentiated germinal layers**, *e.g.,* neural induction by chorda-mesoderm to ectoderm in most primitive chordates and verterbrates or by **mesenchyme**, *e.g.,* epithelio-mesenchymal interaction during organogenesis of kidney, pancreas, skin, thyroid, etc., or by spinal cord.

Embryonic induction in Amphioxus. In *Amphioxus*, developmental potencies of animal hemisphere are influenced by the inductive stimulus from the material of the vegetal hemisphere. The animal half also exerts influence

on the vegetal half of the egg to some extent. The animal hemisphere was rotated 90° to 180° on the vegetal hemisphere at eight-cell stage, without changing the animal-vegetal axis. The shift of original presumptive neural blastomeres to posterior position, normal embryos developed, i.e., blastomeres moved to the anterior position are induced to develop neural structure. In fact, the whole of the presumptive ectoderm of ***Amphioxus*** remains undetermined during cleavage stages, because both nervous system and tail form in normal relationship in spite of shifting the animal hemisphere blastomeres. The vegetal hemisphere (presumptive endoderm-mesoderm) exhibits early self-determination and has an inductive effect on animal hemisphere. (**Tung, Wu** and **Tung**, 1960).

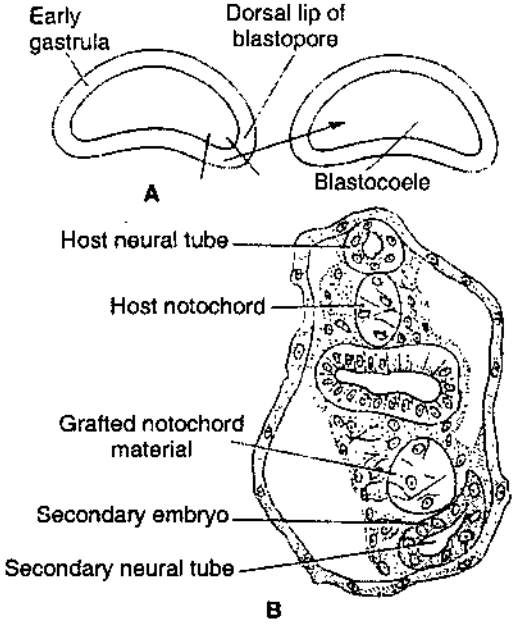


Fig. 1. *Amphioxus belcheri*, Neural induction. A. Cell lying inside dorsal lip of blastopore transplanted into the blastocoele of an embryo of the same stage of development. B. Transverse section of host embryo with a secondary neural tube induced by the grafted notochord material.

The same investigators transplanted pieces of tissue from the inner surface of dorsal blastoporal lip of an early gastrula of ***Amphioxus*** into the blastocoele of another embryo in the same stage of development. They observed that a secondary embryo developed in the vegetal region of the host with a notochord and mesoderm produced by the graft and a neural tube induced from host tissue. Thus, chordal tissue of ***Amphioxus*** gastrula has the power of neural induction. But mesodermal and endodermal tissues have little power of induction.

• 8.4. EMBRYONIC INDUCTION IN VERTEBRATES :

Various types of inductive interactions are found in vertebrates. For example :

1. Induction of vertebrate lens :

Spemann in 1901, conducted certain experiments on some amphibian eggs. According to Spemann at two-celled stage of frog egg, dorsal half differs from vegetal half and it alone has the ability to form a complete embryo. He demonstrated the dependence of eye lens formation upon the presence of an optic vesicle. He found that when presumptive eye rudiment was removed from

grass frog embryos, lens did not form. When an optic vesicle was transplanted under belly ectoderm, it resulted information of a lens instead of belly skin.

Optic cup/lens system explains a number of general points :

(i) Induction is followed by a phase of **organogenesis,** which may involve anyone or several cellular activities. It is followed by a phase of **cytodifferentiation** in which specific proteins are synthesized and cells assume their characteristic differentiated form.

(ii) Induction is the response of tissues to the presence or absence of other tissues. If inducer is (a) present, tissue that lies against will develop according to its presumptive fate. But if inducer is absent tissue will not develop according to its presumptive fate.

(iii) Further, this inducer may be capable of producing the same development in cells whose presumptive fate is different. e.g., formation of lens from presumptive flank epidermis. This type of induction is said to be **instructive**, but where only points (i) and (ii) can be demonstrated, induction may be **permissive**.

Instructive induction shows that responding cells have been directed to develop accordingly which they would not otherwise have followed.

Permissive induction affects only the extent to which an already determined cell can express that state through differentiation in its particular pathway. Several permissive interactions will always succeed an instructive pathway.

Maintenance inductions : If retina is removed from the eye of an adult frog, lens regresses and degenerates. Thus, persistence of lens depends upon lifelong interactions with the adjacent retina. Such interactions are called **maintenance inductions**. They do not initiate cell type, but are required to maintain the population of differentiated cells.

2. Induction of neural tube etc. :

The dorsal blastoporal lip from a salamander or newt early gastrula, when implanted into a ventral or lateral position of another gastrula, it turns inward and develops into a notochord and somites and induces the host ectoderm to form a neural tube. That is, it induces the formation of axial organs of a more or less complete secondary embryo. This secondary embryo accordingly has a

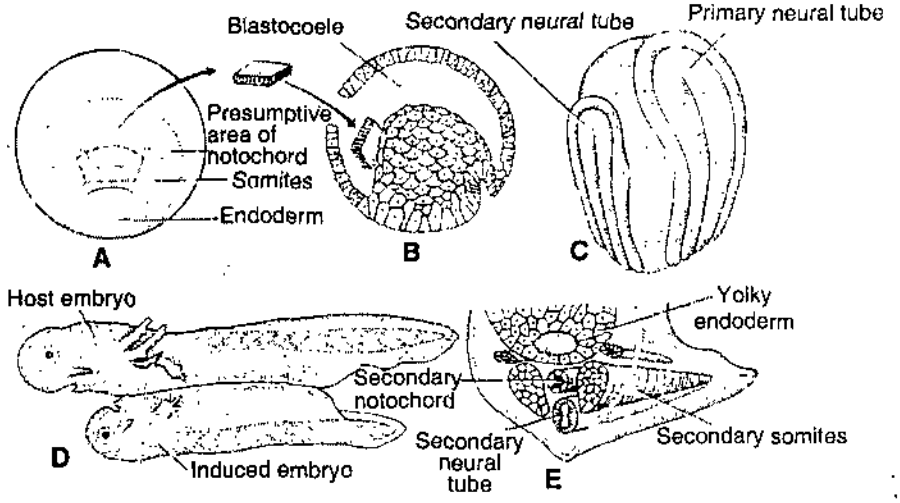


Fig. 2. Induction of a secondary set of axial organs by a graft of future archenteron roof. A and B-Transplantation of a piece of upper blastoporal lip into another gastrula. C.D. and E-Self differentiation plus inductions of the graft into a secondary embryo.

notochord, somites, kidney tubules. Each of these structures is composed partly of grafted, and partly of induced host material. Thus, the graft becomes self differentiated (it had developed notochord, mesoderm, etc., structures that it would have developed in its normal setting), and also induces the adjoining host tissue to form spinal cord and other structures including somites and kidney tubules.

Seeing these results, **Spemann** and **Mangold** named the dorsal lip as the **organizer** or **primary organizer**, because it developed the complete secondary embryo. It is primary in the sense of being first in sequence of inductions. **Elbert** and **Sussex** (1970) referred it as **embryonic induction** or **inductive interaction**, because the organization of secondary embryo results from a series of both inductive interactions and self differentiative changes in the host and donor tissues. Primary organizer of Spemann and Mangold induces the ectoderm to become differentiated into neural tube, hence it is called **neural inductor** or **primary inductor**. Then neural inductor of newt (*Triturus* sp.) is composed of presumptive material for notocnord and dorsal mesoderm (i.e., chorda-mesoderm).

Primary organizer or neural inductor was first discovered in urodele amphibians. It was soon found that dorsal lip of the blastopore and roof of archenteron of other vertebrates has the same function in development. It was found that chorda-mesoderm in all vertebrates induces the nervous system and sense organs. **In lampreys** (cyclostomes) whose cleavage is holoblastic and whose blastula and gastrula resemble those of amphibians, the property of neural induction is present in the presumptive chorda-mesodermal cells of dorsal lip of blastopore.

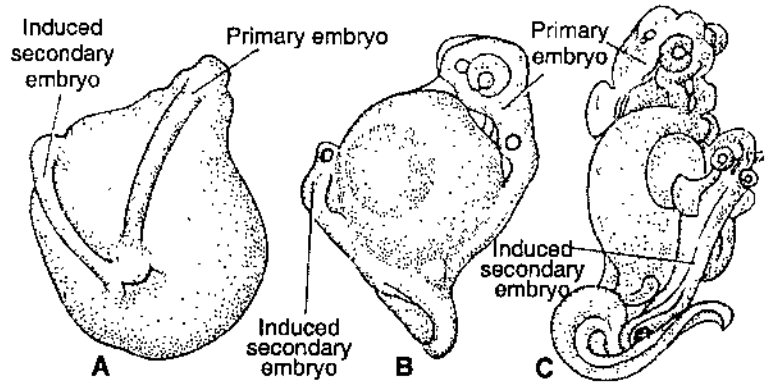


Fig. 3. Induction of secondary embryos by grafting of primary organizer or neural inductor in A-Lamprey and B and C in perch (*Perca*) fish.

In bony fishes, inductions of secondary well developed embryos were produced by transplanting the posterior edge of blastodisc (or transplanting the chorda-mesoderm and endoderm) into the blastocoele of another embryo. Posterior edge of blastodisc corresponds to the dorsal lip of blastopore.

The induction of a secondary embryo can be done by the dorsal lip of blastopore transplanted into the blastocoele of a young gastrula in the same way as in newts and salamanders (**Schotte**, 1930). In reptiles, archenteron has the same inducing activity as in other vertebrates.

In birds, anterior half of primitive streak is found to act as neural inductor (primary organizer). This part in

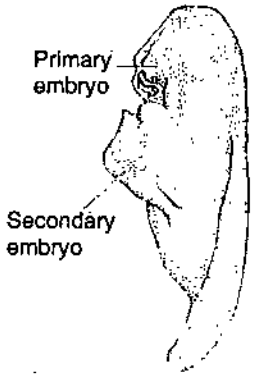


Fig. 4. Induction of secondary embryo by means of a grafted neural inductor in a frog.

birds corresponds to the lips of blastopore in amphibians. For testing the inducing ability of primitive streak, whole blastoderms were removed from the egg in early gastrulation stages and cultivated in vitro on blood plasma clot. Parts of primitive streak from another embryo were then transplanted between epiblast and hypoblast. Thus, inductions of secondary embryos were obtained. Hensen's node also has the inducing ability like the upper part. But the posterior third of primitive streak does not induce neural differentiation, because it corresponds to the latero-ventral lips of blastopore of an amphibian gastrula.

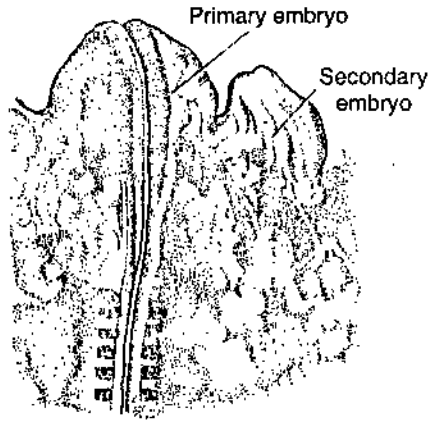


Fig. 5. Induction of secondary embryo by means of a grafted primitive streak in a bird.

A successful neural induction has been performed in a rabbit embryo by cultivating early blastodisc on a plasma clot and implanting the primitive streak of chick as an inductor. This proves that tissues of a mammalian gastrula have the competence for neural induction. It is practically certain that primitive streak and cells migrating from streak (chorda-mesoderm) are the source of the inducing stimuli.

• SUMMARY

One embryonic tissue transmits a stimulus that influences another tissue and produces a structure that was not present earlier, then the former tissue is called organizer or inductor. Induction is the morphogenetic effect of the organizer that produces the structure that was not present earlier. Dorsal lip of blastopore and roof of archenteron of amphibian gastrula are the primary organizer.

Spemann got Nobel prize in 1935 on the discovery of organizer in amphibian gastrula.

Induction is a type of intercellular communication required for differentiation, morphogenesis and maintenance (Graham and Wareing, 1976).

Chemical substance emitted by the inductor that exerts inductive influence on the host to develop a particular structure is called **morphogen**. Tissue on which a morphogen of inductor acts is called as **responsive tissue**.

