# UNIT - I

## GAMETOGENESIS

- The production of gametes is called gametogenesis.
- It occurs in the gonads.
- Gametes are of two types, namely spermatozoon and egg.
- The production of spermatozoon is called spermatogenesis.
- It takes place in the testis.
- The production of egg (ovum) is called oogenesis.
- It occurs in the ovary

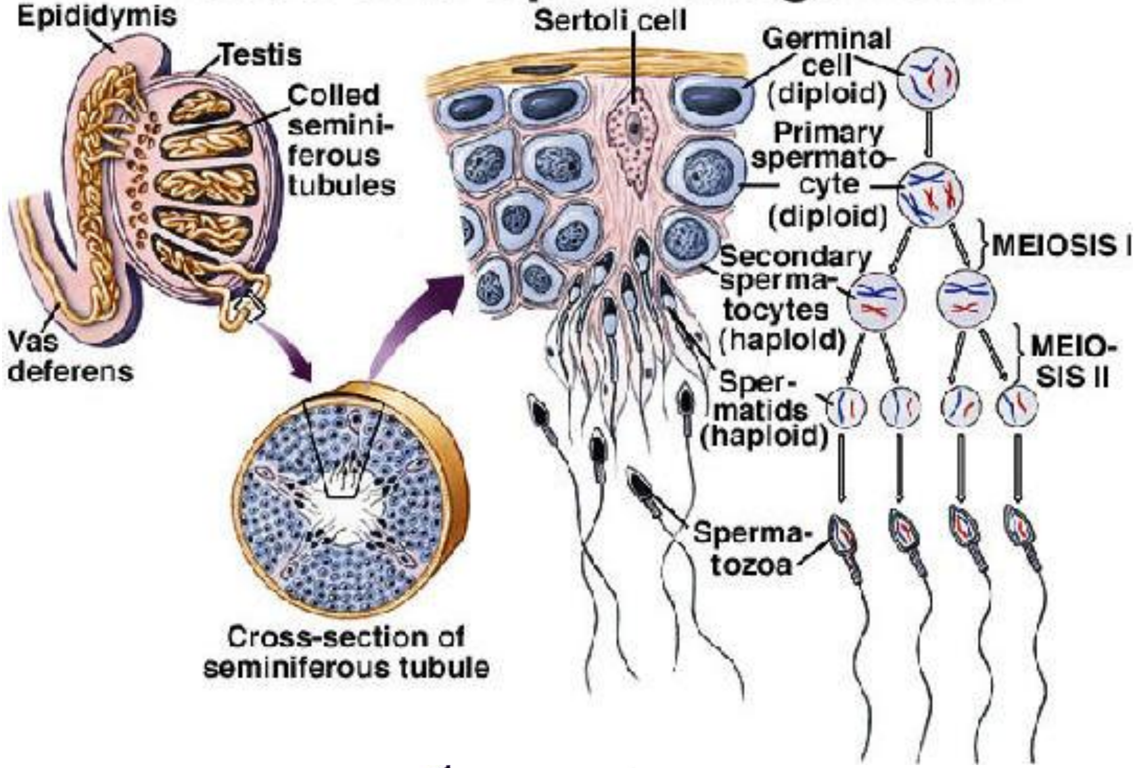
## SPERMATOGENESIS

- It refers to the formation of spermatozoa. Spermatozoa are formed in the testis.
- In each vertebrate, a pair of testes are found.
- Each testis is attached to the dorsal body wall by a connective tissue membrane called mesorchium.
- The testis is formed of thousands of minute tubules called seminiferous tubules.
- They lead into vasa deferentia. The seminiferous tubules are separated by interstitial cells
- Each seminiferous tubule is covered by a basement membrane and lined with germinal epithelium.
- The germinal epithelial cells are separated by giant cells called sertoli cells.
- The germinal epithelial cells develop into spermatozoa and the sertoli cells nourish the developing spermatozoa.
- The entire process of spermatogenesis has two stages, namely
    1. the formation of spermatid and
    2. the spermiogenesis.

### 1. Formation of Spermatid

- The spermatid is formed from the epithelial cells of seminiferous tubules.
- The germinal cells which develop into spermatids are called primordial germ cells.
- There are three phases in the conversion of primordial germ cells into spermatids. They are
    a. Multiplication phase
    b. Growth phase and
    c. Maturation phase

# Testis and Spermatogenesis



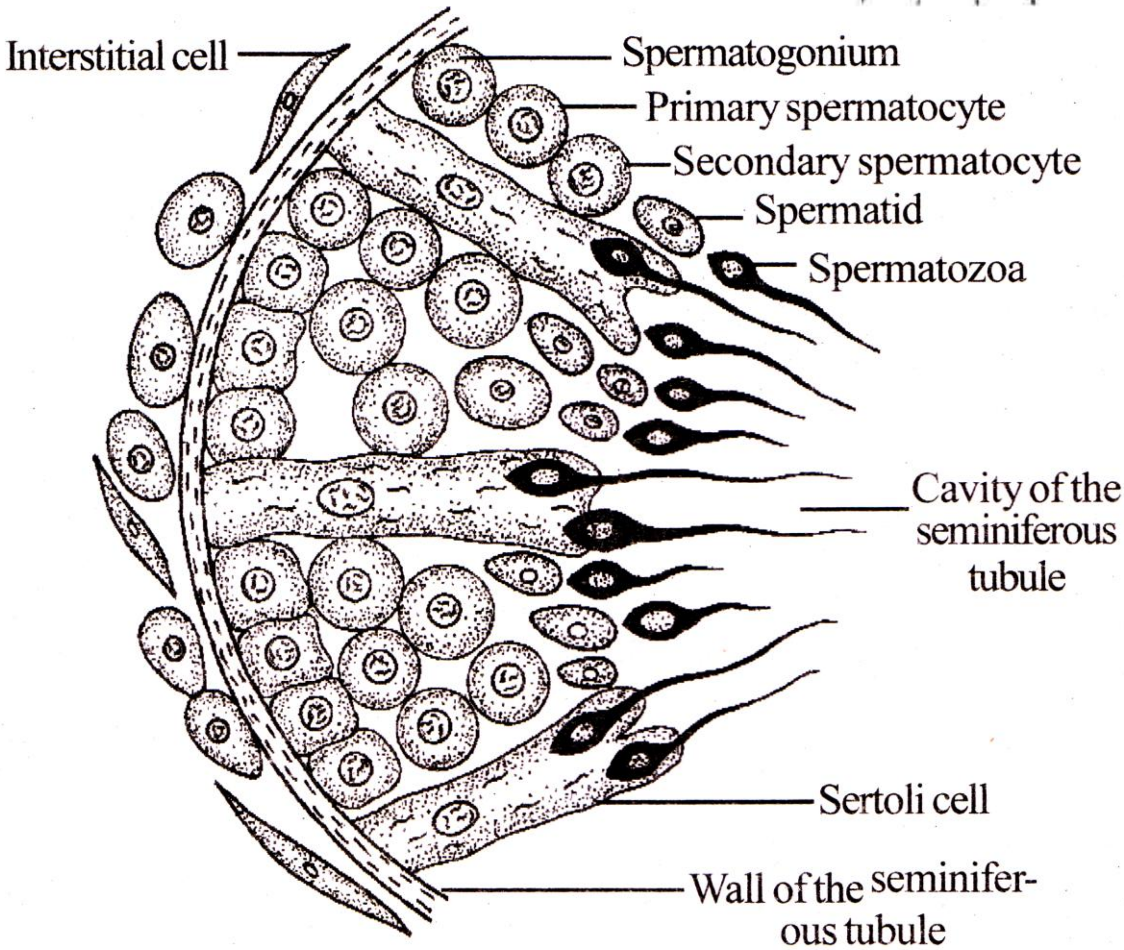



Fig. Transverse section of seminiferous tubule of mammalian testis

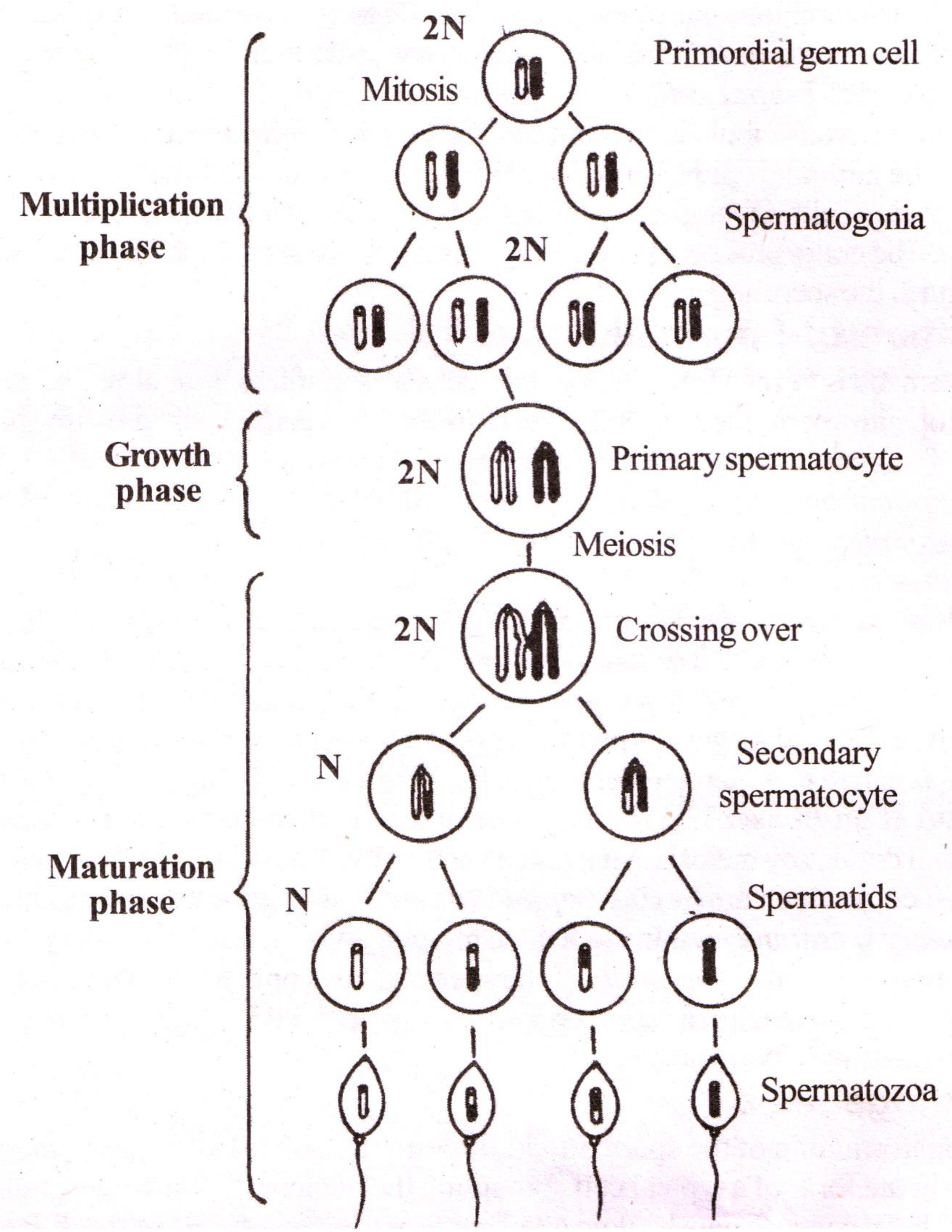



Fig. Spermatogenesis

a. **Multiplication Phase:**
    - ✓ The primordial germ cells are larger in size and their nuclei are distinct.
    - ✓ They undergo repeated mitotic divisions.
    - ✓ The resulting cells are called spermatogonia or sperm mother cells.
    - ✓ Each spermatogonium has a diploid number (2n) of chromosomes

b. **Growth Phase:**
    - ✓ During this phase, the spermatogonium grows.
    - ✓ The volume increases.
    - ✓ Now the cell is called primary spermatocyte.
    - ✓ It is also a diploid (2n) cell

c. **Maturation Phase:**
    - ✓ The primary spermatocyte, then enters the maturation phase where each cell divides by meiosis.
    - ✓ Meiosis consists of two divisions.
    - ✓ The first meiotic division produces two cells which are having haploid (n) number of chromosomes.
    - ✓ These cells are called secondary spermatocytes.
    - ✓ In the second meiotic division, each secondary spermatocyte divides into two cells called spermatids.
    - ✓ The spermatid has only haploid number of chromosomes.
    - ✓ Thus by meiosis each primary spermatocyte is converted into four spermatids.
    - ✓ The spermatids differentiate into spermatozoa

**2. Spermiogenesis**

- The transformation of the spermatid into spermatozoon is called spermiogenesis.
- The spermatid is in the form of a typical cell containing the nucleus, Golgi bodies, mitochondria, centriole, etc. but it contains only haploid number of chromosomes.
- During the differentiation of the sperm, all these organelles undergo changes
    - The nucleus gradually diminishes in its size by losing water
    - The chromosomes become concentrated and are closely packed
    - All the achromatic substances, nucleolus and RNA disappear
    - The nucleus becomes elongated

- The Golgi bodies develop into the acrosome. The small vacuoles gradually fuse together to form large vacuoles. It is now called acroblast. Inside the acroblast, a dense body called proacrosomal granule is developed. The whole of the acroblast spreads over the front part of the nucleus. The proacrosomal granule enlarges to form the acrosomal granule. The acroblast is now called acrosome which forms the cap of the sperm. The acrosome and the nucleus together constitute the head. The remnants of the Golgi body degenerate and eventually get discarded.
- The centrosome of the spermatid contains two centrioles. They move towards the nucleus and occupy a position behind it. One is the proximal centriole and other centriole is called the distal centriole takes up a position behind the proximal centriole.
- The distal centriole develops a filament-like structure called filament. It gradually elongates and forms the tail by developing a cytoplasmic sheath. The distal end of the axial filament is free from the cytoplasmic sheath and it is named endpiece.
- The mitochondria of the spermatid are aggregated together to form a large mass in the region of the centrioles. This is called mitochondrial cloud. This region forms the middle piece
- The cytoplasm flows backwards and forms a thin layer around the nucleus, middle piece and tail
- When the spermatozoa are developing, their heads are embedded in the cvtoplasm of the Sertoli cells and their tails protrude into the lumen of the seminiferous tubules. When they are fully matured, they are released and are carried away by the ciliary movement of the lining of the vasa efferentia to the vas deferens. They are stored in the seminal vesicles to be released when required
- The sperms undergo morphological differentiation in the seminiferous tubules of the testis. By this change they attain the typical structure of a sperm. But they do not possess the ability to fertilize the egg. They attain the ability to fertilize the egg by a process called physiological ripening. In mammals, the physiological ripening of the sperms takes place in the epididymis while they pass through it. In frogs, the sperms undergo physiological ripening in the seminiferous tubules itself

