

MAJOR–IV

DEVELOPMENTAL BIOLOGY

Cr 4(3+1)

Objectives

The course aims to:

- Provide information on transmission of traits from the parents in their gametes, the formation of zygote and its development
- Impart detailed knowledge about cellular basis of morphogenesis, mechanisms of cellular differentiation and induction.
- Provide understanding of the mechanisms of organogenesis, factors controlling growth and oncogenesis.

Course Contents

Introduction: Principal features of development, origin of sexual reproduction, developmental patterns; Spermatogenesis; Oogenesis. Fertilization: Recognition of sperm and egg, fusion of gametes, activation of egg metabolism, rearrangement of egg cytoplasm.

Cleavage: Patterns of embryonic cleavage, mechanism of cleavage. Gastrulation: Fate maps, gastrulation in sea urchin, amphibians, birds and mammals.

Early Vertebrate Development: Neurulation, ectoderm, mesoderm and endoderm.

Cellular Basis of Morphogenesis: Differential cell affinity, cell adhesion molecules.

Mechanism of Cellular Differentiation: RNA processing, translational regulation of developmental process, cell-fate by progressive determinants, autonomous cell specification by cytoplasmic determinants, establishment of body axes and mechanism of teratogenesis; Secondary Induction. Organogenesis: A brief account; Origin and migration of germ cells in vertebrates.

Factors controlling growth and oncogenesis.

Post embryonic Development and metamorphosis

Hormones as mediators of development; Regeneration in vertebrates.

Practicals

1. Study of the structure of gametes in some representative cases, i.e. frog, fish, fowl and a mammal.
2. Study of cleavage and subsequent development from prepared slides and/or whole mounts in various animals i.e., frog, chick etc. Study of fertilization, early development of frog/fish through induced spawning under laboratory conditions.
3. Preparation and study of serial sections of frog or chick embryos.
4. Application of microsurgical techniques on chick embryos *In vitro*. Preparation and staining of histological slides.

Books Recommended

1. Gilbert, S. F. 2012. Developmental Biology, Sinauer Associates, Sunderland, MA.
2. Klaus, K. 2001. Biological Development. 2nd Ed., McGraw Hill.
3. Balinsky, B. I. 1985. An Introduction to Embryology, Saunders.
4. Oppenheimer, S.S. 1984. Introduction to Embryonic Development, Allen and Bacon.
5. Saunders, J. W. 1982. Developmental Biology, McMillan and company.
6. Ham, R. G., Veomett, M. J. 1980. Mechanism of Development. C. V. Mosby Co.

Spermatogenesis

The spermatogenesis is the process of formation of spermatozoa from primordial germ cells (PGCs)/spermatogonia present in the walls of the seminiferous tubules of the testis. The PGCs remain dormant in the seminiferous tubules of testes till puberty. At puberty, they undergo a series of divisions to form spermatogonia. The various stages of spermatogenesis are as under:

1. The PGCs divide by mitosis to form dark type A spermatogonia, which act as stem cells. Each dark type A spermatogonium undergoes mitosis to form one dark A spermatogonium and other light type A spermatogonium. The dark type A spermatogonia are kept in reserve for repetition of the next cycle. The light type A spermatogonium undergoes mitotic division to form two dark type B spermatogonia.
2. The type B spermatogonium undergoes mitotic division to form two primary spermatocytes (largest germ cells). N.B.

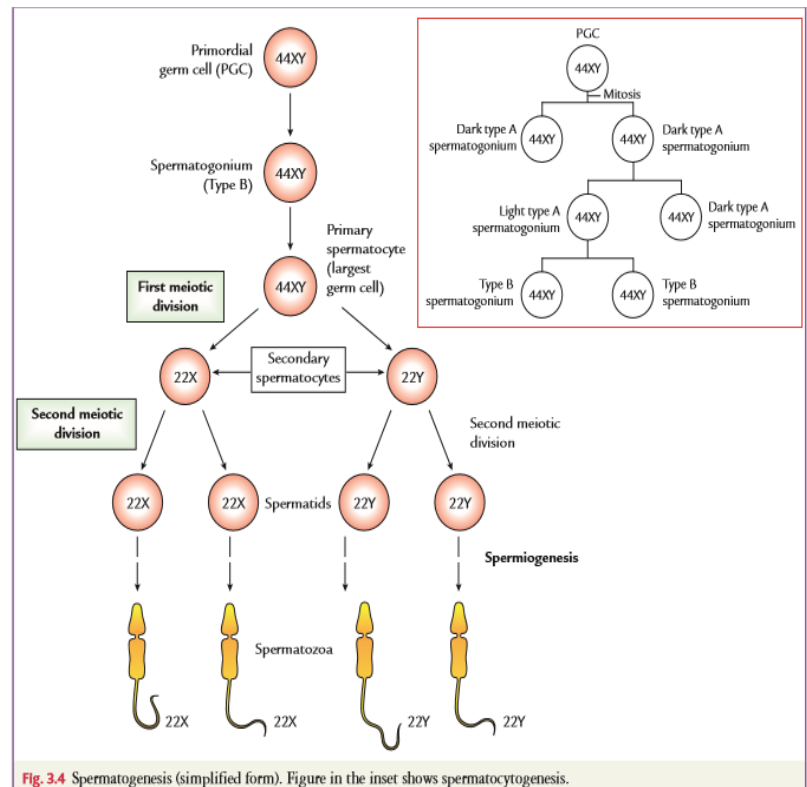


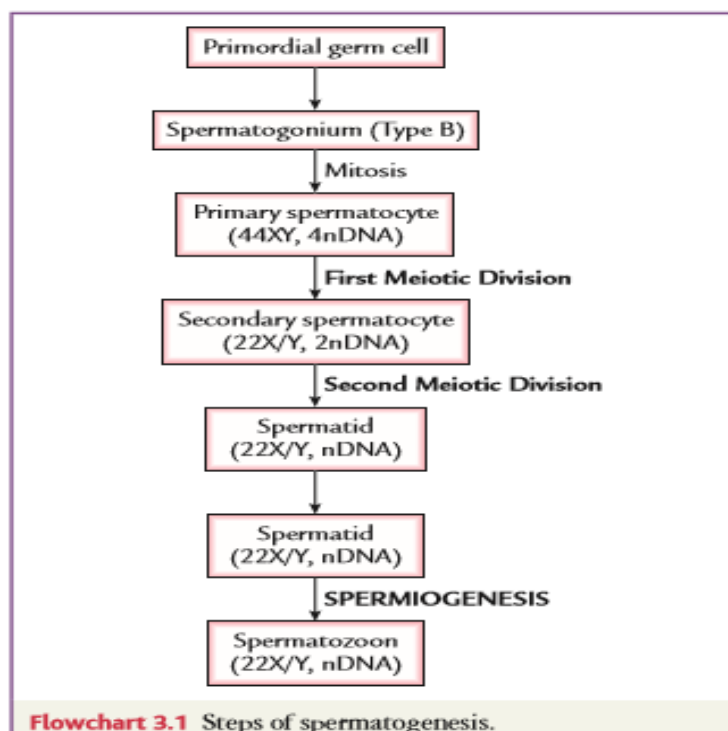
Fig. 3.4 Spermatogenesis (simplified form). Figure in the inset shows spermatocytogenesis.

Spermatocytogenesis: In this process, PGCs undergo a series of mitotic divisions to form a large number of spermatogonia. Depending upon their appearance, three types of spermatogonia are distinguished, viz., (a) dark type A spermatogonia, (b) light type A spermatogonia, and (c) type B spermatogonia.

3. The primary spermatocytes undergo first meiotic division (reductional division) to form two secondary spermatocytes. The secondary spermatocytes thus have haploid number of chromosomes.
4. Each secondary spermatocyte immediately undergoes second meiotic division (i.e., mitotic division) to form two spermatids, each with haploid number of chromosomes. Thus, four haploid spermatids are produced from the meiotic division of one primary spermatocyte. The spermatids are small cells of about half the size of the secondary spermatocyte, and have round and darkly stained nuclei. The spermatids lie close to the lumen of seminiferous tubule.

5. Each spermatid gradually changes its stage to become spermatozoon or sperm. This transformation of circular spermatid into an elongated spermatozoon is called spermiogenesis.

Thus from one primary spermatocyte four spermatozoa are formed; two with 22 autosomes and one X chromosome ($22 + X$, $22 + X$) and two with 22 autosomes and one Y chromosomes ($22 + Y$, $22 + Y$) (Fig. 3.4). The steps of spermatogenesis are summarized in Flowchart 3.1. To understand the process of spermiogenesis, the student must first understand the structure of spermatozoon .



Structure of Spermatozoon

The spermatozoon ($50\ \mu$ in length) consists of head, neck, and tail. The tail is further divided into three parts: middle piece, principle piece, and end piece. Tail forms four-fifth of the length.

The parts of mature sperm are shown on the left side whereas the sections through the head, neck, middle piece, principal piece, and end

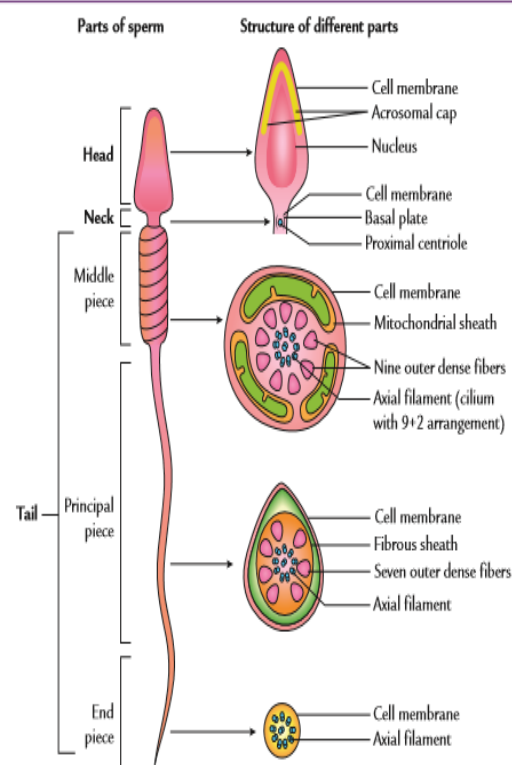


Fig. 3.5 Human sperm. The parts of mature sperm are shown on the left side whereas the sections through the head, neck, middle piece, principal piece, and end piece along with their composition are shown onto the right side.

piece along with their composition are shown onto the right side.

Head The head of sperm appears somewhat like a spearhead in section. It mainly consists of a nucleus that contains the condensed chromatin material (mostly DNA). Anterior two-third of the nucleus is covered by an acrosomal cap that contains various enzymes including hyaluronidase and acrosin.

Neck The neck is narrow. It contains a funnel-shaped basal plate and a centriole. The centriole gives rise to axial filament that extends throughout the tail.

Tail The tail consists of three parts: middle piece, principal piece, and end piece.

1. Middle piece: It contains the axial filament in the center that is surrounded by spirally arranged mitochondrial sheath. At the distal end of the middle piece there is a ring-like structure through which axial filament passes. It is called annulus and is derived from the other centriole.

2. Principle piece: It is made of axial filament covered by seven outer dense fibers.

3. End piece: It is made up of only the axial filament.

N.B

- Structure of the axial filament is very similar to that of the cilium.
- The whole spermatozoon is covered by plasma membrane.
- The axial filament is responsible for the movements of the spermatozoon, while mitochondria supply energy for these movements.

Spermiogenesis

The process by which the spermatids

are transformed into mature spermatozoa is known as spermiogenesis.

Process of Spermiogenesis The spermatid is more or less a circular cell containing a nucleus, golgi apparatus, centrosome, and mitochondria. The spermatid is transformed into the spermatozoon as follows:

1. Nuclear material (chromatin) gets condensed and the nucleus moves towards one pole of the cell to form the head of the spermatozoon.
2. Golgi apparatus forms the acrosomal cap that covers anterior two-third of the nucleus.
3. Centrosome divides into two centrioles. One centriole becomes spherical and

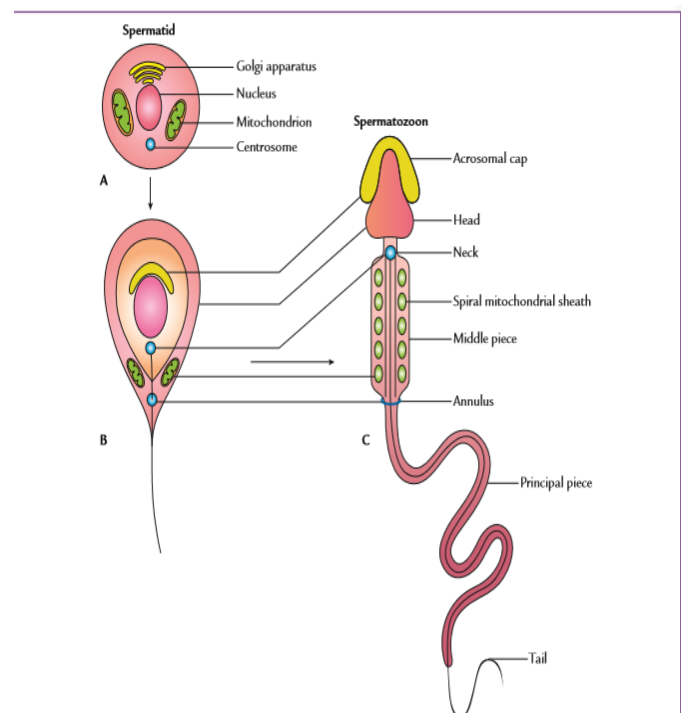


Fig. 3.6 Process of spermiogenesis.

moves towards the posterior end of nucleus to occupy the neck region. It gives rise to the axial filament. The other centriole moves away from the first centriole and becomes ring shaped. It forms an annulus/ring around the distal end of the middle piece through which axial filament passes.

4. The part of the axial filament between the neck and annulus becomes surrounded by the mitochondria, and together with them forms the middle piece.
5. The remaining part of the axial filament elongates to form the principle and end pieces or tail. Most of the cytoplasm of spermatid is shed off but the cell membrane remains, which covers the entire spermatozoon.

The structural components of the spermatid and the spermatozoon are compared in Table 3.2

Table 3.2 Comparison of structural components of the spermatid and the spermatozoon	
Spermatid (round cell)	Spermatozoon (elongated cell)
- Nucleus	- Head
- Golgi apparatus	- Acrosomal cap
- One centrosome	- Two centrioles (a) One lies in the neck and forms axial filament (b) Other forms annulus at the distal end of middle piece
- Mitochondria	- Spirally surround the axial filament between the neck and annulus to form the middle piece; the remaining axial filament forms the tail
- Cell membrane	- Cell membrane

Clinical Correlation

Abnormal sperms: The abnormality of sperms is common as compared to the oocytes. Morphologically for clinicians the sperm consists of two parts of head and tail. Types of abnormalities are as under.

1. Morphological abnormalities (a) Head and tail of sperms may be abnormal (viz., two heads, two tails) (b) Sperms may be giant or dwarf (c) Sperms may be joined

2. Immotility: For potential fertility, 50% sperms should be motile after 2 hours of ejaculation and some should be motile after 24 hours.

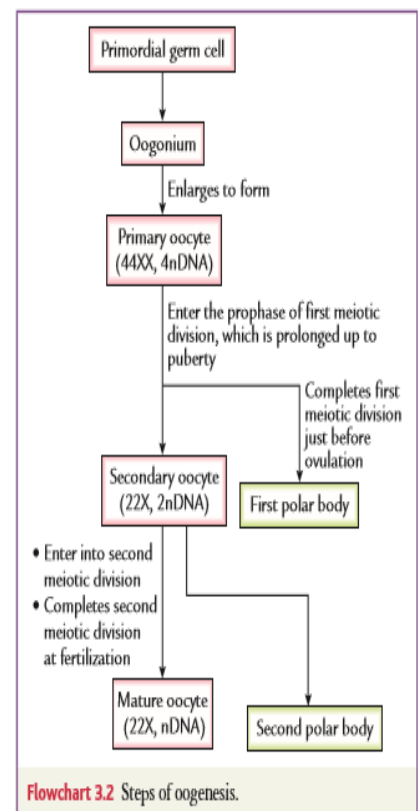
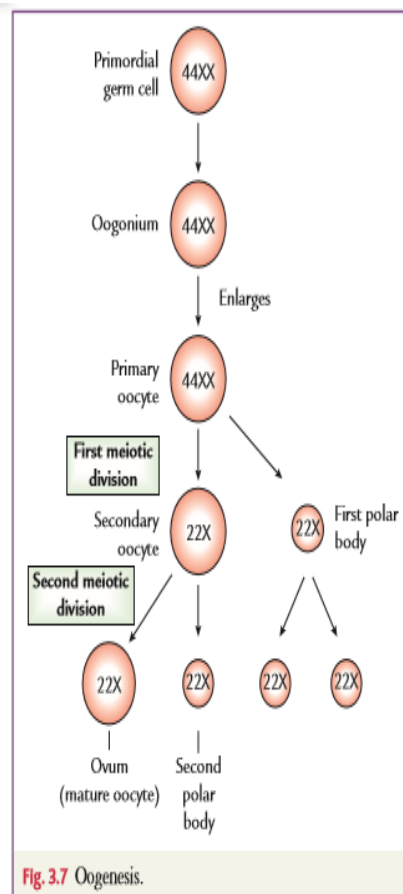
3. Genetic abnormalities: Sperm having abnormal chromosomal content (rare as compared to the oocytes).

Oogenesis

The oogenesis is the process of formation of female gametes—the oocytes from PGCs. The process of oogenesis begins long before birth in the cortex of the ovary.

ion of female gametes—the oocytes from PGCs. The process of oogenesis begins long before birth in the cortex of the ovary.

- The PGCs divide by mitosis to form a large number of oogonia. Each oogonium then enlarges to form a **primary oocyte**. The primary oocyte enters the prophase of first meiotic division before birth. But this division is arrested till puberty due to the presence of an **oocyte maturation inhibitor (OMI)** factor secreted by the follicular cells surrounding the oocyte. The first meiotic division gets completed only when primary oocytes start maturing and are getting prepared for ovulation.



- At puberty in each ovarian cycle, 5–50 primary oocytes re-assume their first meiotic division, which is completed just before the ovulation, forming two daughter cells each with haploid number of chromosomes. The first meiotic division is unequal; most of the cytoplasm goes to one daughter cell forming secondary oocyte, while the other daughter cell receives minimal cytoplasm and forms the **first polar body**.
- The **secondary oocyte** enters the second meiotic division at the time of ovulation, but this division is completed only after the sperm has penetrated the secondary oocyte. The second meiotic division is also unequal so that one daughter cell receives most of the cytoplasm and forms the ovum, while the other daughter cell receives a very small amount of cytoplasm and forms the **second polar body**.
- Thus, one primary oocyte forms only one ovum with 22 autosomes and one X chromosome; and three polar bodies each with 22 autosomes and one X chromosome are formed.
- Oogenesis is accompanied by development and growth of the follicles.