Induction is an interaction between the inductor cells and responsive or competent cells. Mangold had taken a small part of dorsal lip of blastopre from an early gastrula of ***Triturus cristatus*** and grafted it near lateral lip of blastopore of gastrula of ***Triturus taeniatus***. Grafted cells grew in number and spread inside host gastrula to form an additional chordamesoderm, that induced the ectoderm of host gastrula to form an additional neural tube, and additional notochord. As host gastrula developed further that developed into a double embryo united together.

Dorsal lip of blastopore of developing gastrula was able to induce the formation of neural plate in the ectoderm. This is called **neural induction**.

Influence of one structure in the formation of another structure is called **embryonic induction**.

Embryonic induction is of two types : **Endogenous induction** and **exogenous induction**.

When certain embryonic cells gradually take new diversification pattern through inductors which produced them endogenously. This is called endogenous induction. The cells which took new diversification undergo self differentiation or self transformation.

Exogenous induction. An external cell or tissue introduced into an embryo, they exert their influence upon neighbouring cells by a contact induction that is called exogenous induction. Exogenous induction is of two types : homotypic and heterotypic. Homotypic induction develops the same types of cells or tissues, *e.g.* neural ectoderm.

After induction, organogenesis is followed, which involves one or several cellular activities.

Organogenesis is followed by cytodifferentiation in which specific proteins are synthesized.

Inducer, if present, then tissue that lies against will develop according to its presumptive fate.

Dorsal lip of blastopore in amphibian gastrula is the organizer or primary organizer.

Chorda-mesoderm in all vertebrates induces the development of nervous system and sense organs.

• TEST YOURSELF

Long Answer Type Questions

1. What is organizer? Describe an experiment to demonstrate the nature of primary organizer..
2. What is induction? Give an account of structure and function of neural induction in vertebrates.

Short Answer Type Questions

1. What do you understand by transplantation?.
2. What is induction?
3. What is the difference between endognous and exogenous induction?
4. Write the mechanism of neural induction.
5. What is the chemical basis of neural induction?

Very Short Answer Type Questions

1. **Define organizer or inductor.**
   Ans. An embryonic tissue transmits a stimulus that influences another tissue to produce a structure that otherwise would not come into existence, then the former tissue is called inductor or organizer.
2. **Who was awarded a Nobel prize in experiments on embryology ?**
   Ans. Spemann got Nobel prize in 1935
3. **What is morphogen ?**
   Ans. Morphogen is the chemical substance emitted by an inductor.
4. **Write about responsive tissue**
   Ans. The tissue on which morphogen or chemical substance of inductor acts is called responsive tissue.
5. **When the transplantation is to an individual more distantly related then species of one genus, what is the type of transplantation ?**
   Ans. Xenoplastic. When transplantation to an individual is more distantly related than species of one genus, transplantation is called xenoplastic.
6. **What is the basis of genetic neural induction?.**

Ans. DNA, RNA and actinomycin-D are the genetic bases of neural induction.

7. **The embryo from which the tissue is removed for transplantation is the .....**

   Ans. Donor

8. **In homotypic induction, a differentiated cell produces an .....**

   Ans. Inductor

9. **Vital staining experiments of Vogt can be performed with ..... eggs.**

   Ans. Newt.

10. **In ..... the property of neural induction lies in the presumptive chord-mesodermal cells of dorsal lip of blastopore.**

    Ans. cyclostomes.

UNIT

9

CHICK DEVELOPMENT : SALIENT FEATURES

STRUCTURE

LEARNING OBJECTIVES

After going through this unit you will learn :

- Description of hen's egg. Structure and composition of egg.
- To observe the cleavage and blastulation in hen's egg.
- Study of fate map of blastula of hen's egg.
- Gastrulation in hen's egg by epiboly and emboly.
- Formation of primitive streak and its regression in later stage.
- Structure of primitive streak.
- Formation of head process;
- Formation of endoderm or hypoblast.
- Formation of fully formed gastrula.

• 9.1. STRUCTURE AND CHEMISTRY OF HEN'S EGG

Hen's eggs are polylecithal or macrolecithal (eggs having enormous amount of yolk and have a very little cytoplasm) and telolecithal (yolk is more abundant and active cytoplasm and nucleus remain confined to a small cap at the animal pole). Polarity of the egg is well marked; animal pole has very small amount of active cytoplasm in the form of a disc with a nucleus. This is called **germinal disc** or **blastodisc**. It is about 3.0 mm in diameter. The entire egg is filled with yolk. Yolk and blastodisc are bounded by a plasma membrane (oolemma) and around plasma membrane is found a thin non-cellular vitelline membrane, which is primary egg membrane. It is double in origin : inner layer is produced in the ovary and is composed of very rough fibres, and outer layer is finely fibrous layer. The next egg membrane is albumen of the egg.

Yolk. Yolk is deposited in alternating layers of yellow and white yolk. The central mass of white yolk is the **latebra** and around it is present thick layer of darker yellow coloured yolk. Thus, latebra becomes surrounded by alternate concentric rings of yellow and white yolks. Latebra or white yolk extends upward as a narrow yolk, called **neck of latebra**. Just beneath the blastodisc, white yolk spreads slightly to form a plate, the **nucleus of Pander**.

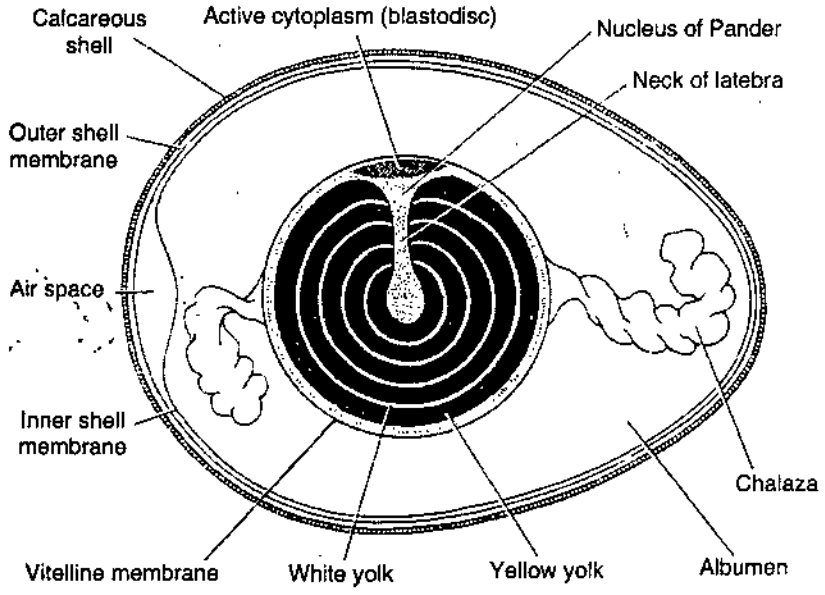


Fig. 1. Longitudinal section of hen's egg (diagrammatic).

Most of the yolk is liquid, but about 23 percent of it is in the form of solid yolk spheres. The yolk contains 48.7% water, 32.6% phospholipids and fats, and 16.6% proteins, 1% carbohydrates and 1.1% other chemical molecules. Proteins are found in the form of phosvitin (phosphoprotein) and lipovitellin or livetin (liquid protein). Fatty portion of yolk is predominantly neutral fat (50%), the rest is in the form of phosphatids, cerebrosides and cholesterol. Lipids are the greater part of the solid constituents of yolk and serve as the major source of food for the embryo. Major carbohydrates of yolk are free glucose and polysaccharides combined with phospholipids, phosphoproteins and cerebrosides.

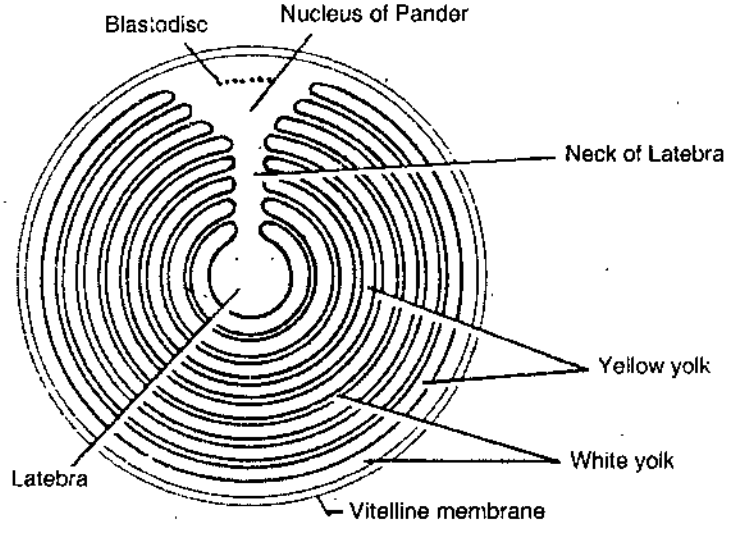


Fig. 2. Structure of ovum or egg.

Albumen. Albumen of the egg is differentiated into two distinct layers : a dense inner layer and a thin outer layer. The inner most denser layer of albumen is **chalaziferous layer** that surrounds the ovum. It is made up of very viscous glycoprotein. It is spirally twisted into cords, at each end of the egg to form chalazae. Beneath this is present layer of dense albumen secreted by magnum part of oviduct . Outer to this layer is present a wide layer of thin albumen. 85% of the egg white is water and the rest (15%) is mixture of several

proteins, mostly albumins that make up 94% of dry weight. The denser part of egg white forms spiral strands, the chalazae on either side of egg (ovum). Albumen provides nutrition to the developing embryo, serves as a water source and also protects the embryo from mechanical and chemical injuries. Albumen also contains a variety of enzymes, vitamins, pigments and phosphorus.

Around the egg white, there is a thin and tough **shell membrane** made up of **keratin** fibres matted together. It is formed of two layers, which are in close contact with each other except at the blunt end of the egg, where they are separated to enclose the **air space**. Inner shell membrane adheres to the albumen and outer membrane adheres to the shell. Shell membrane is secreted in the isthmus part of the oviduct in about 74 minutes.

Shell membrane is surrounded by a hard and porous calcareous **shell**. It allows exchange of oxygen and carbon-dioxide through its fine pores. Shell is soft and flexible when egg is laid but soon it becomes hard and brittle. Yolk and egg white provide food to the developing embryo. Toward the end of incubation, a part of the shell dissolves and calcium carbonate released is utilized to build the developing bones. Shell is pierced by about 7000 fine pores, which are filled by an organic substance related to collagen. Shell is entirely secreted by uterus where the egg remains for about twenty and half hours.

8.2. FERTILIZATION

Fertilization takes place in the upper region of the oviduct. Several sperms surround the oocyte but only one that succeeds first in penetrating through the egg membrane brings effective fertilization. Its nucleus fuses with the egg nucleus. As the sperm enters the egg through the egg membrane it induces the oocyte to undergo two quick maturation divisions. (First polar body is thrown out of the egg when oocyte changes into the ovum, while the second polar body is thrown out of the egg immediately before fertilization.)

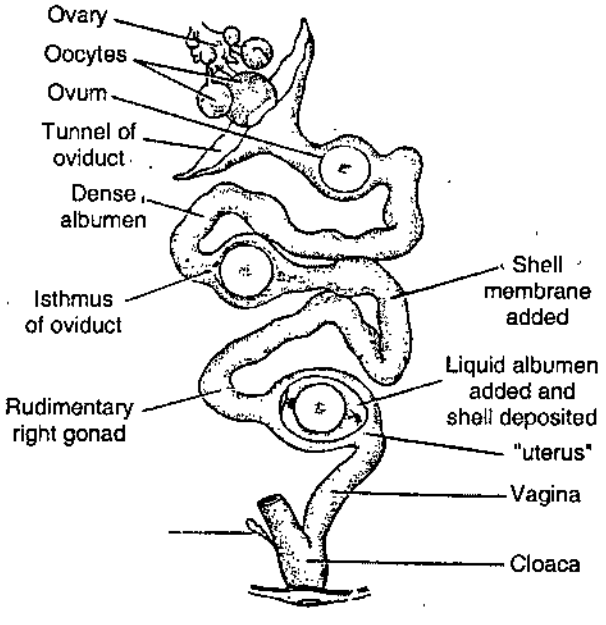


Fig. 3. Egg in the oviduct, passes downward.

Fertilized ovum travels down the oviduct, where albumen, shell membranes and shell are secreted on it. Next day when a new ovum enters the oviduct a few more sperms enter the blastodisc. This process continues till all the six or seven

eggs get fertilized. If sperms are not present in the oviduct the eggs laid will be unfertilized. Ovum from infundibulum goes to magnum part of oviduct. Here albumen is secreted around the ovum. After about three hours, egg enters the isthmus part of the oviduct, where shell membrane is secreted. In the isthmus egg remains for about 75 minutes, then the egg enters the shell gland part of the uterus where shell is secreted. In the oviduct egg remains for about twenty one hours, and then laid as egg. Most of the eggs available in the market are unfertilized.