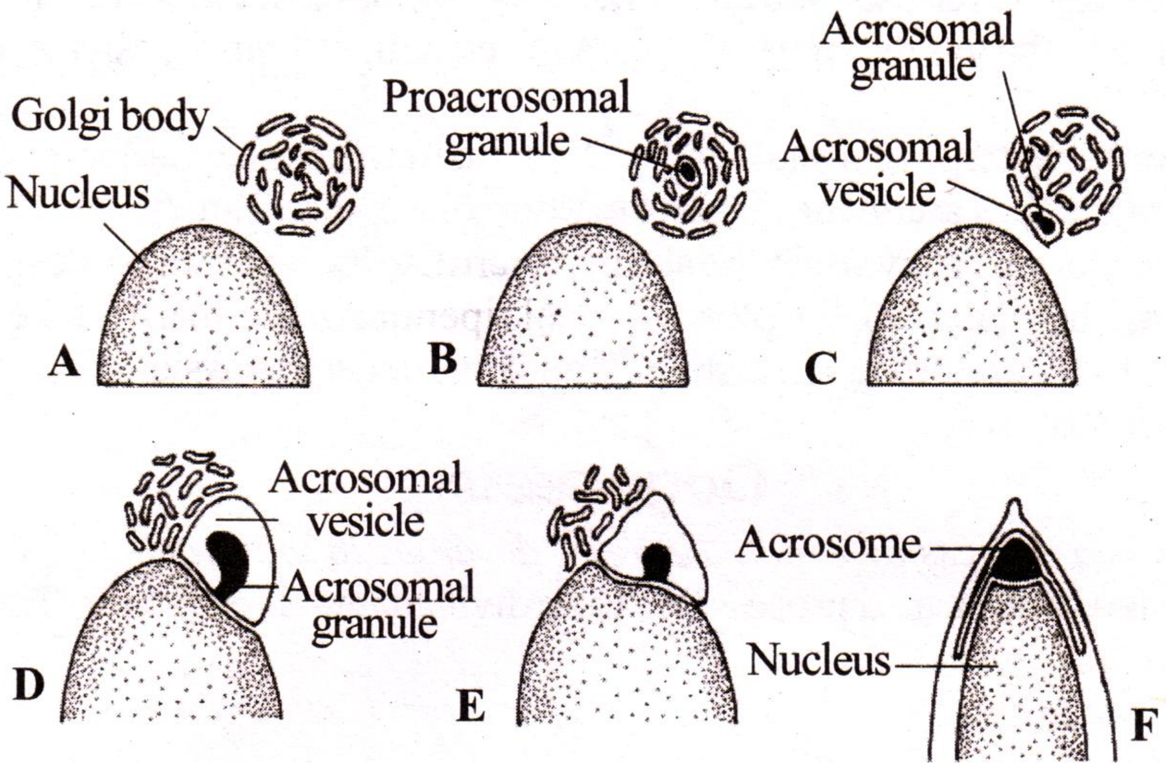



Fig. Spermiogenesis: formation of acrosome and head

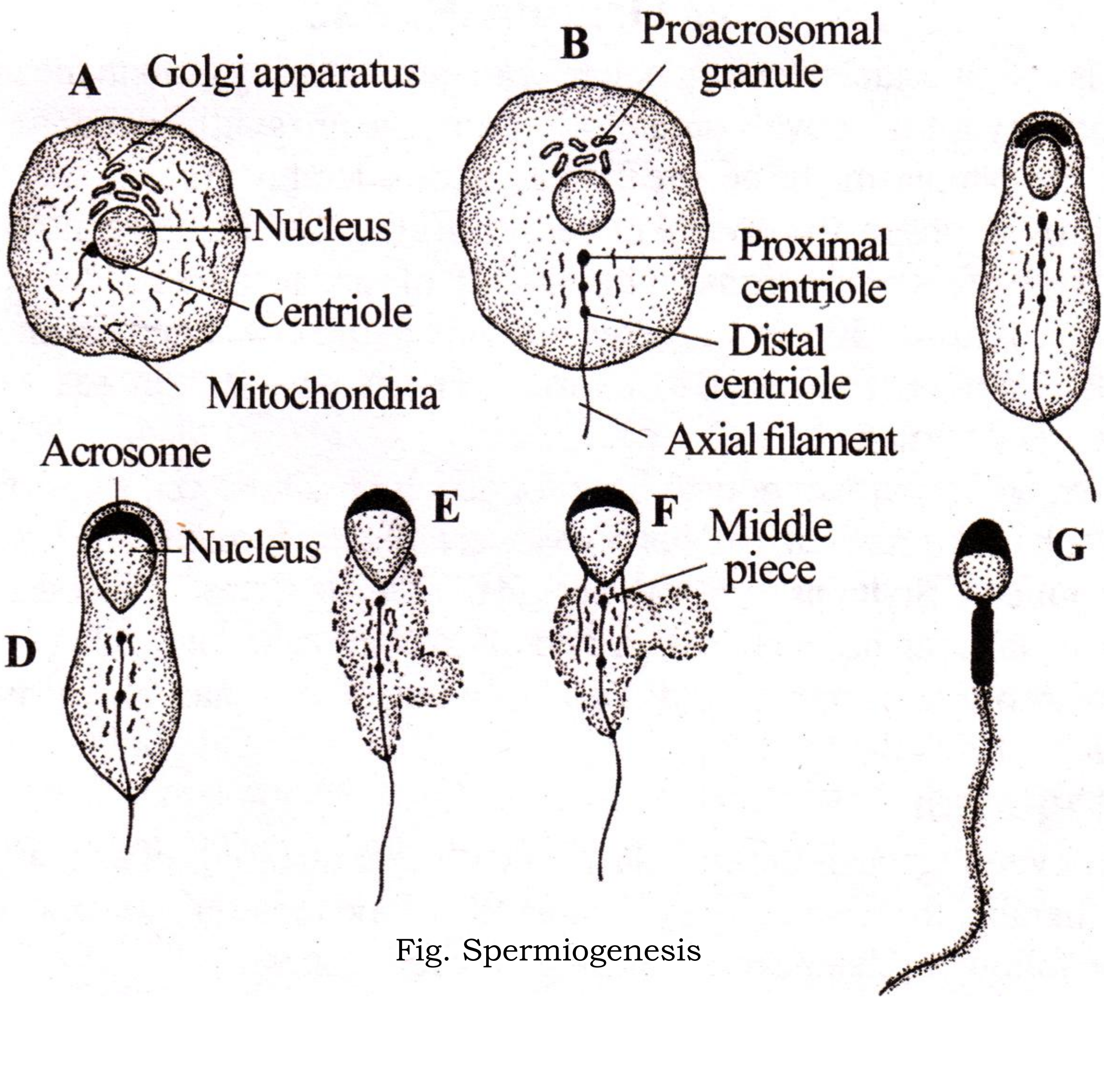



Fig. Spermiogenesis

**TYPES OF SPERM**

- The type of the sperm produced varies from species to species.
- The size of the sperm may be as little as 0.018 mm in *Amphioxus* or as large as 2.25 mm or more in toad.
- The sperm head is species specific.
- It may be spheroidal (teleosts), rod or lance-shaped (amphibians), spoon-shaped (man and many other mammals), or hooked (mouse and rat).
- The sperm types are again divided into two main types found in animals- flagellate spermatozoa, which posses a flagellum or tail like biflagellate (in a toad fish) spermatozoa. The non-flagellate spermatozoa lack flagella and are found in Ascaris, crab etc.

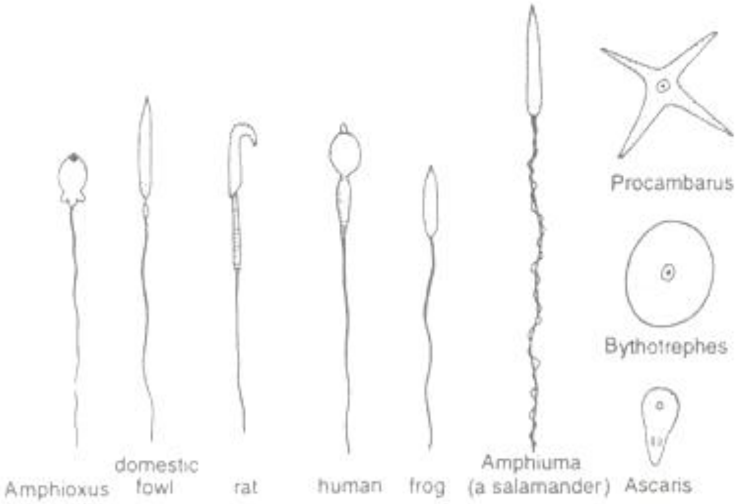



Fig. Sperm of some animals. **Procambarus** and Bythotrephes are **crustaceans.**

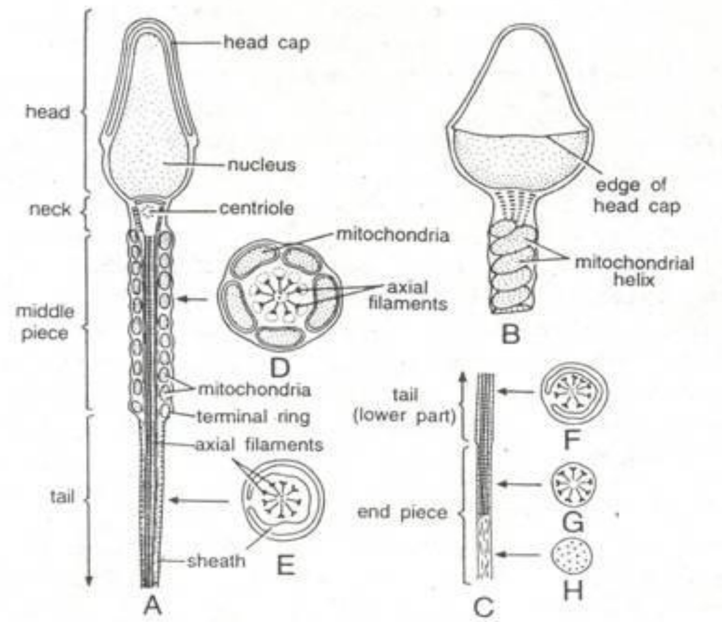



Fig. Electron micrographs of human spermatozoa showing the structure A—L.S. of spermatozoa, B—Head, together with neck and middle piece, C—terminal part of tail proper and endpiece and D-H— highly magnified T.S. of middle piece and tail at various levels.

- Domestic animals are broadly classified as seasonal and non-seasonal breeders depending upon the number of times they breed during a year.
- Seasonal breeders are those that have specified period of time in a year during which they actively breed.

**Significance of spermatogenesis:**

- ✓ provide haploid motile sperm
- ✓ held in causing variations in offspring

# OOGENESIS

- Oogenesis **is** a process by which the ovum develops in the ovary
- Oogenesis is a complicated process. It can be divided into three phases. They are

  I. Multiplication phase

  **II.** Growth phase **and**

  III. Maturation phase

**I. Multiplication phase**

- The germinal epithelial cells of the ovary detach themselves from the surface and enter the cortex of the ovary.
- These cells are called primordial germ cells.
- They divide repeatedly by mitosis and the resultant cells are called oogonia or egg mother cells.
- The oogonia again divide repeatedly by mitosis.
- When the division stops, the cells are named as primary oocytes. The nucleus of primary oocyte is diploid

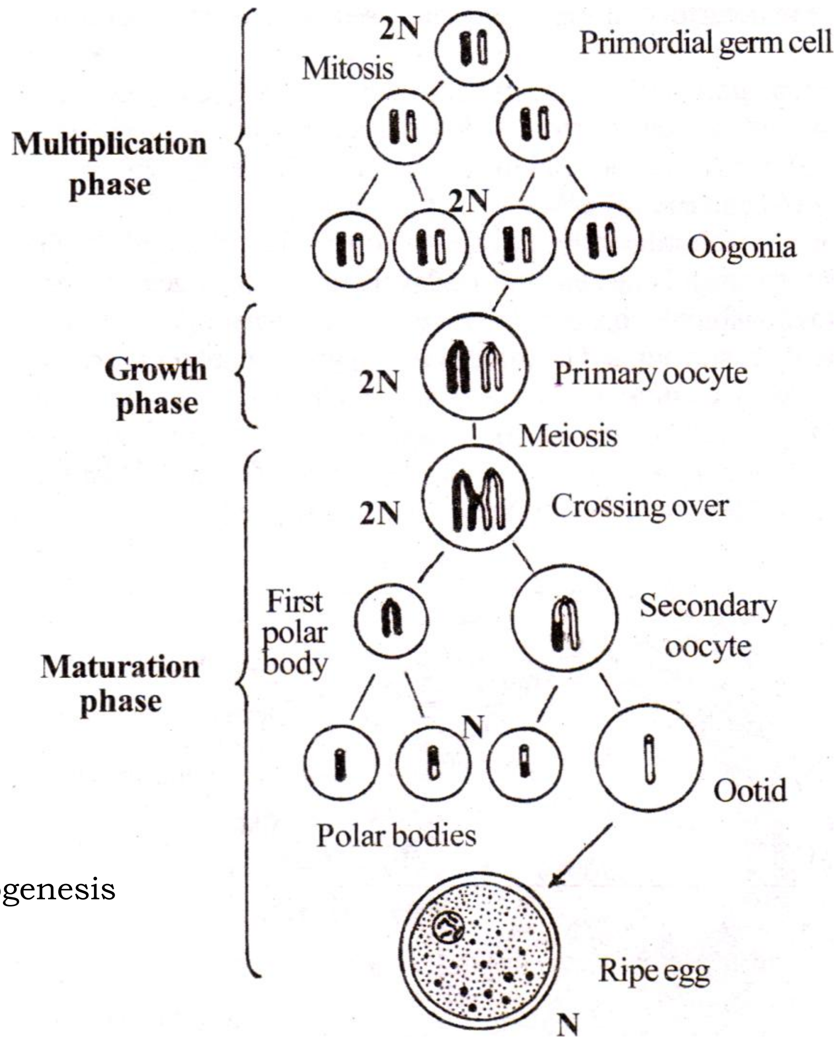
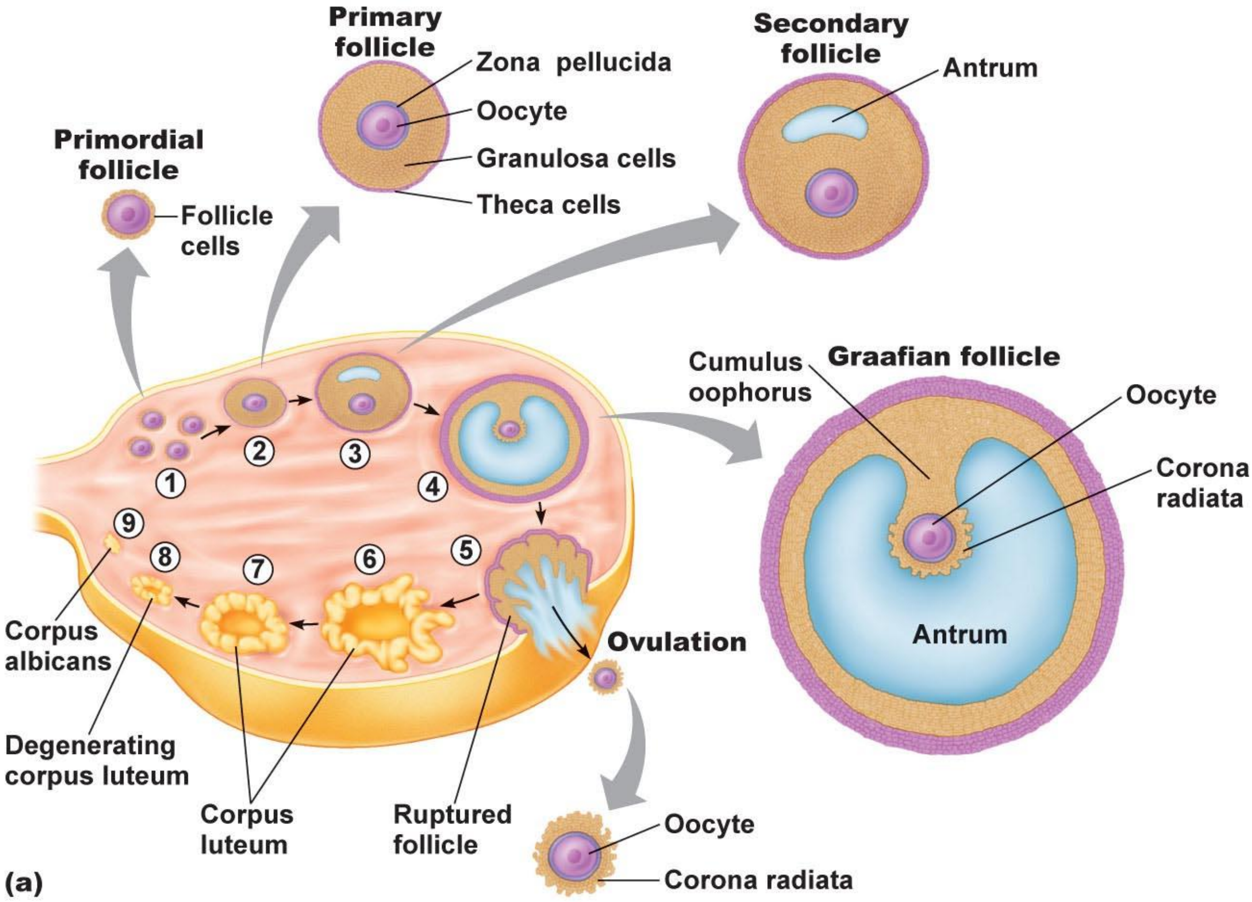