The differences between the male and female gametes

Table 3.3 Differences between male and female gametes		
Features	Sperm (male gamete)	Secondary oocyte (female gamete)
Size	Very small, about $2\mu\text{m}$	Very large, about $120\mu\text{m}$
Length	More	Less
Motility	Highly motile	Immotile
Amount of cytoplasm	Absent/very little cytoplasm	Large amount of cytoplasm
Types	Two types: (a) X-bearing sperms ($22+X$) and (b) Y-bearing sperms ($22+Y$)	Only one type ($22+X$)

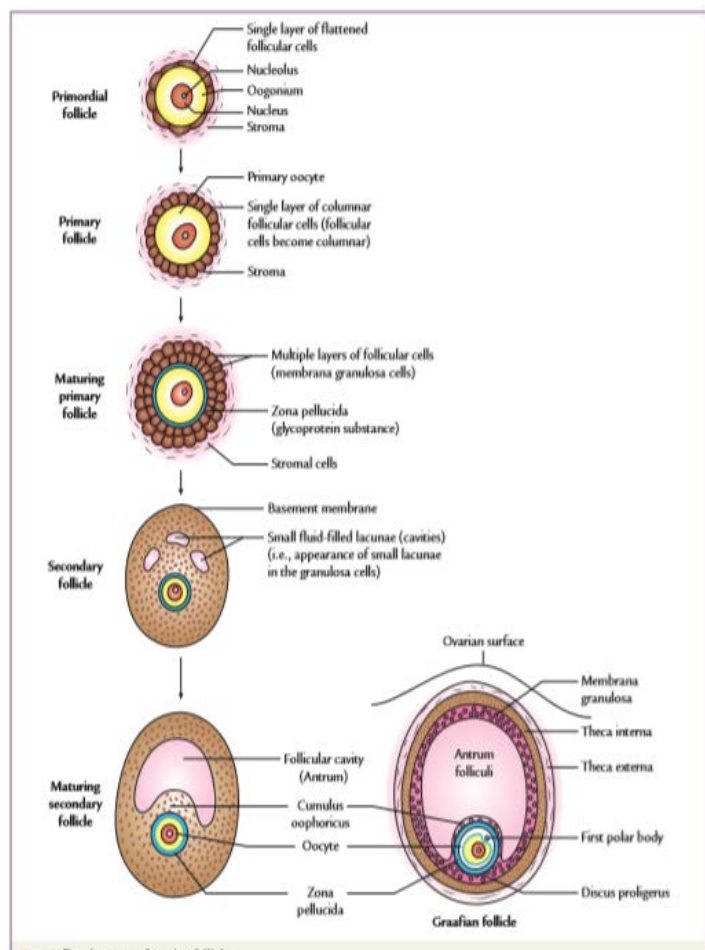
Development of Ovarian Follicles and Ovulation

A. Development of follicles. The various stages of development of ovarian follicles are as follows.

1. The oogonium gets covered by a single layer of flat epithelial cells—the **follicular cells** (which are derived from stromal cells of ovary or from the surface epithelium of the ovary) to form **the primordial follicle**. The oogonium within the follicle contains single large nucleus with prominent eccentric nucleolus.

2. The flattened follicular cells become columnar and form the unilaminar **primary follicle**. The follicular cells proliferate to form several layers for the formation of **membrana granulosa**. The follicular cells are now called **granulosa cells**. The primary oocyte and its granulosa cells secrete a glycoprotein

substance that forms a thick homogeneous membrane between the granulosa cells and the primary oocyte. This membrane is **termed zona pellucida**. The granulosa cells rest on the basement membrane that separates these cells from the surrounding stromal cells. This is **called multilaminar (maturing) primary follicle**.



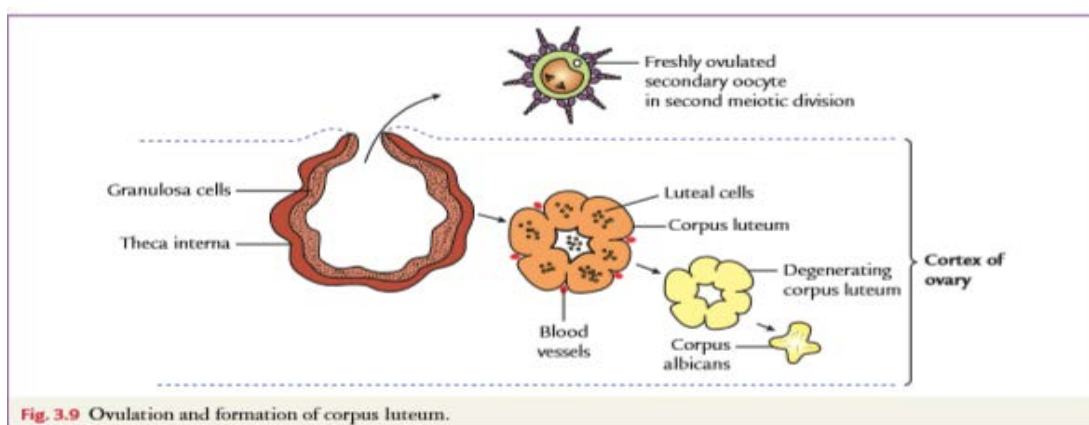
3. The small fluid-filled cavities appear between the follicular cells. These cavities fuse together to form a large cavity—the antral cavity/antrum and the follicle is termed **secondary (vesicular) follicle**.

4. The antrum gradually increases in size and pushes the oocyte towards one side of the follicle. The granulosa cells that surround the oocyte are called **cumulus oophoricus (or cumulus ovaricus)** and those that attach the oocyte to the wall of the follicle are called **discus proligerus**. As the follicle expands the stromal cells surrounding the granulosa cells become condensed to form a covering called **theca interna (theca = covering)**. Outside the theca interna some fibrous tissues get condensed to form another covering of the follicle and is called **theca externa**. The ovarian follicle is now fully matured and is termed **Graafian follicle**.

N.B. Thecal gland: The cells of theca interna later secrete estrogen hormone and together they form the thecal gland.

B. Ovulation: It is a process of shedding off an ovum from the ovary. The Graafian follicle enlarges and becomes so big that it not only reaches the surface of the ovary but also forms a bulge on the surface of ovary. The theca and stroma on this side of follicle become very

thin. An avascular area (stigma) appears in the most convex superficial position of the follicle and, at the same time,



the cells of cumulus oophoricus become loosened by the accumulation of intercellular fluid. Ultimately the follicle ruptures and the ovum is released from the cortex of the ovary (ovulation). The expelled secondary oocyte is surrounded by zona pellucida and one or more layers of follicular cells, which are radially arranged as corona radiata. It is picked up by fimbriated end of uterine tube and put into the lumen of the uterine tube. The empty Graafian follicle is converted into the corpus luteum. If the ovum is not fertilized the corpus luteum lasts for 10–12 days, and for 2–3 months if the ovum is fertilized and pregnancy continues. The cells of Graafian follicle secrete the estrogen while the cells of corpus luteum secrete the progesterone.

Structure of the Female Gamete (Secondary Oocyte)

The secondary oocyte is a very large cell and measures more than 100 μm in diameter. The structure of secondary oocyte shed from the ovary is as follows:

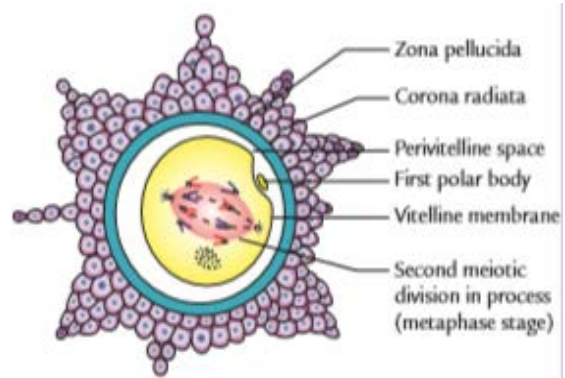


Fig. 3.10 Structure of an ovum (female gamete).

1. It undergoes a second meiotic division to shed off the second polar body.
2. No nucleus is seen as nuclear membrane dissolves for second meiotic division. Spindle and chromosomes attached on it in the equatorial plane (metaphase stage) are seen.
3. It is surrounded by the zona pellucida, which in turn is surrounded by the cells of corona radiata.
4. A distinct space is present between the cell membrane called vitelline membrane and zona pellucida. It is termed **perivitelline space**. It contains first polar body when it was derived from the ovum during the first meiotic division.

Corpus Luteum

After ovulation the wall of the ruptured follicle (consisting of granulosa cells and cells of theca interna) collapses and gets transformed into a glandular structure known as corpus luteum. Under the influence of LH (secreted by the pituitary gland), the yellowish pigment develops in the cells of corpus luteum, which are now called lutein/luteal cells. These cells secrete progesterone and some estrogen. Under the influence of progesterone together with some estrogen, the uterine endometrium enters into secretory phase in preparation for the implantation of embryo. The corpus luteum lasts only for 10–14 days if pregnancy does not occur. Thereafter it degenerates and is gradually transformed into a mass of fibrous tissue called corpus albicans (white body). This corpus luteum is called **the corpus luteum of menstruation**.

The corpus luteum persists for 3–4 months if the ovum is fertilized (i.e., pregnancy occurs) under the influence of HCG secreted by the trophoblast of embedded blastocyst in the endometrium. It is called the **corpus luteum of pregnancy**. Progesterone secreted by the corpus luteum maintains the pregnancy for initial 3–4 months and thereafter the pregnancy is maintained by progesterone secreted by the **placenta**.

Fate of Ovarian Follicles

In each ovarian cycle, a number of ovarian follicles begin to develop but only one reaches maturity. The fate of ovarian follicles is as under:

1. One that reaches maturity, ruptures and sheds off a secondary oocyte. The wall of empty follicle collapses to form corpus luteum (vide supra).
2. The follicles that fail to reach maturity, contrary to what one might expect, do not persist in the next ovarian cycle. They undergo degeneration. The oocyte and granulosa cells of each follicle disappear. However, the cells of theca interna proliferate to form interstitial gland (corpora atretica). These glands secrete estrogen for some period of time and then degenerate to form a mass of fibrous tissue similar to the corpus albicans.

The occurrence of abnormal oocyte is rare as compared to sperms. The various types of abnormalities of oocyte are:

Clinical Correlation

1. Oocytes may be binucleated or trinucleated. Although such oocytes may give rise to twins or triplets, but they usually degenerate before reaching the maturity.
2. Oocytes with abnormal chromosomal contents. It may occur due to nondisjunction of chromosomes in meiosis I or meiosis II. The abnormal oocyte instead of having 23 chromosomes may contain 24 chromosomes or 22 chromosomes.
 - If oocyte with 24 chromosomes is fertilized by a normal sperm (23 chromosomes), a zygote with 47 chromosomes is produced (i.e., trisomy). The trisomy 21 or Down's syndrome is most common type of trisomy. Similarly, if an ovum with 22 chromosomes is fertilized by a normal sperm (23 chromosomes), a zygote with 45 chromosomes will be produced, i.e., monosomy, e.g., Turner's syndrome.

GOLDEN FACTS TO REMEMBER

Ø Largest germ cells in the seminiferous tubules	Primary spermatocytes
Ø Secondary oocyte completes its second meiotic division fertilization	Soon after
Ø Usual period of viability of sperm after ejaculation	4 8 hours (but may service up to 4 days in female genital tract)
Ø Usual period of viability of an unfertilized secondary oocyte is	24 hours (but may service up to 2 days)
Ø Three oocyte barriers are	(a) Corona radiata (b) Zona pellucida (c) Vitelline membrane

Ø Number of spermatozoa formed from one primary spermatocyte	4	
Ø Number of secondary oocyte formed from one primary oocyte	1	
Ø A person is likely to be sterile if number of healthy sperms per ml is less than	10 million	
Ø Number of ovarian follicles that undergo ovulation during entire life of a woman (i.e., 12–50 years)	400–500	reproductive
Ø Number of ovarian follicles present at puberty	40,000	
Ø Number of primary oocytes that get matured and complete their meiotic division before ovulation	5–30	first
Ø Total period required for the process of spermatogenesis	60 days	
Ø State of secondary oocyte at the time of ovulation	In the state of metaphase of second meiotic division	

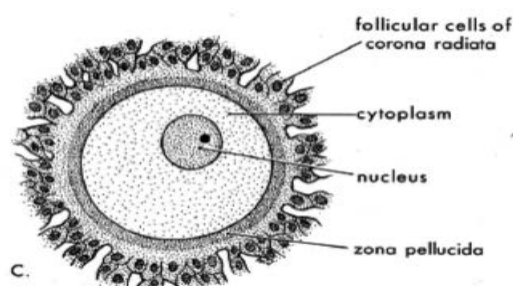
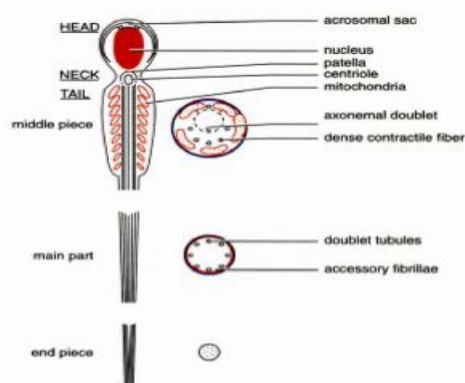
FERTILIZATION

Introduction:

Special features of the gametes for fertilization:

Both egg and sperm acquire structural specializations for fertilization. Eggs are non-motile, surrounded by protective egg coverings. These serve to recognize the sperm specifically and prevent fertilization by more than one sperm (polyspermy). The mammalian egg has zona pellucida layer around the plasma membrane beneath which cortical granules are present. The zona pellucida layer makes the egg impenetrable to more than one sperm.

Sperms are highly motile cells consisting of nucleus, mitochondria to provide energy source



and a flagellum for movement. The anterior end of the sperm is highly specialized

which aids in penetration of the egg. Sperms are typically designed to activate the egg and to deliver their nuclei into the egg cytoplasm.

Basic requirements of fertilization:

Fertilization requires a fluid medium in most animals. It may be seawater in marine forms, fresh water in fresh water forms and body fluid in viviparous animals. To increase the probability of fertilization, the number of sperms must exceed the number of eggs. Moreover the lifespan of gametes is limited; therefore fertilization must take place within a short duration of time. Eggs that are shed in water like that of most invertebrates, fishes and amphibians, have shorter life span while those fertilized within the body of female, generally have longer life span.

Site of fertilization:

Fertilization may be either external or internal. In external fertilization the gametes are discharged in the aquatic medium and the fertilization occurs outside the body of both male and female parents, as in most invertebrates and some vertebrates (fish and frog). The aquatic medium for external fertilization may be either seawater or fresh water. When fertilization occurs inside the body of female parent, it is internal fertilization, as in *Drosophila*, birds and mammals.

Mechanism of fertilization:

The process of fertilization has been mostly studied in invertebrates such as sea urchins and in vertebrates like amphibians and mammals. Fertilization begins with the approach of the sperm to the egg and ends up with the formation of diploid zygote. The process of fertilization requires five general events:

- Recognition of egg and sperm (approach of spermatozoan to the egg, attachment and binding)
- Acrosome reaction and penetration
- Fusion of plasma membranes of egg and spermatozoa.
- Activation of egg
- Fusion of egg and sperm pronuclei

1. Recognition of Egg and Sperm: Encounter of spermatozoa and ova:

The meeting of sperm and egg is not a simple task. Most animals accomplish close approximation of gametes through special devices or a particular behaviour.

Among fresh water animals the timing of spawning of eggs by females and shedding of sperms by male parent are very specific. The sperms are delivered directly to the eggs immediately after laying.

In marine forms the time interval between shedding of gametes may be longer by weeks or months. The task of meeting sperm and egg is further intensified as they release their gametes into the open sea, where they are readily dispersed. For this reason, a large number of gametes may be produced to maintain a sufficient gamete concentration in the water. Moreover, the aquatic medium is shared by other species that may shed their gametes at the same time. In aquatic medium the movement of spermatozoa may either be entirely at random or the consequence of directed movements caused by some attractive force associated with the egg.

During internal fertilization, such as in mammals, the gametes of both sexes are deposited in the female reproductive tract. The fluid movements within the reproductive tract, assist in transporting the gametes to the site of fertilization.

Sperm attraction: In many animals, sperms are attracted towards eggs of their species by “chemotaxis” i.e. following a gradient of a chemical secreted by the egg. Chemotaxis has been demonstrated in cnidarians, molluscs, echinoderm and urochordates (Miller, 1985; Yoshida et al, 1993). Similarly in the egg jelly of the sea urchin, chemotactic factors are present for sperm attraction. A chemotactic factor **called resacet**, a 14 – amino acid peptide, has been isolated from the egg jelly of sea urchin *Arbacia punctulata* (Ward et al 1985). A peptide of 10 – amino acid in the jelly of *Strongylocentrotus purpuratus* and *Hemicentrotus pulcherimus* has been named as **speract** (Garbers et al 1982). They diffuse readily into seawater and are species specific.

Fertilizin and Anti fertilizin interactions:

Factors that mediate sperm– egg interactions even before they make contact were identified by F.R. Lillie (1912). He proposed the first theory of physiology of fertilization called fertilization theory. He observed that the egg water (seawater surrounding unfertilized sea urchin eggs), agglutinated the sperm and activated their motility. The reaction was species specific. This factor **called fertilizin** came from the egg jelly coat. It slowly dissolved as in sea water. Fertilizin was later shown to be the constituent of both jelly coat and egg membrane such as vitelline membrane and plasma membrane. Fertilizin is a proteoglycan. Both the amino acids and monosaccharides of fertilizin vary from one species to another so that each species possesses its specific type of fertilizin. Each molecule of fertilizin has more than one ‘active group’ so that one fertilizin particle may attach to two or more sperms and bind them together.

The receptor sites for fertilizing are present on the sperm plasma membrane called **anti fertilizin**. These are acid proteins. Adhesion of spermatozoa to the surface of the egg is brought about by linking of fertilizin molecule with antifertilizin molecules. The reaction between fertilizin and antifertilizin is similar to antigen – antibody reaction. In both cases, a chemical lock is formed between two complimentary substances. It has been suggested that the main function of fertilizin – antifertilizin is to thin out the number of spermatozoa around the egg, so that the chances of two or more spermatozoa fusing with the egg at the same time are diminished.

2. Acrosome reaction and penetration:

The acrosomal reaction in sea urchin Once the sperm makes contact with the egg, then it has to penetrate surface coats that surround the egg. The penetration is facilitated by the acrosome reaction in which the membrane enclosing the acrosome is shed, releasing the contents of acrosome. The acrosomal reaction involves two processes:

- a) exocytosis of acrosomal vesicle and
- b) extension of acrosomal process.

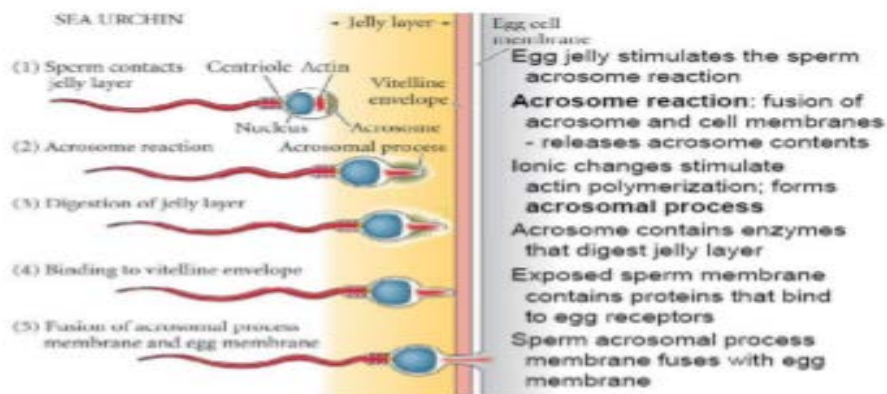
a) Exocytosis of acrosomal vesicle: Contact of the sperm with the egg jelly coat component, a fucose containing polysaccharide triggers the acrosome reaction and causes influx of calcium into the sperm head. This initiates fusion of the outer acrosomal membrane with sperm plasma membrane and ultimate breakdown of acrosomal vesicle. Hydrolytic enzymes called lysins present in the acrosomal vesicle, are released. Lysins digest the egg envelope locally and clear the path for spermatozoa to reach the egg surface (vitelline membrane). The exocytosis of acrosomal vesicle is thus caused by calcium – mediated fusion of acrosomal membrane with the adjacent sperm plasma membrane. The egg jelly factors that stimulate the acrosome reaction are highly species specific.

b) Extension of acrosomal process:

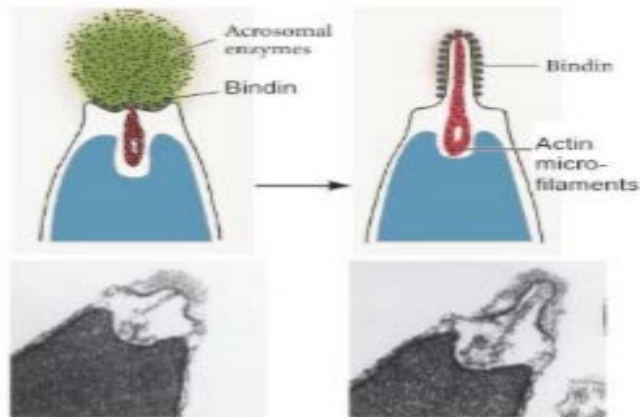
The second part of the acrosomal reaction involves the extension of the acrosomal process / tube / filament. It is formed by polymerization of globular active molecules into actin filaments.