• 8.3. CLEAVAGE AND BLASTULATION

Cleavage of fertilized hen's egg (ovum) is started inside the oviduct, i.e., before the egg is laid. Segmentation occurs only in the blastodisc and yolk does not undergo cleavage. At the time of first cleavage, the blastodisc is about 3mm in diameter and 0.5 mm thick.

Cleavage is **meroblastic** or partial. This type of cleavage is also said to be **discoidal**, because it is confined only in the blastodisc. As the egg is laid, it is in the blastula stage.

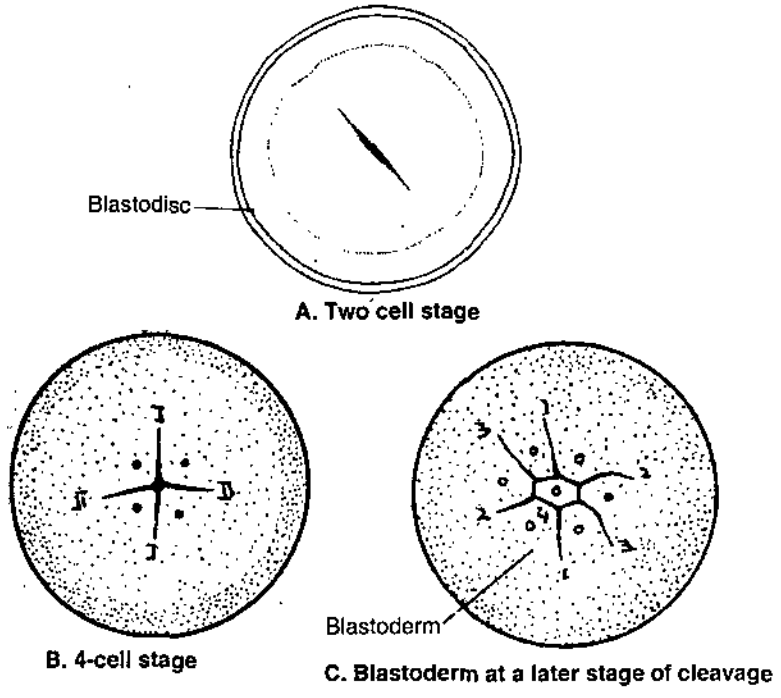


Fig. 4. Early cleavage in chick.

First cleavage or furrow appears in the middle of blastodisc and does not extend upto the margin of the blastodisc. It also does not extend completely upto the lower margin of the blastodisc. This results in two incompletely separated blastomeres. Cytoplasm of both the regions, *i.e.*, around the edge and beneath the furrow is continuous.

Second cleavage furrow is roughly perpendicular to the middle of first furrow and also extends upto the same depth. This furrow also does not extend upto the margin and the lower edge of the blastodisc.

Third cleavage furrows are vertical and cut across the second cleavage firrows. These are roughly parallel to the first cleavage furrow. A horizontal cleavage also appears in the blastodisc that cuts off the upper blastomeres from the underlying yolk while marginal cells remain in contact with yolk.

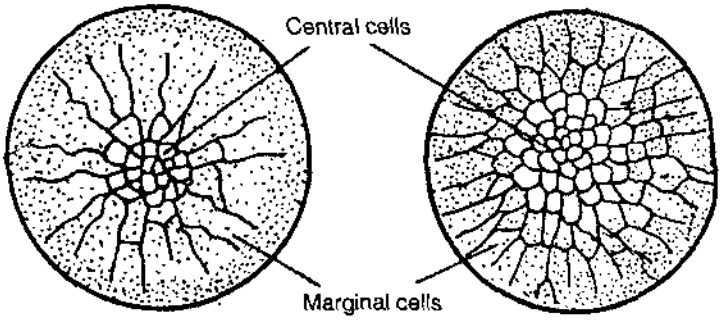


Fig. 5. Later cleavages in blastoderm in surface view.

Fourth cleavage furrow is also vertical and circular, cutting the blastomeres into two regions : eight **central blastomeres** and eight **marginal blastomeres.** Thus meridonial and vertical cleavages separate the daughter blastomeres from each other, but not from the underlying yolk. Thus, central blastomeres are continuous with the underlying yolk at their lower ends, while the marginal cells are continuous with the uncleaved cytoplasm at their outer edges. As the process of cleavage goes on, the marginal cells are constantly cut off and are added to the central cells. At the same time, marginal cells undergo peripheral and radial divisions that finally reach the margin of the disc. In this way central segmented area continually increases in diameter. In later stages of cleavage, central cells of the blastodisc become cut off from all sides, but for some time at the edge there is syncytial region where cells still remain in

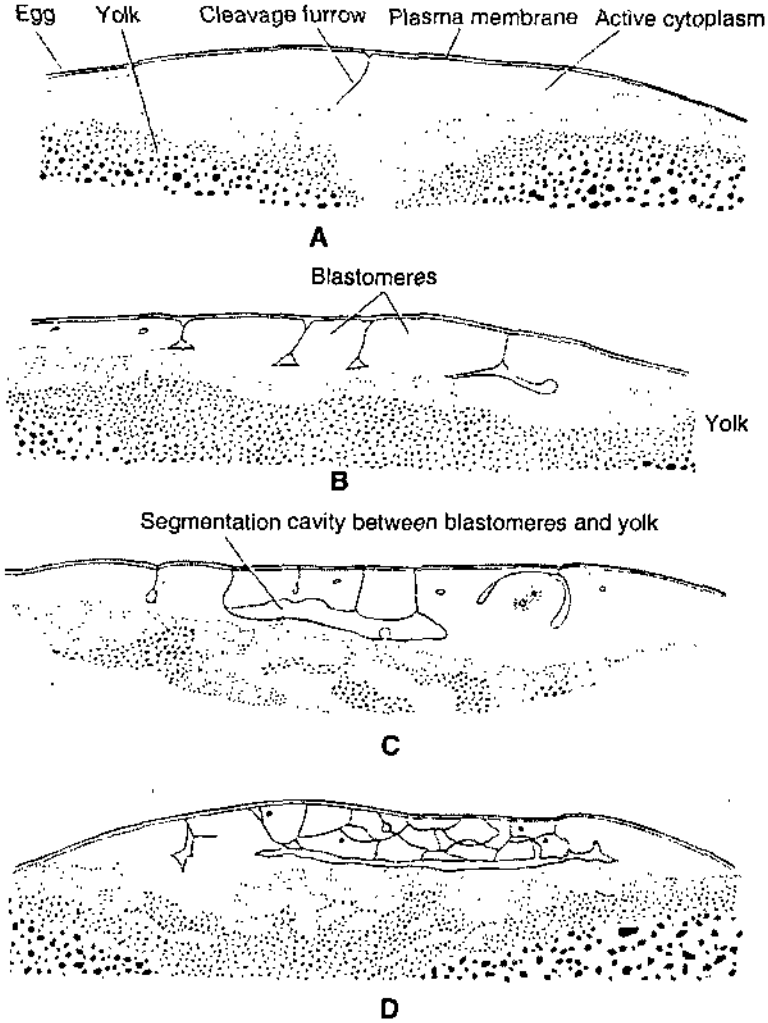


Fig. 6. T.S. of germinal disc of hen's egg showing early cleavage.

communication with each other. A peripheral rim of unsegmented cytoplasm surrounding the central cellular region of the disc is called **periblast**. It is slightly darker in colour than the central region and is about 0.5 mm wide.

Further cleavage produces a round disc, 5 to 6 cells deep in the center, but only one to two cells deep at the periphery. This results into a compact plate having a thickness of several cells, this is called **blastoderm**.

Thus, the central cells become free from the yolk, creating a **subgerminal cavity** that is filled with liquified yolk. This cavity at this stage may be regarded as equivalent of the blastocoel and the embryo is now in the **blastula stage.** Blastula of chick is in the form of a disc, hence called **discoblastula**.

• 8.4. FATE MAP OF DISCOBLASTULA

For the study of fate map, the blastoderm of chick is stained by vital stains like fine carmine or carbon particles or radioactive thymidine. It shows that blastomeres of area opaca (or area vitellina) do not form the embryo proper. They form the extra-embryonic membranes.

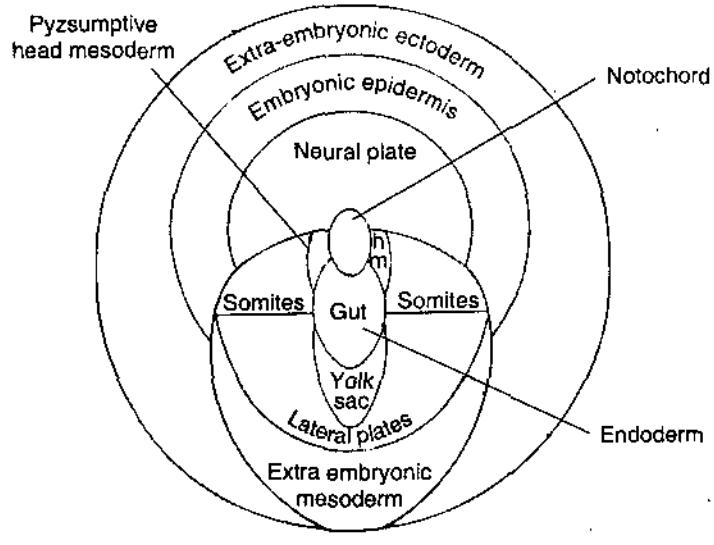


Fig. 7. Fate map of epiblast of a bird.

Epiblast and hypoblast of area pellucida have different fates in the embryonic development. Epiblast has blastomeres for ectoderm, mesoderm and endoderm. Hypoblast has blastomeres only for endoderm. Fate map of chick blastula has been prepared by radioactive thymidine, showing epiblast only.

1. In the center of area pellucida lies a small area that will produce the notochord

2. Posterior to this, in the middle lies an elongated oval area, of presumptive endoderm that will form the gut and its other parts.

3. Toward the posterior edge of area pellucida lies extra-embryonic endoderm that will form lining of yolk sac.

4. Right and left of presumptive notochord and endoderm and posterior to presumptive yolk sac lie various subdivisions of presumptive mesoderm.

5. *Near the notochordal area is present prechordal or head mesoderm* area.

6. Posterior and lateral to head mesoderm, notochord and also lateral to anterior half of gut is present the presumptive somite areas: Posterior to these is present presumptive lateral plate mesoderm area.

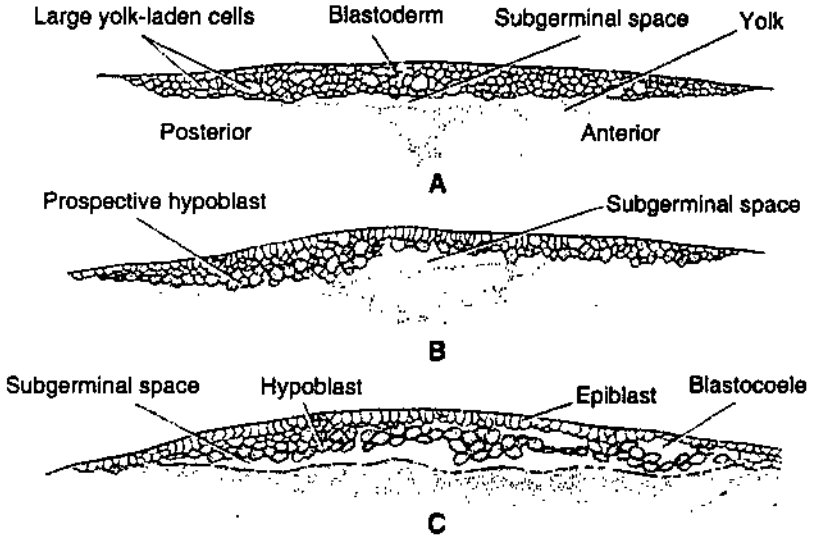


Fig. 8. Sections through blastoderm, origin of hypoblast and blastocoele.

7. Toward the posterior edge of area pellucida is present a large crescentic area which will form extra-embryonic mesoderm (that is, area vasculosa).

8. Presumptive ectoderm occupies the anterior and lateral parts of area pellucida. Thus, roughly a semicircular area anterior to notochordal material is the presumptive neural plate area. Around neural plate area is the presumptive embryonic epidermis. Outward to embryonic epidermis, forming a roughly complete ring at the outer edge of area pellucida lies extra-embryonic ectoderm.

• 8.5. GASTRULATION

Gastrulation in chick includes two types of morphogenetic movements : Emboly and epiboly.

1. Emboly : It includes convergence, invagination and involution or ingression of the mesentoblast cells, i.e., progenitors of endoderm and mesoderm. Thus, by emboly formation and elongation of primitive streak and later its regression and also formation of head process is included. Thus, formation of embryonic endoderm, mesoderm and notochord is due to embolic process of gastrulation.