Fig. Oogenesis



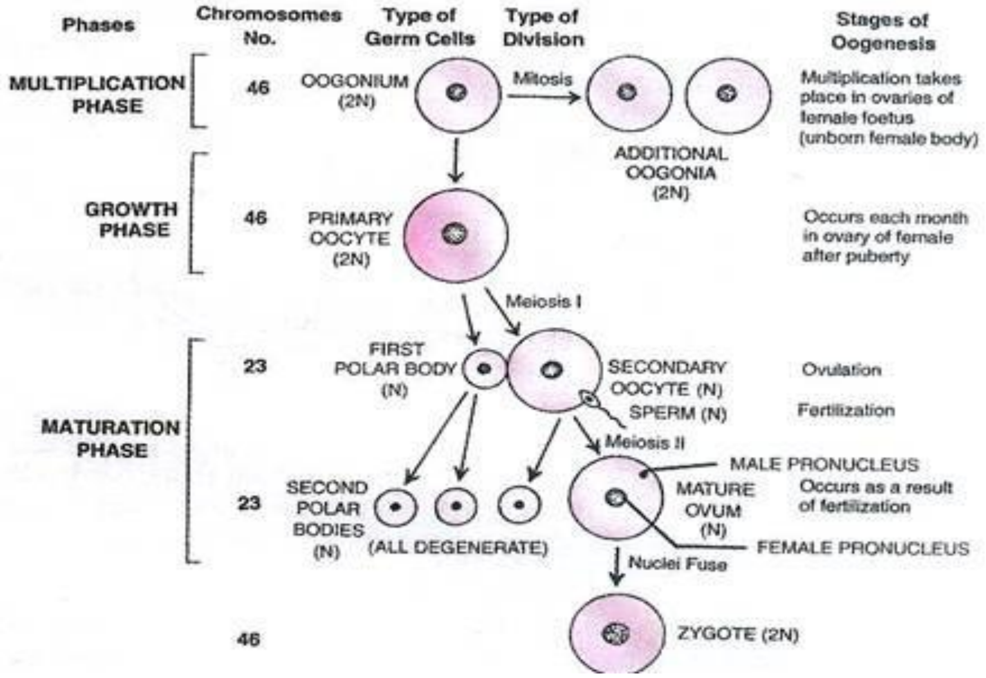




**Fig. Stages in oogenesis**

**II. Growth phase**

- Growth is an important phenomenon in oogenesis.
- During growth the nutrients and other materials necessary for the development of the embryo are synthesized.
- As these substances accumulate in the cytoplasm, the oocyte increases considerably in size
- During growth phase, the oocyte increases in size.
- The size is proportional to the amount of food reserved in the cytoplasm.
- In frogs, the young oocyte is about 50µ in diameter and the mature egg ranges from 1000 to 2000µ in diameter; so the oocyte increases 20 to 40 times. The hen's oocyte increases 200 times and in mouse it increases 43 times
- The duration of growth of oocyte is prolonged.
- It lasts for 3 years in frog
- In the new born human baby all her oocytes are already formed.
- The first ovum is released at the time of puberty.
- So it has a growth period of 12 to 14 years.
- The last egg released at the age of 45.
- The growth phase of oocyte is divided into two stages, namely previtellogenesis and vitellogenesis

**i. Previtellogenesis**

- During previtellogenesis the cytoplasm and nuclear materials of primary oocyte grow and increase considerably in volume.
- The yolk and other food materials are not synthesized during this phase.
    - The following changes occur during previtellogenesis
- The nuclear sap is produced in large amount. As a result, the nucleus increases in size.
- The large nucleus of the oocyte is called germinal vesicle
- Homologous chromosomes pair together
- In amphibians, the chromosomes of primary oocytes acquire a characteristic shape; thin loops or threads appear on the sides of the chromosomes. These loops give a brush-like appearance to the chromosomes. Hence the chromosomes are called lamp-brush chromosomes. The loops of these chromosomes are actively involved in the synthesis of mRNA
- The ribosomal RNAs are produced in a remarkable amount. They are produced by the nucleolus. As a result the nucleolus increases greatly in size

- The genes producing the rDNA are multiplied several times to facilitate the rapid synthesis of rDNA. This increase in the number of genes is called amplification
- In many cases, the production of RNA is increased further, by the development of a greater number of nucleoli (a small dense spherical structure in the nucleus of a cell during interphase). This phenomenon is more common in the egg of the amphibian.
- The mitochondria increase in number
- Cortical granules are manufactured by the cistemae of Golgi complex
- The growing oocytes are surrounded by special kinds of nutritive cells.
- These cells immensely assist the growth of oocytes in various ways.
- There are two types of nutritive cells, namely follicle cells and nurse cells
- In the ovary of chordates, the developing oocyte is surrounded by follicle cells
- **In mammals**, the follicle cells and the developing ovum together constitute a Graafian follicle.
- Each Graafian follicle consists of a cavity called antrum filled with a fluid called liquor folliculi.
- The cavity is surrounded by three layers, namely an outer theca externa, a middle theca interna and an inner membrana granulosa.
- The oocyte lies inside the antrum.
- It is surrounded by a few layers of follicle cells called corona radiata.
- The oocyte is attached to the membrana granulosa on the side by a stalk of cells called discus proligerus.
- The oocyte is surrounded by a transparent membrane called zona pellucida

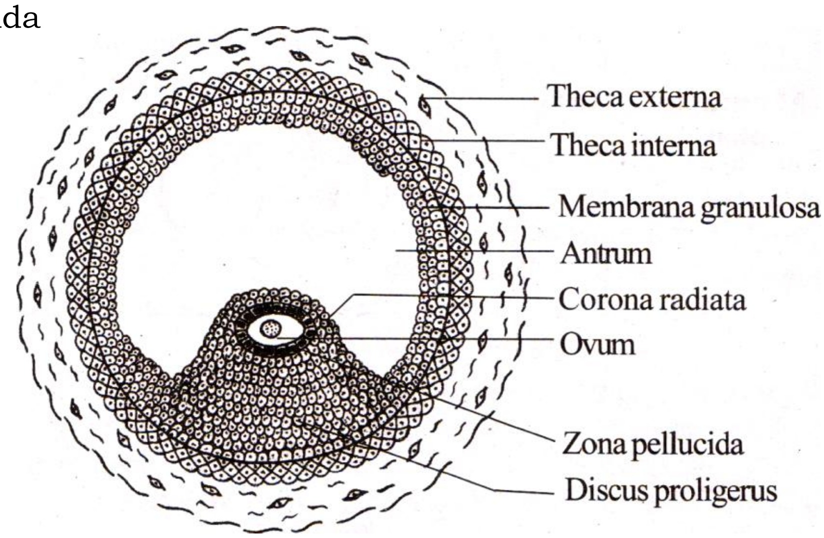



Fig. A mature graafian follicle

### ii. Vitellogenesis

- The process of formation and deposition of yolk in the oocyte is called vitellogenesis.
- Yolk is the nutritive material of the ovum.
- It is present in the form of platelets or granules

**Origin of Yolk**

- Initially when an oocyte starts developing, it does not contain any nutrients.
- The nutrients are formed only during growth phase of oogenesis.
- There are two views regarding the place of the origin of yolk
    - **Insitu Origin:** A very small amount of yolk is synthesized in the oocyte cytoplasm. For example, in vertebrates less than 1% is synthesized by the oocyte cytoplasm
    - **Exogenous Origin:** A major part of the yolk is synthesized outside the oocyte. In vertebrates, it is synthesized in the liver; in insects it is synthesized in the fat body

**Transportation of Yolk**

- In vertebrates, the yolk synthesized in the liver, is in a soluble state.
- It is transported by the blood stream to the follicle cells present around the oocyte.
- The follicle cells deposit the yolk in the oocyte.
- The transport of yolk to the oocyte is facilitated by the development of finger-like structures called microvilli by the oocyte and macrovilli by the follicle cells
- The deposit of yolk and other nutrients like the lipid, glycogen, etc. begins close to the oocyte surface and fills the peripheral cytoplasm first.
- Gradually, the entire cytoplasm is deposited with yolk except the perinuclear zone. Thus the cytoplasm is progressively occupied from the peripheral zone inward

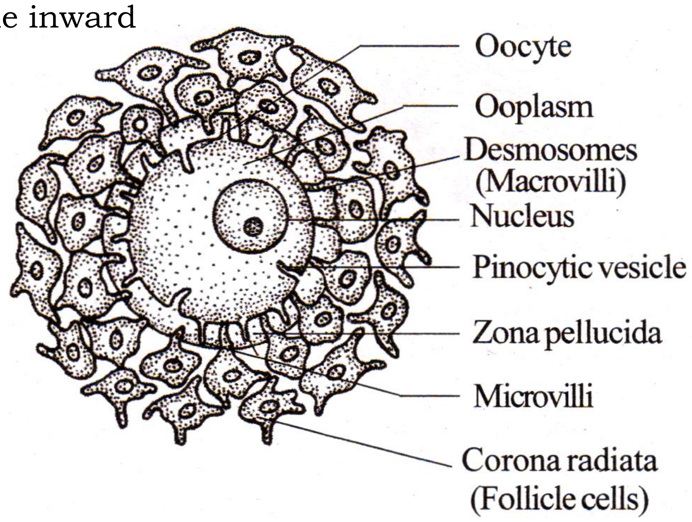



Fig. Developing mammalian oocyte with follicle cells

**The Processing of Yolk by the Oocyte**

- The liver does not produce the yolk directly.
- But it synthesizes a precursor called vitellogenin for the yolk.
- When it is brought to the oocyte, the vitellogenin is transformed into actual yolk
- The Golgi complex and the endoplasmic reticulum transport the yolk-components to the mitochondria.
- In mitochondria, the soluble yolk-components are made insoluble by a mitochondrial enzyme called proteinkinase.
- This enzyme crystallizes the soluble yolk into insoluble yolk granules or yolk platelets.
- During this process of crystallization, the mitochondrial cristae become dislodged and their membrane ultimately become arranged in concentric layers while the whole mitochondrial space is occupied by the main body of the yolk platelet

**III. Maturation phase**

- The primary oocyte contains a diploid number of chromosomes.
- The diploid chromosome number is reduced to haploid number by meiosis or reduction division and the primary oocyte is changed into the ovum or egg.
- This is called maturation
- The meiotic divisions are unequal in oogenesis.
- As a result of the first meiotic division the primary oocyte divides into a very small and a large cell each with a haploid number of chromosomes.
- The smaller cell is always formed at the animal pole and is called the first polar body or polocyte.
- It contains only a negligible amount of cytoplasm.
- The other cell contains the main bulk of the primary oocyte and is called the secondary oocyte.
- In the second meiotic division also, the secondary oocyte divides unequally into a small cell and a large cell.
- This small cell is called the second polar body or secondpolocyte.
- The larger cell is the ovum.
- As the secondary oocyte is dividing, the first polar body divides into two polar bodies
- Thus in oogenesis only one egg is produced along with the three polar bodies. The polar bodies disintegrate later
- The time of maturation varies in the different species of animals.
- It may occur after fertilization or at the time of fertilization or even before fertilization.
- In sea urchin, maturation division occurs before fertilization.

- In all the vertebrates, the second maturation division occurs only after fertilization.
- In ascidians, in some molluscs and in some annelids, the maturation division occurs at the time of fertilization.
- In extreme cases, the maturation division occurs only after fertilization

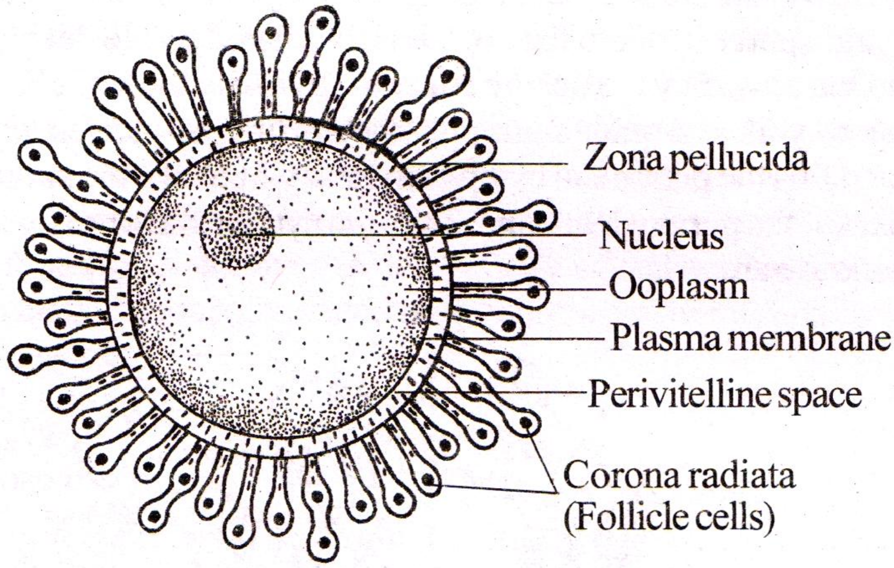


Fig. Human ovum

**Significance of Oogenesis:**

(i) One oogonium produces one ovum and three polar bodies.

(ii) Polar bodies have small amount of cytoplasm. It helps to retain sufficient amount of cytoplasm in the ovum which is essential for the development of early embryo. Formation of polar bodies maintains half number of chromosomes in the ovum.

(iii) During meiosis first crossing over takes place which brings about variation.

(iv) Oogenesis occurs in various organisms. Therefore, it supports the evidence of basic relationship of the organisms.

# PATTERNS OF EGGS

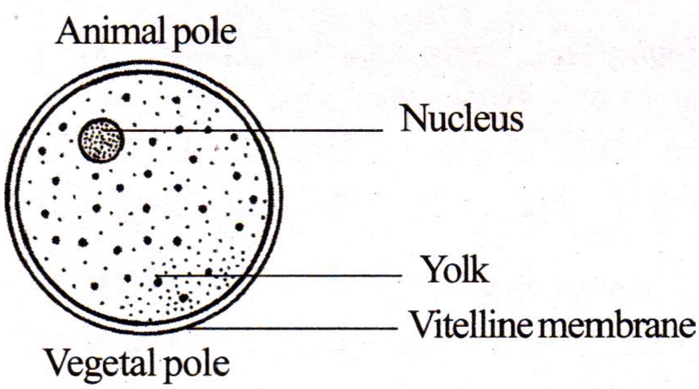
- Eggs are classified mainly on the basis of
  1. **Amount of yolk**
  2. **Distribution of yolk**
  3. **Presence or absence of shell** *and*
  4. **Type of development**

## 1. On the Basis of the Amount of Yolk

- Eggs are grouped into three types on the basis of the amount of yolk

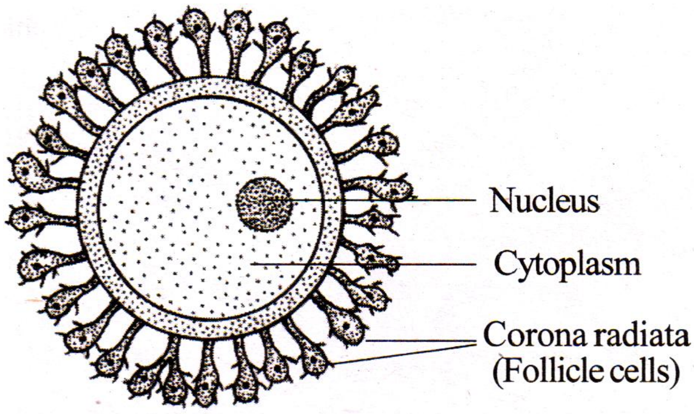
### i. Alecithal Egg

- ✓ When the egg contains no yolk, it is called alecithal egg.
- ✓ Eg. The eggs of eutherian mammals



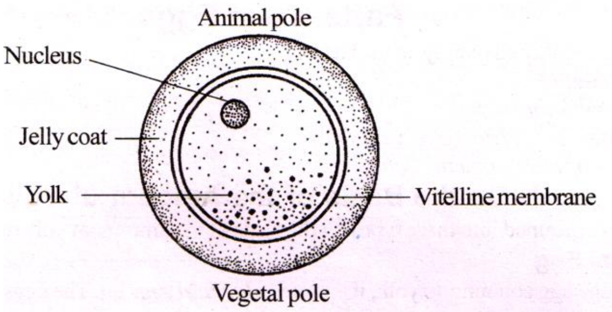

### ii. Microlecithal Egg

- ✓ When the egg contains small or negligible amount of yolk, it is said to be microlecithal
- ✓ Romer (1962) and Balinsky (1970) named these eggs as oligolecithal eggs. Eg. Amphioxus