Gamete binding and species specific recognition in sea urchins: Once the sea urchin sperm has penetrated the egg jelly, the acrosomal process of the sperm contacts the vitelline envelope of the egg. The attachment between the acrosomal process and the vitelline envelope is species - specific. The specificity is due to interactions between sperm bindin present on the acrosomal process and a specific sperm receptor on the vitelline envelope. Bindin is located specifically on the acrosomal process and it binds to species - specific bindin receptors present on the egg vitelline membrane.

Sea Urchin Acrosome Reaction



Gamete binding and recognition in mammals:



a) Capacitation

In mammals fertilization is internal. The reproductive tract plays a very active role in fertilization. The differentiated sperms are unable to undergo the acrosome reaction without residing for some time in the female reproductive tract where they undergo physiological changes. The change in the mammalian spermatozoan, which makes it capable of fertilizing the egg, is called capacitation. There are four sets of molecular changes, which take place during capacitation:

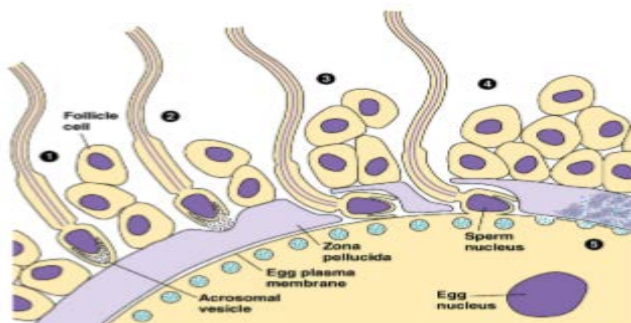
- Albumin proteins, present in the female reproductive tract, remove the cholesterol thereby altering the fluidity of sperm plasma membrane.
- Certain proteins or carbohydrates on the sperm surface are lost during capacitation.
- Membrane potential of the sperm becomes more negative, as potassium ions leave the sperm. This change in membrane potential opens up the calcium channels and allows calcium to enter the sperm facilitating the process of membrane fusion during acrosomal reaction.
- Protein phosphorylation occurs. However it not known whether these events are independent of one another and to what extent each one of them causes sperm capacitation.

b) Gamete binding

The mammalian egg is surrounded by extracellular envelope called zona pellucida. Around zona pellucida is a layer of cumulus cells (corona radiata) embedded in a cementing substance, hyaluronic acid. Hyaluronidase activity on the surface of the sperm head helps it to penetrate this layer. Next, sperm must bind to zona pellucida before they make contact with the surface of egg itself. The zona pellucida in mammals plays a role analogous to that of vitelline envelope in lower vertebrates and invertebrates. The zona pellucida is a glycoprotein matrix synthesized and secreted by the growing oocyte. It plays two important roles in fertilization. It binds the sperm and initiates acrosome reaction.

c) Acrosome reaction in mammals

Binding of the sperm to zona triggers the acrosome reaction, which allows the sperm to penetrate the zona. Acrosome reaction in mammals involves the fusion of the outer membrane of the acrosome with the sperm plasma membrane. After the fusion, the acrosomal membrane vesiculates which results in the release of acrosomal contents. Subsequently, the outer portion of the acrosomal membrane disappears and only the inner portion adjacent to the nucleus remains intact. When the acrosomal contents are exocytosed, several enzymes are released. These enzymes allow the sperm to approach the egg plasma membrane. The mammalian acrosome reaction differs from that of sea urchin in that no acrosomal process is formed.



3. Gamete

fusion & Prevention of polyspermy Sperm & egg plasma membrane fusion

After penetration of the extracellular layers by sperm, there occurs the fusion of sperm plasma membrane with that of the egg. In sea urchin, all regions of the egg plasma membrane are capable of fusing with sperm. The cytoplasm of the egg bulges forward at the point of contact producing a process of hyaline cytoplasm called the fertilization cone. Sperm egg fusion appears to cause the polymerization of actin into microfilaments and extension of several microvilli to form the fertilization cone. Fertilization cone and microfilaments facilitate sperm entry. The sperm and egg membrane join together forming cytoplasmic bridge. The sperm nucleus and tail pass through the cytoplasmic bridge, which is widened by the actin polymerization.

In mammals, after penetrating the zona, the sperm enters the perivitelline space surrounding the egg and lands on the egg plasma membrane, where fusion begins at the equatorial region of the sperm head. The plasma membrane of egg and sperm become continuous forming a cytoplasmic bridge through which the sperm nucleus enters the egg cytoplasm. Usually the entire sperm including the nucleus, centriole, mitochondria and flagellum enters the egg cytoplasm. Once the sperm enters the egg, fertilization cone is formed as in sea urchin. Fertilization cone is extension of the egg cytoplasm around the spermhead. A sperm protein **fertilin**, is thought to be involved in mediating fusion of sperm egg membrane.

The prevention of polyspermy The entry of the sperm into the egg activates the egg. Although many sperm attach to the egg surface, it is important that only one sperm enters the egg(monospermy). Entry of more than one sperm polyspermy) may result in several abnormalities such as polyploidy, abnormal mechanism of chromosomal separation during cell division and ultimate death of the embryo. Organisms have evolved ways to prevent the union of more than two haploid nuclei.

In fishes the sperm can enter into the egg only through the narrow opening, the micropyle, the rest of the egg being covered by impermeable chorion.

In sea urchin and mammals, there is restriction on the number of sperm that are able to penetrate the extra cellular coats and fuse with the egg. In mammals, the sperm has to migrate the long female reproductive tract to reach the egg and further, structural changes in zona pellucida block polyspermy.

The most common way to prevent polyspermy is to prevent the entry of more than one sperm into the egg. The polyspermy is blocked in many animals as soon as the first sperm fuses with the egg plasma membrane. The sea urchin egg has evolved two mechanisms to avoid polyspermy, a) fast reaction that is accomplished by an electric change in the egg plasma membrane and b) a slower reaction caused by exocytosis of the cortical granules.

a) The fast and temporary block to polyspermy

The fast block is a temporary measure, which is mediated by a transient depolarization of the egg plasma membrane, caused by sperm-egg fusion. Within 1-3 seconds after entry of the first sperm the electrical membrane potential across the egg plasma membrane shifts from -70 mV to $+20\text{ mV}$. This change is caused by a small influx of sodium ions into the egg & lasts for about 60 seconds after which the membrane potential returns to its original level. Some acrosomal proteins of sperm open the sodium channel in the egg that causes influx of sodium ions into the egg & depolarizes the egg membrane. This results in the fast block to polyspermy.

b) The slow & permanent block to polyspermy.

The fast block to polyspermy is for a very short duration (about a minute only). This brief period is not sufficient to prevent polyspermy. Therefore, the fast block to polyspermy is supplemented by a second mechanism, known as cortical reaction. It is a slower mechanical block to polyspermy. Sperm entry into the sea urchin egg results in the release of intracellular calcium ions that are stored in the endoplasmic reticulum in egg cortex. The calcium ions are first released at the site of sperm entry and within a minute, a wave of calcium ions traverses the entire egg. This wave of released calcium ions initiates cortical reaction. The cortical reaction consists of a wave of exocytosis of cortical granules, which are present just beneath the plasma membrane in the mature egg. The cortical granules fuse with the egg plasma membrane and release their contents into the perivitelline space. This space lies between the plasma membrane and the vitelline envelope. Several proteins are released by this cortical granule exocytosis, which are as follows :

- Proteolytic enzymes (proteases) released, break the bonds that bind the vitelline envelope to the egg plasma membrane. This creates a perivitelline space. These enzymes also clip off the binding receptors and any sperm attached to it.
- Mucopolysaccharides (glycosaminoglycans) released, produce an osmotic gradient that causes water to rush into the perivitelline space. As a result, vitelline envelope expands and is elevated. It now becomes fertilization envelope.
- A third protein, a peroxidase enzyme released during cortical reaction, hardens the fertilization envelope by cross-linking tyrosine residues on adjacent proteins of fertilization envelope.
- Finally, cortical granule protein, hyaline, forms a coating around the egg. The egg plasma membrane adheres to this protein and the hyaline provides a support for blastomeres during cleavage. Both fertilization envelope and hyaline layer prevent further sperm from binding to the egg plasma membrane. In mammals, the cortical reaction is same as in sea urchin except that a fertilization envelope is not formed. Exocytosis of cortical granules causes release of hydrolytic enzymes into perivitelline space. These enzymes modify the zona pellucida sperm receptors so that sperm can no longer bind to zona pellucida. The changes in the zona pellucida are called the zona reaction. A block to polyspermy thus allows only one sperm to fuse with the egg and deliver its nucleus into the egg cytoplasm.

4. The activation of egg metabolism:

After the sperm penetrates the egg a series of diverse cytoplasmic reactions are initiated. The response of the egg to the sperm can be divided into “early” responses, which occur

within seconds of the cortical reaction and “late” responses which take place several minutes after fertilization begins.

Early responses:

The early responses to the activation are the prevention of polyspermy, consisting of two major mechanisms. The fast block, which is initiated by sodium influx into the cell, and the slow block initiated by the intracellular release of calcium ions. Within one second, the membrane potential of egg rises and sperm – egg fusion takes place within 6 seconds followed by cortical vesicle exocytosis within 15 – 60 seconds.

Late responses:

Late responses include many metabolic changes, which are as follows:

- Increased rate of respiration due to utilization of glycogen and other food stuffs for getting energy ATP molecules.
- Activation of NAD kinase and increase in NADH and NADPH: NAD kinase converts NAD to NADP, a co enzyme for lipid biosynthesis, which is essential in formation of new cell membrane during cleavage.
- Ionic changes: certain intracellular changes occur in the concentration of cations such as sodium, potassium and calcium. There is increase in pH (remains high). The change in calcium ion concentration has great significance in the metabolic activation of the egg.
- Activation of protein and DNA synthesis: there is increase in the rate of protein synthesis by utilizing mRNA already present in the oocyte cytoplasm. After 5 minutes of fertilization, the rate of protein synthesis increases three to twelve folds. About 20 minutes after fertilization DNA synthesis is initiated.
- Resumption of meiosis: in most animals meiosis is arrested at a particular stage and resumes only after fertilization. The time of fertilization varies from species to species. It has been found that spermatozoan may enter the egg at different stages of maturation in different animals .
- Initiation of mitosis: the initiation of mitosis occurs because (a) the rate of DNA synthesis increases after fertilization and (b) by the contribution of centriole by sperm to the egg, which is needed for proper mitosis.

To summarize, late responses include many metabolic changes such as the activation of potassium ions and amino acid transport, an increase in the rate of protein synthesis, initiation of DNA replication and several major regulatory events. These events include production of inositol triphosphate, diacylglycerol, release of cytoplasmic free calcium ions and rise in hydrogen ions concentration .

5. Fusion of genetic material in sea urchins and mammals:

In sea urchins, the sperm nucleus enters the egg perpendicular to the egg surface. After entry, the sperm nucleus and its centriole separate from the mitochondria and flagellum. The mitochondria and the flagellum disintegrate inside the egg. Second meiotic division is completed after the entry of the sperm and the resulting haploid egg nucleus is known as female pronucleus. The sperm nucleus once inside the egg, undergoes several changes and becomes male pronucleus. Its nuclear envelope becomes vesicular. De-condensation of chromatin and the formation of pronuclear envelope take place. The male pronucleus inside the egg rotates at 180 degrees so that the sperm centriole comes to lie between male pronucleus and the female pronucleus. The female pronucleus is located in the center of the egg and male pronucleus is in the cortical region of egg at the site of sperm entry. For fusion the male pronucleus has to travel a considerable distance through the egg cytoplasm to reach female pronucleus. The sperm asters mediate this movement. The sperm aster is a complex of long microtubules that radiate from the sperm centriole. The sperm centriole acts as a microtubule organizing center for sperm aster. The microtubules of the sperm aster push the male pronucleus towards the center of the egg. The astral microtubules also make contact with female pronucleus and pull it towards the male pronucleus. Thus the two pronuclei migrate towards each other and fuse to form the diploid zygote nucleus. The fusion of male and female pronuclei is called **amphimixis**.

In mammals the entry of sperm is tangential to the eggs surface. Once inside the egg, the sperm nucleus becomes male pronucleus. The sperm centrosome produces asters and contacts the female pronucleus. Male & female pronuclei migrate towards each other, become apposed but do not fuse. They remain adjacent to each other; their nuclear envelopes break down but instead of forming a zygote nucleus the chromatin condenses into chromosomes orienting them on a common mitotic spindle. Thus, only after completion of the first division of fertilized egg, the paternal and maternal chromosomes become enclosed by a common nuclear membrane to form the nuclei of two blastomeres.

Rearrangement of egg cytoplasm:

One of the consequences of egg activation during fertilization is reorganization of egg cytoplasm. In mammals or sea urchin eggs, the cytoplasmic movements are not obvious but in several other animals these cytoplasmic displacements are crucial for the determining

cell fate during development. Most spectacular cytoplasmic movements have been observed in ascidian, *Styela partita* and in frog. In both these animals, a bilateral symmetry is established in the cytoplasm of fertilized egg. In *Styela partita*, the different regions of the cytoplasm have distinct pigmentation. In the mature egg, a layer of cortical cytoplasm containing yellow lipid granules surrounds a central gray cytoplasm. After sperm entry in vegetal hemisphere of the oocyte, the yellow cortical cytoplasm and the clear cytoplasm derived from the breakdown of the oocyte nucleus starts moving vegetally towards the sperm. As the male pronucleus penetrates deeper into the cytoplasm and moves towards a female pronucleus, the clear and the yellow cytoplasm move with it. Just before the first cleavage, the yellow cytoplasm forms a crescentic area (mesodermal crescent) just below the equator of the egg.

Simultaneously a crescent of light grey cytoplasm (notochordal crescent) appears subequatorially on the opposite side of the egg. As a result of cytoplasmic displacements four different kinds of cytoplasmic regions are seen:

- i) the yellow cytoplasm on one side,
- ii) the light cytoplasm on the other side. These two together form a belt surrounding the egg just below the equator. Below the zone towards the vegetal pole
- iii) The cytoplasm containing abundant yolk granules and
- iv) the clear cytoplasm in the animal hemisphere. This state of cytoplasmic localization before the first division displays asymmetrical distribution of cytoplasm that will be irreversibly fixed after the first division with each cell receiving different cytoplasmic constituents.

Unfertilized egg of frog is radially symmetrical about animal – vegetal axis. A single sperm can enter anywhere on the animal hemisphere of the egg. After sperm entry, the cortical cytoplasm rotates 30 degrees towards the sperm entry point, relative to the inner cytoplasm. As a result of this rotation, the underlying cytoplasm located near the equator on the opposite side of sperm entry point contains diffuse pigment granules and therefore appears gray. This region has been referred to as **gray crescent**.

The rotation of the cortical cytoplasm is important as the grey crescent area, which is exposed, marks the region where gastrulation is initiated in amphibian embryos. The rotation of cortical cytoplasm with respect to inner cytoplasm also causes marked changes within the inner cytoplasm. The cytoplasm of the future dorsal side becomes different from the future ventral side where sperm enters. Thus radially symmetrical embryo is transformed into bilaterally symmetrical embryo. These cytoplasmic movements are also responsible for laying down dorsal – ventral axis of the future embryo.

Preparation for cleavage:

Before fertilization, the egg, which has been under metabolic arrest, is released from this arrest on the entry of the sperm. This initiates the process of development by active protein and DNA synthesis in the egg leading to the beginning of cleavage. The first cleavage is not random but tends to be specified by the point of sperm entry and the subsequent rotation of egg cytoplasm.

Cleavage/ Cellulation / Segmentation

Cleavage

Cleavage is a series of rapid cell divisions without cell growth or gene expression which occurs in early embryogenesis.

Explanation:

The process of cleavage remains one of the earliest mechanical activities in the conversion of a single celled egg into a multicellular embryo. Cleavage of human zygote occurs within the fallopian tube. It is holoblastic, i.e., it divides the zygote completely into daughter cells or blastomeres. The first cleavage takes place about 30 hours after fertilization. It divides zygote longitudinally into two blastomeres (one slightly larger than the other). The second cleavage occurs within forty hours after fertilization. It is at right angles to the plane of the first resulting in four blastomeres. The third cleavage takes place about 72 hours after fertilization. During these early cleavages, the young embryo moves slowly down the fallopian tube towards the uterus.

At the end of the fourth day, the embryo reaches the uterus. It looks like a mulberry and is known as **morula**. This solid ball like morula has thirty two cells. In human zygote the cleavage is radial (blastomeres are arranged in radial plane around the polar axis) and indeterminate type (fate of each blastomere is not predetermined).

Background:

The first cleavage of frog's egg was observed by Swammerdam in 1738. The entire process of cleavage in frog's egg was studied by Prevost and Dumas in 1824.

Cleavage: Functions & Events

☐ Initial divisions of zygote to form the multicellular embryo called "Blastomeres"

☒ The cleavage

☐ They are special mitotic divisions

☐ Since they are mitotic divisions, they maintain the 2N complement

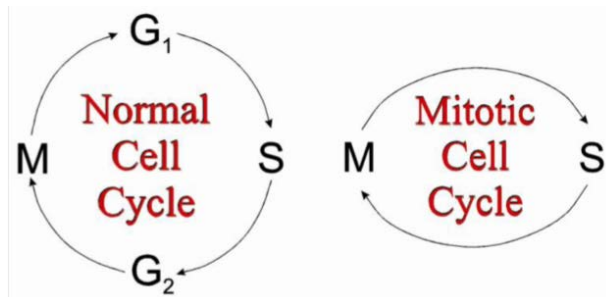
☐ They are rapid cell divisions with no intervening growth (G1 & G2) phases

☐ During cleavage G1 and G2 stages are by-passed so cells simply progress from S (DNA synthesis) to M (mitosis) without the intervening growth phases

☐ At the blastocyst stage the transition occurs to a normal cell cycle: G1, S, G2, M.

The

☐ Converts



Purposes of Cleavage

unicellular zygote to multicellular embryo ☐

Maintains diploid complement of cells--all are genetically identical

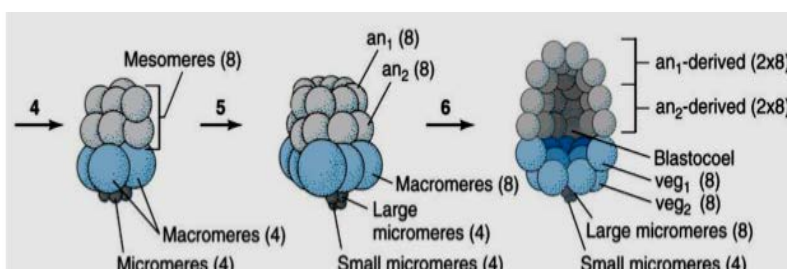
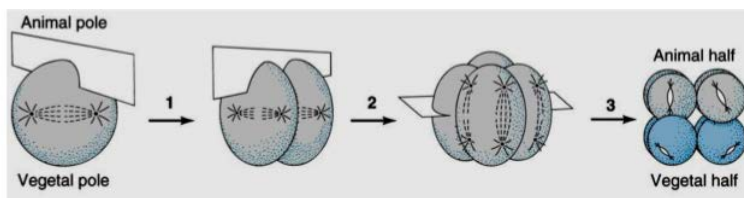
☐ Human cleavage is not synchronous; all of the cells do not cleave at precisely the same time, as a result embryos with odd numbers of cells can be seen at various times ☐ Slow

cleavage; takes approximately 12-24h between each cell division

☐ No growth occurs during early cleavage, so the total embryo will remain ~100 microns (0.1mm) in diameter

☐ Cleavage brings about the distribution of cytoplasm among the blastomeres.

The planes of cleavage



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. The cleaved cells may be unequal in size.

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. It is also called as transverse or horizontal cleavage.

Influence of yolk on cleavage

Yolk is needed for embryonic development. However the fertilized egg has to undergo all stages of development and result in a suitable 'young form' initiating next generation. Somehow with all the influences of yolk the developmental procedures are so adapted and modified that a well formed embryo will result. The initial influence of yolk is felt during the process of cleavage. The amount of the yolk and its distribution affect the process of cleavage. Accordingly several cleavage patterns have been recognized

A. Holoblastic or 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 in size

Holoblastic Cleavage is of four types:

1. Radial Cleavage

Radial cleavage is defined as a type of cleavage that is present in deuterostomes, which is characterized by the arrangement of the blastomeres. They are arranged in a position that blastomeres of each upper tier are directly over those of the next lower tier.