2. Epiboly : Overgrowth of ectoderm and extra-embryonic endoderm around the yolk sac is due to epiboly.

• 8.6. FORMATION OF PRIMITIVE STREAK

Prospective mesodermal and endodermal cells of the epiblast migrate toward the posterior border of area pellucida forming a conical thickening, called the beginning of primitive streak. Primitive streak appears after 6 to 7 hours of incubation. Primitive streak grows forward due to proliferation of cells as well as addition of cells that migrate to it from various parts of area pellucida due to convergence. After 12 to 13 hours of incubation, primitive streak, extends upto the center of area pellucida from its posterior margin. At the same time a longitudinal groove appears in the middle of primitive streak called **primitive groove** bordered by **primitive folds**. Meanwhile the most anterior portion of groove thickens into a depressed knot of cells, called **primitive knot** or **Hensen's node**. The depression of the primitive knot changs into a funnel-shaped **primitive pit**. Primitive streak is now sharply delimited in the

middle of area pellucida, that extends upto three-fourth length of area pellucida. This is called **definitive primitive streak,** which is completed in about 18 to 19 hours of incubation. Now area pellucida also changes in shape, it becomes pear-shaped from circular shape.

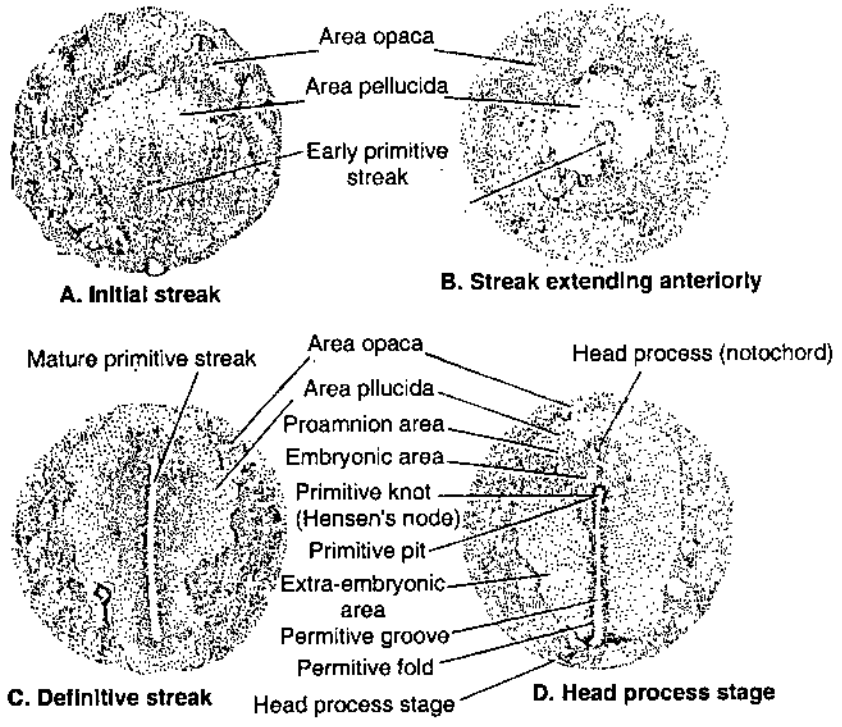


Fig. 9. Development of primitive streak and head process (gastrulation).

Epiblast cells of primitive groove become flask-shaped and invaginate into the subgerminal cavity. The basal ends of flask-shaped epiblast cells are highly motile and their apical ends remain fixed in the surface by junctional contacts. In this way they pull neighbouring cells into groove, migrating as a cohesive sheet. Ingressing cells detach from the inner layers in the groove and migrate into the subgerminal cavity between epiblast and hypoblast (endoderm). Immigrating cells are replaced by more epiblast cells converging toward the streak area. These immigrating progenitor cells of mesoderm and endoderm make contact with the previously migrating mesoderm and hypoblast cells. Thus, the entire primitive streak is a mass of moving cells. These migrating mesoderm and endoderm cells spread out sideways and forward from anterior end of primitive streak.

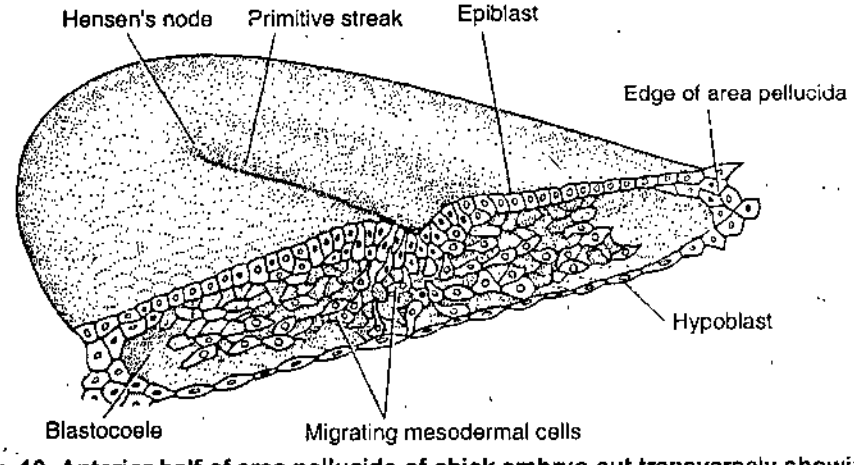


Fig. 10. Anterior half of area pellucida of chick embryo cut transversely showing migration of mesodermal and endodernal cells from the primitive streak.

The future notochordal cells migrate from anterior region of primitive streak, i.e., primitive pit. Endoderm cells invaginate through the streak which lies just behind Hensen's node. **Mesodermal cells of somites** just follow the path of endoderm cells. **Lateral plate mesoderm cells** invaginate through middle section of primitive streak, but after the disappearance of endoderm from area pellucida. **Extra-embryonic mesoderm** of yolk sac migrates through the posterior part of primitive streak.

During the same period, some hypoblast cells expand into area opaca forming extra-embryonic endoderm, which forms the lining of yolk sac. Thus, the combined effect is involution of epiblast cells over the lips of primitive groove. Due to expansion pressure in the epiblast the groove becomes narrow and extended anteriorly.

• 8.7. FORMATION OF HEAD PROCESS

Prospective notochordal cells which converge on the node, sink down through the node and then migrate forward as a tongue of tissue, known as the **head process** or **notochordal process**.

Gastrulation is completed at about 20 to 25 hours of incubation and at this stage blastoderm is in head process stage. The notochordal tissue develops as rigid rod anterior to receding primitive streak. As the primitive streak regresses posteriorly, the embryo develops anterior to it. Head process consists of a thicker central mass of cells and more diffuse lateral wings. Thicker central portion represents the definitive notochord, whereas lateral wings contribute to somatic mesoderm that flanks the notochord. Later on notochord becomes detached from the hypoblast below, but at the extreme anterior end it is fused with the endoderm.

Disappearance of primitive streak : Elongation of primitive streak stops when no more endodermal, notochordal and mesodermal cells are available to replenish the streak area. Primitive streak then begins to shrink; anterior end with Hensen's node recedes posteriorly. Primitive streak finally becomes reduced to a fragment in the tail bud and becomes cloaca of the embryo.

• 8.8. COMPLETION OF ENDODERM

The first cells that migrate into the interior through the anterior part of streak form the endoderm. As Hensen's node shifts backward and notochordal process elongates anteriorly, presumptive endoderm of middle and posterior part of gut located behind the node involute and invaginate as an endodermal strip beneath the notochord. In chick, archenteron is not formed during gastrulation.

• 8.9. STRUCTURE OF FULLY FORMED GASTRULA

Process of gastrulation ends when primitive streak completely disappears. Now beginning of neurulation (development of nerve cord) takes place. Fully formed gastrula consists of three germ layers : ectoderm, chorda-mesoderm and endoderm. Ectoderm and chorda-mesoderm are in continuity along the axis of primitive streak and the endoderm is also united with the mesoderm and ectoderm at the anterior end and at the posterior end of streak.

• SUMMARY

1. Hen's egg is macrolecithal or polylecithal, i.e., it contains enormous amount of yolk and cytoplasm is very little.

2. Germinal disc or blastodisc is a small active cytoplasmic disc at the animal pole over the yolk.

3. Yolk and blastodisc are bounded by inner plasma membrane and outer vitelline membrane. Vitelline membrane is a primary egg membrane.

4. Vitelline membrane is double in origin : inner layer is produced in the ovary and outer finely fibrous layer, other egg membrane outer to ovum is albumen : Inner denser layer of albumen is chalaziferous layer surrounding the ovum, it is spirally twisted into cords at each end of the egg to form the chalazae. Outer to this is dense albumen secreted by magnum of the oviduct. Outer to this layer is thin albumen.

5. Composition of albumen is 85% water and 15% is mixture of proteins, mostly albumins that make up 94% of dry weight.

6. Outer to egg membrane is shell membrane made up of keratin fibres matted together. Egg membranes are two closely applied with each other. At the blunt end both the layers are separated from each other to enclose air space.

7. Outer to shell membrane is calcareous shell that is pierced by about 7000 fine pores filled up by an organic substance.

8. Fertilization takes place in the upper region of the oviduct, by a single sperm. As the sperm enters the egg through egg membrane it induces oocyte to undergo two maturation divisions producing two polar bodies and an ovum.

9. Cleavage starts inside the oviduct before the egg is laid..

10. Cleavage is meroblastic or partial because it occurs only in the blastodisc.

11. Egg is laid in the blastula stage. First, second and third cleavages are meridonial or vertical and confined only in the central region of the blastodisc. These do not reach upto the lower portion of the blastodisc, that is, upto yolk.

12. Fourth cleavage furrow is also vertical and circular, cutting blastomeres into two regions : eight central blastomeres and eight marginal blastomeres whose peripheral boundaries do not exist.

As cleavage continues, inner ends of marginal blastomeres are cut off and are added to central cells, thus, central cells increase in number and in area, while marginal cells also divide peripherally and radially.

13. Cellular blastodisc is now called blastoderm.

14. Beneath the central cells lies the subgerminal cavity filled up with liquified yolk. Embryo at this stage is called blastula (discoblastula).

15. Gastrulation is by epiboly and emboly.

Epiboly is overgrowth of ectoderm and extra-embryonic endoderm around yolk sac.

Emboly by convergence, invagination and involution or ingrssion/ or progenitors of endoderm and mesoderm cells.

16. Primitive streak is formed by the aggregation of endodermal and mesodermal cells from the blastoderm in the center of the blastoderm.

Definitive primitive streak is formed at about 18 to 19 hours of incubation.

Primitive streak occupies about three-fourth part of area pellucida. It is present in the middle of area pellucida.

At the anterior end of the streak is present a knot-like structure, called Hensen's node or primitive knot. It contains cells of future notochord. Centre of knot is excavated to form the primitive pit.

After fully formed streak, area pellucida only contains the epiblast cells.

17. Cells of Hensen's node (notochordal cells) migrate inward and then extend forward as rod-like tissue to form the head or notochordal process. It lies in between upper ectoderm and inner endoderm.

18. Primitive streak regresses posteriorly due to non-availability of endodermal, mesodermal and notochordal cells.

19. Area pellucida is of circular shape in the beginning, but at the end of gastrulation, it becomes pear-shaped.

• QUESTIONS

Long Answer Type Questions

1. Describe the structure and chemistry of a hen's egg.
2. Describe the formation and development of primitive streak.
3. Describe the development of chick upto the formation of primitive streak.
4. Discuss the process of gastrulation in chick.

Short Answer Type Questions

1. Write the chemical composition of yolk.
2. What are the functions of albumen in egg ?
3. Write about the formation of primitive streak.
4. Write a note on the Hensen's node.

Very Short Answer Type Questions

1. **In which part of the oviduct fertilization takes place?**
   Ans. Upper part of the oviduct.
2. **For how many hours egg remains in the oviduct?**
   Ans. Twenty one hours.
3. **In how many hours a fully developed streak is formed?.**
   Ans. 18 to 19 hours of incubation..
4. **What is the type of hen's egg?**
   Ans. Polylecithal or macrolecithal or teolecithal.
5. **Write the names of various layers of egg,**
   Ans. Vitelline membrane around the egg, albumen, shell membrane and shell.
6. **What is the type of cleavage in hen's egg ?**
   Ans. Meroblastic or discoidal
7. **What are the two regions of blastoderm?**
   Ans. Area pellucida and area opaca.
8. **Write name of blastula in birds.**
   Ans. Discoblastula because blastula in chick is in the form of disc.