**iii. Macrolecithal or Megalecithal Egg**

- ✓ When the egg contains large amount of yolk, it is said to be macrolecithal or megalecithal egg.
- ✓ It is also called polylecithal egg. Eg. Bony fishes, amphibians, reptiles, birds, etc
- ✓ In amphibians, Dipnoi and Petromyzon, the amount of yolk present is not very high. Hence these eggs are also named as mesolecithal eggs

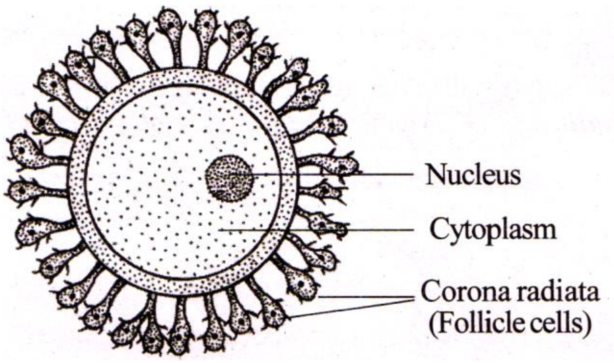



**2. On the Basis of the Distribution of Yolk**

- ➢ Eggs are classified into three types on the basis of the distribution of yolk in the egg. They are as follows

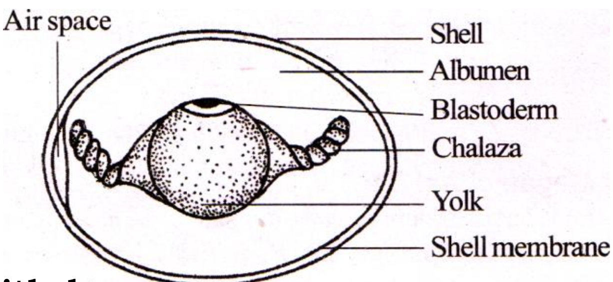
**i. Homolecithal or Isolecithal**

- ✓ In isolecithal egg, the yolk materials are uniformly distributed throughout the eggs. Eg.Echinoderms and Amphioxus



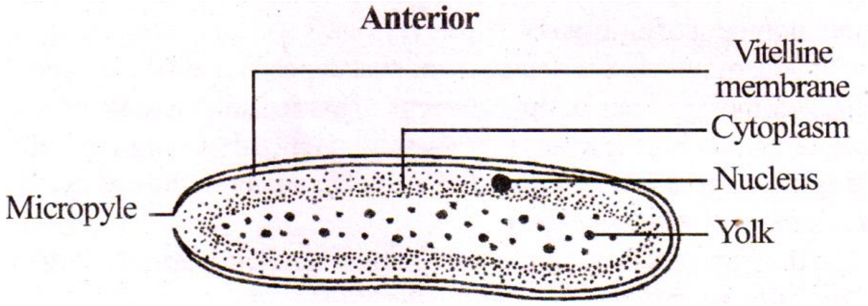

**ii. Telolecithal**

- ✓ Here the yolk is highly concentrated towards the vegetal pole.
- ✓ The amount of yolk gradually decreases from the vegetal pole towards the animal pole. Eg. Fishes, amphibians, reptiles and birds




**iii. Centrolecithal**

- ✓ In centrolecithal egg, the yolk takes up a central position and is surrounded by a thin layer of cytoplasm. Eg. Insects




### 3. On the Basis of the Presence or Absence of Shell

- ➢ According to the presence or absence of shells, eggs are classified into two types. They are as follows

#### i. Cleidoic Egg

- ✓ The eggs with hard shell are called cleidoic eggs. Eg. Reptiles, Birds, etc

#### ii. Non-cleidoic Egg

- ✓ The non-cleidoic eggs are not protected by shells.
- ✓ This type of egg is in animals in whose case the development is internal. Eg. Mammals

### Polarity

- ➢ The unequal distribution of egg components creates the animal pole and vegetal pole in the egg, it is called polarity.

### Gradient

- ➢ A gradient is defined as a proportional rise and fall of certain factors in the embryo, the interaction of which brings about normal development.

**Significance:**

1. The process leads to formation of germ cells or gametes.

2. The normal body cells known as somatic cells are diploid (2n) where as the germ cells are haploid (n).

3. During fertilization one halpoid sperm unites with one haploid ovum to form a normal diploid somatic cell thus keeping the chromosome number constant generation after generation.

4. During first maturation division, the reshuffling of paternal and maternal genes take place resulting in variation.

# UNIT - II

# FERTILIZATION

- ✓ Fertilization is the **union of spermatozoon and egg resulting in the formation of a zygote.**

## PHYSICAL FACTORS INVOLVED IN FERTILIZATION

### 1. External Fertilization

- ✓ In majority of aquatic animals, sperms and ova are released into the water where fertilization takes place.
- ✓ It is called external fertilization.
- ✓ It is the primitive type of fertilization. Eg. Fishes, Frog*, etc.*

### 2. Internal Fertilization

- ✓ In amniotes, sperms are introduced into the female's genital tract, where fusion takes place.
- ✓ It is called *internal fertilization.* It is the advanced type. Eg. *Man.*

### 3. Life-span of the Gametes

- ✓ Generally, the eggs fertilized externally, have shorter life-span than those which are fertilized internally.
- ✓ For example, the human egg can live for more than twenty-four hours after ovulation.
- ✓ Spermatozoa have a comparatively long-life span.
- ✓ For example, the spermatozoa of bat can live for 135 days in the female reproductive tract.
- ✓ The cock spermatozoon can live for two weeks in the oviduct.
- ✓ The life time of the human spermatozoon in the female genital tract is about 24 hours.

### 4. Production of Enormous Number of Sperms

- ✓ The meeting of gametes is enhanced by the production of enormous numbers of sperms

*Table: The duration of survival of sperms in the female genital tract.*

| Sl. No. | Animal | Duration |
|---|---|---|
| 1. | Turtle | 4 years |
| 2. | Aquarium fish, guppy | 1 year |
| 3. | Horse | 144 days |
| 4. | Bat | 135 days |
| 5. | Garter snake | 4 months |
| 6. | Hen | 2 weeks |
| 7. | Guinea pig | 41 hours |
| 8. | Man | 24 hours |
| 9. | Rat | 17 hours |
| 10. | Rabbit | 14 hours |

5. **Random Collision**

- ✓ The egg and sperm are brought together by random collision.
- ✓ This is favoured by the large size of the egg and the enormous number of sperms

6. **Mechanical Juxtaposition of Gametes**

**(Juxtaposition is an act or instance of placing two elements close together or side by side)**

- ✓ Animal's provide a number of mechanical agencies to bring together the eggs and spermatozoa. In mammals, the spermatozoa are injected deep into the female genital duct by ***copulation***
- ✓ In birds, the spermatozoa are introduced into the cloaca of female by a process called ***'cloacal kiss***
- ✓ In cephalopods **(Sepia, Loligo,)**, one of the arms in the male modified to transfer the spermatozoa into the female genital duct. This arm is said to be ***hectocotylus.*** During courtship, the male carries a bundle of spermatophores from the genital duct in his hectocotylus arm and places it either in the mantle cavity or in the seminal receptacle of the female

7. **Synchrony in Production and Release of Gametes**

- ✓ The male and female gametes are produced at a particular time.
- ✓ In certain animals, eggs are released only after ovulation.
- ✓ This prevents wastage of sperms

8. **Capacitation**

- ✓ Capacitation is a process where the spermatozoa acquire the capacity to fertilize the eggs.
- ✓ After capacitation, the spermatozoa develop the ability to penetrate the membranes surrounding the egg.
- ✓ The spermatozoa obtain capacitation by the following methods
  - The spermatozoa gets capacitation by remaining in the female genital tract for some time. The duration is six hours in man and one hour in mouse
  - In some animals, sperms obtain capacitation by passing through ***epididymis***
  - During capacitation the coating substances on the surface of the sperm are removed. This helps the receptor sites on the sperm to recognize signals coming from the egg

## 9. Entry of Sperm into the Egg

- ✓ In some animals, such as molluscs, echinoderms, insects and fishes, the egg is surrounded by a tough membrane called ***chorion.***
- ✓ This membrane cannot be easily penetrated by the spermatozoa.
- ✓ Hence, the eggs are provided with one or more-minute openings called ***micropyles.***
- ✓ The spermatozoa enter the eggs only through these openings

## 10. Time of Fertilization

- ✓ The sperm fertilizes the egg at different stages of maturation in different species.
- ✓ It may occur after maturation or at the time of maturation or before maturation.
- ✓ In sea urchin, fertilization occurs after maturation divisions.
- ✓ In all vertebrates, the egg is fertilized after maturation division
- ✓ In ascidians, some molluscs and some annelids the egg is fertilized at the time of first maturation division.
- ✓ In nematodes and annelids, the egg is fertilized before the commencement of maturation division

**CHEMICAL FACTORS INVOLVED IN FERTILIZATION**

**1. Chemotaxis**

- ✓ In certain animals, sperms are attracted towards the eggs by chemicals.
- ✓ This happens in coelenterates, fishes, insects, etc.
- ✓ In fishes and insects, this chemical is present in the *chorion* lining the *micropyle.*
- ✓ When the chorion is removed from the egg, the activity of sperm slows down

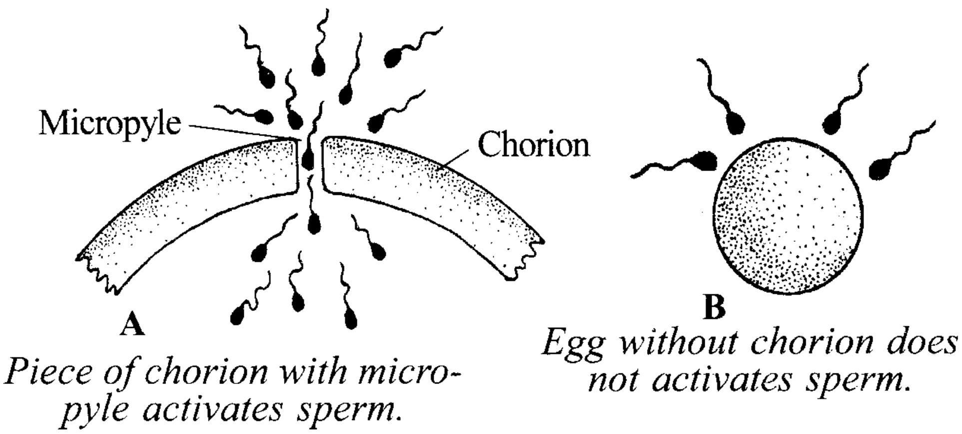



Fig. Chemotaxis in the egg of fish

**2. Fertilizin-Antifertilizin Reaction**

- ✓ The sperm identifies the egg by the reaction between  ertilizing and antifertilizin
- ✓ ***Fertilizin:***
  - o The egg contains on its surface a chemical substance called *ertilizing.*
  - o It is *a glycoprotein.*
  - o It has a molecular weight of 3,00,000.
  - o The  ertilizing molecule has *many receptor* or *binding sites* for antifertilizin.
  - o Fertilizins are *species specific* and there may be different fertilizins for different species
- ✓ ***Antifertilizin:***
  - o The surface of the sperm contains a chemical substance called *antifertilizin.*
  - o It is an *acid protein.*
  - o It is smaller than  ertilizing.
  - o It has a molecular weight of 10,000.

- o There may be different antifertilizins in the different species

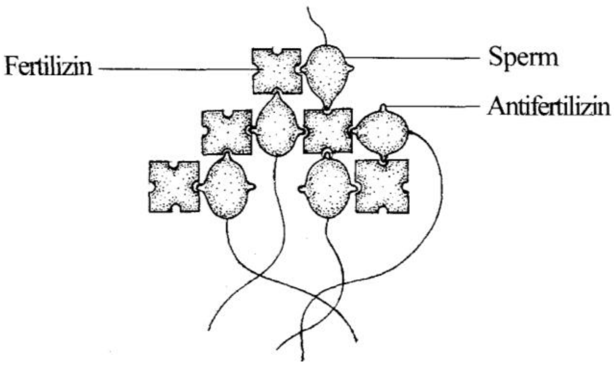



*Fig.: Fertilizin-antifertilizin reaction.*

- ✓ ***Reaction:*** Fertilizin reacts with the antifertilizin in a manner comparable to the reaction between antigen and antibody.
- ✓ This reaction can also be compared to the ***lock*** and ***key system***

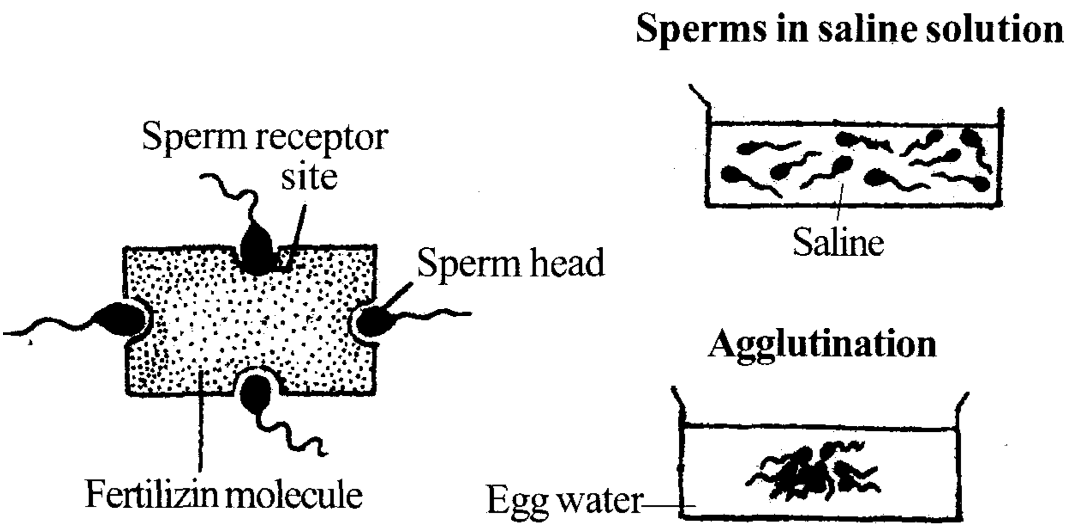


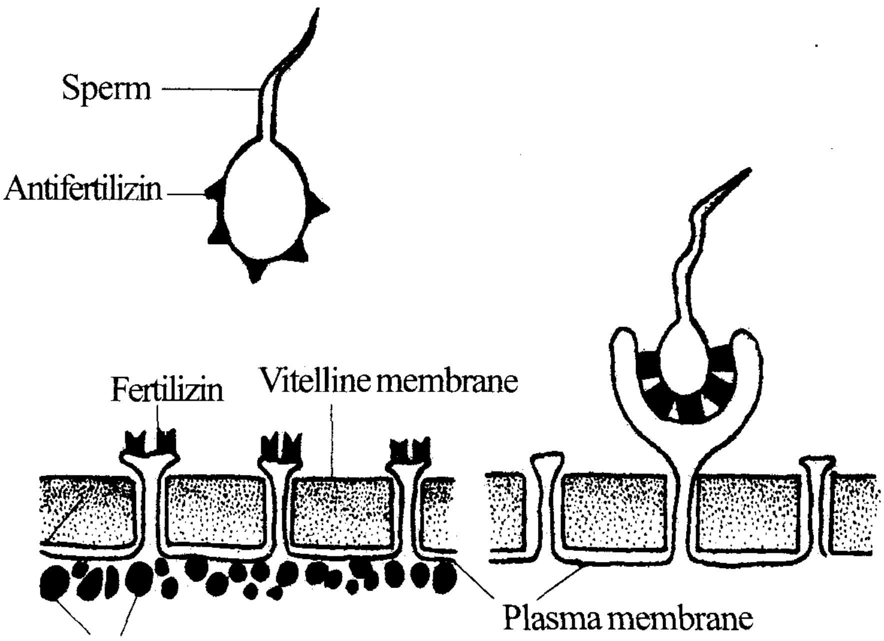

Fig.: Fertilizin and Antifertilizin reaction

- ✓ The ertilizing molecule has many *receptor sites* or *binding sites*. These receptor sites are complementary to the antifertilizin molecules
- ✓ When eggs and sperms are released in the water, the ertilizing particles embrace the antifertilizin particles. As a result, the sperms agglutinate or clump together. This reaction is strictly species specific

- ✓ ***Functions of Fertilizin-Antifertilizin Reaction:***

  - The sperm identifies the egg by ertilizing antifertilizin reaction
  - The initial attachment of the sperm to the egg is the result of the linking between ertilizing particles and antifertilizin particles
  - Certain amount of ertilizing is released from the egg into the surrounding water.