2. Spiral Cleavage

It is typically present in protostomes. It is mainly the arrangement of the blastomeres of each upper tier over the cell junctions that are present in the lower tier, result in making the blastomeres arranged spirally around the pole to pole axis of the embryo.

3. Bilateral cleavage

The first cleavage results in bisection of the zygote into left and right halves. The following cleavage planes are centered on this axis and result in the two halves being mirror images of one another. In bilateral holoblastic cleavage, the divisions of the blastomeres are complete and separate.

4. Rotational cleavage

Rotational cleavage involves a normal first division along the meridional axis, giving rise to two daughter cells. The way in which this cleavage differs is that one of the daughter cells divides meridionally, whilst the other divides equatorially.

B. Meroblastic cleavage

This occurs in polylecithal eggs in which only the small germinal disc lying at the animal pole consisting of clear cytoplasm and a nucleus, undergoes a series of incomplete divisions forming an area of cells at the animal pole, the large yolky portion beneath the germinal disc remains unsegmented, e.g., teleosts, reptiles, birds and egg-laying mammals.

Meroblastic cleavage may be of two types.

1. Discoidal cleavage

Since the macrolecithal eggs contain plenty of yolk, the cytoplasm is restricted to the narrow region in the animal pole. Hence cleavage furrows can be formed only in the disc-like animal pole region. Such a cleavage is called discoidal meroblastic cleavage. Eg: birds and reptiles.

2. Superficial cleavage

This type of incomplete cleavage is found in centrolecithal eggs, e.g., insects and many arthropods. The nucleus lying in the centre of the egg yolk surrounded by an island of cytoplasm undergoes cleavage, and each nucleus is surrounded by small amount of cytoplasm. They later move towards the periphery in the peripheral cytoplasm. Here their cytoplasm fuses with the peripheral cytoplasm. Later the peripheral cytoplasm becomes subdivided by furrows extending inward from the surface, thus, a layer of peripheral or superficial cells is formed which surrounds the central undivided yolk.

☐ **Determinate and Indeterminate Cleavage**

Determinate cleavage also called mosaic cleavage is in most protostomes. It results in the developmental fate of the cells being set early in the embryo development. Each blastomere produced by early embryonic cleavage does not have the capacity to develop into a complete embryo.

Indeterminate or Regulative cleavage It is characteristic of deuterostomes. The original cell in a deuterostome embryo divides, if the two resulting cells can be separated, and each one can individually develop into a whole organism.

Mechanism of Cleavage

Cleavage involves the division of the cell cytoplasm and the nucleus. What are the mechanisms of these processes? A ring of microfilaments, probably composed of the protein actin, can be observed just below the cell surface of many eggs. These filaments form a contractile ring that separates the cytoplasm in much the same way as pulling purse strings decreases the size of the purse opening. Experiments have shown that a drug, Cytochalasin B, disrupts cytoplasmic microfilaments. If the drug is removed, cell division resumes as the microfilaments reappear. Such experiments suggest that microfilaments are involved in cytoplasmic division.

There is evidence that the spindle-or, more correctly, the asters- initiates cytoplasmic division by producing a diffusible factor that acts on the contractile ring. This was indicated by experiments in which asters were removed or separated. Asters must be present in areas where the cleavage furrow occurs. Asters, therefore, may set up conditions that are required for the organization of a constriction mechanism.

The mitotic spindle apparatus involved with nuclear division is composed mainly of the protein tubulin A and B. These proteins are present in the egg and blastomeres (in subunit form) even when mitotic spindle is not present. At the proper time for spindle formation, these subunits probably triggered to come together (polymerize), forming the visible mitotic apparatus.

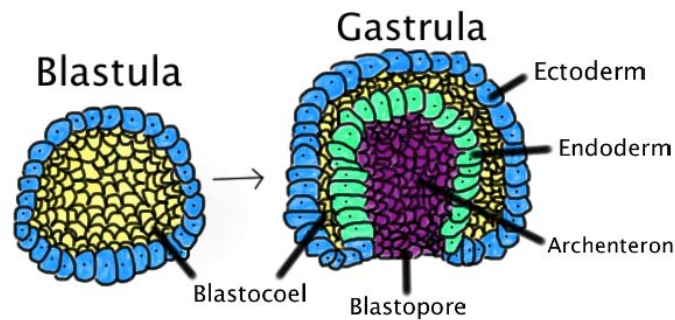
GASTRULATION

Gastrulation is a phase early in the embryonic development of most animals, during which the single-layered blastula is reorganized into a multilayered structure known as the gastrula. Before gastrulation, the embryo is a continuous epithelial sheet of cells; by the end of gastrulation, the embryo has begun differentiation to establish distinct cell lineages, set up the basic axes of the body (e.g. dorsal-ventral, anterior-posterior), and internalized one or more cell types including the prospective gut.

In triploblastic organisms the gastrula is trilaminar ("three-layered"). These three germ layers are known as the ectoderm, mesoderm, and endoderm. In diploblastic organisms, such as Cnidaria and Ctenophora, the gastrula has only ectoderm and endoderm. The two layers are also sometimes referred to as the hypoblast and epiblast.

The molecular mechanism and timing of gastrulation is different in different organisms. However, some common features of gastrulation across triploblastic organisms include:

- (1) A change in the topological structure of the embryo, from a simply connected surface (sphere-like), to a non-simply connected surface (torus-like);
- (2) the differentiation of cells into one of three types (endodermal, mesodermal, and ectodermal)
- (3) the digestive function of a large number of endodermal cells.



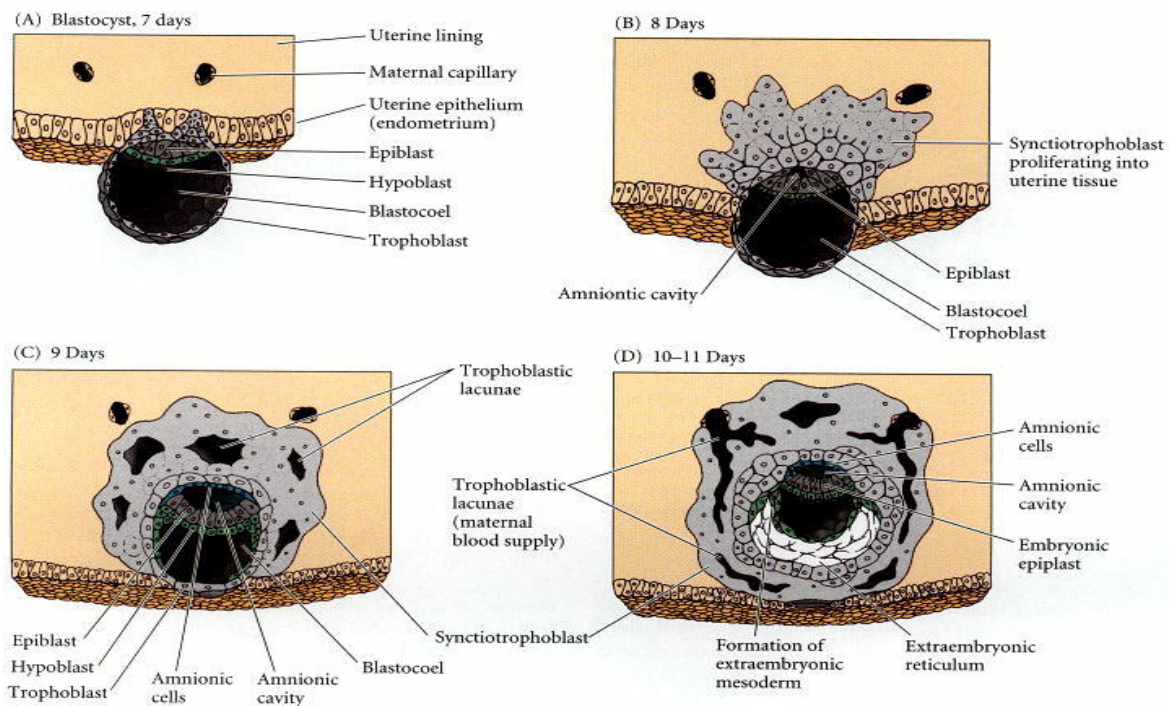
- **Gastrulation in Mammals**

the gastrulation movements of reptilian and avian embryos, which evolved as an adaptation to yolky eggs, are retained even in the absence of large amounts of yolk in the mammalian embryo.

The mammalian embryo obtains nutrients directly from its mother and does not rely on stored yolk. This adaptation has entailed a dramatic restructuring of the maternal anatomy (such as expansion of the oviduct to form the uterus) as well as the development of a fetal organ capable of absorbing maternal nutrients. This fetal organ—the **chorion**—is derived primarily from embryonic trophoblast cells, supplemented with mesodermal cells derived from the inner cell mass. The chorion forms the fetal portion of the placenta. It will induce the uterine cells to form the maternal portion of the placenta, the **decidua**. The decidua becomes rich in the blood vessels that will provide oxygen and nutrients to the embryo.

The first segregation of cells within the inner cell mass results in the formation of the hypoblast (sometimes called the primitive endoderm) layer. The hypoblast cells delaminate from the inner cell mass to line the blastocoel cavity, where they give rise to the extraembryonic endoderm, which forms the yolk sac. The remaining inner cell mass tissue above the hypoblast is now referred to as **the epiblast**. The epiblast cell layer is split by small clefts that eventually coalesce to separate the embryonic epiblast from the other epiblast cells, which form the amnionic cavity. Once the lining of the amnion is completed, it fills with a secretion called amnionic (amniotic) fluid, which serves as a shock absorber for the developing embryo while preventing its desiccation. The embryonic epiblast is believed to contain all the cells that will generate the actual embryo.

Gastrulation begins at the posterior end of the embryo, and this is where the node forms. Like the chick epiblast cells, the mammalian mesoderm and endoderm migrate through a primitive streak, and like their avian counterparts, the migrating cells of the mammalian epiblast lose E-cadherin, detach from their neighbors, and migrate through the streak as individual cells. Those cells migrating through the node give rise to the notochord.

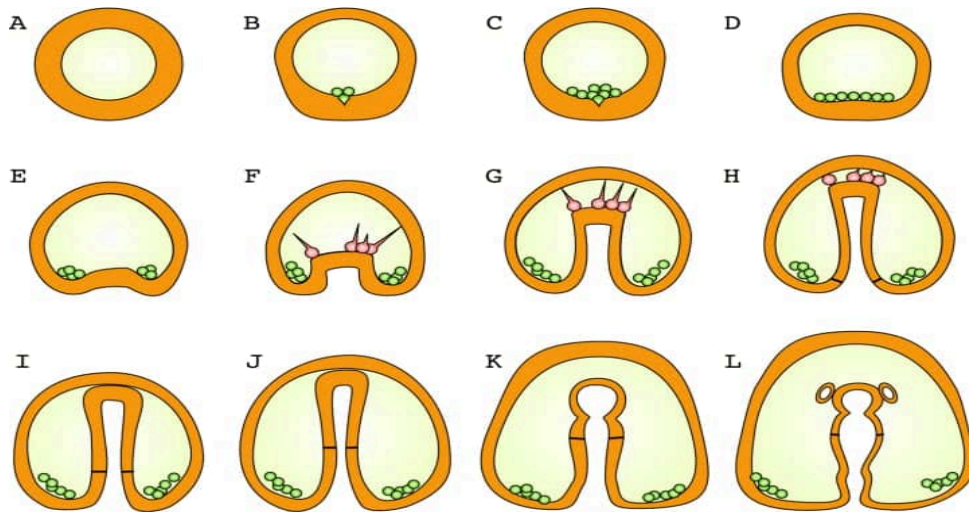


Tissue formation in the human embryo between days 7 and 11. (A, B) Human blastocyst immediately prior to gastrulation. The inner cell mass delaminates hypoblast cells that line the blastocoel, forming the extraembryonic endoderm of the primitive yolk. (C) Meanwhile, the epiblast splits into the amnionic ectoderm (which encircles the amnionic cavity) and the embryonic epiblast. The adult mammal forms from the cells of the embryonic epiblast. (D) The extraembryonic endoderm forms the yolk sac.

GASTRULATION IN SEA URCHIN

Processes of sea urchin gastrulation have been conventionally divided into two distinct phases, primary and secondary invagination. In this review, the processes are divided into four steps for convenience. Preceding the occurrence of invagination, the cells around the vegetal pole become elongated and give rise to a thickened vegetal plate (Step 1). Then the thickened vegetal plate bends inwardly and gives rise to a short stub-like gut rudiment (Step 2, primary invagination). During a couple of hours after primary invagination, the gut rudiment scarcely elongates. Meanwhile, another population of mesodermal cells, secondary mesenchyme cells (SMC), appears at the archenteron tip (Step 3). After such a pause of archenteron elongation, the gut rudiment rapidly elongates until its tip reaches the inner surface of the apical plate (Step 4, secondary invagination).

Step 1: ingression of primary mesenchyme cells and formation of the vegetal plate.



(A) **Hatching-blastula stage.** Embryos are composed of a monolayered epithelium and spherical in shape.

(B) **Early mesenchyme blastula stage.** The vegetal plate thickens. A small number of primary mesenchyme cells (PMC) appear in the blastocoel.

(C) **Middle mesenchyme blastula stage.** Ingression of PMC culminates.

(D) **Late mesenchyme blastula stage.** Most PMC have entered the blastocoel. The vegetal plate becomes somewhat thinner at this stage than the preceded stages.

(E) **Beginning of primary invagination.** The vegetal plate bends inwardly.

(F) **Early gastrula stage.** Primary invagination completed, and a stub-like gut rudiment forms. Secondary mesenchyme cells (SMC) appear at the tip of the gut rudiment.

(G) **Mid to late gastrula stage.** The gut rudiment is stretched along the animal-vegetal axis by contraction of SMC filopodia.

(H) **Late gastrula stage.** The archenteron cells are rearranged, and slender archenteron forms.

(I) **Very early prism stage.** The ectodermal layer begins to expand, and the cells near the blastopore are pulled into the base of the archenteron.

(J) **Early prism stage.** Recruitment of the archenteron cells continues as the ectodermal layer expands.

(K) **Mid prism stage.** A constriction appears between the esophagus and stomach.

(L) **Late prism stage.** Late recruitment of endodermal cells completed. The cells that had invaginated by the end of secondary invagination occupy the esophagus and the anterior half of the stomach. PMC and SMC are colored in green and pink, respectively. The short lines in (H-L) indicate the boundary of the cells that have invaginated by the end of secondary invagination.

Step 2: primary invagination

Unlike in amphibian embryos, bottle cells are arranged in a circle at the central region of the vegetal plate in *Strongylocentrotus purpuratus*. Kimberly & Hardin (1998) showed that

elimination of a 90–180 degree arc of bottle cells markedly retards invagination, while ablation of other types of cells does not cause the significant delay. Thus, it is clear that bottle cells trigger the initial inward bending of the vegetal plate.

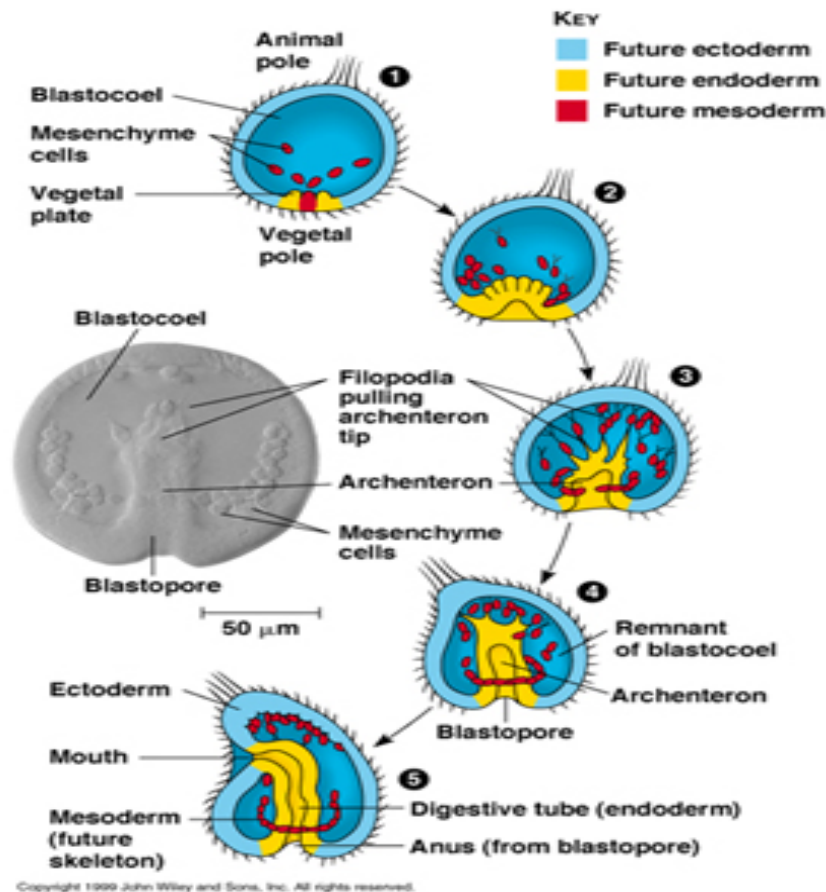
Step 3: lag phase in archenteron elongation

During a couple of hours after primary invagination, the gut rudiment scarcely elongates. Meanwhile, another population of mesodermal cells, secondary mesenchyme cells (SMC), appears at the archenteron tip.

Step 4: Secondary invagination

A short stub like gut rudiment is converted into a slender archenteron through a series of morphogenetic movements called secondary invagination. It has been thought that contraction of the filopodia connecting the archenteron tip and the apical plate pulls the gut rudiment upward. In general, the height (length along the animal–vegetal axis) and width of embryos become larger during gastrulation due to the expansion of the blastocoele wall. A part of the ectoderm layer to which SMC filopodia adhere is flattened or depressed. Further, elongation of the archenteron is blocked when the pseudopodia are broken by expanding the blastocoel, the length of the archenteron reaches two thirds to three quarters of the full length of the archenteron formed in normal embryos.

Ettensohn (1985) found that a dozen cells are initially observed on a cross section of the gut rudiment, but the number decreases to 7–8 after secondary invagination completes laser beam. These observations and experiments clearly indicate that the pseudopodia exert contractile force for archenteron elongation. This explicitly indicates that the archenteron cells are rearranged during this morphogenetic process. Together with the force exerted by SMC filopodia, cell rearrangement leads to the formation of a slender archenteron.



GASTRULATION IN BIRDS

It is characterised by movement and rearrangement of cells in embryo. During gastrulation the blastoderm splits into two layers: an upper layer of cells called **epiblast** and a lower layer of cells called **hypoblast**. **Epiblast** is mainly presumptive ectoderm and mesoderm. **Hypoblast** is mainly presumptive endoderm because hypoblast cells grow outward over the surface of yolk, then downward around it to form the endodermal lining of a yolk cell. At this stage, the central cells of blastoderm can be separated from the yolk, under these central cells a pool of fluid develops rising them of the yolk and giving the area an translucent appearance (**area pellucidae**) while the peripheral part of blastoderm where the cells lie unseparated from the yolk is termed as **AREA OPACA**, the white area that transmits light. The upper layer of the blastoderm consists of the presumptive mesoderm and ectoderm.