UNIT

10

EXTRA-EMBRYONIC MEMBRANES OF CHICK

STRUCTURE

LEARNING OBJECTIVES

After going through this unit you will learn :

- What is extra-embryonic membrane.
- Which part of blastoderm forms the foetal membranes.
- Names of the various foetal membranes in chick and mammal.
- Development of foetal membranes.
- Functions of foetal membranes.

10.1. EXTRA-EMBRYONIC MEMBRANES

Extra-embryonic membranes are present in reptiles, birds and mammals. These membranes do not enter into the formation of the embryo. These membranes are external to the developing embryo and are devoted to the care and maintenance of the developing embryo. Extra-embryonic membranes or foetal membranes or foetal sacs protect the developing embryo against desiccation and shock, and serve in respiration, excretion, etc. These membranes do not enter into the formation of embryo.

Types of Extra-embryonic Membranes :

During development of the embryo, the extra-embryonic membranes formed are : Yolk sac, amnion, chorion and allantois. The appearance of these membranes during evolutionary development allows higher vertebrates (reptiles, birds and mammals) to eliminate the vulnerable larval stages from their life cycle thereby ensuring the survival of the adult (**Phillips**, 1995).

These membranes are composite structures formed of two germ layers. Amnion and chorion are formed of extraembryonic ectoderm and somatic layer of mesoderm, both are collectively known as **somatopleure,** while yolk sac and allantois are composed of extra-embryonic endoderm and splanchnic mesoderm. Both are collectively called **splanchnopleure**.

Development of Extra-embryonic Membranes :

During development (after neurulation) somatopleure and splanchnopleure gradually spread peripherally over the yolk mass beyond the forming embryonic body. As the body of embryo grows, it becomes separated from the extra-embryonic membranes by the formation of limiting folds, i.e., cephalic or head fold, caudal or tail fold and lateral folds. Thus, in the anterior region of growing embryo, cephalic fold rolls beneath the head area, separating it from the underlying yolk, forming **subcephalic pocket**. Similar folds appear on both sides of the body of embryo, called **lateral folds**. On about third day of

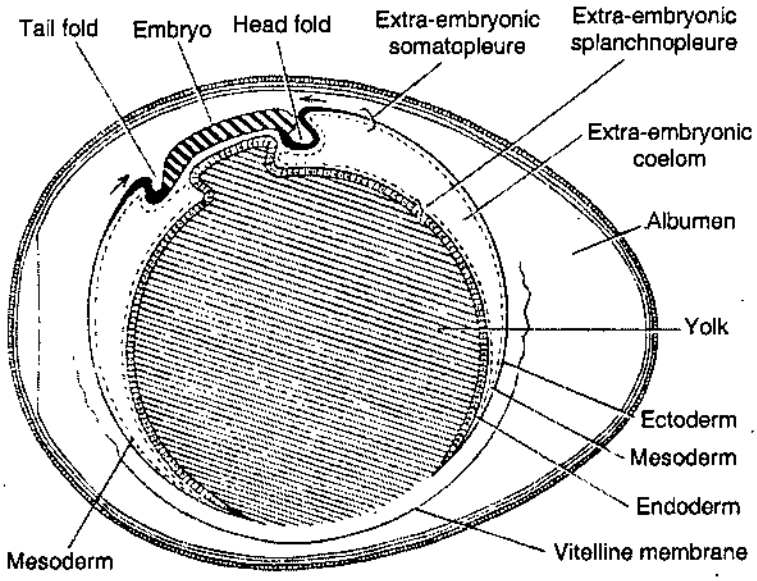


Fig. 1. Early chick embryo showing body folds delimiting embryo from extra-embryonic areas.

incubation, **caudal fold** appears behind the tail part of embryo. These body folds (cephalic, laterals and caudal) delimit the body of embryo from the extra-embryonic region.

Development of Yolk Sac :

At the time of neurulation (nerve cord formation), gut region of embryo has a dorsal roof and sides, but has no floor. The ventrally open gut lies over the yolk mass. The splanchnopleure closely applied to yolk extends peripherally and eventually surrounds the entire yolk, thus, forming the **yolk sac**. It forms first among extra-embryonic membranes. Later on, all the body folds move towards one another beneath the body of embryo, forming the floor of the gut leaving a small region of the midgut. These body folds do not unite completely, and a small aperture remains open into the yolk sac, called **yolk duct**. When complete walled gut is formed in the embryo, yolk sac cavity communicates with the gut cavity through yolk duct that runs through **yolk stalk** of yolk sac. The endoderm of the yolk sac is thrown into folds, **yolk sac septa,** which penetrate the yolk mass. Endodermal cells secrete digestive enzymes, which digest the yolk and are absorbed by the endoderm and transported to the

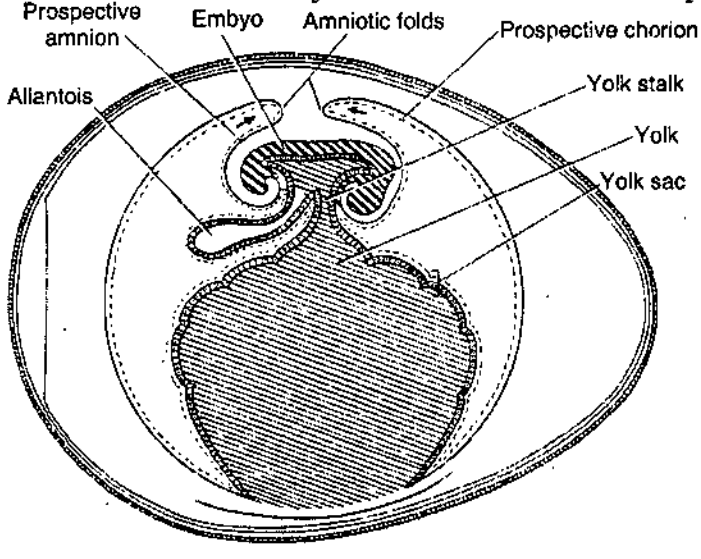


Fig. 2. Development of the extra-embryonic membranes of chick (Early stage)

embryo by **vitelline vein**. Upto this time, blood circulation develops within mesoderm layer of the yolk sac : vitelline arteries and veins (omphalomesenteric blood vessels) network develops in the yolk sac. Constriction of yolk stalk region brings paired vitelline arteries and veins closer. Thus, digested yolk is collected by left and right vitelline veins, which open into unpaired **ductus venosus** that finally opens into sinus venosus of heart. Yolk is completely absorbed by the embryo and shortly before hatching yolk sac is retracted into the abdominal cavity of the embryo, and walls of abdominal cavity close behind it.

Development of Amnion and Chorion :

Origin of amnion and chorion is considered together, because they are formed together from the extra-embryonic somatopleure (ectoderm and mesoderm). About 30 hours of incubation, extra-embryonic somatopleure elevates over the embryo in the form of folds. An elevation or fold arises anterior of the head end of embryo, called **cephalic amniotic fold**. Cephalic amniotic fold extends backward and fuses with the **lateral amniotic folds**, both arch over the embryo. A similar fold arises behind the tail region of the embryo which extends anteriorly covering the tail part of the embryo, this is **called caudal aminiotic fold**. All these amniotic folds finally converge over the developing embryo in two sheets of somatopleure. The region where these folds unite with each other is called **sero-amniotic connection**. The point of the folds leaves a scar, called **sero-amniotic raphe**.

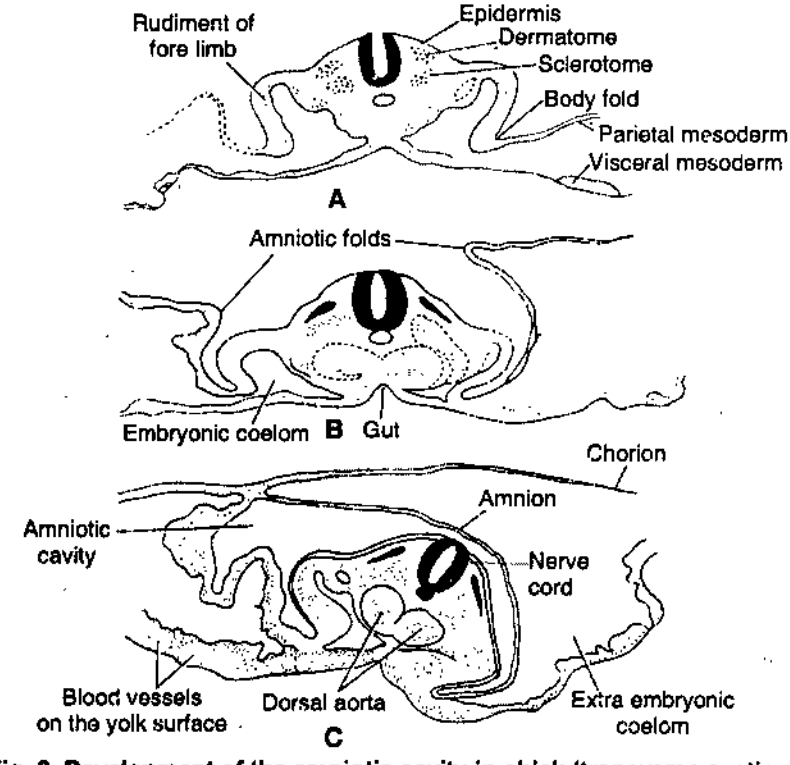


Fig. 3. Development of the amniotic cavity in chick (transverse sections).

The fusion of aniotic folds produces two sac-like cavities enclosed by somatopleure. Inner sheet of somatopleure is called **amnion** that encloses the embryo within fluid-filled cavity, called **amniotic cavity**. Amnion consists of extra-embryonic ectoderm on the inner side and extra-embryonic mesoderm on the outer side.

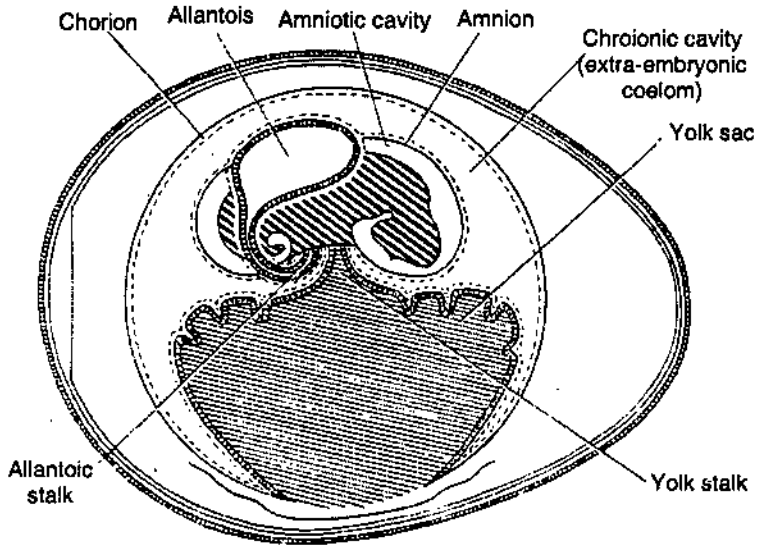


Fig. 4. Development of the extra-embryonic membranes of the chick (Later stage)

The outer layer of somatopleure is called **chorion** which consists of outer ectoderm and mesoderm on the inner side. Chorion is continuous with the yolk sac. The cavity present between chorion and amnion is **chorionic cavity**, lined by mesoderm on the inner side and ectoderm on the outer side. Chorionic cavity is called **extra-embryonic coelom**, which is continuous with the coelom of embryo. Both amnion and chorion continuously grow peripherally and laterally and cover the embryo.

Functions of Amnion and Chorion :

Amnion. Embryo floats freely in the salty fluid of the amniotic cavity. Amnion protects the embryo from desiccation.

Amniotic cavity fluid is a good shock absorber and protects the soft, collapsible and skeletonless early embryo from mechanical shocks.

Amnion with fluid-filled amniotic cavity protects the embryo from adhesion to the shell or from friction against shell.

During later stages of development, mesoderm of amnion forms muscle cells which contract rhythmically, thus rocking the embryo within amniotic fluid. Rocking causes circulation of amniotic fluid that moves parts of the embryo and thus, prevents them from adhesion.

Amnion isolates the embryo from shell of the egg and thus, protects the embryo from adhesion to the shell and also friction from shell.

In later development, mesoderm of amnion forms muscle cells, which contract rhythmically and thus, rocking the embryo within amniotic fluid.

Functions of chorion. Fluid of chorionic cavity (extraembryonic cavity) also provides protection to the embryo. Allantois also expands into the chorionic cavity (extra-embryonic coelom),

Mesoderm of allantois and chorion join with each other and play an important role in gaseous exchange through porous shell.