- These released fertilizins combine with the sperms.
- This leads to the *agglutination* of sperms.
- As a result of this, only a few sperms reach the surface of the egg. This prevents *polyspermy*
- As the reaction between ertilizing and the antifertilizin is *species-specific,* fertilization between different species of eggs and sperms is prevented
- Sperms contain *lytic* substances which can break down the egg-coat.
- By holding together many sperms on the surface, the ertilizing-antifertilizin reaction ensures the production of sufficient quantities of lytic enzymes to dissolve the egg coverings
- It leads to the capacitation of sperm
- Fertilizin activates the *sperms* and initiates *acrosome reaction*




*Fertilizin of egg and antifertilizin of sperm.*

*Linking of fertilizin and antifertilizin.*

Fig. Fertilizin-Antifertilizin reaction

**3. Sperm Penetration**

✓ The mechanism of penetration is *chemical*

✓ The spermatozoon liberates an enzyme called *sperm-lysin.* It is produced by the *acrosome* of the spermatozoa. It dissolves the egg-membrane and makes way for the entry of spermatozoa

✓ In mammals at the time of ovulation, the egg is surrounded by follicle cells. These cells are cemented together by a substance called *hyaluronic acid.* The mammalian sperm secretes a lytic enzyme called *hyaluronidase.* This enzyme dissolves the hyaluronic acid and the follicle cells are loosened. This paves the way for the entry of spermatozoa

**CYTOLOGICAL FACTORS INVOLVED IN FERTILIZATION**

**1. Monospermy**

- ✓ Generally, only one spermatozoon enters the egg and fuses with it.
- ✓ Such a fertilization is said to be ***monospermy.***
- ✓ Monospermy is common in majority of animals like coelenterates, annelids, echinoderms, bony fishes, frogs, etc

**2. Polyspermy**

- ✓ In some animals, normally many sperms enter the egg.
- ✓ Such a fertilization is said to be ***polyspermy.***
- ✓ Eventhough many sperms enter the egg, only one sperm nucleus fuses with the egg nucleus. Other sperm nuclei degenerate
- ✓ Polyspermy naturally occurs in animals with heavily yolked eggs.

Eg. ***Arachnids, some insects, elasmobranchs, urodeles, reptiles, birds,*** etc.

This is known as ***physiological polyspermy***

**3. Acrosome Reaction**

- ✓ When the spermatozoon comes in contact with the egg, tremendous changes occur in the acrosome of sperm.
- ✓ All these changes constitute the ***acrosome reaction.***
- ✓ The acrosome reaction has been studied extensively in a variety of animals. ***Colwin*** (1967) demonstrated the acrosome-reaction in the enteropneust ***Saccoglossus***

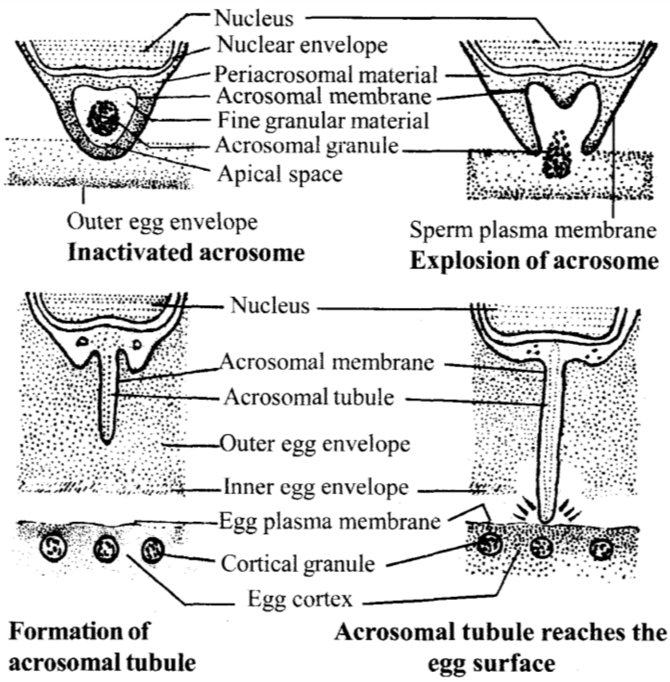



Fig. Fertilization: Changes in the spermatozoon of Saccoglossus during fertilization

- ✓ When the spermatozoon's tip makes contact with the egg envelope, the sperm-plasma membrane and the acrosomal membrane rupture at the point of contact

- ✓ The acrosomal membrane then joins with the plasma membrane around the margin of the opening
- ✓ The ***acrosomal granule*** is released on the egg-envelope
- ✓ The acrosomal granule contains the ***sperm lysin*** which dissolves the egg envelope. Thus the egg surface is exposed at this spot
- ✓ Now, the centre of acrosomal membrane, nearer to the nucleus, grows towards the egg surface as a thin tube called ***acrosomal tubule***
- ✓ As the acrosomal tube grows further, its tip comes in contact with the plasma membrane of the egg
- ✓ In some cases, more than one acrosomal tubes develop. Eg. ***Hydroides*** (Polychaete)

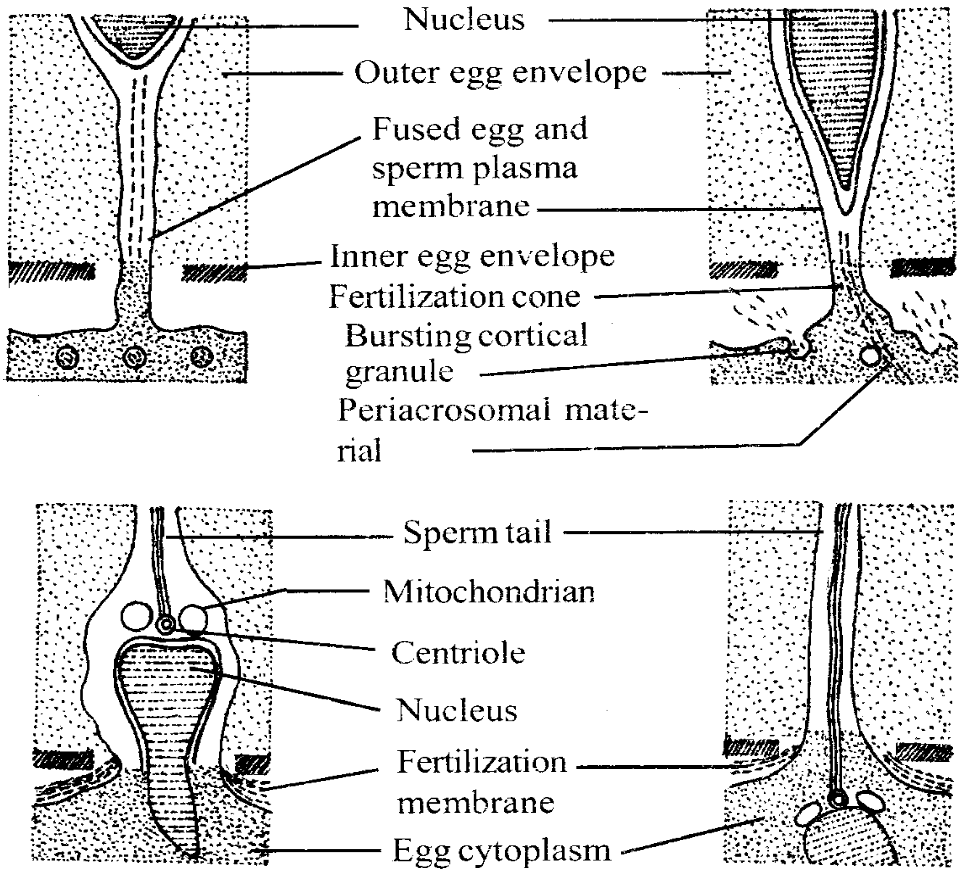
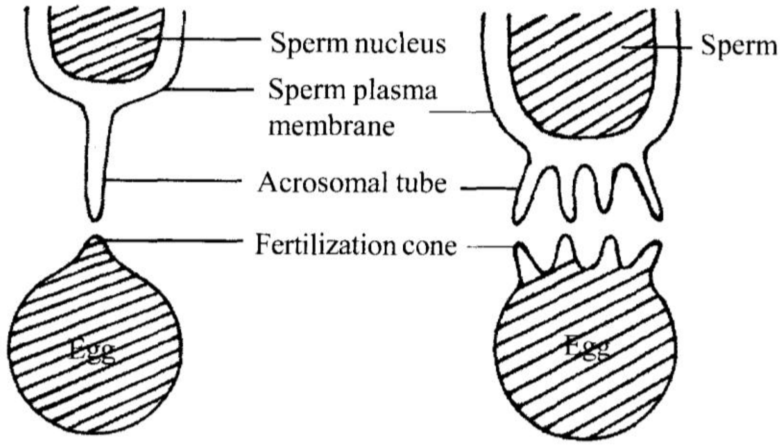



Fig.: Fertilization. Enlargement of spermatozoon by the fertilization cone.




| | |
|---|---|
| Fig. An acrosomal tube and a fertilization cone | Fig. Many acrosomal tubes and fertilization cones level opening during fertilization in Hydroides |

**4. Cytoplasmic Fusion**

- ✓ As the acrosomal tube comes in contact with the egg-surface, the egg is activated.
- ✓ At the point of contact, the plasma membranes of the egg and sperm fuse and the acrosomal tube opens into the egg-cytoplasm.
- ✓ As the fusion is made, the egg-cytoplasm at this point bulges out as a conical projection called ***fertilization cone.***
- ✓ In ***Saccoglossus,*** only one fertilization cone develops.
- ✓ But in ***Hydroides*** many cones develop as there are many acrosomal tubes. The fertilization cone gradually engulfs the spermatozoon into the interior of the egg
- ✓ In mammals, the entire spermatozoon (nucleus, middle piece and tail) is engulfed by the egg-cytoplasm.
- ✓ In majority of animals, the nucleus and the middle piece enter the egg and the tail is excluded.
- ✓ In ***Nereis,*** the sperm nucleus along with the proximal centriole alone is engulfed; the tail and the middle piece are discarded

**5. Cortical Reaction**

- ✓ As the sperm enters the egg, the egg becomes activated.
- ✓ First of all, changes occur in the cortex (surface) of the egg.
- ✓ These changes, constitute the *cortical reaction.*
- ✓ The cortical reaction in the egg of sea urchin can be summarized as follows
    - o The colour of the egg-surface gradually changes from yellow to white. The change starts from the point of attachment of the sperm and gradually spreads over the surface of the egg.

Fig.: Colour changes in the egg of sea urchin after fertilization

- o The vitelline membrane gets lifted off. This membrane is then called ***fertilization membrane.*** The space between it and the surface of the egg is called ***perivitelline space.*** It is filled with a fluid called ***perivitellinefluid.***
- o The cortical granules swell rapidly and explode. The cortical granules release three important components. They are,

**a. Lamellar Folded Bodies:**

These are dark and dense bodies. On release, they unfold and fuse with the inner surface of the fertilization membrane. Thus the fertilization membrane is strengthened by the lamellar bodies.

**b. Globules:**

The globular structures fuse together and form a new surface layer just outside the plasma membrane. This layer is called ***hyaline layer.*** It helps to keep the blastomeres intact during cleavage.

**c. Liquid Component:**

The cortical granules contain mucopolysaccharides. They absorb water and become liquified. This liquid is released into the perivitelline space, and it is called ***perivitellinefluid.*** By imbibing more and more water, it assists in lifting the fertilization membrane still further.

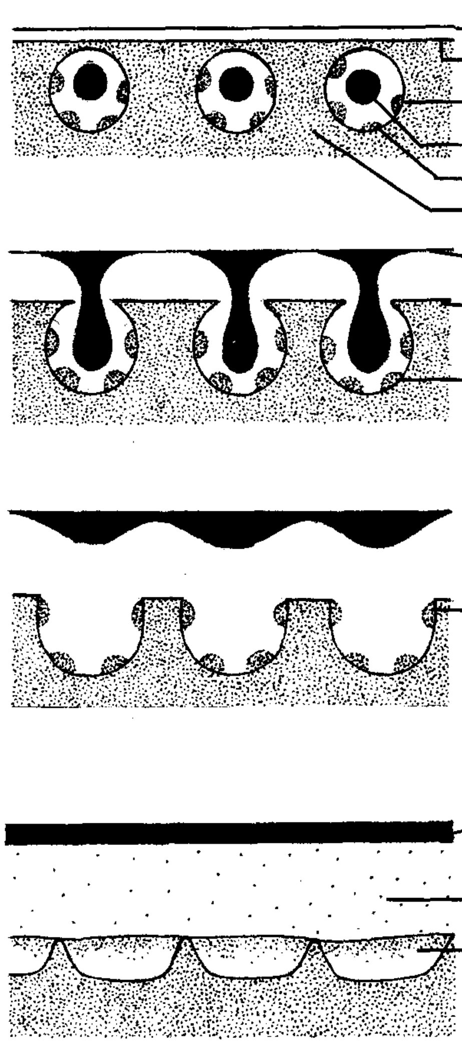



Fig.: Cortical reaction and the formation of fertilization membrane

## 6. Sperm nucleus

When the sperm is engulfed by the fertilization cone, it moves towards the ***female pronucleus.*** The sperm nucleus is followed by the centriole, the middle piece and the tail. Soon rotation of the nucleus and centriole occurs. As a result, the centriole comes to occupy infront of the sperm nucleus. The other parts of the spermatozoon (the middle piece and the tail) get disconnected from the nucleus. They later disintegrate in the egg-cytoplasm

The sperm nucleus is now termed as the ***male pronucleus.*** It moves through the egg- cytoplasm towards the female pronucleus. As the male pronucleus moves inwards, the path of the male pronucleus may be marked by pigment granule trailing along its path. The path taken by the sperm in the periphery of the egg (cortex) is called ***penetration path.*** Inside the egg the direction of movement of the sperm is slightly changed and the nucleus moves towards the female pronucleus. This changed path is called ***copulation path.***

7. **Amphimixis**

- ✓ ***Amphimixis*** refers to the *fusion of male and female pronuclei.*
- ✓ In sea urchin and in all vertebrates, the two nuclei come in contact, the nuclear membranes at the point of contact disappear and the contents of the two nuclei unite into one mass surrounded by a common nuclear membrane.
- ✓ In *Ascaris,* molluscs and annelids, only after the completion of first cleavage, the male and female pronuclei fuse together.
- ✓ In *Cyclops,* the paternal and maternal nuclear components remain separate even after cleavage has started. As a result, each blastomere has a double nucleus consisting of two parts lying side by side, each surrounded by its own nuclear membrane

## PHYSIOLOGICAL CHANGES IN FERTILIZATION

### Fertilizin-Antifertilizin Reaction:

- ✓ The egg releases a substance called ***fertilizin.***
- ✓ The sperm contains a chemical substance called ***antifertilizin.***
- ✓ The fertilizin binds to the antifertilizin.
- ✓ This reaction is called ***fertilizin-antifertilizin reaction.***
- ✓ This reaction helps to attract the sperm near the egg.