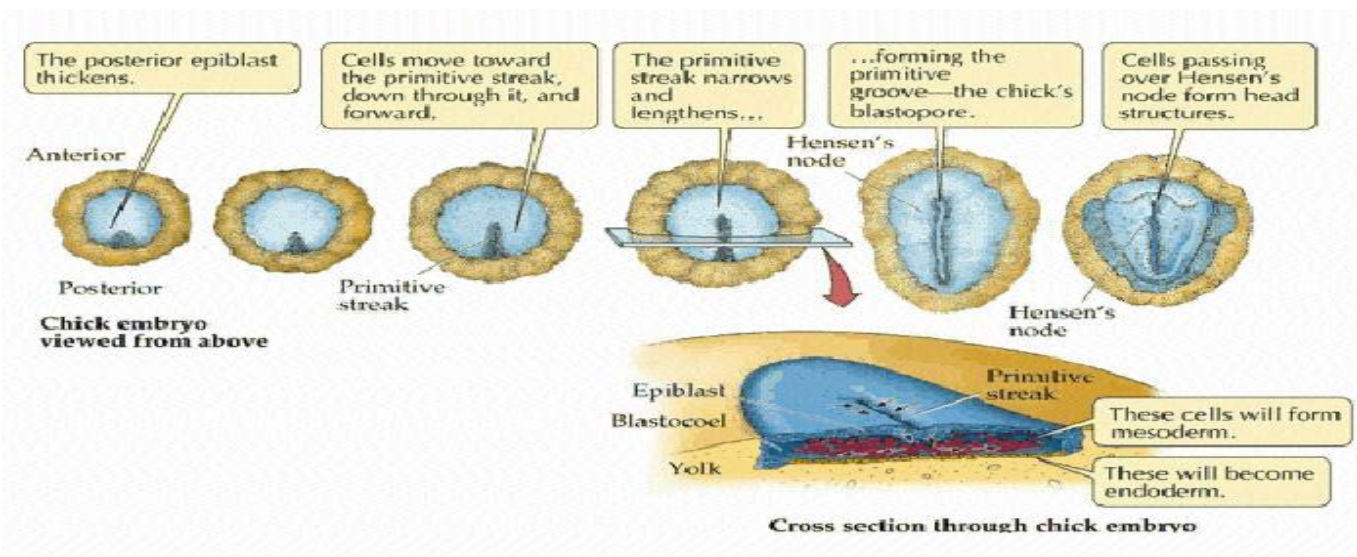
- 1) Formation of Primitive Streak, Primitive Groove, Primitive Ridges and Hensen's Node.

In chick, the mesodermal cells migrate medially and caudally to form a mid line thickening called **primitive streak**. Presumptive mesodermal cells continue migrating and the length of primitive streak grows and finally, the shape of blastoderm changes from circular to pear. The primitive streak elongates almost half of the length of ectoderm. The anterior end of the primitive streak is occupied by an aggregation, the **primitive node or notochordal cells** while the rest of the cells are **mesodermal cells**. The cells continue to migrate between

epiblast and hypoblast and form mountain like ridges called **primitive ridges** and the groove between those ridges is called **primitive groove**. Primitive ridges and primitive groove are formed from primitive streak. Primitive node is called **Hensen's node**, in birds, which is a thickening at the top end of primitive streak.

2) Formation of Notochord, Primitive Gut and Somites

After this, the cells start pushing in from the region of Hensen's node and form a rod like structure, beneath the ectoderm, called **notochord**. Notochord is a prominent feature in a chick embryo of 18 hours. The ectoderm becomes a coherent layer of cells merging with yolk and marginal area and forms **germ wall**, where the expanding germ layers merge with underlying yolk. The cavity between the yolk and the endoderm, which was previously called **gastrocoele** is now termed as **primitive gut**. Hensen's node form the dorsal mesoderm which form the **somites**. The groove between them is called neural groove. Remember that primitive groove and ridges were in primitive streak (in almost one half)



while neural groove and somites are formed above the notochord (in almost other half). Mesoderm gets split in somatic mesoderm and splanchnic mesoderm and the space between them is called coelom. Somites are seen in 25-26 hours embryo

Gastrulation in Amphibians (Frog):

The amphibian embryo undergoes a midblastula transition during which the cell cycle slows down (as a result of acquisition G1 and G2 phases of the cell cycle), cell division becomes a synchronus, the cells gain the ability to move from their original positions, and the transcription of new mRNA is seen from the nucleus for the first time in the animal's life. In **Xenopus**, this transition occurs immediately after the twelfth cleavage. There occur three types of morphogenetic movements in amphibian gastrulation.

1. Invagination:

In frog embryos, gastrulation is initiated at the future dorsal side of the embryo, just below the equator in the region of the grey crescent. Here the marginal endodermal cells sinks into the embryo thus forming a slit like blastopore. These cells now change their shape and become flask shaped.

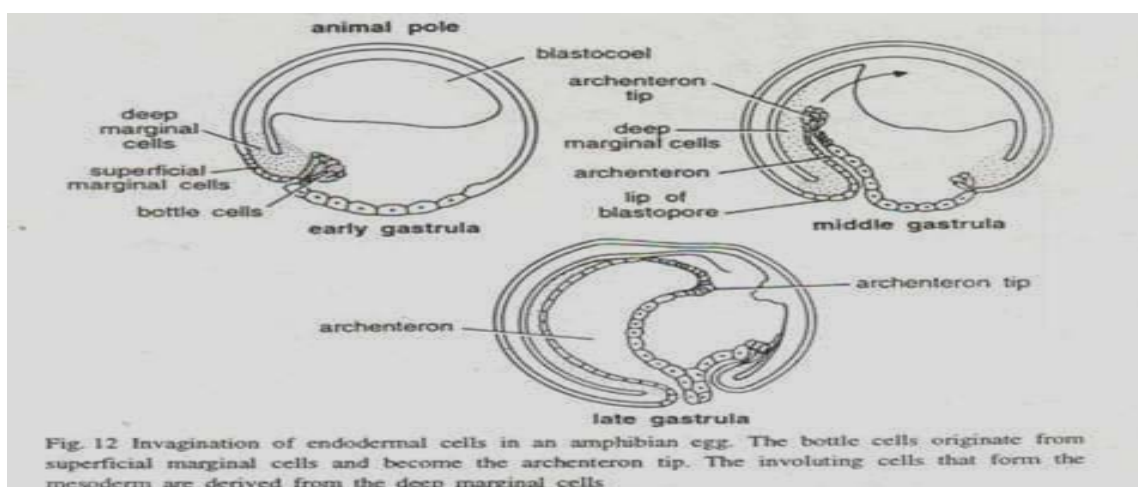
These are called as bottle cells. The bottle cells maintain the contact with the outer surface with the help of cytoplasmic strands whereas their main body is displaced towards the inside of the embryo. Therefore in frog, gastrulation begins in the marginal zone near the equator of the blastula.

Furthermore, this deep layer of cells appears to be responsible for the continued migration of cells into the embryo.

2. Involution:

The next phase of gastrulation involves the involution of the marginal zone cells, while the animal cells undergo epiboly and converge at the blastopore. On reaching the tip of the blastopore, the marginal cells turn inward and travel along the inner surface of the outer cells sheets. Thus, the cells constituting the lip of blastopore are constantly changing. The first cells to form the dorsal lip are endodermal cells that invaginated to form the leading edge of the archenteron.

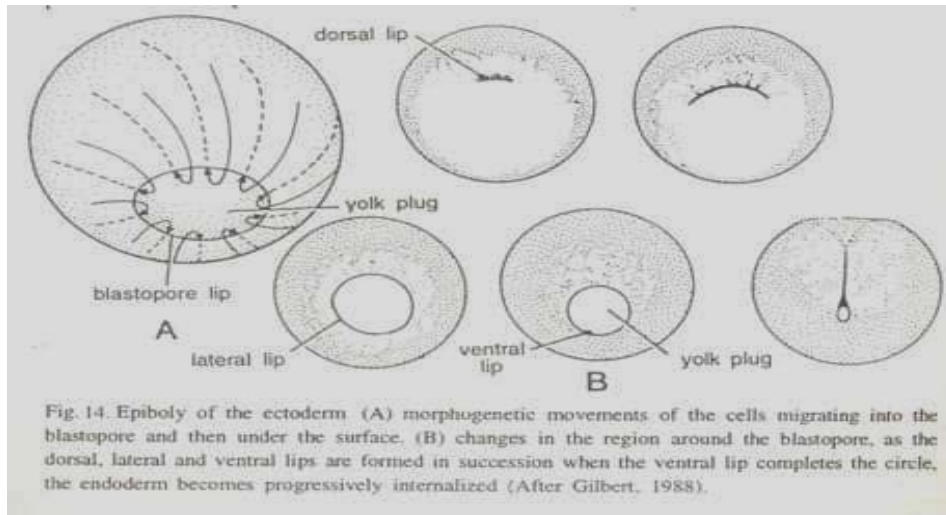
These cells later become the pharyngeal cells of foregut. As these first cells pass into the interior of the embryo, the blastopore lip becomes composed of involuting cells that are precursors of the head mesoderm. The next cells involuting over the dorsal lip of the blastopore are called the chorda mesoderm cells. These cells will form the Notochord, a transient mesodermal “back bone” that is essential for initiating the differentiation of nervous system.



3. EPIBOLY

As the new cells enter the embryo, the blastocoel is displaced to the side opposite the

dorsal blastoporal lip. Meanwhile, the blastopore is displaced vegetal and widens as more animal hemisphere cells converge at the blastopore lip. The widening blastopore develops lateral lips and finally a ventral lip over which the additional mesodermal and endodermal precursor cells pass. With the formation of the ventral lip, the blastopore has formed a ring around the large endodermal cells that remain exposed on the surface. The remaining patch of the endoderm is called the yolk plug, and it too, is eventually internalized (Fig). At this point, all the endodermal precursors have been brought into the interior of the embryo, the ectoderm has encircled the surface and the mesoderm has been brought between them.



The Cellular Basis of

Morphogenesis

Morphogenesis arises because of changes in the cellular structure or how cells interact in tissues cell sorting.

“The changes in tissues cause the elongation, thinning, folding or separation of one tissue into distinct layers. This is often referred as cell sorting. (Cell sorting is the ability to separate cells according to their properties.)” Cell "sorting out" consists of cells moving so as to sort into clusters that maximize contact between cells of the same type.

Cellular basis of morphogenesis has following mechanisms;

➤ Cell-Cell Adhesion:

During embryonic development, cells are restricted to different layers due to differential affinities. One of the ways this can occur is when cells share the same cell- to-cell adhesion molecules.

Example:

In one experiment, Scientists prepared single cell suspension from each of the three germ layers of amphibian embryos soon after the neural tube had formed. When the PH of the solution was normalized, the cells adhered to one another, forming aggregates on agar coated Petri dishes. By using embryos from species having cells of different sizes and colors,

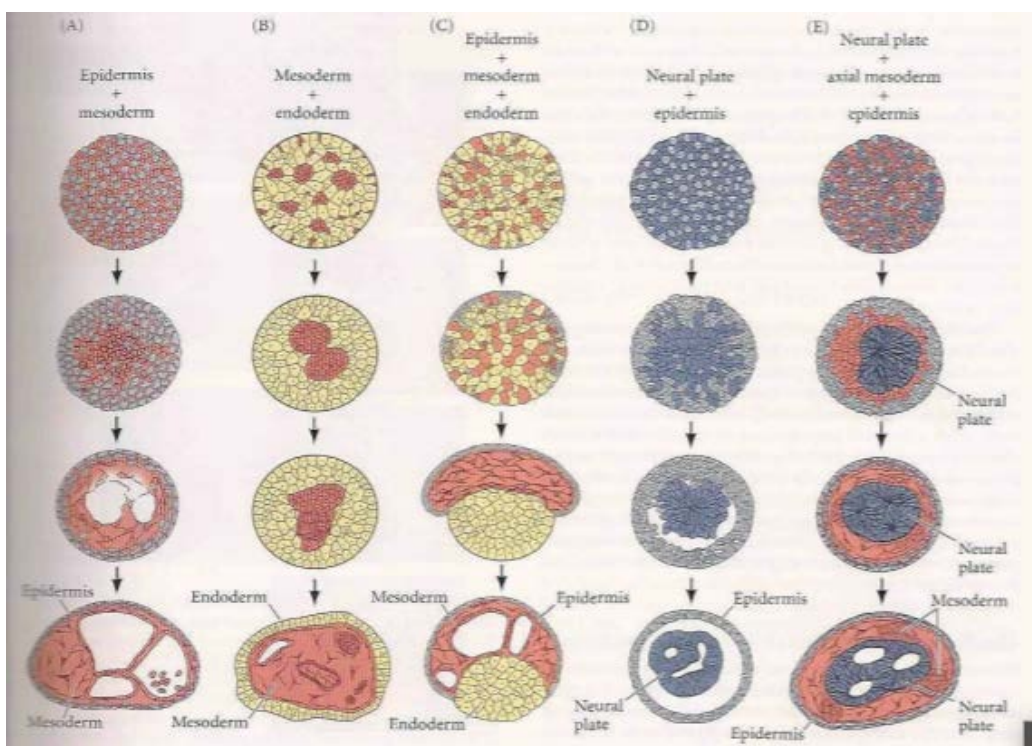
they were able to follow the behavior of the recombined cells. The results of their experiment were striking.

First, they found that reaggregated cells become spatially segregated. That is, instead of two cell types remaining mixed, each type sorts out into its own region. Thus, when epidermal (ectodermal) and mesodermal cells are brought together in a mixed aggregate, the epidermal cells move to the periphery of the aggregate and the mesodermal cells move to the inside. In the recombined cells, one tissue type completely envelops the other.

Second, the researchers found that the final position of the reaggregated cells reflect their respective positions in the embryo. The reaggregated mesoderm migrates centrally, adhering to the inner epidermal surface (**Figure 1.1 A**). The mesoderm also migrates centrally with respect to the endoderm (**1.1 B**). However, when the three germ layers are mixed together, the endoderm separated from the ectoderm and mesoderm and is then enveloped by them (**1.1 C**). In the final configuration, the ectoderm is on the periphery, the endoderm is internal and the mesoderm lies in the region between them.

Researchers interpreted this finding in terms of **Selective affinity**. The inner surface of the ectoderm has a positive affinity for mesodermal cells and a negative affinity for the endoderm, while the mesoderm has positive affinities for both ectodermal and endodermal cells.

Mimicry of normal embryonic structure by cell aggregate by cell aggregate is also seen in



the recombination of epidermis and neural plate cells (**Figure**) . The presumptive epidermal cells(Figure-sorting out and reconstruction of spatial relationships in aggregates of embryonic amphibian cells, after Townes and Holtfreter 1955) migrate to the periphery , the neural plate cells migrate inward, forming a structure similar to the neural tube.

When axial mesoderm cells are added to a suspension of presumptive epidermal and presumptive neural cells, cell segregation results in an external epidermal layer, a centrally located neural tissue, and a layer of mesodermal tissue between them(Figure 1.1 E).Somehow, the cells are able to sort out to their proper embryonic positions.

The **third** of researcher was that selective affinities change during development because embryonic cells do not retain a single stable relationship with other cell types. For development to occur, cells must interact differently with cell populations at specific times. Such changes in cell affinity are extremely important in the process of morphogenesis. When tissues from later stage mammalian and chick embryos were made into single cell suspensions, the cells reaggregated to form tissue like arrangements.

➤ **Differential Adhesion Hypothesis:**

The ability of cells to do this has been proposed to arise from differential cell adhesion by Malcolm Steinberg through his Differential Adhesion Hypothesis. It is a model that sought to explain patterns of cell sorting based on thermodynamic principles.

Definition:

“According to DAH, cells move to be near other cells of similar adhesive strength in order to maximize the bonding strength between cells and produce a more thermodynamically stable structure.”

Explanation:

Using cells derived from trypsinised embryonic tissues, Steinberg showed that certain cell types migrate centrally when combined with some cell types, but migrate peripherally when combined with others.

Example: The interactions between pigmented retina cells and neural retina cells.

➤ **Cell Adhesion Molecules:**

The molecules responsible for adhesion are called cell adhesion molecules (CAMs).

Several types of cell adhesion molecules are known and one major class of these molecules are **cadherins**. As their name suggests, cadherins are calcium-dependent adhesion molecules. They are transmembrane proteins that interact with other cadherins on adjacent cells. Cadherins bind to other cadherins in a like-to-like manner.

Major types of Cadherin:

E-Cadherins is expressed on all early mammalian embryonic cells, even at the zygote stage.

P-cadherin is found on the placenta, where it helps the placenta stick to the uterus.

N-cadherin becomes highly expressed on the cells of the developing central nervous system, and it may play roles in mediating neural signals

R-cadherin is critical in retina formation.

The ability to sort cells based on the amount of cadherin.

➤ **Role of Extracellular Matrix:**

The extracellular matrix (ECM) is a collection of extracellular molecules secreted by cells that provides structural and biochemical support to the surrounding cells.

The extracellular matrix (ECM) is involved in:

- keeping tissues separated
- providing structural support
- Providing a structure for cells to migrate on.

ECM Molecules:

Collagen, Laminin and fibronectin are major ECM molecules that are secreted and assembled into sheets, fibers, and gels.

Multi subunit transmembrane receptors called integrins are used to bind to the ECM.

Example:

A well-studied example of morphogenesis that involves ECM is mammary gland ductal branching.

➤ **Cell Contractility:**

Tissues can change their shape and separate into distinct layers via cell contractility.

Just like in muscle cells, myosin can contract different parts of the tissue to change its shape or structure.

Example:

Typical examples of myosin-driven contractility in tissue morphogenesis occur during the separation of, drosophila and zebra fish germ layers.

Often, during embryonic morphogenesis, cell contractility occurs via periodic pulses of contraction.

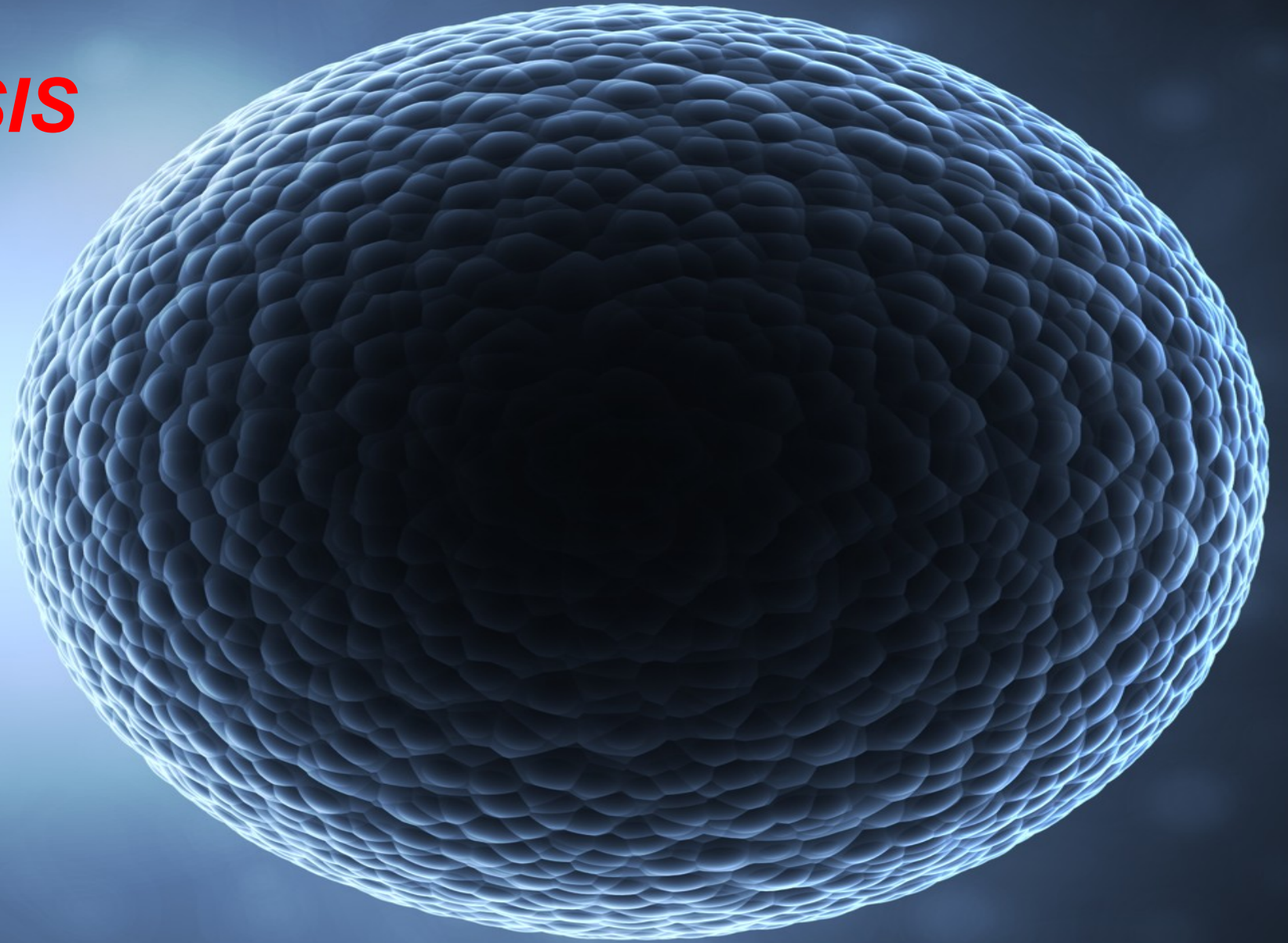
➤ **The Epithelial- Mesenchymal Transition:**

“The epithelial-mesenchymal transition (EMT) is a process by which epithelial cells lose their cell polarity and cell-cell adhesion, and gain migratory and invasive properties to become mesenchymal stem cells”

Mesenchymal cells typically leave the epithelial tissue as a consequence of changes in cell adhesive and contractile properties.