Development of Allantois :

Allantois develops as a ventral out growth from the hindgut. It consists of outer mesoderm and inner endoderm (splanchnopleure). Allantois grows rapidly and expands into the extraembryonic coelom, i.e., spsce in between yolk

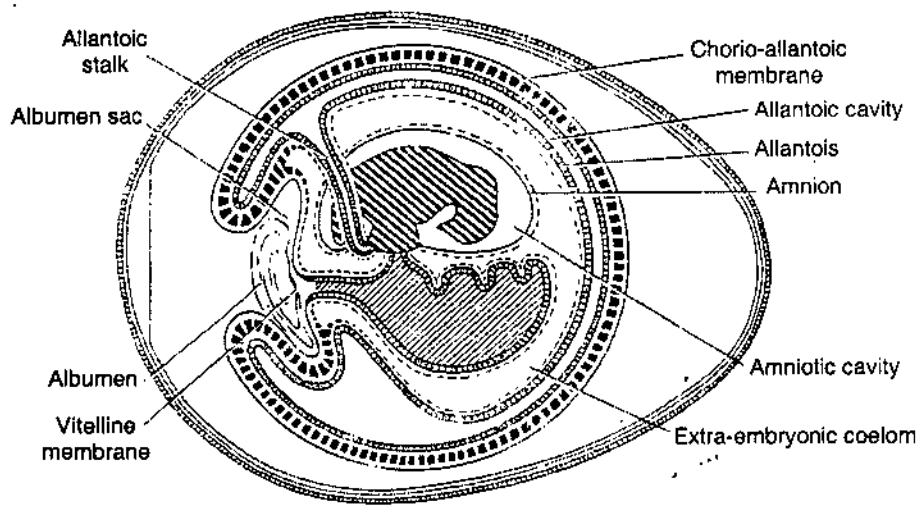


Fig. 5. Fully formed extra-embryonic membranes of chick.

sac and amnion and chorion. Proximal part of allantois forms a narrow neck, **allantoic stalk** through which it remains connected with the hindgut of the embryo. Distal part of embryo expands into the extra-embryonic coelom, space between yolk sac and amnion and chorion. Proximal part of allantois remains connected with hindgut of embryo by a narrow **allantoic stalk**. When body folds contract, separating the embryo from extra-embryonic parts, allantoic stalk and stalk of yolk sac come closer and enclose together to form an **umbilical cord**.

As allantoic vesicle (distal part of allantois) enlarges and spreads in between yolk sac and amnion on one side and chorion on the other side, the splanchnic mesoderm of allantois comes in contact with somatic mesoderm of chorion and fusess with each other to form **chorio-allantoic membrane**. Thus, it consists of ectoderm on the outer side, combined mesoderm of allantois and chorion, and endoderm on the inner side. The expanding chorio-allantois bursts through the vitelline membrane of the egg and pushes outward towards the shell membrane and shell. Gradually chorio-allantois envelopes the albumen forming a sac filled with albumen, called **albumen-sac**. It helps in the absorption of water and albumen.

Network of blood vessels develops in the allanto-chorionic mesoderm, which communicates with the embryonic circulation through umbilical cord. This communication persists until chick breaks the egg shell and starts breathing air. Now umbilical vessels close, circulation stops and allantois dries up and separates from the chick's body.

Functions of allantois :

1. Cavity of allantois serves as a urinary bladder, it stores the embryonic excretory wastes such as crystals of uric acid (water insoluble), upto the time of hatching.

2. Vascular chorioallantoic membrane lies in close proximity to the inner surface of porous shell. Thus, it serves as a respiratory surface for gaseous exchange.

3. Allantois with chorion surrounds the albumen to form albumen sac and thus helps in the absorption of albumen.

• SUMMARY

1. Extra-embryonic membranes are found in reptiles, birds and mammals.

2. These do not enter into the formation of embryo.

3. Extra-embryonic membranes are yolk sac, amnion, chorion and allantois.

4. Amnion and chorion are formed of somatopleure, i.e., extraembryonic ectoderm and somatic mesoderm.

5. Yolk sac and allantois are formed of splanchnic mesoderm and endoderm.

6. Subcephalic pocket is the space beneath the head part of the embryo formed by the cephalic fold anterior to the head end of embryo.

7. Yolk sac is the first formed extraembryonic membrane and it is formed of splanchnopleure (endoderm and mesoderm). Mesoderm is richly supplied with blood vessels.

8. Yolk sac septa are the folds of endoderm of yolk sac dipped into the yolk.

9. Amnion and chorion develop together and are formed of ectoderm and mesoderm (somatopleure). Extraembryonic folds are cephalic, laterals and caudal.

These folds join with each other over the embryo to enclose it completely. All these folds are double. Inner layer of somatopleure around the embryo is called amnion that encloses the embryo within fluid-filled cavity called amniotic cavity.

10. Seroamniotic raphe is the scar left after the union of body folds over the embryo.

11. Chorion on the outer side of amnion is also formed of somatic mesoderm and ectoderm. Its mesoderm later unites with the mesoderm of allantois to form a composite membrane which is richly supplied with blood vessels.

12. Allantois is the extension of hindgut of embryo formed of endoderm and splanchnic mesoderm (splanchnopleure). It spreads in the space in between yolk sac and amnion and chorion. Proximal part of allantois is narrow called allantoic stalk.

13. Allantoic stalk and stalk of yolk sac are enclosed together forming umbilical cord.

14. Amion protects the embryo from desication, outer mechanical shocks, friction against shell and protects the embro from adhesion against the shell.

15. Allantois serves as urinary bladder. Excretory wastes of embryo are stored in the allantoic vesicle. Allantochorion serves as respiratory surface for the gaseous exchange of the embryo.

16. Absorption of albumen also takes place through allantochorion membrane.

• TEST YOURSELF

Long Answer Type Questions

1. What are extra embryonic membranes? Describe the origin and development and functions of amnion and chorion.
2. Justify why allantois is known as urinary bladder of the developing chick. Discuss the importance of chorioallantois in chick
3. What are embryonic membranes? In what way are they different from egg membranes?

Short Answer Type Questions

1. What are foetal membranes ?
2. Compare the formation of amnion and chorion in chick.
3. How does allantois develop in chick?
4. What are the functions of amnion and chorion?
5. What is umbilical cord? What are its functions? What is the role of albumen sac in chick?

Very Short Answer Type Questions

1. **What are foetal membranes?**

   Ans. Foetal membranes are yolk sac, aminion. Chorion and allantois. These are essential for the development of embryo.

2. **What is sometopleure and splanchnopleure?**

   Ans. Somatopleure consists of extra embryonic ectoderm and somatic mesoderm. **Splanchnopleure** consists of extra embryonic endoderm and splanchnic mesoderm.

3. **Write about amnion**

   Ans. Amnion encloses the developing embryo in a salty fluid-filled cavity amnitic cavity. Amnion consists of ectoderm and somatic mesoderm.

4. **What is the function of amnion?**

   Ans. Amnion protects the embryo from desication in a fluid-filled cavity amniotic cavity. It also protects from mechanical shocks, adhesion to the shell etc..

5. **What is chorion?**

   Ans. Chorion develops along with amnion. It is outer to amnion and encloses extra embryonic coelom which is continuous with embryonic coelom.

6. **What is umbilical chord?**

   Ans. Allantoic stalk and yolk stalk are enclosed together in a membrane to form umbilical chord.

7. **What is chorio-allantoic membrane?**

   Ans. Somatic mesoderm of chorion and splanchnic mesoderm of allantois fuse with each other to form a composite membrane which is richly vascular and is called chrio-allantoic membrane.

8. **What is albumen sac?**

   Ans. Chorioallantoic progressively envelopes the albumen forming a sac filled with albumen, called albumen sac.

UNIT

11

PLACENTA IN MAMMALS

STRUCTURE

LEARNING OBJECTIVES

After going through this unit you will learn :

- Study of implantation of blastocyst (developing embryo) in the wall of uterus.
- Study of two basic types of placenta; Chorio-vitelline placenta and chorio-allantoic placenta
- Study of chorio-vitelline placenta (chorionic placenta or yolk sac placenta)
- Study of chorio-allantoic placenta; its morphological and histological study.
- Study of embryonic nutrition in mammals.
- Functions of placenta or physiology of placenta.

11.1. PLACENTA

Placenta is a Greek word it means a **flat cake**. Human placenta is flat, rounded mass, whose shape is more or less like a pancake. Placenta is formed jointly by the extra-embryonic membranes of the foetus (embryo) and maternal tissues. The developing embryo (foetus) of viviparous mammals obtains its nourishment from the maternal uterine tissue. Placenta allows the maternal and foetal blood to come in close proximity for mutual exchange of substances. Placenta allows some materials to enter the foetal body, which are chemically quite similar to others that are rejected.

Placentation is the mode of formation of placenta and its fusion to the uterine wall. In all **viviparous animals**, development of young one takes place inside the mother's uterus, because amount of yolk stored in the egg is not sufficient for the development of an embryo. Therefore, the developing embryo depends on the mother for nourishment and oxygen supply, etc.

Besides eutherian mammals, placenta is also found in ***Peripatus*** (Onychophora), ***Salpa*** (Tunicata), ***Mustelus laevis*** (elasmobranch fish) and certain lizards. But the nature of tissues entering into the formation of placenta is not same in all cases. In placenta of fishes and reptiles, yolk sac comes in close relation with maternal blood stream and vitelline circulation carries the materials to and from the embryo.

• 11.2. IMPLANTATION

The process of attachment of blastocyst into the uterus and start getting nutrition from the mother is called **implantation.** In implantation, blastocyst is closely held against uterine mucosal epithelium. Uterine wall and uterine capillaries come closer to the embryo and become more permeable. Now mucosal lining of uterus (*i.e.*, **endometrium**) around the embryo shows sign of **decidual wall reaction** (DCR) : Epithelium becomes disrupted and loosely packed fibroblast-like cells of stroma are transformed into large rounded glycogen filled cells, which are tightly bound with each other. Number of cells increases and vascularization area also increases. Thus, decidual cells form an **implantation chamber** around the embryo. In all species, DCR is not a precondition for implantation. Study of implantation in rats and mice showed that before implantation uterine wall is prepared by two hormones of ovary, progesterone and oestrogen. The immediate stimulus for sensitization is given by a small amount of oestrogen that acts on uterus on the morning of fourth day of pregnancy. After a period of several days progesterone is produced by developing corpora lutea. It is the dominant hormone. Progesterone acts on uterine cells which are sensitized by oestrogen hormone. Thus, progesterone and oestrogen hormones are of special importance in preparing uterus for implantation of blastocysts, in maintaining pregnancy and regulating the accessory organs during reproductive cycle.

Types of Implantation : Pattern of implantation of blastocyst is of the following types :

1. **Superficial or central implantation :** In most ungulates (pig), carnivores (lion) and monkey, implantation is superficial. In this type, blastocyst remains unembeded in the uterine cavity.

2. **Interstitial implantation :** In hedgehog, guinea pig, some bats, ape and man, blastocyst burrows into endometrium.

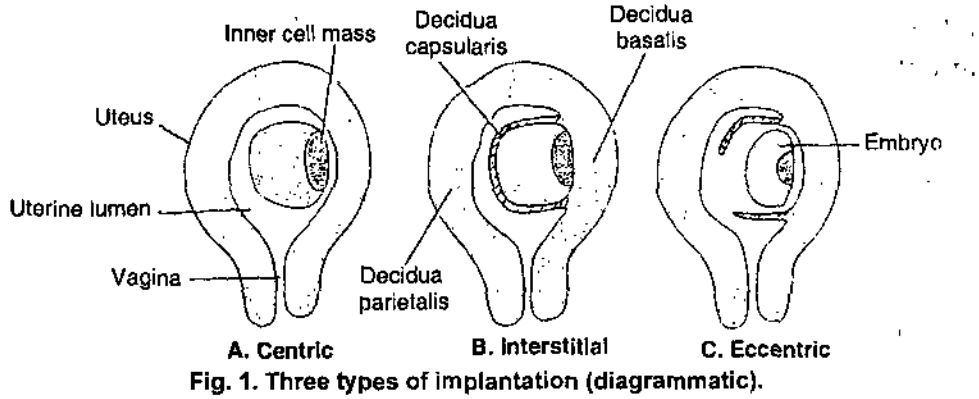


Fig. 1. Three types of implantation (diagrammatic).

3. **Eccentric implantation :** In beaver, rat, squirrel and some rodents, chorionic sac lies for a time in a fold or pocket, which looses off from the main cavity. Implantation is primarily eccentric. Later it becomes interstitial. In human female, maternal component consists of that part of uterine wall (endometrium) that sloughs off at the termination of pregnancy. The name 'decidua' meaning to shed refers to pregnant endometrium.

Parts of endometrium

1. **Decidua basalis :** Part of endometrium that is directly lying beneath the embryo. Part of the embryo which is in contact with decidua basalis contribute to the placenta.

2. **Decidua capsularis :** This is thin portion of endometrium on the lumen side of uterus that covers the implantation site.