### Acrosome Reaction:

- ✓ When the sperm comes in contact with the egg, the acrosome of the sperm explodes and releases ***sperm lysin.***
- ✓ The sperm lysin dissolves the egg membrane and makes a hole for the entry of sperm
- ✓ The acrosomal membrane of sperm grows out as a tube called ***acrosomal tube.***
- ✓ The acrosomal tube elongates and reaches surface of the egg.
- ✓ Here the plasma membrane of the egg and the acrosomal membrane fuse together and the acrosomal tube opens into the egg cytoplasm

### Fertilization Cone:

- ✓ When the acrosomal tube opens into the egg cytoplasm, the egg cytoplasm at this point bulges out as a conical projection called***fertilization cone.***
- ✓ The fertilization cone gradually engulfs the sperm

### Elevation of Fertilization Membrane:

- ✓ The vitelline membrane is lifted off from the surface of the egg.
- ✓ This membrane is now called ***fertilization membrane.***
- ✓ It is strengthened by the deposition of cortical granule materials on its inner surface.
- ✓ A fluid called ***perivitellinefluid*** gradually accumulates in the space between the surface of the egg and the fertilization membrane.
- ✓ The fertilized egg freely rotates inside the perivitelline fluid

### Explosion of Cortical Granules:

- ✓ When the sperm enters the egg, the cortical granules explode and release their contents.
- ✓ The cortical granules synthesize the ***fertilization membrane, perivitelline fluid*** and the ***hyaline layer***

### Cytoplasmic Movements:

- ✓ The contact of sperms sets in the egg elaborate cytoplasmic movements.
- ✓ The movement of egg-cytoplasm is reflected in the violent activity of the pigment granules on the surface
- ✓ The cytoplasmic movement is best illustrated in amphibian eggs.
- ✓ As the sperm penetrates through the cortex of the egg, a trail of dark pigment from the egg's periphery flows in after the ***sperm.***
- ✓ This initial path of the sperm constitutes the ***penetration path***
- ✓ Then the sperm changes its direction and begins to travel towards the female pronucleus.

- ✓ This secondary path is the ***copulation path***
- ✓ On the surface of the egg opposite to the point of sperm entry, the peripheral area of the egg becomes lighter in colour and assumes a grey appearance. This area is ***crescentic*** in shape and is known as the ***grey crescent***

**Permeability of Plasma Membrane:**

- ✓ The permeability of plasma membrane increases in the case of the molecules of water, ethylene, glycol, phosphate, etc

**Phosphorylation of Coenzymes:**

- ✓ At fertilization, the coenzyme NAD is phosphorylated into NADP and NADPH in the presence of the enzyme ***NAD kinase***

**Rate of Oxygen Consumption:**

- ✓ The rate of oxygen utilization may increase or decrease or may not change.
- ✓ In frog and toad, there is a pronounced drop in ***respiratory quotient.***
- ✓ In sea urchin and lamprey, oxygen consumption is increased during fertilization.
- ✓ In teleost fish, there is no change in the rate of oxygen consumption

**Rate of Protein Synthesis:**

- ✓ In the unfertilized egg of sea urchin, there is no protein synthesis.
- ✓ During fertilization the rate of protein synthesis increases

**Initiation of Mitosis:**

- ✓ Fertilization initiates mitosis in the egg resulting in cleavage. Mitosis requires the development of mitotic apparatus.
- ✓ It is produced exclusively by the centriole introduced by the sperm.
- ✓ The introduction of sperm centriole is a must for the egg to form a mitotic spindle.
- ✓ Thus, the sperm stimulates the first mitotic division (cleavage) of the fertilized egg by contributing its centriole to the egg

**Breakdown of Polysaccharide:**

- ✓ Immediately after fertilization a rapid breakdown of ***polysaccharide*** takes place.
- ✓ There is a corresponding increase in ***lactic acid***

**Hexose Phosphate:** It increases considerably after fertilization

**Dehydrogenase:** This enzyme increases after fertilization

**ACTIVATION**

- ✓ The process of initiating the development in an egg is called ***activation.***
- ✓ It is initiated or stimulated by the sperm. The egg responds to the sperm by forming fertilization cone and by undergoing the various surface and internal changes. All these changes collectively constitute activation.
- ✓ During activation the following changes occur in the egg.

1. The egg surface produces *fertilization cone.*
2. The vitelline membrane is lifted and is converted into *fertilization membrane.*
3. The cortical granules explode.
4. The cytoplasm exhibits movements.
5. The permeability of plasma membrane increases.
6. The coenzyme NAD (Nicotinamide adenine dinucleotide) is phosphorylated.
7. The rate of ***protein synthesis*** increases.
8. Mitosis is initiated.
9. The breakdown of polysaccharide occurs.
10. The enzyme ***dehydrogenase*** increases.

**Significance of Fertilization**

- ✓ Fertilization maintains the diploid number of chromosomes in the race
- ✓ Fertilization produces genetic variation by bringing together chromosomes from two different parents
- ✓ Fertilization activates the egg and thus development is initiated

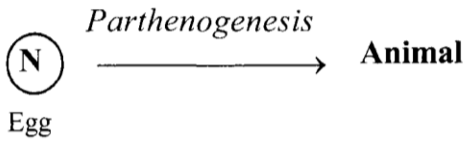
## PARTHENOGENESIS

Parthenogenesis is the development of an egg without fertilization.

(Gr. Parthenos =virgin; genesis = birth)

The individuals formed by parthenogenesis are called parthenotes

Parthenogenesis may be of two types. They are natural parthenogenesis *and* artificial Parthenogenesis




### Natural Parthenogenesis

***When parthenogenesis occurs spontaneously*** it is called ***natural parthenogenesis.*** Parthenogenesis is a regular natural phenomenon in a few groups of animals. Some animals reproduce exclusively by parthenogenesis. In some other species, parthenogenesis alternates with sexual reproduction.

Natural parthenogenesis is divided into two groups, namely

1. *complete parthenogenesis and 2. incomplete parthenogenesis.*

### 1. Complete Parthenogenesis

In certain animals, parthenogenesis is the only method of reproduction. This type of parthenogenesis is called ***complete*** or ***total*** or ***obligatory*** parthenogenesis. Populations exhibiting total parthenogenesis consist entirely of females. There are no males. Eg. ***Lacerta*** (lizard).

### 2. Incomplete Parthenogenesis

In some animals, parthenogenetic reproduction and sexual reproduction occur alternately. This is called ***incomplete*** or ***cyclical*** parthenogenesis.

***Example:***

**1.** In gallflies, parthenogenetic reproduction alternates with sexual reproduction; there is one parthenogenetic reproduction and one sexual reproduction per year.

**P** → **S** → **P** → **S** → **P** → **S**

**2.** In aphids, daphnids and rotifers, one sexual reproduction occurs in summer after many parthenogenetic reproductions

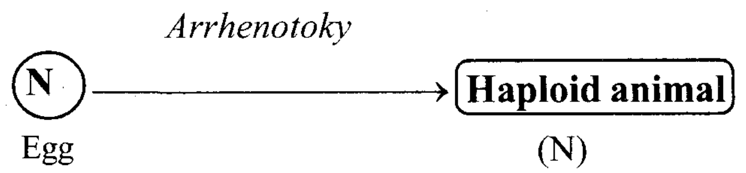
$$P \to P \to P \to P \to P \to \boldsymbol{S} \to P \to P \to P \to P \to P \to \boldsymbol{S} \to P \to P$$

Natural parthenogenesis is further classified into two types.

They are haploid parthenogenesis or ***arrhenotoky*** and diploid parthenogenesis or ***thelytoky***

**1. Arrhenotoky**

Arrhenotoky is a type of parthenogenesis.
In arrhenotoky, a haploid egg develops into a haploid animal without fertilization.
It is also called haploid parthenogenesis.




It is found in insects, arachnids and rotifers.
In honeybee, parthenogenetic eggs develop into males and fertilized eggs develop into females.
Hence the males are haploid and the females are diploid.
The males are fertile.
During spermatogenesis, the reduction division is omitted.
Hence, the spermatozoa contain the haploid number of chromosomes as the parent.
The females contain diploid number. The eggs are formed by meiosis; hence, they are haploid.

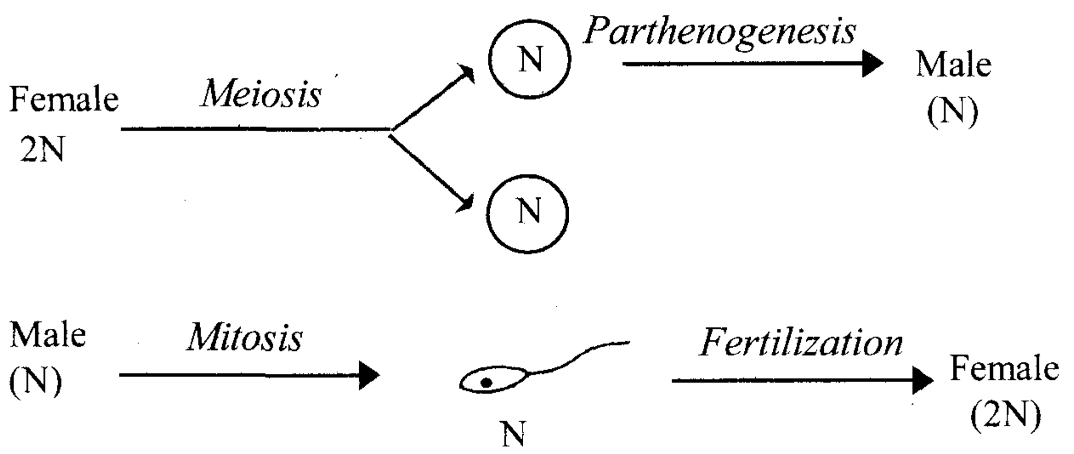
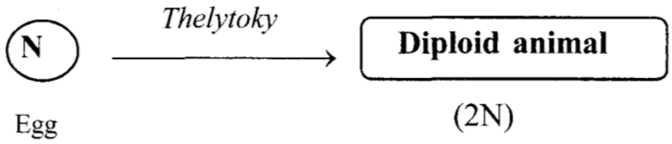



Fig.: Haploid parthenogenesis in honey bee

## 2. Thelytoky

Thelytoky is a type of parthenogenesis.
In thelytoky, an unfertilized egg develops into a diploid animal.
It is also called ***diploid parthenogenesis.***




It is found in crustaceans and insects.
The diploid condition is maintained either by omitting the reduction division or by the fusion of one of the polar bodies with the haploid egg. Based on this, thelytoky is classified into two types, namely ameiotic thelytoky and meiotic thelytoky.

(i) Ameiotic Thelytoky

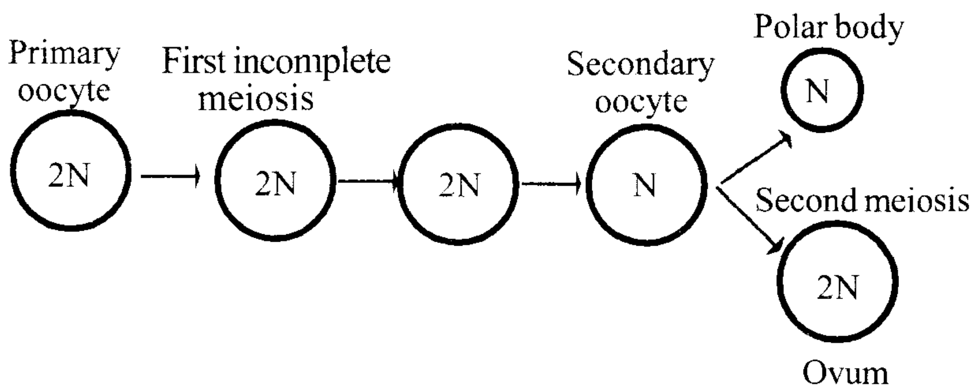
In ameiotic thelytoky, meiosis is suppressed. The egg is produced by simple mitosis. As a result, the egg is diploid. It generally occurs in crustaceans and insects.

(i) Meiotic Thelytoky

In meiotic thelytoky, meiosis occurs during oogenesis and the haploid eggs are produced. The diploid condition is achieved by the doubling of the chromosome. The doubling of the chromosome is called diplosis. Diplosis occurs in any one of the two ways, namely restitution and autofertilization.

**a. Restitution**: In this process, the first meiotic division is incomplete. The second meiotic division occurs normally. This results in the production of two diploid cells.

One cell is smaller and is called polar body. The other cell is large and it develops into the egg. This diploid egg develops into the embryo without fertilization. Eg. Lepidopteran insects.




***b.Autofertilization:*** In this process, meiosis occurs normally during oogenesis and haploid egg is produced.

The haploid egg then becomes diploid by fusing with one of the polar bodies. This process is called ***autofertilization.*** In ***Artemia salina*** (brine shrimp), diplosis occurs by the fusion of egg with the second polar body

## Artificial Parthenogenesis

Parthenogenesis produced experimentally in the laboratory is called artificial parthenogenesis. This has been demonstrated in annelids, molluscs, echinoderms, amphibians, birds and mammals. Parthenogenesis may be produced by mechanical or chemical means.

Chemical Means: Artificial parthenogenesis is induced by treating the eggs with the following chemical agents:

- Hypotonic or hypertonic seawater
- Chloroform
- Blood serum
- Fatty acids like lactic acid, butyric acid, etc.
- Fat solvents like ether, acetone, etc.
- Urea
- Chlorides of K, Ca, Na, Mg, etc.

Mechanical Means: Artificial parthenogenesis is induced by the following mechanical methods:

- High or low temperature
- Ultraviolet light.

## Significance of Parthenogenesis

- ✓ **As a Means of Reproduction:** Parthenogenesis is a simple and easier means of reproduction. It ensures the continuity of a race when fertilization fails to occur.
- ✓ **Rapid Multiplication**: Parthenogenesis favours the production of large number of individuals within a short period.
- ✓ **Persistence of Advantageous Characters**: Sometimes advantageous characters appear in the parthenotes by mutations.
- ✓ **Elimination of Disadvantageous Characters**: Sometimes mutation produces unfavourable characters. These are not useful to the possessor. Such organisms cannot compete the fittest animals. Hence, parthenotes with unfavourable adaptations are eliminated from the population.
- ✓ **Elimination of Variety**: As the offspring are formed by the contribution of only one animal, the possibility for the development of variation is reduced. Hence, parthenogenesis cannot produce new varieties.
- ✓ **As a Means of Sex Determination**: As the sex is determined by chromosomes, parthenogenesis is very useful in determining the ratio of males and females in a population. For example, in aphids all the unfertilized eggs develop into males and fertilized eggs into females.
- ✓ **Prevention of Wastage of Energy**: Mating is an essential phenomenon in sexual reproduction. It involves the wastage of energy. Parthenogenesis does not require mating. Hence, most of the energy can be saved.
- ✓ **Polyploidy**: Parthenogenesis favours polyploidy.
- ✓ **Fertility**: Parthenogenesis avoids sterility' in the races.
    - ✓ **Inheritance**: Parthenogenesis supports the chromosome theory of inheritance.

# CLEAVAGE

Fertilization results into the formation of zygote. The process of segmentation (cleavage) immediately follows fertilization or any other process which activates the egg. Cleavage consists of division of the zygote into a large number of cellular entities. The cells which are produced during segmentation are called blastomeres.

**Salient Features of Cleavage**

- ✓ The fertilized egg divides repeatedly by ***mitosis*** to produce thousands of cells called ***blastomeres.***
- ✓ Cleavage produces a solid mass of cells called ***morula.*** Morula develops only in certain animals. Eg. ***Mammals.***
- ✓ In majority of animals, cleavage produces a hollow sphere of cells called ***blastula.***
- ✓ Cleavage provides an adequate number of cells for the construction of tissues and organs.
- ✓ During cleavage, the embryo does not grow.
- ✓ The shape of the embryo does not change during cleavage.
- ✓ During cleavage the amount of DNA increases.
- ✓ Cleavage brings about a fixed proportion between nuclear and cytoplasmic materials.

**Cleavage can be characterized as that period of development in which:**

1. The unicellular fertilized egg is transformed by consecutive mitotic divisions into a multicellular complex.
2. No growth occurs.
3. The general shape of the embryo does not change, except for the formation of a cavity in the interior—the blastocoele.
4. Apart from transformation of cytoplasmic substances into nuclear substance, qualitative changes in the chemical composition of the embryo in cleavage are limited.
5. The constituent parts of the cytoplasm of the egg are not displaced to any great extent and remain on the whole in the same positions as in the egg at the beginning of cleavage.
6. The ratio of nucleus to cytoplasm, very low at the beginning of cleavage, is, at the end, brought to the level found in ordinary somatic cells.

**Chemical Changes during Cleavage:**

Although there is no growth during the period of cleavage, chemical transformations do occur, and at least some are markedly intensified, as compared with the conditions in the unfertilized egg.