Following epithelial-mesenchymal transition, cells can migrate away from an epithelium and then associate with other similar cells in a new location.

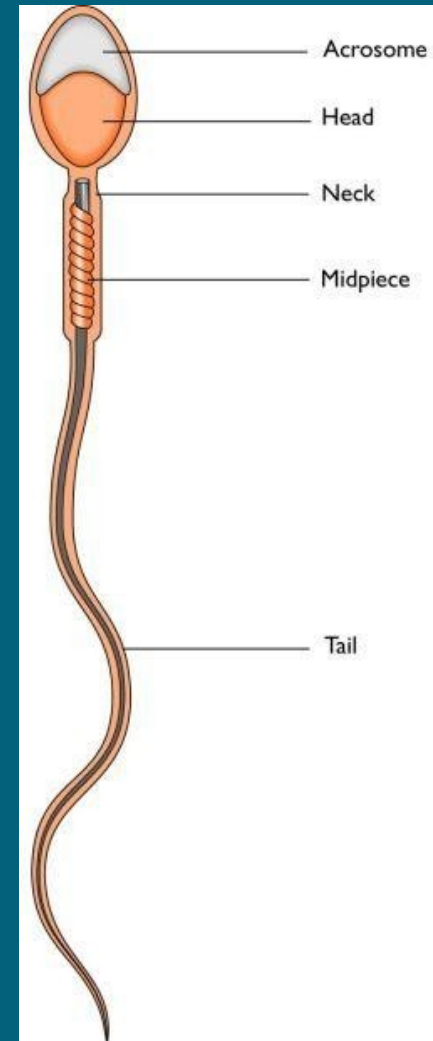
SPERMATOGENESIS AND OOGENESIS



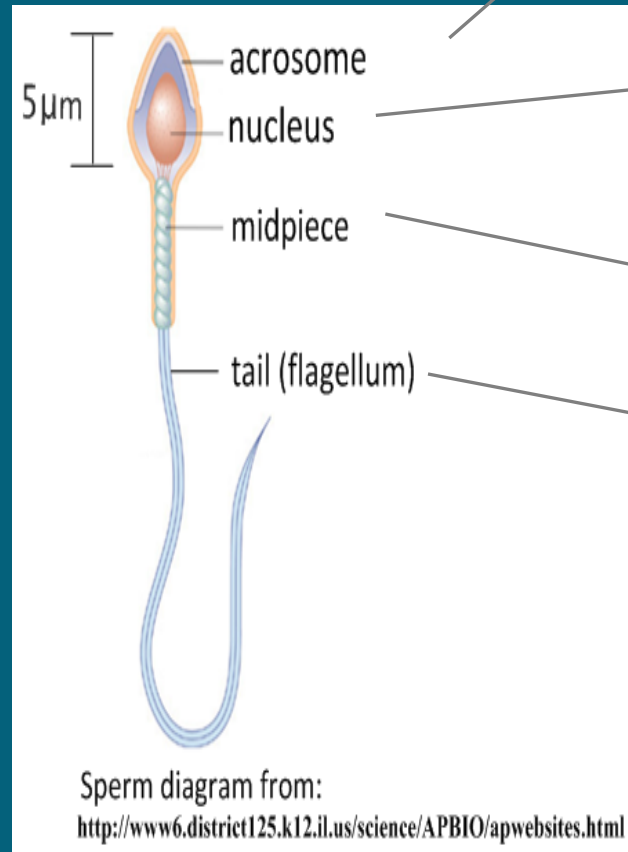
Structure of a Mature Spermatozoon

Spermatozoon Consists of

- Head
- Neck
- Middle piece
- Principal piece = Tail
- An axial filament passes through the middle piece and extends to the tail
- Measures about (60 μ m) in length



Structure of the mature sperm



Contains enzymes which can digest the zona pellucida

Haploid (n), contains 23 chromosomes to be passed from father to child

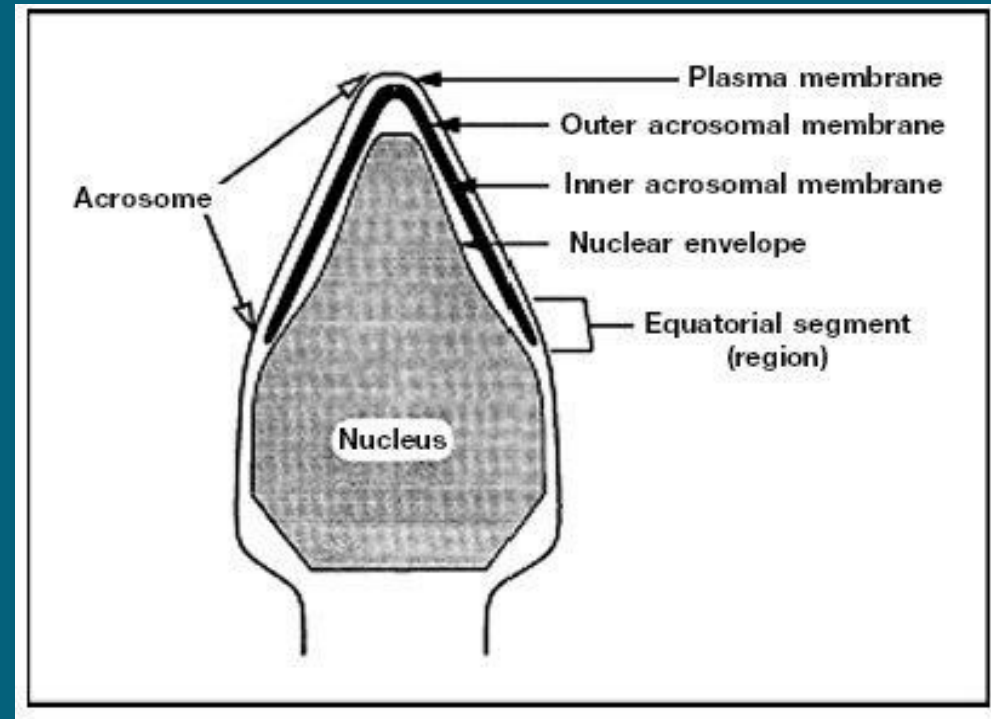
Possesses helical mitochondria which provide the ATP (energy) for swimming (and other processes)

Contains protein fibres and microtubules to strengthen and allow the tail to move respectively.

The Head

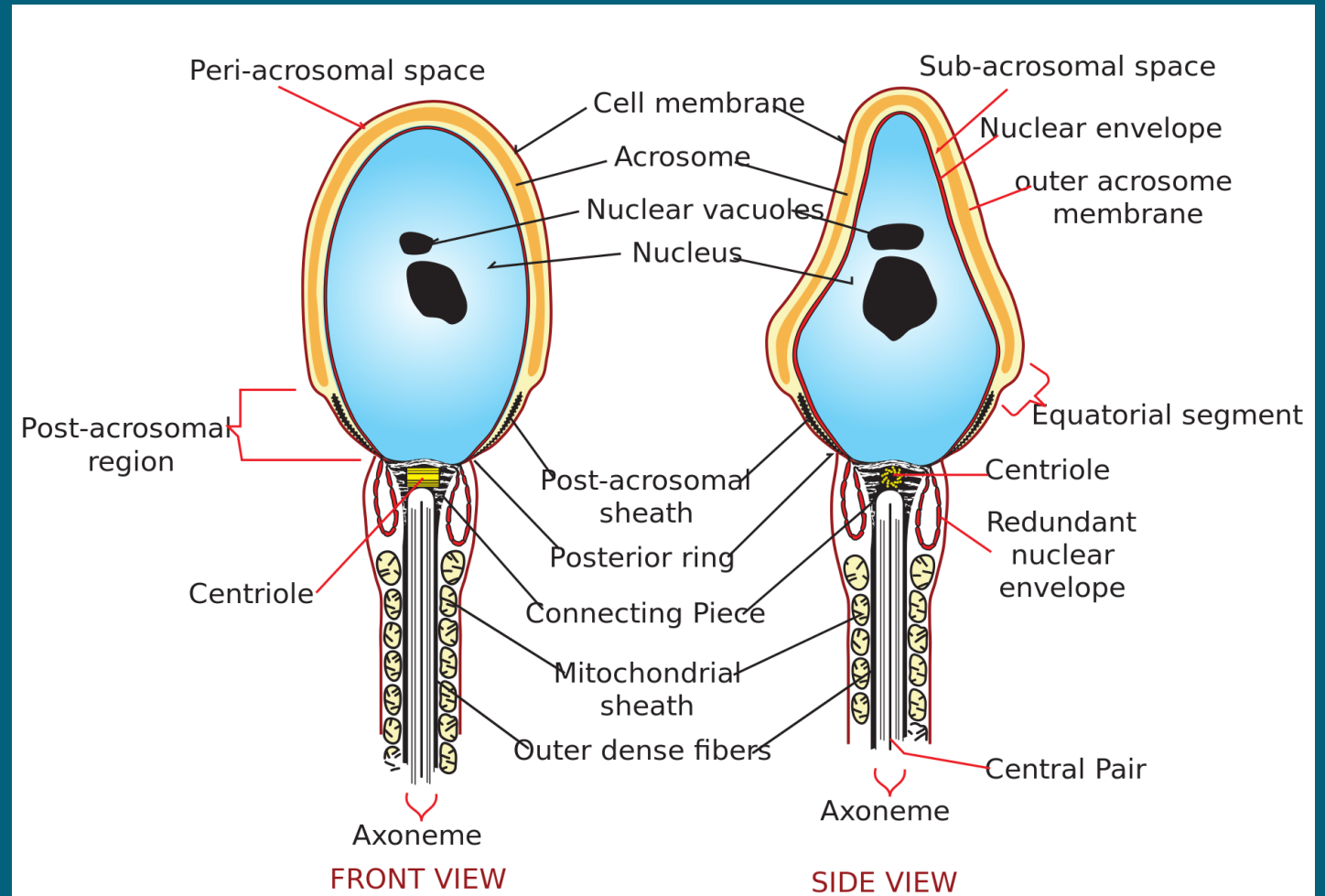
Spermatozoon

- ❑ Piriform in shape.
- ❑ Measures (4 μm) in length.
- ❑ Derived from the nucleus.
- ❑ ***Contains (23) highly condensed chromosomes.***
- ❑ Covered by a cap-like structure called (acrosome)= acrosomic cap = galea capitis.
- ❑ The acrosome contains enzymes that help in penetration of the spermatozoon into the ovum during fertilization

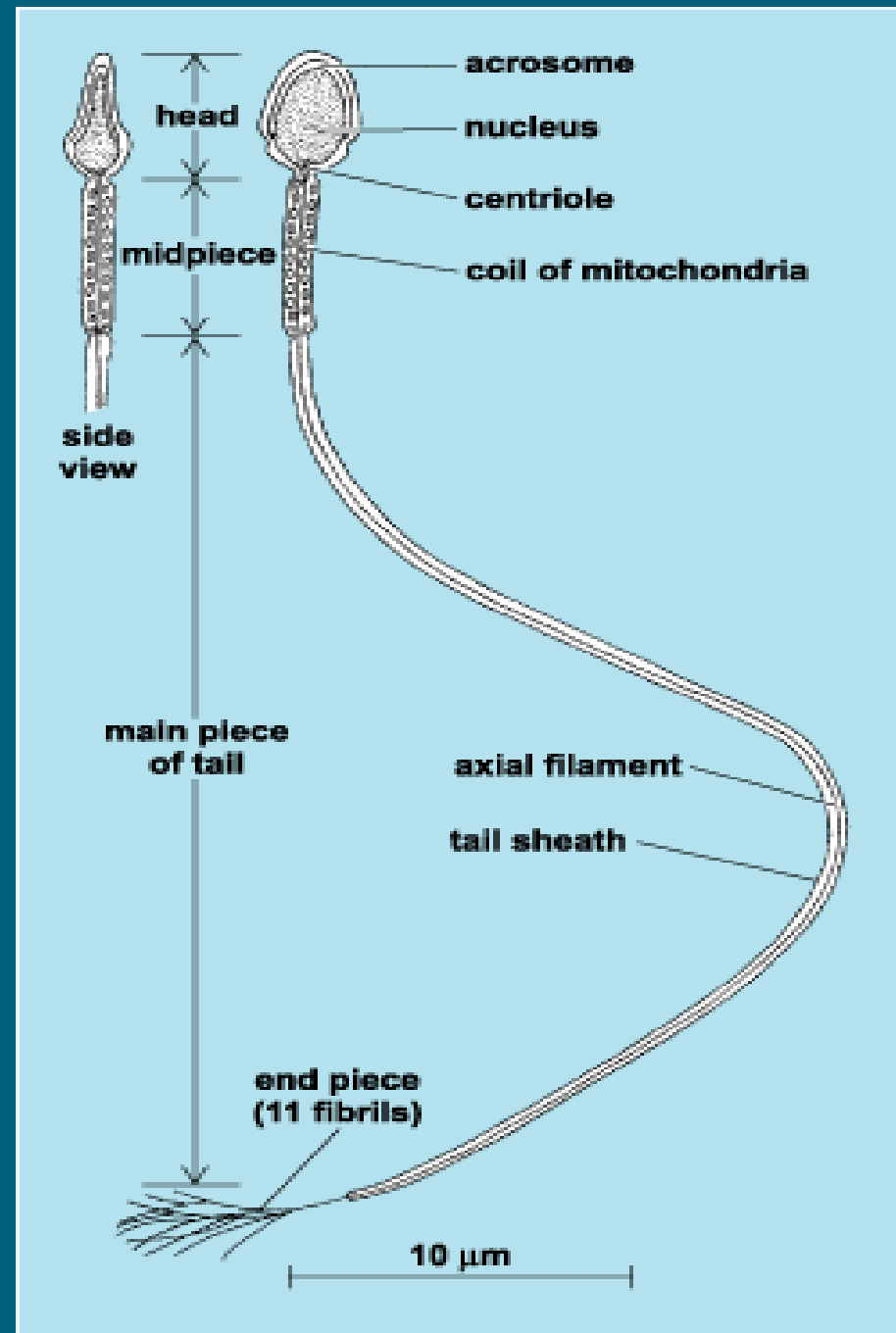


The Neck

- ❖ Narrow.
- ❖ Contains a funnel-shaped basal body and a spherical centriole.
- ❖ The basal body is also called the connecting piece because it helps to establish an intimate connection between the head and the remainder of the spermatozoon.



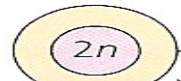
The Axial Filament



■ The Axial Filament

- Begins just behind the centeriole.
- Passes through the middle piece and most of the tail.
- **Passes through the annulus** (ring like structure at the point where the middle piece joins the tail).
- In the middle piece it is surrounded by a spiral sheath made up of mitochondria.
- **Is composed of several fibrils.** There is a pair of central fibrils, surrounded by nine pairs (doublets) arranged in a circle around the central pair.
- The whole system of fibrils is kept in position by a series of coverings.

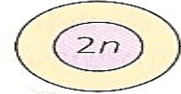
Primordial germ cell in embryo
Differentiation



Spermatogonium

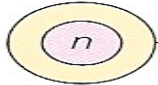
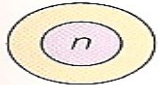
Mitotic division,
producing large numbers of spermatogonia

Differentiation and
onset of meiosis I



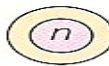
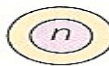
Primary spermatocyte
(in prophase of meiosis I)

Meiosis I completed



Secondary spermatocyte

Meiosis II



Early spermatids

Spermatids
(at two stages of
differentiation)

Differentiation
(Sertoli cells provide
nutrients)



**Sperm cells
(spermatozoa)**

Epididymis

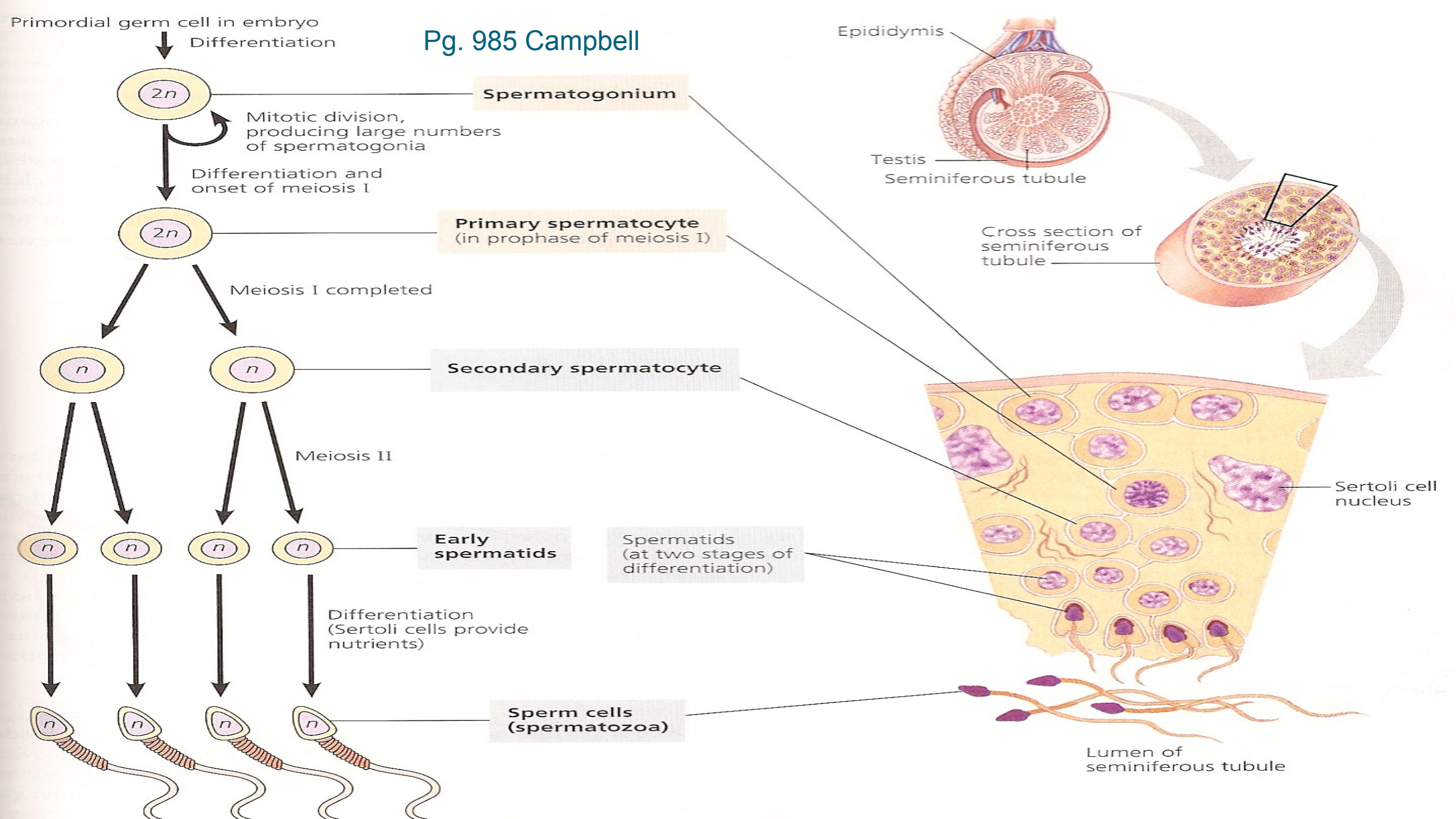
Testis

Seminiferous tubule

Cross section of
seminiferous
tubule

Sertoli cell
nucleus

Lumen of
seminiferous tubule



■ Spermiogenesis

- The process by which a spermatid becomes a spermatozoon.
- The spermatid is a more or less circular cell containing a nucleus, Golgi apparatus, centriole and mitochondria.

all these components take part in forming the spermatozoon:

1. The nucleus forms head
 2. The Golgi apparatus is transformed into acrosomic cap
 3. The centriole divides into two parts the axial filament appears to grow out of them
- The process of Spermatogenesis , including Spermiogenesis, requires about two months for its completion

■ Maturation and Capacitation of Spermatozoa

- *When 1st seen in seminiferous tubules Spermatozoa :*
- *are immature.*
- *Non-motile.*
- *Incapable of fertilizing an ovum.*
- *Stored in the epididymis (undergo **maturation**).*
- *Most of the cytoplasm is shed, but the cell membrane persists as a covering for the spermatozoon.*
- *After maturation they acquire some motility (become fully motile only after ejaculation WHEN THEY GET MIXED WITH SECRETION OF THE PROSTATE GLAND AND SEMINAL VESICLES).*

- Acquire the ability to fertilize an ovum only after they have been in the female genital tract for some time.
- This final step is called *capacitation* (occurs in the uterus or uterine tube under the influence of substances secreted by female genital tract).
- *The glycoprotein coat and seminal proteins lying over the surface of the spermatozoon are altered.*
- **Acrosome reaction:**

When spermatozoon becomes in contact with the zona pellucida, changes take place in the membrane of the acrosome and enable the release of lysosomal enzymes.
- **Zona reaction:**

some enzymes help in digesting the zona pellucida and in penetration of the spermatozoa through it.