3. **Decidua parietalis :** Part of endometrium lining the uterus other than at the implantation site.

11.3. TYPES OF PLACENTA

From the point of origin of placenta, it consists of two parts : **a foetal placenta** formed by the extra-embryonic membranes of foetus and a **maternal placenta** formed by the uterine endometrium.

In mammals, there are two different types of placentae :

1. Chorio-vitelline placenta
2. Chorio-allantoic placenta

1. Chorio-vitelline placenta or Chorionic placenta or Yolk sac placenta

It is primitive type of placenta found in some marsupials, *e.g., Didelphis* (opossum) and *Macropus* (kangaroo). In yolk sac placenta, allantois remains relatively small and never makes contact with chorion. Yolk sac becomes very large and fuses with the chorion. This type of placenta develops from the trophectoderm (single layered) that encloses the blastocoele. This is chorionic placenta. Later it becomes two layered because blastocoele acquires endodermal lining and hence it is called **yolk sac placenta**. Later in many species, it becomes three layered, due to the development of extra-embryonic mesoderm and its vascular supply between trophectoderm and endoderm, i.e., mesoderm. Now it is called **chorio-vitelline placenta**. Thus, in this type of placenta, allantois remains small and never makes contact with chorion. Chorion remains in close apposition with vascular uterine lining the endometrium.

In rodents and rats, distal part of yolk sac breaks down due to which endoderm of proximal part of yolk sac lies in close proximity with uterine epithelium. This is called **inverted yolk sac placenta,** because endoderm of yolk sac faces the uterine endometrium. It coexists with chorio-allantoic placenta till parturition.

Chorio-Allantoic Placenta :

In some marsupials like *Perameles* and *Dasyurus,* and all eutherians yolk sac remains rudimentary and allantois becomes well developed and vascularized. It fuses with chorion and provides blood supply. Such placenta is called **chrio-allantoic placenta**. Chorion bears root-like processes, called the villi which develop from chorion to enter the adjacent maternal tissue. In this type of placenta chorion is lined with allantois and associated with uterine wall

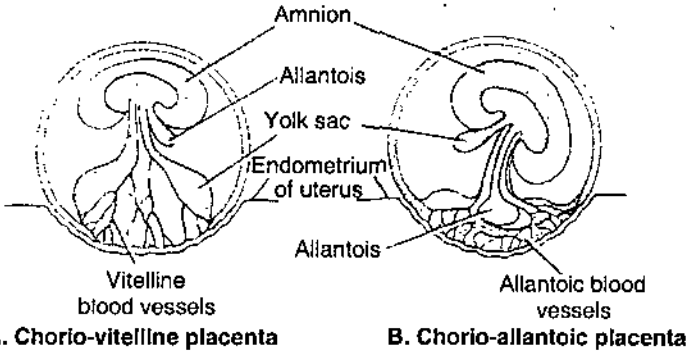


Fig. 2. Two types of mammalian placentae.

during gestation period. As the embryo develops, it separates from the foetal membrane and only umbilical cord connects the foetus with the placenta.

This placenta consists of chorionic villi and each villus has an outer chorionic ectoderm, inner allantoic endoderm and in between these two is present vascularized mesodermal connective tissue layer. Mesoderm is a composite membrane or layer formed of somatic mesoderm and splanchnic mesoderm. Ectoderm cells have microvilli which increase the absorptive area for the absorption of nutrients.

Maternal part of placenta consists of endometrial epithelium and stroma of endometrium with glands and uterine blood vessels

• 11.4. CLASSIFICATION OF CHORIO-ALLANTOIC PLACENTA

Its classification is based on its morphology, arrangement of villi and histology.

A. Morphological Classification of Placenta

1. Non-deciduate placenta : Blastocyst or embryo lies in the cavity of uterus in contact with uterine wall. From blastocyst arise finger-like outgrowths, villi which penetrate into depressions in the wall of uterus. These chorionic villi have blood vessels either of branches of allantoic blood vessels or vitelline blood vessels, in case of chorio-vitelline placenta.

At the time of birth (parturition), chrionic villi are drawn out from depressions in the wall of uterus. Thus, maternal and foetal tissues are separated without any damage to the uterine wall and no bleeding takes place. This type of placenta is found in pigs, cattle and some other mammals.

2. Deciduate Placenta (Placenta vera) : In deciduate placenta the intimacy between maternal and foetal tissues becomes more. Walls of uterus are eroded to various degrees by the action of trophoblast and embryonic tissues penetrate into uterine wall resulting into more close contact that facilitates passage of substances from the mother to the foetus and from foetus to the mother.

Here chorionic villi fuse with eroded uterine mucosa, hence placenta is called **placenta vera** (true placenta).

At the time of birth, foetus with its membranes (including chorion) is removed, extensive haemorrhage from uterine wall takes place. Such placenta is found in higher mammals.

After parturition, haemorrhage is normally stopped by the contraction of muscular wall of uterus, that constricts blood vessels and gradually slows down the blood flow.

3. Contra-deciduate placenta : In this type of placenta, there is loss of maternal tissue and foetal part of the placenta. Both these tissues are absorbed in situ by maternal leucocytes. It is found in *Perameles* and *Talpa* (mole).

B. Classification of Placentae on the Basis of Distribution of Villi on Chorion :

The pattern of distribution of villi on chorion varies in different mammals. On the basis of distribution of villi, following kinds of placentae have been found:

1. Diffuse placenta : The villi are found scattered on the surface of chorion. Such placentae are called diffuse, and are found in ungulates, e.g., pig, mare etc.

2. Cotyledonary placenta : In this type, villi are found in groups over the chorion and the remaining surface remains smooth. These groups of villi are called **cotyledons.** This is found in ruminants ungulates such as cattle, sheep, goat, deer, etc.

3. Intermediate placenta : In camel and giraffe, isolated villi are found scattered between the cotyledons. Thus, blastocyst has cotyledons and also interspersed single villi.

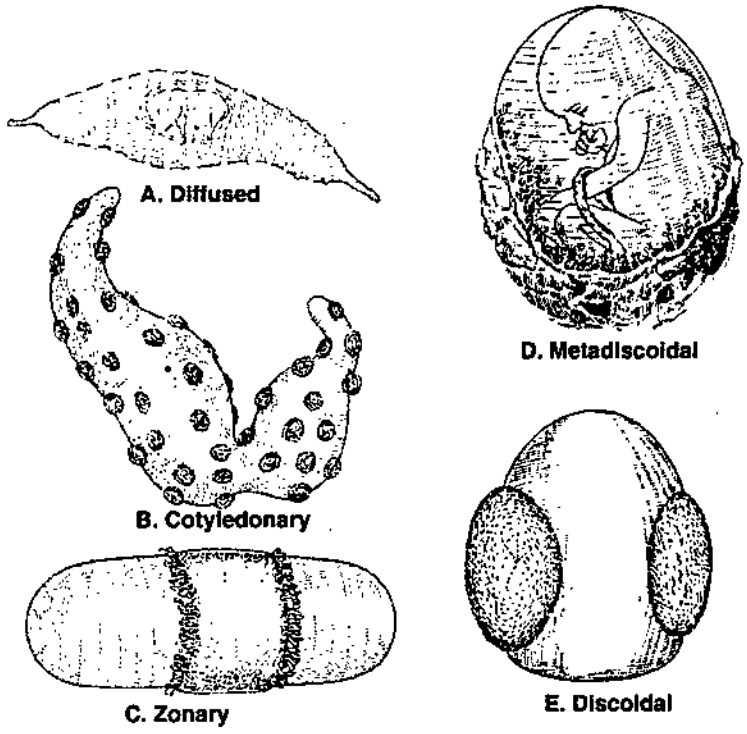


Fig. 3. Various types of placentae on the basis of morphology.

4. Zonary placenta : In this type, villi are developed in the form of girdle-like band around the middle of blastocyst (chorion). Such placenta is found in carnivores, e.g., cats, dogs, lion, tiger, fox, mongoose etc.

5. Discoidal placenta : In this case, villi are restricted on a circular disc or plate on the dorsal surface of blastocyst. It is found in insectivores, bats, rodents (rat, guinea pig, porcupine) and rabbit. In monkeys, placenta consists of two discs and hence placenta is called **bidiscoidal.**

6. Metadiscoidal placenta : It is found in human beings and anthropoid apes. The early blastocyst is at first all covered with villi, but later on it becomes restricted to a small disc-shaped area that is attached to the uterine wall.

C. Histological Types of Placenta

Foetal placenta has allantoic blood vessels in chorio-allantoic placenta and blood vessels run to and from the foetus. On the other side, maternal blood vessels run to and from the maternal placenta present in the uterine endometrium. These two circulations come very close together, but not fuse with each other. Foetal blood does not circulate in the mother and maternal blood also does not circulate in the foetus. All the nutrients, oxygen, carbon doxide and excretory product (urea) pass from mother circulation to the foetus and vice versa from foetus to mother through tissue barriers present between blood of mother and of foetus.

Thickness of partition between foetal and maternal blood decreases by removal of some layers of tissue (barriers) present between the two bloods. On the basis of which layers have disappeared, placentae are of the following types:

1. Epitheliochorial placenta : This is most primitive type of placenta. In this type six tissues (membranes) called barriers are present in between foetal and maternal blood streams :

1. Endothelium of maternal blood vessels
2. Endometrial connective tissue
3. Uternine epithelium

] Maternal part of placenta

4. Ectoderm of chorion (chorionic epithelium)
5. Chorionic connective tissue (foetal mesenchyme)
6. Endothelium of foetal blood vessels.

] Foetal part of plancenta

In this case immediate contact of the two tissues (mother and foetus) involves chorionic epithelium and uterine epithelium, hence placenta is called epithelio-chorial placenta. Villi of foetal placenta penetrate in the wall of uterus (pocket-like depressions of uterine wall). It is found in marsupials, ungulates (pig, horse sow, cattle, etc) and lemurs.

2. Syndesmo-chorial placenta : In this type of placenta, uterine epithelium disintegrates, and hence, foetal and maternal components are intimately fused. The chorion comes in contact with connective tissue of uterine mucosa. It is found in ruminant ungulates (sheep etc).

3. Endothelial-chorial placenta : Uterine mucosa is reduced and thus, chrionic epithelium comes in contact with endothelial wall of uterine blood vessels. In this type, thus, only four barriers between foetal and maternal blood streams are present. This is found in carnivores.

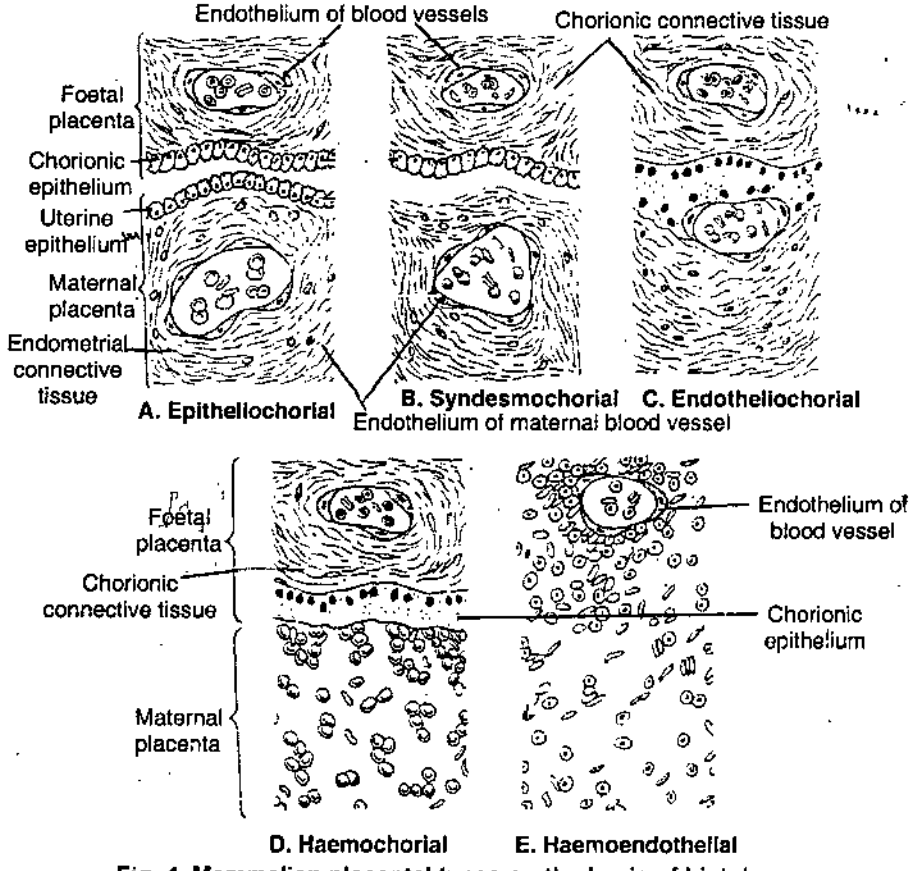


Fig. 4. Mammalian placental types on the basis of histology.