The most obvious change observed during cleavage is a steady increase of nuclear material at the expense of cytoplasm. The number of nuclei is of course doubled with every new division of the blastomeres, and this doubling is accompanied by an increase of nuclear substance, which involves an increase of deoxyribonucleic acid—the amount per nucleus of the latter remaining constant.

The increase in the chromosomal deoxyribonucleic acid, at least during the earlier phases of development, must be at the expense of materials contained in the egg. There are several possible sources of such materials. First, the nucleic acids present in the cytoplasm of the eggs should be mentioned.

In sea urchin eggs there is a large amount of ribonucleic acid in the egg cytoplasm, and this gradually disappears later in development. When sea urchin embryos are supplied with radioactively tagged uridine, some of it is later incorporated into the DNA.

Such incorporation is made possible by the presence in developing sea urchin eggs of an enzyme, ribonucleotide reductase, which converts ribonucleotides into deoxyribonucleotides. DNA may, however, be synthesized in the cleaving egg directly from low molecular weight precursors. This has been proved by supplying such precursors, labeled with radioactive atoms, to cleaving eggs of sea urchins and amphibians.

When cleaving sea urchin eggs were kept in seawater containing 14C-labeled glycine (which may be used for the synthesis of purine groups in the nucleic acid molecule), it was found that the radioactive carbon atoms were incorporated in large amounts into the deoxyribonucleic acid, bypassing the cytoplasmic ribonucleic acid. Also, when 14C-labeled glycine was injected into fertilized frog eggs, some of it was incorporated into deoxyribonucleic acid.

The second important aspect of metabolism during cleavage is the synthesis of ribonucleic acids, which is believed to be very limited, although not absent altogether. In frogs, ribosomal RNA apparently is not produced at all until after completion of cleavage.

As the nucleolus is the site of synthesis of rRNA, this organelle is completely lacking in these animals during cleavage. It reappears in the nuclei at the onset of gastrulation simultaneously with the resumption of ribosomal RNA synthesis.

In the sea urchin there is very little ribosomal RNA produced during cleavage, but in both the amphibians and sea urchins synthesis increases drastically at the onset of gastrulation. Messenger RNA and transfer RNA, on the other hand, are synthesized during cleavage, or at least in the later stages of cleavage.

Synthesis of RNA, however, does not seem to be necessary for cleavage, since eggs which are treated with actinomycin D and in which presumably DNA dependent RNA synthesis is suppressed continue cleaving normally. It is concluded, therefore, that any messenger RNA produced during cleavage remains inactive or “masked,” similar to the messenger RNA in unfertilized eggs.

Fertilization in sea urchins leads to a spectacular increase in protein synthesis, and this is continued throughout the period of cleavage. In other animals, such as amphibians, protein synthesis does not markedly change after fertilization; a certain amount of protein synthesis, however, takes place throughout the period of cleavage. The amount of active cytoplasm increases. One indication of this is the steady increase of respiration throughout the period, which is generally attributed to an increase in the amount of active cytoplasm.

Much of the protein newly produced during cleavage is directly involved in the process of cell multiplication. One group of such proteins is the nuclear histones, which are needed for the chromosome replication in the same degree as additional quantities of DNA. In mid-cleavage of sea urchin embryos as much as 50 per cent of the newly synthesized protein is located in the nucleus.

The mRNA for these proteins is transcribed during cleavage and contrary to other mRNA’s, does not become masked, but is immediately used for translation into protein. This exception is probably due to the need for rapid synthesis of large quantities of nuclear histones. Some mRNA for nuclear histones is, however, present in the egg before fertilization.

Another protein synthesized during cleavage is tubulin, the constituent protein of microtubules—the fibers of the achromatic figures appearing during the mitotic divisions of cleavage cells. Tubulin is synthesized on messenger RNA already present in the egg. In the course of cleavage, tubulin is synthesized in increasing quantities, presumably as a result of progressive "unmasking" of the corresponding mRNA.

A third protein synthesized during cleavage is the enzyme ribonucleotide reductase, which in sea urchin embryos converts cytoplasmic ribonucleotides into deoxyribonucleotides, and thus provides a source of material for the replication of the chromosomal DNA.

The messenger RNA for ribonucleotide reductase is present in the unfertilized egg, but becomes active (is unmasked) after fertilization. A fourth protein necessary for chromosomal replication, the DNA polymerase, is already present in necessary quantities in the egg, and its quantity does not increase during early development.

If cleaving eggs are treated with puromycin, which inhibits RNA dependent protein synthesis, cleavage stops immediately, thus showing that protein synthesis is indispensable for cleavage to take place. This is in marked contrast to cleavage being able to proceed in the presence of actinomycin D which effectively prevents the production of new RNA and particularly of new messenger RNA.

Possibly the most important of the proteins which have to be synthesized for cleavage to proceed are those which are used in the replication of the chromosomes – the nucleohistones, actually incorporated into the chromosome structure, and the ribonucleotide reductase, without which the cells are unable to use the supplies of RNA in the cytoplasm for replication of the nuclear DNA.

The synthesis of tubulin seems to be less important, as asters may be formed in the cytoplasm in the presence of puromycin, but do not lead to cell division. Presumably there is enough tubulin in the egg for aster formation, without new synthesis.

**Patterns of Cleavage:**

The way in which the egg is subdivided into the daughter blastomeres is usually very regular. The plane of the first division is, as a rule, vertical; it passes through the animal-vegetal axis of the egg. The plane of the second division is also vertical and passes through the animal-vegetal axis, but it is at right angles to the first plane of cleavage.

The result is that the first four blastomeres all lie side by side. The plane of the third division is at right angles of the first two planes and to the animal-vegetal axis of the egg. It is therefore horizontal or parallel to the equator of the egg. Of the eight blastomeres, four lie on top of the other four, the first four comprising the animal hemisphere of the embryo, the second the vegetal hemisphere.

If each of the blastomeres of the upper tier lie over the corresponding blastomeres of the lower tier, the pattern of the blastomeres is radially symmetrical. This is called the radial type of cleavage.

In many animals, however, the upper tier of blastomeres may be shifted with respect to the lower tier, and the radially symmetrical pattern becomes distorted in various degrees. The distortion may sometimes be due to individual variation, but there are certain groups of animals in which distortion always takes place and is the result of a specific structure of the egg.

In the annelids, molluscs, nemerteans, and some of the planarians (the Poly-cladida), all the blastomeres of the upper tier are shifted in the same direction in relation to the blastomeres of the lower tier, so that they come to lie not over the corresponding vegetal blastomeres, but over the junction between each two of the vegetal blastomeres.

This arrangement comes about not as a result of secondary shifting of the blastomeres, but because of oblique positions of the mitotic spindles, so that from the start the two daughter cells do not lie one above the other. The four spindles during the third cleavage are arranged in a sort of spiral. This type of cleavage is therefore called the spiral type of cleavage.

The turn of the spiral as seen from above may be in a clockwise direction or in a counterclockwise direction. In the first case the cleavage is called dextral; in the second case it is called sinistral. Since the cleavage planes are at right angles to the spindles, they also deviate from the horizontal position found in radial cleavage, and each cleavage plane is inclined at a certain angle.

The spiral arrangement of the mitotic spindles can be traced even in the first two divisions of the egg; the spindles are oblique and not vertical as in radial cleavage. However, the resulting shifts in the position of the blastomeres are not as obvious as after the third cleavage.

During the subsequent cleavages the spindles continue to be oblique, but the direction of spiraling changes in each subsequent division. Dextral spiraling alternates with sinistral, so that the spindle of each subsequent cleavage is approximately at right angles to the previous one.

Note that the type of cleavage of the egg as a whole, whether dextral or sinistral, depends on the direction of spiraling occurring during the third division of the egg.

Peculiarities of the cleavage pattern can also be introduced by differences in the size of the blastomeres. Of the four blastomeres in the four-cell stage of eggs having a spiral type of cleavage, one blastomere is often found to be larger than the other three.

This allows us to distinguish the individual blastomeres. The four first blastomeres are denoted by the letters A, B, C, D, the letters going in a clockwise direction (if the egg is viewed from the animal pole) and the largest blastomere being denoted by the letter D.

In some animals which otherwise have an approximately radial type of cleavage, two of the first four blastomeres may be larger than the other two, thus establishing a plane of bilateral symmetry in the developing embryo. Subsequent cleavages may make the bilateral arrangement of the blastomeres still more obvious (as in tunicates and in nematodes, although in a different way). The resulting type of cleavage is referred to as the bilateral type.

A very special type of cleavage showing bilateral symmetry is found in nematodes. The first division produces two unequal cells – a slightly larger cell designated as cell AB and a smaller cell $P_1$. The two cells divide next in mutually perpendicular planes, so that the blastomeres in the four-cell stage are placed in the form of a letter T.

The transverse shaft of the T is made up of blastomeres A and B (descendants of the cell AB), and the vertical shaft is made up of the offspring of blastomere $P_1$. The cells are designated as EMSt (abbreviation for endoderm, mesoderm, stomodeum—which shows the destiny of this cell) and as $P_2$. The “T” arrangement is, however, only temporary, the $P_2$ cell soon shifting toward the B cell.

The blastomeres are then arranged in a rhomboid figure. Next, the third division enhances the bilateral symmetry of the embryo, because the blastomeres A and B each divide into a right and left daughter cell, while the other two blastomeres produce a group of four cells lying one behind the other in the median plane. The blastomeres of this group are designated Mst, E, $P_3$, and C, respectively.

The cleavage in nematodes is also an example of determinate or mosaic cleavage in which definite blastomeres give rise to specific parts of the embryo. Thus, blastomeres A, B, and C give rise to the skin of the animal, blastomere E gives rise to the endoderm of the alimentary tract, blastomere MSt gives rise to the mesoderm and the stomodeum, and blastomere $P_3$ eventually produces the reproductive cells.

From the stage of eight cells a slight asymmetry is noticeable between the right and the left halves of the embryo.

The yolk, which is present in the egg at the beginning of cleavage in greater or lesser quantities, exerts a very far-reaching effect on the process of cleavage. Every mitosis involves movements of the cell components—the chromosomes, parts of the cytoplasm constituting the achromatic figure, the mitochondria, and the surface layer of the cell—the activity of which along the equator of the maternal cell leads to the eventual separation of the daughter cells.

During these movements, the yolk granules or platelets behave entirely passively and are passively distributed among the daughter blastomeres. When the yolk granules or platelets become very abundant, they tend to retard and even to inhibit the process of cleavage.

As a result, the blastomeres which are richer in yolk tend to divide at a slower rate and consequently remain larger than those which have less yolk. The yolk in the uncleaved egg is more concentrated toward the vegetal pole of the egg. It is therefore at the vegetal pole of the egg that cleavage is most retarded by the presence of yolk, and where the blastomeres are of the largest size.

A good example of the effect of the yolk on cleavage is provided by the frog's egg. The yolk's influence may be detected even during the first division of the fertilized egg. During the anaphase of the mitotic division, a furrow appears on the surface of the egg which is to separate the two daughter blastomeres from each other.

This furrow, however, does not appear simultaneously all around the circumference of the egg, but at first only at the animal pole of the egg, where there is less yolk. (It has been indicated that the first cleavage plane is vertical and therefore passes through the animal and vegetal poles of the egg.)

Only gradually is the cleavage furrow prolonged along the meridians of the egg, until, cutting through the mass of yolk-laden cytoplasm, it eventually reaches the vegetal pole and thus completes the separation of the first two blastomeres.

The same process is repeated during the second cleavage. During the third cleavage, when the plane of separation of the daughter blastomeres is horizontal, the furrow appears simultaneously over the whole circumference of the egg, for it meets everywhere with an equal resistance from yolk.

A further accumulation of the yolk at the vegetal pole of the egg causes still greater delay in the cell fission at this pole, so that the cleavage becomes inhibited more and more. This can be clearly traced in a series of various ganoid fishes, whose eggs possess an increasing amount of yolk.

In Acipenser the cleavage is complete, as in the amphibians, but the difference between the micromeres of the animal hemisphere and the macro-meres of the vegetal hemisphere is much greater than in amphibians. In Amia cleavage starts at the pole, and the cleavage furrows reach the vegetal pole, but they are so retarded that subsequent divisions begin at the animal pole before the preceding furrows cut through the yolk at the vegetal pole.

In Lepidosteus the cleavage starts at the animal pole as in Amia, but the cleavage furrows never reach the vegetal pole, so that the vegetal hemisphere of the egg remains un-cleaved, resulting in what is called incomplete cleavage. The type of cleavage during which the whole of the egg becomes subdivided into blastomeres is called holoblastic (complete) cleavage.

The type of cleavage in which only a part of the egg is subdivided into blastomeres is called meroblastic (incomplete) cleavage. As the result of meroblastic cleavage the egg is divided into a number of separate blastomeres, and a residue, which is a continuous mass of cytoplasm, usually with some nuclei scattered in it.

Sometimes the terms holoblastic and meroblastic are applied also to the eggs having a particular type of cleavage; thus, one finds in the literature the term “holoblastic eggs,” meaning eggs having holoblastic cleavage, and also the term “meroblastic eggs” for eggs having meroblastic cleavage.

In eggs in which the yolk is segregated from the active cytoplasm (elasmobranchs, bony fishes, birds, and reptiles), the cleavage, right from the start, is distinctly recognizable as meroblastic or incomplete. At first, all the cleavage planes are vertical, and all the blastomeres lie in one plane only.

The cleavage furrows separate the daughter blastomeres from each other but not from the yolk, so that the central blastomeres are continuous with the yolk at their lower ends, and the blastomeres lying on the circumference are, in addition, continuous with the un-cleaved cytoplasm at their outer edges. As the nuclei at the edge divide, more and more cells become cut off to join the ones lying in the center, but the new blastomeres are also in continuity with the un-cleaved yolk underneath.

In a later stage of cleavage, the blastomeres of the central area become separated from the underlying yolk in one of two ways – either slits may appear beneath the nucleated parts of the cells, or else the cell divisions may occur with horizontal (tangential) planes of fission. In the latter case one of the daughter cells, the upper one, becomes completely separated from its neighbors, while the lower blastomere retains the connection with the yolk mass.

The marginal cells, which remain continuous with one another around their outer edges, are also continuous with the mass of yolk and hence with the lower cells resulting from tangential divisions. All these blastomeres eventually lose even those furrows which partially separated them from one another and fuse into a continuous syncytium with numerous nuclei but no indication of individual cells.

**2. Planes of Cleavage:**

During early cleavage, distinct geometrical relationships exist between the blastomeres, i.e., each plane of cell-division bears a definite relationship with each other.

**The planes of division are:**

**a. Meridional plane of cleavage:**

When a furrow bisect both the poles of the egg passing through the median axis or centre of egg it is called meridional plane of cleavage. The median axis runs between the centre of animal pole and vegetal pole.

**b. Vertical plane of cleavage:**

When a furrow passes in any direction (does not pass through the median axis) from the animal pole towards the opposite pole.

**c. Equatorial plane of cleavage:**

This type of cleavage plane divides the egg halfway between the animal and vegetal poles and the line of division runs at right angle to the median axis.

**d. Latitudinal plane of cleavage:**

This is almost similar to the equatorial plane of cleavage, but the furrow runs through the cytoplasm on either side of the equatorial plane.

**3. Types of Cleavage:**

Considerable amount of reorganisation occurs during the period of cleavage and the types of cleavage depend largely upon the cytoplasmic contents.

**Different types of cleavage encountered in different eggs are catalogued below:**

**a. Holoblastic op total cleavage:**

When the cleavage furrows divide the entire egg.

**It may be:**

**Equal:**

When the cleavage furrow cuts the egg into two equal cells. It may be radially symmetrical, bilaterally, symmetrical, spirally symmetrical or irregular.

**Unequal:**

When the resultant blastomeres become unequal ir size.

**b. Meroblastic cleavage:**

When segmentation takes place only in a small portion of the egg resulting in the formation of blastoderm, it is called meroblastic cleavage. Usually the blastoderm is present in the animal pole and the vegetal pole becomes laden with yolk which remains in an tihcleaved state, i.e., the plane of division does not reach the periphery of blastoderm or blastodisc.

**c. Transitional cleavage:**

In many eggs, the cleavage is atypical which is neither typically holoblastic nor meroblastic, but assumes a transitional stage between the two.