OÖGENESIS

- Ovary is the female gonad.
- It has an outer part called cortex.
- It has inner part called medulla.
- The cortex contains many oogonia.
- All oogonia are produced at a very early stage (possibly before birth) and do not multiply thereafter.
- OÖGENESIS is similar to spermatogenesis. However there important differences as well.

Structure of the mature egg

Provides nutrients to support the early development of fertilised egg

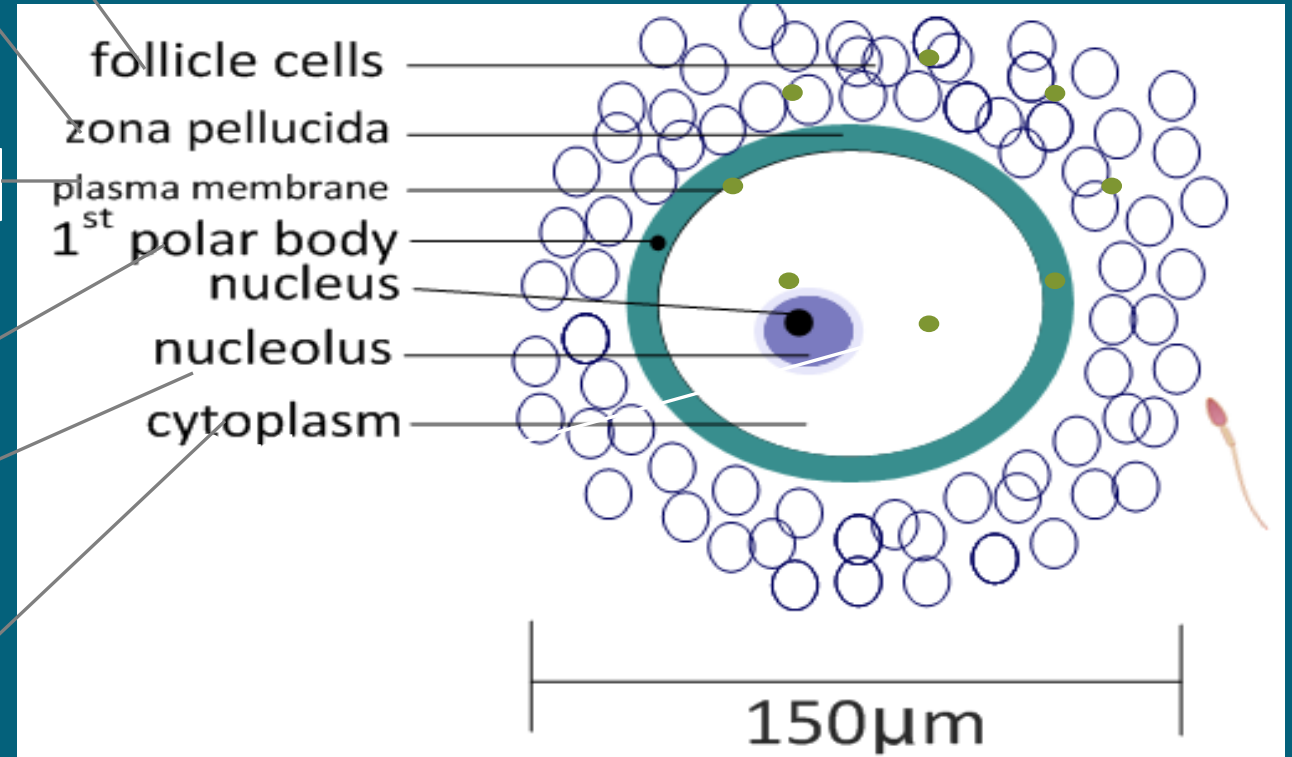
Consists of a glycoprotein that protects the egg and prevents the entry of sperm.

Not required – will break down

Haploid (n) contains 23 chromosomes to be passed from mother to child

Contains nutrients to support the early development of fertilised egg

Makes the zona pellucida impenetrable to sperm (after fertilisation) to prevent polyspermy*



Oogenesis

1

during fetal development large numbers of oogonia are formed by mitosis.

2

oogonia enlarge (growth) and undergo meiosis, but stop in prophase I (until puberty). They are now termed primary oocytes and are held in primary follicles.

3a

(at puberty) some follicles develop each month in response to FSH:

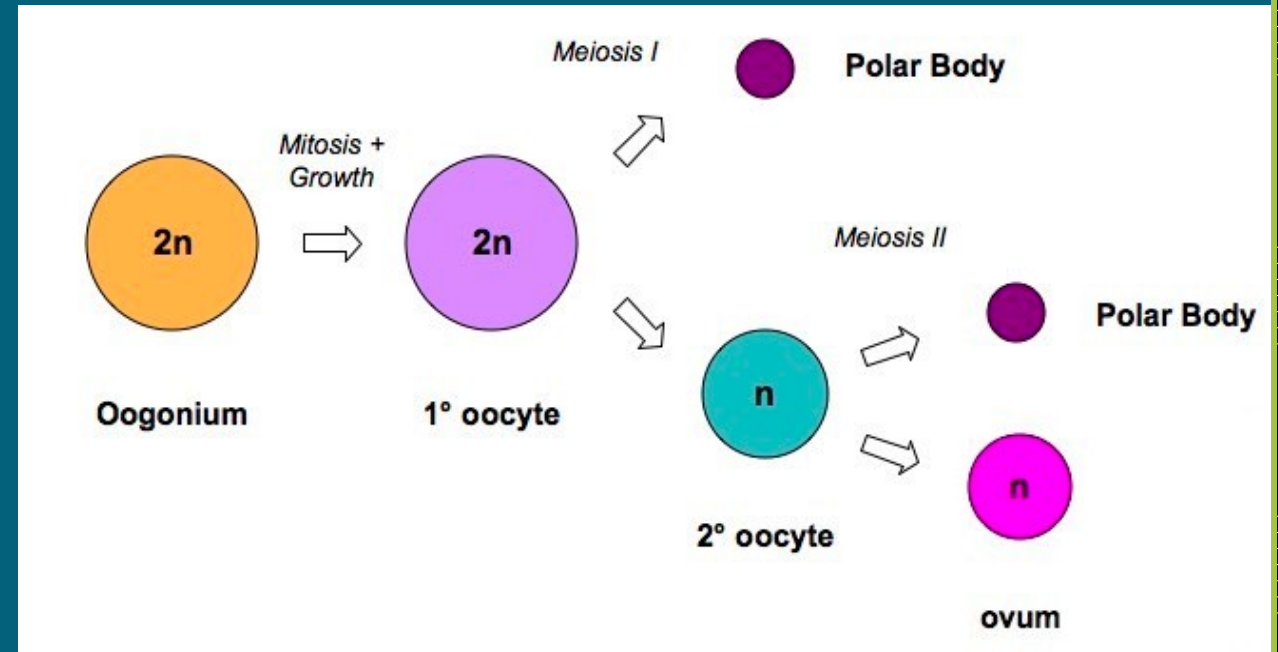
- the oocyte completes the first meiotic division
- Division of the cytoplasm is unequal creating a polar body
- the secondary oocyte continues into meiosis II and halts at prophase II

3b

Secondary oocytes develop along with the follicle. When the follicle is mature it ruptures to release the secondary oocyte with a small number of cells (the mature egg) into the fallopian tube. The remaining follicle cells remain in the ovary to form the corpus luteum (which secretes progesterone).

5

polar bodies eventually degenerate



4

The oocyte completes meiosis II (forming the ovum) if the cell is fertilized and another polar body

Compare and contrast the processes of spermatogenesis and oogenesis


	Oogenesis	Spermatogenesis
Cell division	Begin with mitosis and later on involve meiosis	
Growth	Involve cell enlargement before meiosis	
Product	Haploid cells (gametes)	
Differentiation	Produce specialised gametes	
Location	Eggs/ova produced in the ovaries	Sperm produced in the testes
Initiated	During development of fetus	During puberty
Pauses	During prophase I and between prophase II and metaphase II	None
cytokinesis	Unequal, producing polar bodies	Equal
Number of gametes	One ova, polar bodies degenerate	Four sperm
Release	14 th day, midpoint of the menstrual cycle	Continuous production, released during sexual intercourse
Ceases	At the menopause	Continuous until death

Further Details !!!!!!!!!!!!!!!!!!!!!!!

- Only 400 ova are discharged During the entire reproductive life of a female , (400 out of 40,000).
- 5 to 30 primary oocytes start maturing each menstrual cycle, but only one of them reaches the maturity and is ovulated.
- In late fetal period primary oogonia enlarged to form primary oocytes.
- At the time of birth all primary oocytes are in the prophase of the 1st meiotic division. There number is about 40,000.
- The primary oocytes remain in prophase and do not complete their 1st meiotic division until they begin to mature and are ready to ovulate.



- The reproductive period of a female is between 15 to 50 years of age.
- With each menstrual cycle, a few primary oocyte (about 5 to 30) begin to mature and complete 1st meiotic division shortly before ovulation.
- The 1st meiotic division of primary oocyte produces two unequal daughter cells. Each cell has the haploid number of chromosomes (23). The large cell is called the secondary oocyte, and the smaller one called the 1st polar body.
- The secondary oocyte immediately enters the second meiotic division. Ovulation takes place while the oocyte is in metaphase. *The secondary oocyte remains arrested till fertilization occurs.*

- 
- The second meiotic division is completed only if the fertilization occurs.
 - This division results in two unequal daughter cells.
 - The smaller cell is called the 2nd polar body. The 1st polar body may also divide during the 2nd meiotic division.
 - If fertilization does not occur, the secondary oocyte fails to complete the 2nd meiotic division, and degenerates in about 24 hours after ovulation

Formation of Ovarian Follicles

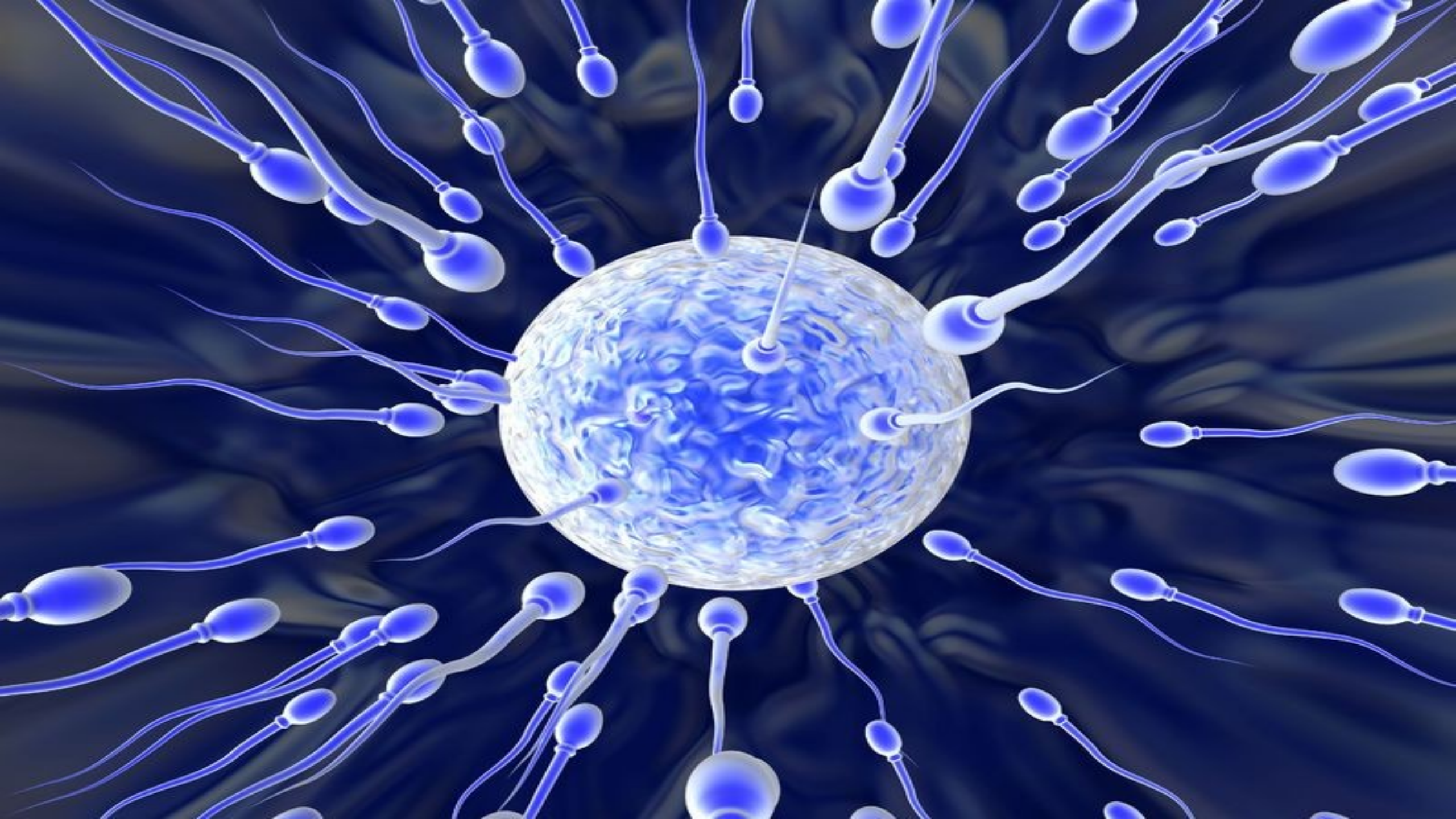
- Ova develop from oogonia.
- Then oogonia are surrounded other cells that form the stroma.
- These stromal cells form ovarian or graafian follicles that surround ova and protect them.
- **The stages in the formation of follicle are as follows:**
 1. Follicular cells: some cells of the stroma become flattened and surround an oocyte. These flattened cells form the ovarian follicle and, therefore, called follicular cells.
 2. **Primordial cells: the flattened follicular cells become columnar.**

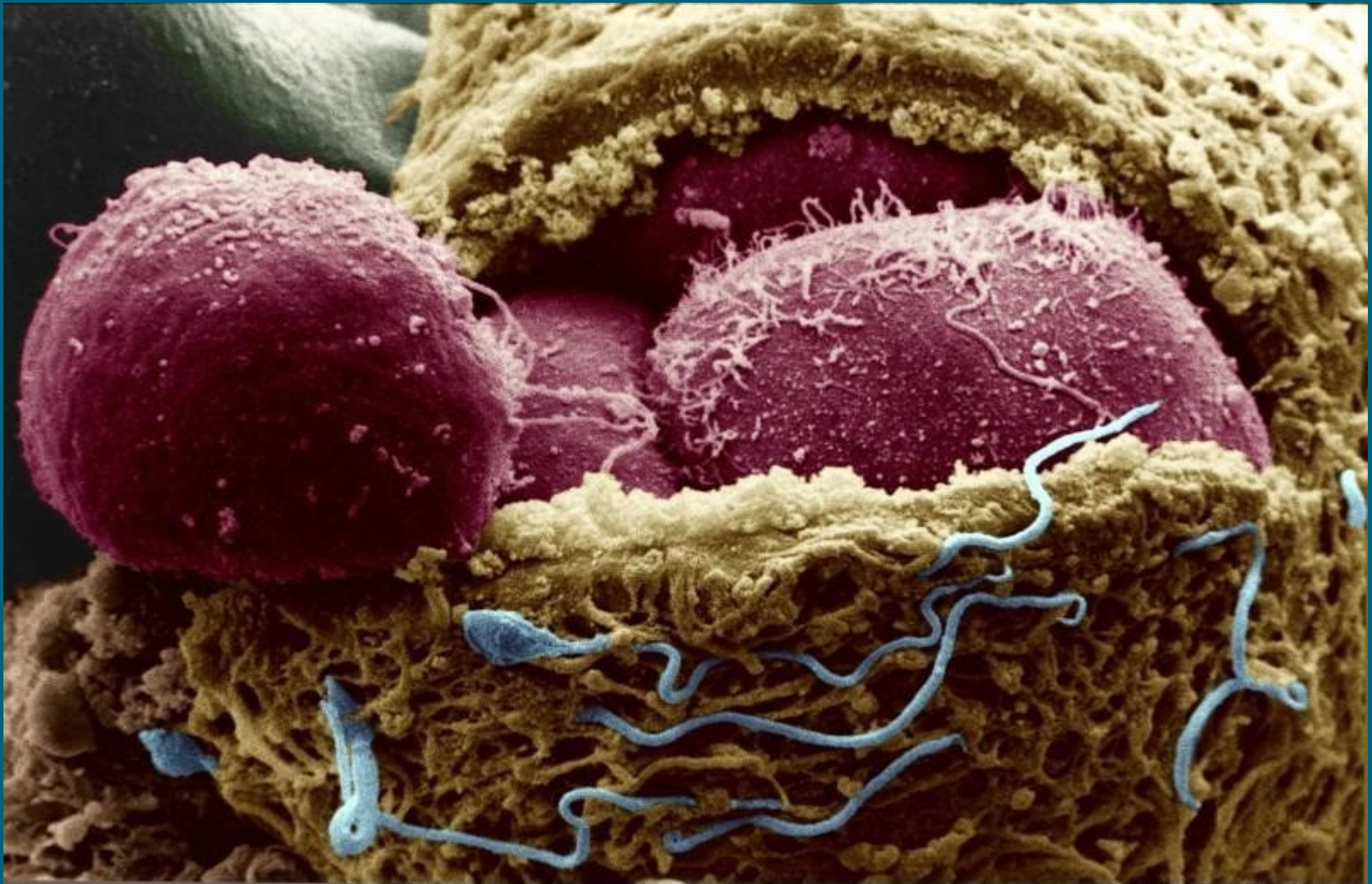


1. **Zona pellucida:** a homogenous membrane appears between follicular cells and oocyte.
2. **Membrana granulosa:** the follicular cells proliferate to form several layers these constitute the Membrana granulosa and the cells called granulosa cells.

FACTORS LEAD TO OVULATION

1. High LH (luteinizing hormone) concentration.
2. Increase activity of *collagenase* enzyme.
(digest the collagen fibers surrounding the follicle).
3. Increase prostaglandins concentration
(results in smooth muscle contraction).
4. Increase pressure of fluid in the follicular cavity.
5. Enzymatic digestion=
the main factor responsible for ovulation.