4. Haemochorial placenta : In this type of placenta, endothelial walls or uterine blood vessels also disappear and chrionic epithelium is directly bathed in maternal blood. Chorionic villi are surrounded by sinuss (spaces) devoid of endothelial lining into which maternal blood enters through arteries of uterus and from sinuses blood flows into uterine vein. This placenta is found in primates, insectivores (moles, shrews) and chiropters (bats).

5. Haemo-endothelial placenta : In this type of placenta, number of barriers between maternal and foetal blood streams is reduced to two. Chorionic villi loose their epithelial and mesenchymal layers to such a degree that, in most places, bare endothelial lining of their blood vessels alone separates the foetal blood from maternal tissues. It is found in mouse, rat, guinea pig, rabbit etc.

Embryonic Nutrition in Mammals

Mammalian egg has no yolk, hence during the first few days embryo metabolizes its endogenous reserves of glycogen and glucose for its energy needs. Thus, embryo weight diminishes.

Before implantation, embryo receives its nutrition from the mother. Uterine milk serves as embryonic food until implantation. Uterine milk is a mixture of secretion from endometrial glands and tissue debris. After implantation, in many species decidual cells may have a nutritive value before the formation of a true placenta.

After the development of a true placenta, nutrients are taken up by diffusion, active transport and endocytosis by microvilli of trophoblast epithelium covering the chorion. Glycogen and glucose may be the principal source of energy for the developing embryo.

Physiology of Placenta
(Functions of placenta)

1. Nutritive Function : Foetus gets its nutrients from the maternal blood. These nutrients are : Glucose is the principal source of energy of foetus; lipids (triglycerides, fatty acids and cholesterol) are for foetal growth and development of foetus; amino acids for the synthesis of proteins by the foetus; water and electrolytes (sodium. potassium, chloride, calcium, phosphorus, iron and iodine) cross through foetal membrane by simple diffusion and active transport; water soluble vitamins transferred by active transport and fat soluble vitamins by simple diffusion; hormones.

2. Respiratory Function : Intake of oxygen and output of carbon dioxide takes place by simple diffusion through placental barrier. Oxygen from maternal blood diffuses into foetal blood, similarly carbon dioxide from foetal blood diffuses into maternal blood through placenta.

3. Excretory Function : Waste products of foetus like urea, uric acid and creatinine are excreted from foetus to maternal blood through placenta by simple diffusion.

4. Numerous enzymes such as diamine oxidase,: Oxytocinase and phospholipase-A are produced by the placenta.

5. Endocrine Function : Mammalian placenta acts temporarily as an endocrine organ. In rat on 12th day of pregnancy, placenta secretes **rat chorionic mammoluteotrophin** that maintains corpora lutea and later **progesterone** that is responsible to continue pregnancy upto birth. Similarly,

horse placenta secretes **pregnant mare serum gonadotrophin**, progesterone and oestrogens.

Human placenta produces various proteins and steroid hormones.

A. Protein hormones : These are oestrogens and progesterones.

HCG is secreted by syncytiotrophoblast of the placenta about ten days after ovulation. It stimulates the corpus luteum to sustain the secretion of progesterone. Traces of HCG hormone can be detected in maternal serum or urine after 7 to 10 days of fertilization. HCG contents greatly increase between sixty to seventy days of pregnancy.

Progesterone helps in the maintenance of pregnancy and prevents parturition.

Relaxin is produced by placenta and causes the relaxation of the symphysis and sacro-iliac joints during pregnancy and also reduces tension of cervix.

Barrier Functions of Placenta :

Substances of high molecular weight of more than 500 daltons are held by the placenta. But **leucocytes** are more numerous in the blood of umbilical vein than in that of umbilical artery. It is possible that they migrate from the maternal blood through the placental barrier into foetal capillaries.

Antibodies and antigens are found to traverse across the placental barrier in both directions. Thus, antibodies developed in mother's blood against diseases like diphtheria, measles, small pox and scarlet fever are passed from mother's blood into foetus and develop immunity against such diseases. Similarly anti-Rh factor (Rh-antibody) of Rh-negative mother enters in the blood circulation of Rh-positive foetus, and develops anaemia by destroying red blood cells of foetus.

During pregnancy, maternal infection by **viruses** of rebella or German measles, chicken pox, measles, mumps and poliomyelitis; **bacteria** (*Treponema pallidum* (syphilis, *Tubercle bacillus*) or **protozoa** of malarial parasite may be transmitted to foetus across the placental barrier and affect the foetus *in utero*.

Any drug used in pregnancy can cross placental barrier and may cause harmful effect on foetus. For instance, sleeping drug, **thalidomide** used as a sedative and to avoid nausea and morning sickness during early pregnancy (25 to 44 days) causes deformity in limb development in foetus, perforation of anus, and development of defective heart.

• SUMMARY

1. Placenta is a Greek word, meaning a flat cake. Human placenta is flat, rounded mass, shape more or less like a pancake.

2. Placenta is jointly formed by extra-embryonic membranes of foetus and maternal tissues.

3. Placenta allows maternal and foetal blood to come in close proximity for mutual exchange of substances like nutrients, oxygen and carbon dioxide and urea etc.

4. Process of attachment of blastocyst (embryo) into the uterus and start getting nutrition from the mother is called implantation.

5. Before implantation the uterine wall is prepared by two hormones of ovary, progesterone and oesrtogen. These two hormones are responsible for preparing uterus for implantation of blastocyst (embryo), in maintaining pregnancy.

6. Implantation is of three types : superficial, interstitial and eccentric.

7. Endometrium (uterine wall) has three layers : decidua basalis lying beneath the ovary, decidua capsularis and decidua parietalis.

8. Placenta is of two types – foetal placenta formd by extra-embryonic membranes of foetus and maternal placenta formed by uterine endometrium.

9. Placenta is of two types : Chorio-vitelline placenta and chorio-allantoic placenta.

Chorio-vitelline placenta has small allantois and never makes contact with chorion and yolk sac is very large fuses with chorion. Chorio-allantoic placenta has rudimentary yolk sac but allantois becomes well developed and vascularized.

10. Morphologically placenta is of three types : Non-deciduate, deciduate and contra-deciduate.

In non-deciduate placenta, embryo lies in the cavity of uterus in contact with uterine wall. Blastocyst gives off finger-like villi which penetrate into pits in the wall of uterus. At time of parturition, chorionic villi are withdrawn from pits without damaging the uterine wall, e.g., pigs, cattle etc.

In deciduate placenta, maternal and foetal tissues are very intimate, chrionic villi fuse with eroded tissues of uterus. At parturition, foetus with its membranes including chorion is removed causing extensive damage to the uterine wall rsulting into haemorrhage. In contra-deciduate placenta, loss of maternal tissue and foetal part of placenta takes place, which are absorbed in situ by maternal leucocytes.

11. On the basis of distribution of villi on chorion, placenta is of six types : diffuse, cotyledonary, intermediate, zonary, discoidal and metadiscoidal..

12. On the basis of histology of placenta, it is of five types : Epithelio-chorial, syndesmo-chorial, endothelio-chorial, haemochorial and haemoendothelial.

13. Six types of tissues take part in the formation of placenta : three belong to foetus and three belong to mother.

Maternal placenta is formed of endothelium of maternal blood vessels, endometrial connective tissue and uterine epithelium. Foetal part of placenta involves ectoderm of chorion, chorionic connective tissue and endothelium of foetal blood vessels.

14. In epitheliochorial placenta, all the six tissues are present. It is found in marsupials, ungulates and lemurs.

15. In syndesmochorial placenta, uterine epithelium destroys, found in ruminant ungulates.

16. in endotheliochorial placenta, uterine epithelium and uterine connective tissue are destroyed, chorionic epithelium comes in contact with endothelium of uterine blood vessels.

17. Haemochorial placenta is found in primates, insectivores and bats. In it all the tissues of maternal placenta are destroyed, chorionic villi of foetal placenta lie in sinuses of uterine tissue filled with blood.

18. In haemo-endothelial placenta, along with destroyed uterine tissues, chorionic epithelium and connective tissue are also destroyd.

QUESTIONS

Long Answer Type Questions

1. Define placenta. Describe its formation and structure.
2. Classify the different types of plancenta. What are its functions ?
3. What are the various functions of plancenta ?

Short Answer Type Questions

1. Write about implantation.

Very Short Answer Questions

1. What is the meaning of placenta?

Ans. Placenta means a flat cake. This name is received from human placenta.

2. Define human placenta.

Ans. Human placenta is a flat rounded mass shaped like a pancake.

3. Define placentation.

Ans. Mode of formation and fusion of placanta with the uterine wall is called placentation.

4. What is implantation?

Ans. Process of attachment of embryo (blastocyst) to the uterine wall and start getting nutrition from the mother is called implantation.

5. What are the various parts of endometrium?

Ans. There are three layers in endometrium : innermost decidua basalis contributes to placenta, decidua capsularis lies on the lumen side, covering the implantation site and decidua parietalis lining the uterus beyond the implantation site.

6. Write about pattern of implantation of blastocyst.

Ans. 1. Superficial or central implantetion : Blastocyst remains unembeded in uterine cavity.

2. Interstitial implantation : Blastocyst burrows into endometrium.

3. Eccentric implantation : Chorionic sac lie in a fold or pocket for some time which looses off from the main cavity.

7. What are the two types of plancenta ?

Ans. Chorio-vitelline placenta and chorio allantoic plancenta.

8. Write the difference between the chorio-vitelline placenta and chorio allantoic placenta.

Ans. In chorio-vitelline placenta, allantois remains small and never makes contact with chorion and yolk sac becames very large, fuses with chorion.

In **chorio allantoic placenta,** yolk sac is rudimentary and allantois becomes well developed and richly vascularized, and fuses with chorion. Mesoderm of allantois and chorion fuse with each other and becomes vascularized.

9. On the basis of structure (morphology) what are the types of placentae?

Ans. Three types : Non-deciduate placenta, deciduate placenta and contra-deciduate placenta.

Non deciduate placenta. Blastocyst lies in the cavity of uterus in contact with uterine wall. Villi arise from blastocyst chorion penetrate into the depressions in the uterine wall. At the time of birth chorionic villi are withdrawn from the uterine wall pits without causing any damage to the uterine wall. Examples. Horse, ass, zebra and tapir.

10. On the basis of distribution of villi on blastocyst chorion, write the types of placentae.

Ans. 1. Diffuse in ungulates (pig, mare, etc.)

2. Cotyledonary in ruminants, ungulates (cattle, sheep, goat, deer etc.)

3. Intermediate in camel, giraffe.

4. Zonary in carnivores.

5. Discoidal in insectivores, bat, bear, rodents (rat, guinea pig, porcupine) and rabbit. In monkey bidiscoidal placenta.

6. Metadiscoidal in human beings

11. What types of placentae are found in mammals on the basis of histology of placenta?

Ans. Five types of placentae are found in mammals these are :

1. Epitheliochorial placenta found in marsupials, ungulates (pig, horse, sow, cattle) and lemurs.

2. Syndesmo-chorial placenta found in ruminant, ungulates (sheep cow)

3. Endothelio-chorial placenta found in carnivores.

4. Haemochorial placenta found in primates, insectivores and bats.

5. Haemoendothelial placenta found in mouse, rat, guinea pig, rabbit etc.

12. What are the various tissues (maternal and foetal) found in placenta?

Ans. Maternal and foetal tissues found in placenta are : Maternal tissues are endothelium of maternal blood vessels, endometrial connective tissue and uterine epithelium.

Foetal tissues are : Chorionic epithelium, chorionic connective tissue and endothelium of foetal blood vessels.

13. In which type of placenta, all the six tissues of mother and foetus placenta are present?

Ans. In epithelio-chorial placenta, all the six tissues are present.

14. Which type of placenta is found in carnivores and human beings ?

Ans. In carnivores endothelio-chorial placenta and in human beings haemochorial placenta are found.

15. Write about embryonic nutrition.

Ans. Uterine milk is the embryonic food until implantation. Uterine milk is a mixture of secretion from endometrial glands

After development of placenta nutrients are taken up by diffusion, active transport and endocytosis by villi of trophoblast epithelium (chorion). Glycogen and glucose may be the principal source of energy.

16. What are the various functions of placenta?

Ans. Placenta function is nutritive. Nutrients like glucose, lipids, amino acids etc from the mother reach the foetus through placenta.

Intake of oxygen and output of carbon dioxide takes place by diffusion through placental barrier.

Urea, urice acid and creatinine are excreted from foetus to maternal blood through placenta by diffusion.

Placenta also secretes certain hormones like progesterone, oestrogen and relaxin.