**4. Effects of Yolk in Cleavage:**

The fertilized egg in most cases contains yolk, which are inert bodies. During division these bodies exert mechanical influences. In the egg of Amphioxus, the yolk is thin and remains uniformly distributed. Therefore the division is complete and early divisions occur at a very quicker rate.

The amphibian egg contains yolk which is localised at the vegetal pole. Here division initiates from the animal pole and extends towards the vegetal pole, where the progress of cleavage slows down considerably.

Consequently, the animal pole divides faster than the vegetal pole. The eggs of reptiles and birds are fully laden with large masses of yolk, thus restricting the cytoplasm and nucleus on the periphery as a circular disc on the animal pole. Here the lines of cleavage divide only the small animal pole region. Such effects of yolk on cleavage pattern influence the pattern of further development.

**5. Mechanism of Cleavage:**

The incidence of cleavage provides unique opportunity to study the mechanism of cell division and specially the role of different cell organelles during division.

**Opinions differ regarding the accumulation of force for the initiation of cleavage and following factors are believed to be responsible for controlling the cleavages:**

(a) Localised expansion of cortex.

(b) Increased stiffness of the cortical cytoplasm.

(c) Increase of tangential force activity in the cortex.

(d) Contractile nature of the regions near the cortex and

(e) Formation of new cell membrane from the subcortical cytoplasm.

Though the abovementioned factors are not clearly understood, it is evident that three structures present within the cell: Cortical layer, Spindle structures and Chromosomes play the important part.

The energy which is required during the process is supplied by the metabolic activity of the developing egg. Besides the factors involved in segmentation, there are cleavage laws which govern the behaviour of the cells during cleavage.

**Sach's rules:**

The blastomeres tend to divide into identical daughter cells and a cleavage furrow tends to cut the previous cell at right angles.

**Hart wig's laws:**

The position of nucleus is vital and it tends to lie at the centre of the protoplasmic content of the cell. The nucleus exerts influence on cleavage. The long axis of mitotic spindle usually coincides with the long axis of the protoplasmic content. During cleavage the long axis of the protoplasm has the tendency to cut transversely.

**Balfour's law:**

The rate of cleavage is inversely proportional to the amount of yolk material present in the egg.

**6. Chemical Changes during Cleavage:**

Significant chemical changes go on in the fertilized egg during cleavage.

**They are:**

**Increase of nuclear material:**

During cleavage a steady increase in nuclear material (predominantly DNA) is observed. Cytoplasm of the egg is the source of such nuclear material. Cytoplasmic DNA contained in mitochondria and yolk platelets are available.

**RNA synthesis:**

During cleavage messenger RNA (mRNA) and transfer RNA (tRNA) are synthesised during cleavage, especially in late stages.

**Synthesis of proteins:**

Throughout the period of cleavage there is steady and spectacular increase in protein synthesis.

**7. Cleavage in Different Chordates:**

The pattern of cleavage differs in different animals. The following account will give an idea of the process of cleavage in different chordates.

**a. Amphioxus:**

The cleavage in Amphioxus is typically holoblastic (Fig. ). The first cleavage is meridional. The second cleavage is also meridional but at right angle to the first one. Four equal blastomeres are produced. The third cleavage is latitudinal and occurs slightly above the equatorial plane resulting in the production of eight blastomeres—four are smaller called the micromeres and four are larger known as the macromeres.

The micromeres are situated towards the animal pole and the macromeres towards the vegetal pole. The fourth cleavage is meridional which involves all the eight cells resulting in the formation of eight micromeres and eight macromeres. The fifth cleavage planes are latitudinal.

Each micromere is divided into an upper and lower micromere and each macromere likewise divides to form an upper and lower macromere. The fifth cleavage planes produce thirty-two blastomeres. The sixth cleavage planes are nearly meridional involving all the thirty-two cells resulting in sixty-four cells.

At the 64-cell stage a conspicuous space is produced at the centre and this space becomes filled with a fluid. When the eighth cleavage planes take place, the blastula becomes pear- shaped and the blastocoel becomes large.

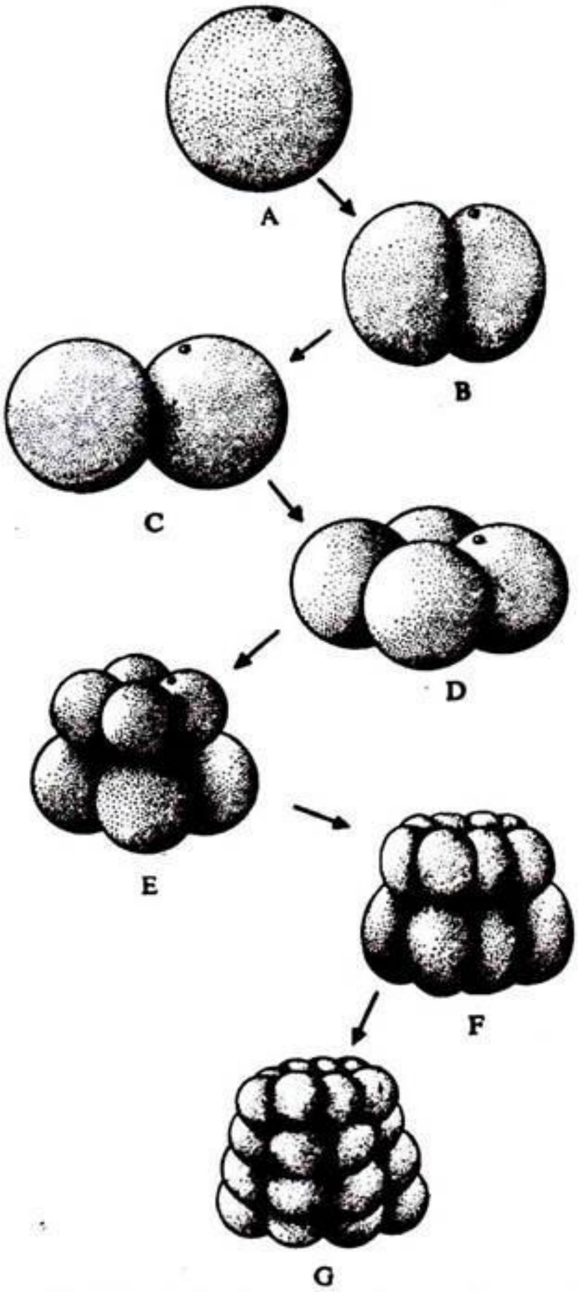


Fig.: Early cleavage pattern in the egg of Amphioxus. A.Fertilized egg. B-C. First cleavage. D. Second cleavage. E.3rd cleavage. F.4th cleavage G.Morula stage.

**b. Frog:**

The egg of frog is telolecithal with a considerable amount of yolk localized towards the vegetal pole. The cleavage is holoblastic in nature, but differs considerably from that of Amphioxus because of larger quantity of yolk.

The first cleavage plane is meridional which occurs at about 3-3½ hours after fertilization. But the time depends largely on extrinsic factors. The first cleavage starts at the animal pole and gradually travels towards the vegetal pole. Thus the egg is bisected along the poles. Two blastomeres of equal size are produced. The second cleavage is almost meridional but oriented at right angles to the first cleavage plane (Figure).

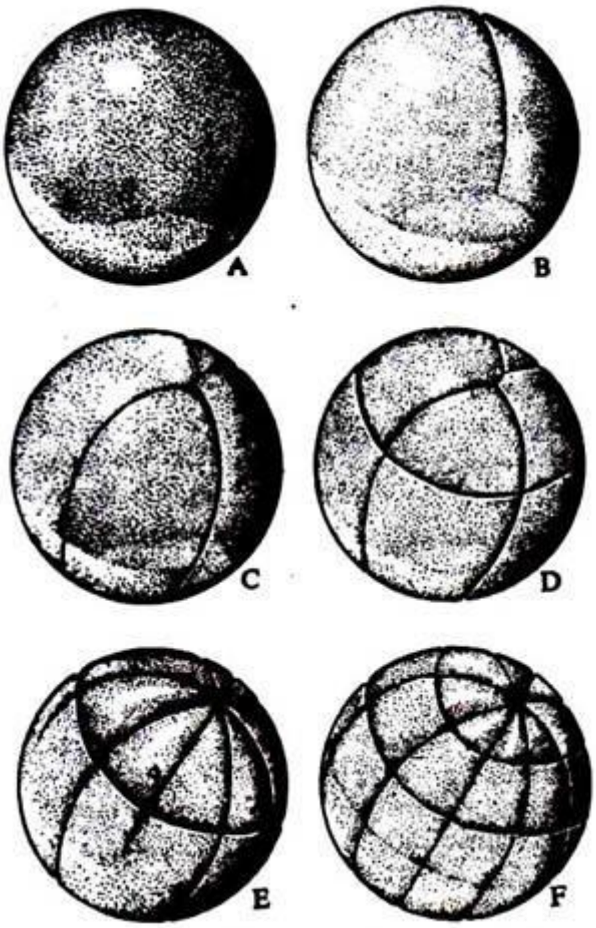



Fig. Semidiagrammatic representation of the cleavage pattern in the egg of frog. A.Fertilized egg. B-C. First cleavage. D. Second cleavage. E.3rd cleavage. F.4th cleavage G.Morula stage.

The four blastomeres thus produced are not qualitatively identical, because the grey crescent material is present in two of the four blastomeres. Each blastomere contains dark pigment at the animal pole and yellowish yolk towards the vegetal pole. The third cleavage is latitudinal and occurs at right angles to previous cleavage planes but passes slightly above the equator.

The furrow produces eight unequal blastomeres, four micromeres in the animal hemisphere and four macromeres in the vegetal part. The fourth cleavage planes are meridional which involve the micromeres first and pass on slowly towards the yolk-laden macromeres of the vegetal pole.

In Amphioxus, the cleavages occur in a synchronous fashion, while in frog considerable degree of irregularities (asynchronism) appear in later stages. But it is certain that the micromeres always continue to divide at a faster rate than do the macromeres.

At the eight-celled stage, a small space makes its appearance between the four micromeres. As development goes on, this space becomes conspicuous and forms the blastocoel. The floor of the blastocoel is formed of macromeres. The blastocoel (or segmentation cavity) is eccentrically located and becomes displaced towards the animal pole as development proceeds.

**c. Chick:**

Typical meroblastic cleavage occurs in chick, where the segmentation activity is restricted only at the blastodisc or germinal disc (Fig.). Thus the cleavage is incomplete.

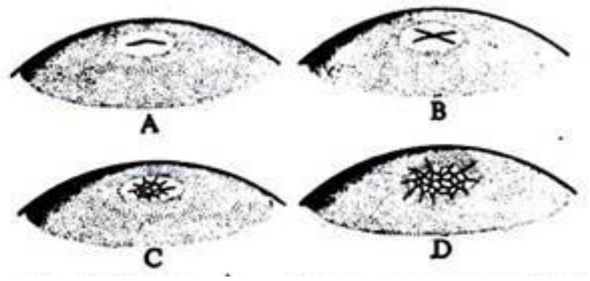



Fig. Side view of cleavage pattern of the egg of chick. A. First cleavage. B. Second cleavage. C-D.Subsequent cleavage.

The first cleavage starts as a meridional furrow near the centre of the blastodisc at about 4½ hours after fertilization when the egg reaches the isthmus of oviduct. This furrow cuts across the blastodisc and passes towards the vegetal pole but does not reach the pole. The second cleavage is also meridional, but approximately at right angles to the first one. The third cleavage is vertical.

The fourth cleavage is also vertical but the division is not synchronous. As a consequence eight central cells encircled by twelve marginal cells are produced. From this point onward the cleavage becomes irregular and a disc containing smaller cells appears.

This disc remains firmly connected with the underlying yolk. Soon a cleft appears which separates the disc in the middle from the underlying yolk. The new cavity in between is known as sub-germinal space (Fig.).

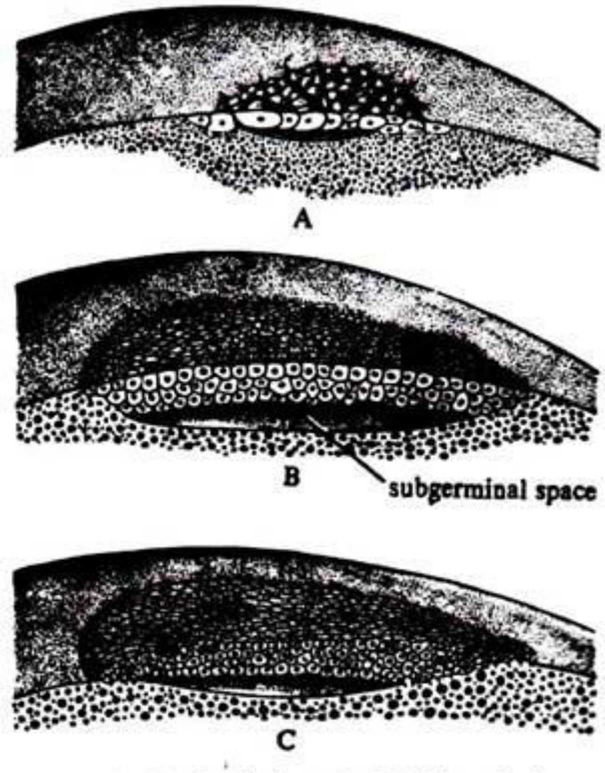



**Fig. 5.13. Sectional view of chick blastula showing the formation (A–C) of subgerminal space (after Huettner).**

Fig. Sectional view of chick blastula showing the formation (A-C) of submerged space (after Huettner)

Thus at the end of segmentation, the disc contains many-layered small cells which are connected with the yolk only at the periphery. This disc is then termed as blastoderm, the cells of which still continue to divide.

The peripheral part which lies in contact with yolk possesses granular cells called area apaca and the inner layer having clear portion is called area pellucida. At one end of area opaca, aggregation of cells takes place. This denotes the formation of future posterior side.

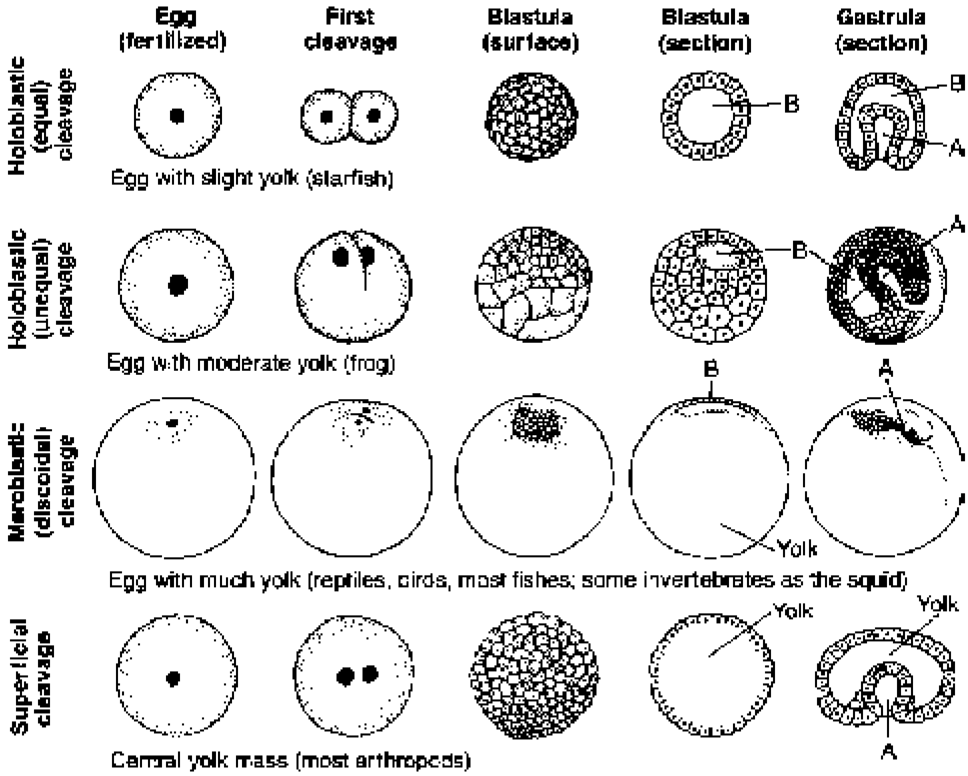



Fig. Types of cleavage