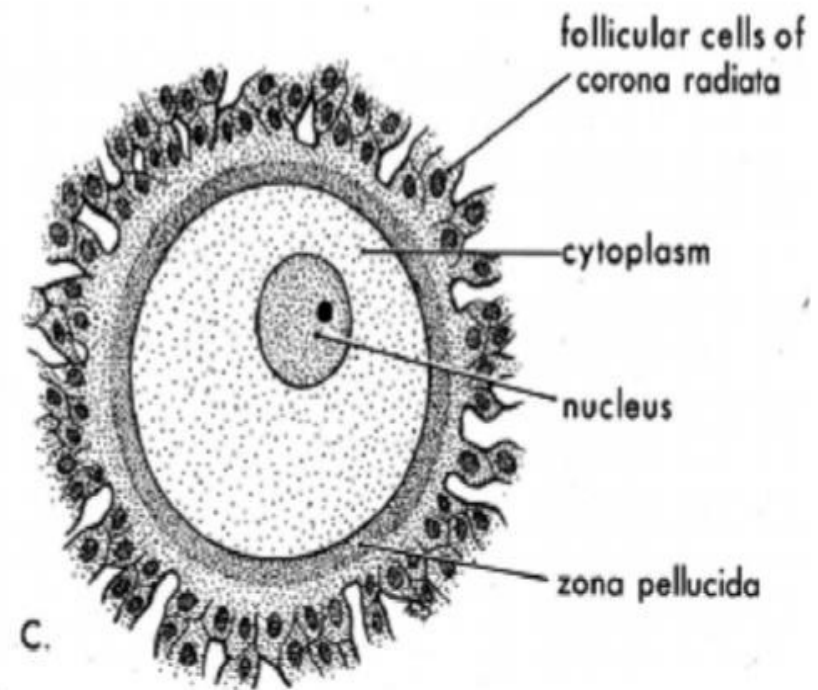
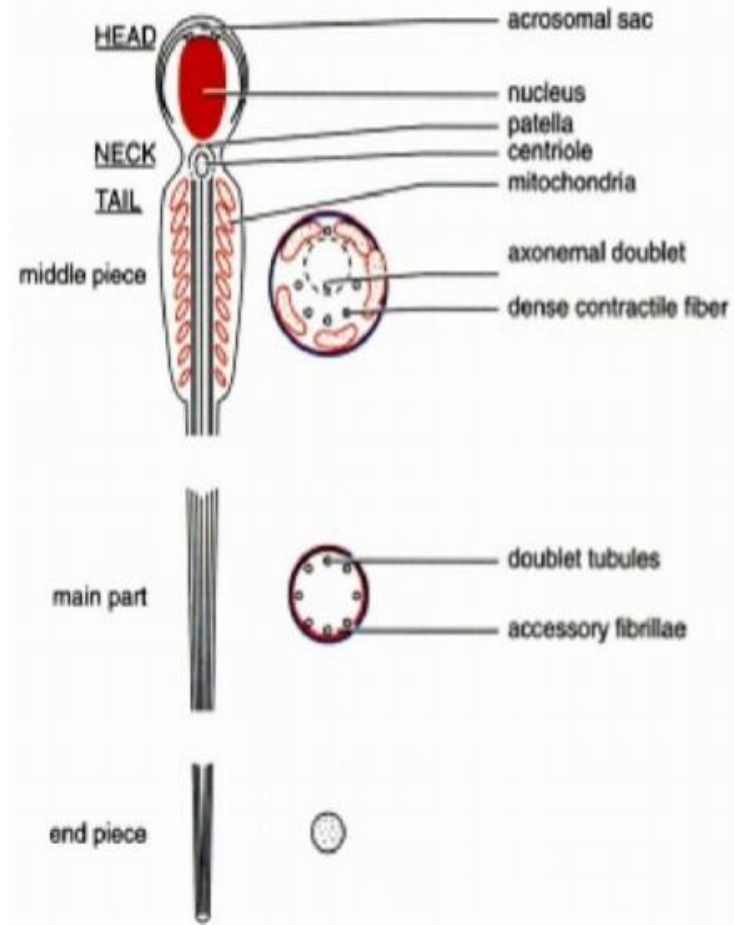




Fertilization

Introduction

- Eggs are non motile surrounded by protective egg coverings
- Mammalian egg has zona pellucida layer around the plasma membrane
- Sperms are highly motile consisting of nucleus, mitochondria and a flagellum
- Sperms activate the egg and deliver their nuclei into egg cytoplasm



Requirement of fertilization

- Fertilization requires a fluid medium in most animals.
- It may be seawater in marine forms, fresh water in fresh water forms and body fluid in viviparous animals.
- To increase the probability of fertilization, the number of sperms must exceed the number of eggs.
- Moreover the lifespan of gametes is limited; therefore fertilization must take place within a short duration of time.

Site of fertilization

- Fertilization may be either external or internal.
- In external fertilization the gametes are discharged in the aquatic medium and the fertilization occurs outside the body of both male and female parents, as in most invertebrates and some vertebrates (fish and frog).
- When fertilization occurs inside the body of female parent, it is internal fertilization, as in *Drosophila*, birds and mammals.

Mechanism of fertilization

- Recognition of egg and sperm (approach of spermatozoan to the egg, attachment and binding)
- Acrosome reaction and penetration
- Fusion of plasma membranes of egg and spermatozoa.
- Activation of egg
- Fusion of egg and sperm pronuclei

Recognition of egg and sperm

- Among fresh water animals the timing of spawning of eggs by females and shedding of sperms by male parent are very specific.
- The sperms are delivered directly to the eggs immediately after laying.
- During internal fertilization, such as in mammals, the gametes of both sexes are deposited in the female reproductive tract.
- The fluid movements within the reproductive tract, assist in transporting the gametes to the site of fertilization.

Sperm attraction

- In many animals, sperms are attracted towards eggs of their species by “chemotaxis” i.e. following a gradient of a chemical secreted by the egg.
- Chemotaxis has been demonstrated in cnidarians, molluscs, echinoderm and urochordates.

Fertilizin interaction

- Factors that mediate sperm– egg interactions even before they make contact were identified by F.R. Lillie (1912). He observed that the egg water (seawater surrounding unfertilized sea urchin eggs), agglutinated the sperm and activated their motility.
- The reaction was species specific. This factor called fertilizin came from the egg jelly coat. It slowly dissolved as in sea water.

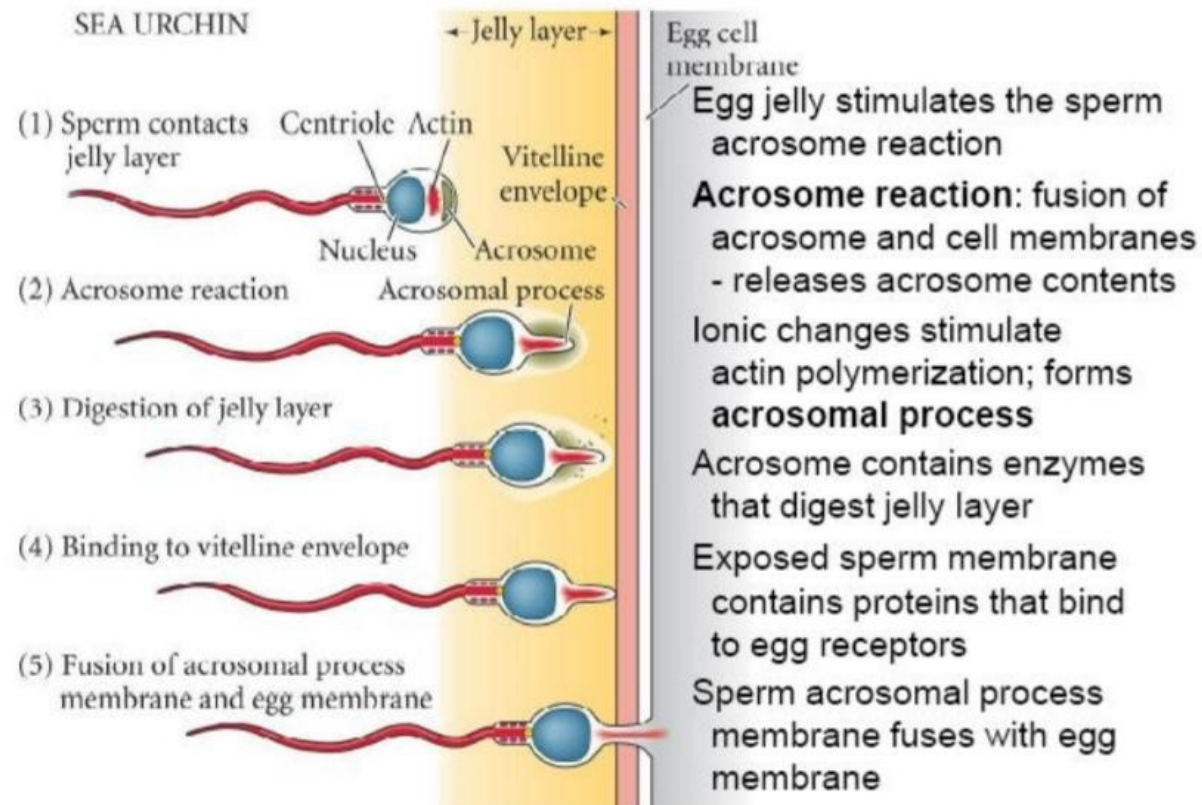
Anti fertilizin interaction

- The receptor sites for fertilizing are present on the sperm plasma membrane called anti fertilizin.
- These are acid proteins. Adhesion of spermatozoa to the surface of the egg is brought about by linking of fertilizin molecule with antifertilizin molecules.

Acrosome reaction in sea urchin

- Once the sperm makes contact with the egg, then it has to penetrate surface coats that surround the egg.
- The penetration is facilitated by the acrosome reaction in which the membrane enclosing the acrosome is shed, releasing the contents of acrosome.
- The acrosomal reaction involves two processes:
 - a) exocytosis of acrosomal vesicle and
 - b) extension of acrosomal process.

Sea Urchin Acrosome Reaction



Gamete binding in sea urchin

- Once the sea urchin sperm has penetrated the egg jelly, the acrosomal process of the sperm contacts the vitelline envelope of the egg.
- The attachment between the acrosomal process and the vitelline envelope is species - specific.
- The specificity is due to interactions between sperm bindin present on the acrosomal process and a specific sperm receptor on the vitelline envelope.

In mammals : Capacitation

- Albumins in female reproductive remove cholesterol altering fluidity of sperm plasma membrane .
- Proteins or carbohydrates on sperm surface are lost .
- Potassium ions leaves the sperms allowing calcium to enter the sperms facilitating the process of membrane fusion during acrosomal reaction.
- Protein phosphorylation occurs.

Gamete binding

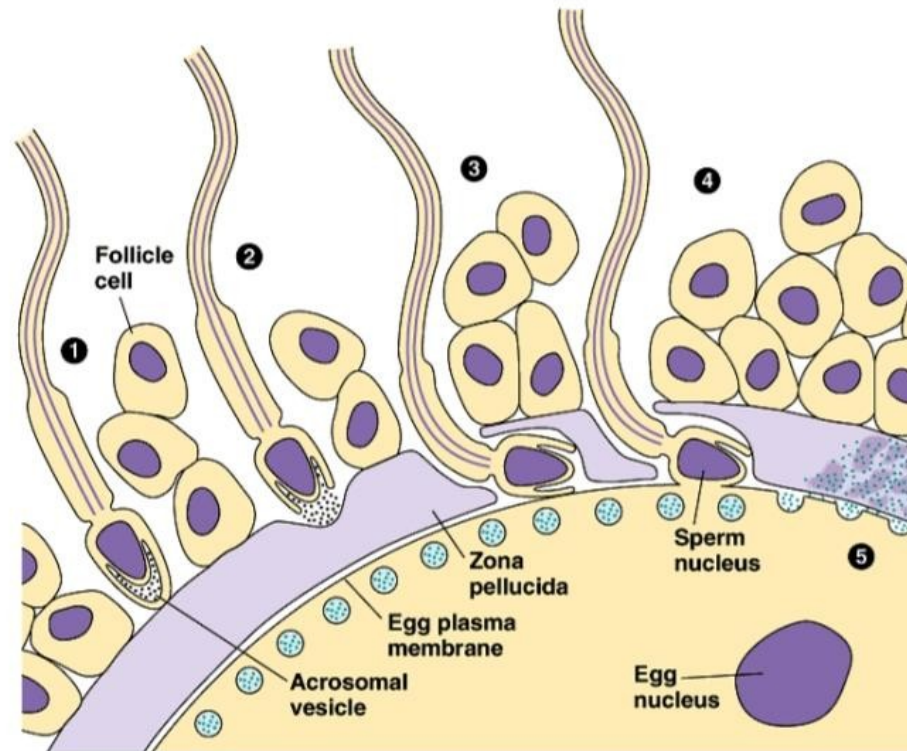
- The mammalian egg is surrounded by extracellular envelope called zona pellucida.
- Around zona pellucida is a layer of cumulus cells (corona radiata) embedded in a cementing substance, hyaluronic acid.
- Hyaluronidase activity on the surface of the sperm head helps it to penetrate this layer.
- Next, sperm must bind to zona pellucida before they make contact with the surface of egg itself.

Acrosome reaction

- Binding of the spermatozoa triggers the acrosome reaction, which allows the sperm to penetrate the zona.
- Acrosome reaction in mammals involves the fusion of the outer membrane of the acrosome with the sperm plasma membrane.
- After the fusion, the acrosomal membrane vesiculates which results in the release of acrosomal contents.
- Subsequently, the outer portion of the acrosomal membrane disappears and only the inner portion adjacent to the nucleus remains intact.

■ Sperm and egg plasma membrane fusion

- In mammals, after penetrating the zona, the sperm enters the perivitelline space surrounding the egg and lands on the egg plasma membrane, where fusion begins at the equatorial region of the sperm head.
- The plasma membrane of egg and sperm become continuous forming a cytoplasmic bridge through which the sperm nucleus enters the egg cytoplasm.
- Usually the entire sperm including the nucleus, centriole, mitochondria and flagellum enters the egg cytoplasm.



Prevention of polyspermy


- The most common way to prevent polyspermy is to prevent the entry of more than one sperm into the egg.
- The polyspermy is blocked in many animals as soon as the first sperm fuses with the egg plasma membrane.
- The sea urchin egg has evolved two mechanisms to avoid polyspermy, a) fast reaction that is accomplished by an electric change in the egg plasma membrane and b) a slower reaction caused by exocytosis of the cortical granules.

Fast and temporary block

- Within 1-3 seconds after entry of the first sperm the electrical membrane potential across the egg plasma membrane shifts from -70 mV to $+20\text{ mV}$.
- This change is caused by a small influx of sodium ions into the egg & lasts for about 60 seconds after which the membrane potential returns to its original level.
- Some acrosomal proteins of sperm open the sodium channel in the egg that causes influx of sodium ions into the egg & depolarizes the egg membrane.

Slow and permanent block

- Proteolytic enzymes (proteases) released, break the bonds that bind the vitelline envelope to the egg plasma membrane. This creates a perivitelline space.
- These enzymes also clip off the binding receptors and any sperm attached to it.
- Mucopolysaccharides (glycosaminoglycans) released, produce an osmotic gradient that causes water to rush into the perivitelline space.
- As a result, vitelline envelope expands and is elevated. It now becomes fertilization envelope.

- 
- A peroxidase enzyme released during cortical reaction, hardens the fertilization envelope by cross-linking tyrosin residues on adjacent proteins of fertilization envelope.
 - Finally, cortical granule protein, hyaline, forms a coating around the egg. The egg plasmamembrane adheres to this protein and the hyaline provides a support for blastomeres during cleavage.
 - Both fertilization envelope and hyaline layer prevent further sperm from binding to the egg plasma membrane.

Activation of egg metabolism


- After the sperm penetrates the egg a series of diverse cytoplasmic reactions are initiated.
- The response of the egg to the sperm can be divided into “early” responses, which occur within seconds of the cortical reaction and “late” responses which take place several minutes after fertilization begins.

Early responses

- The early responses to the activation are the prevention of polyspermy, consisting of two major mechanisms.
- The fast block, which is initiated by sodium influx into the cell, and the slow block initiated by the intracellular release of calcium ions.
- Within one second, the membrane potential of egg rises and sperm – egg fusion takes place within 6 seconds followed by cortical vesicle exocytosis within 15 – 60 seconds

Late responses

- Increased rate of respiration due to utilization of glycogen and other food stuffs for getting energy ATP molecules.
- Activation of NAD kinase and increase in NADH and NADPH: NAD kinase converts NAD to NADP, a co enzyme for lipid biosynthesis, which is essential in formation of new cell membrane during cleavage.
- Ionic changes: certain intracellular changes occur in the concentration of cations such as sodium, potassium and calcium. There is increase in pH (remains high).

- 
- After 5 minutes of fertilization, the rate of protein synthesis increases three to twelve folds. About 20 minutes after fertilization DNA synthesis is initiated.
 - The time of fertilization varies from species to species. It has been found that spermatozoan may enter the egg at different stages of maturation in different animals.
 - Initiation of mitosis :
 - (a) the rate of DNA synthesis increases after fertilization and
 - (b) by the contribution of centriole by sperm to the egg, which is needed for proper mitosis.

Fusion of genetic material in sea urchin

- The sperm aster is a complex of long microtubules that radiate from the sperm centriole.
- The sperm centriole acts as a microtubule-organizing center for sperm aster. The microtubules of the sperm aster push the male pronucleus towards the center of the egg.
- The astral microtubules also make contact with female pronucleus and pull it towards the male pronucleus.
- Thus the two pronuclei migrate towards each other and fuse to form the diploid zygote nucleus. The fusion of male and female pronuclei is called amphimixis.

Fusion of genetic material in mammals

- Male & female pronuclei migrate towards each other, become apposed but do not fuse.
- They remain adjacent to each other; their nuclear envelopes break down but instead of forming a zygote nucleus the chromatin condenses into chromosomes orienting them on a common mitotic spindle.
- Thus, only after completion of the first division of fertilized egg, the paternal and maternal chromosomes become enclosed by a common nuclear membrane to form the nuclei of two blastomeres.

■ Rearrangement of egg cytoplasm

- Yellow cytoplasm on one side
- The light cytoplasm on other side
- Cytoplasm containing abundant yolk granules and clear cytoplasm in the animal hemisphere

Gray crescent

- A single sperm can enter anywhere on the animal hemisphere of the egg.
- After sperm entry, the cortical cytoplasm rotates 30 degrees towards the sperm entry point, relative to the inner cytoplasm.
- As a result of this rotation, the underlying cytoplasm located near the equator on the opposite side of sperm entry point contains diffuse pigment granules and therefore appears gray. This region has been referred to as gray crescent.

Preparation for cleavage

- Before fertilization, the egg, which has been under metabolic arrest, is released from this arrest on the entry of the sperm.
- This initiates the process of development by active protein and DNA synthesis in the egg leading to the beginning of cleavage.
- The first cleavage is not random but tends to be specified by the point of sperm entry and the subsequent rotation of the egg cytoplasm.

The background of the slide is a complex, abstract composition. It features a dark blue to black gradient. Overlaid on this are various elements: glowing, semi-transparent spheres in shades of purple, pink, and blue, some of which appear to be molecular models or cells. Faint, white chemical structures, including hexagons and lines representing bonds, are scattered across the background. Some of these structures are labeled with chemical symbols like 'H₂O', 'C₂H₄', and 'C₂'. The overall effect is a high-tech, scientific aesthetic.

Cleavage/ Cellulation / Segmentation Its Pattern and Mechanish

What is Cleavage ?




A series of rapid cell divisions without cell growth or gene expression which occurs in early embryogenesis.

Cleavage: Functions & Events

- Initial division of zygote to form multi-cellular embryo.
- During cleavage, cells are named blastomeres.
- They are special *mitotic divisions*, hence maintain **2N** complement.
- They lack intervening growth (G1 & G2)phases.
- During cleavage, **G1** & **G2** phases are by-passed, so cells enter from **S** phase to directly into **M** phase.

Purposes of Cleavage

- Converts unicellular zygote to multicellular embryo.
- Maintains **2N** complement of cells, all are genetically identical.
- No growth during early cleavage, so total embryo remains 0.1mm in diameter.
- Cleavage brings about distribution of cytoplasm among the blastomeres.


- 
- Slow cleavage; takes approximately **12-24** hours b/w each cell division.
 - Human cleavage is not synchronous; all of the cells don't cleave at precisely the same time. **As** a result embryos with odd numbers of cells can be seen at various times.

■ Mechanism of Cleavage

- Cleavage involves division of **cytoplasm** and **nucleus**.
- Division of cytoplasm is called cytokinesis.
- Division of nucleus is called karyokinesis.

■ Cytokinesis

- A ring of microfilaments (of actin protein) can be observed just below cell surface of many eggs.
- These filaments form a contractile ring that separate the cytoplasm
- Experiments have shown that a drug, **Cytochalasin B**, disrupts cytoplasmic filaments. If drug is removed, cell division resumes as microfilaments reappear. This suggests that microfilaments are involved in cytokinesis.

- 
- ❑ There is an evidence that asters initiate cytoplasmic division by producing a diffusible factor that act on contractile ring.
 - This was indicated by experiments in which asters were removed or separated.
 - This suggested that asters must be present in the area where cleavage furrow occurs.

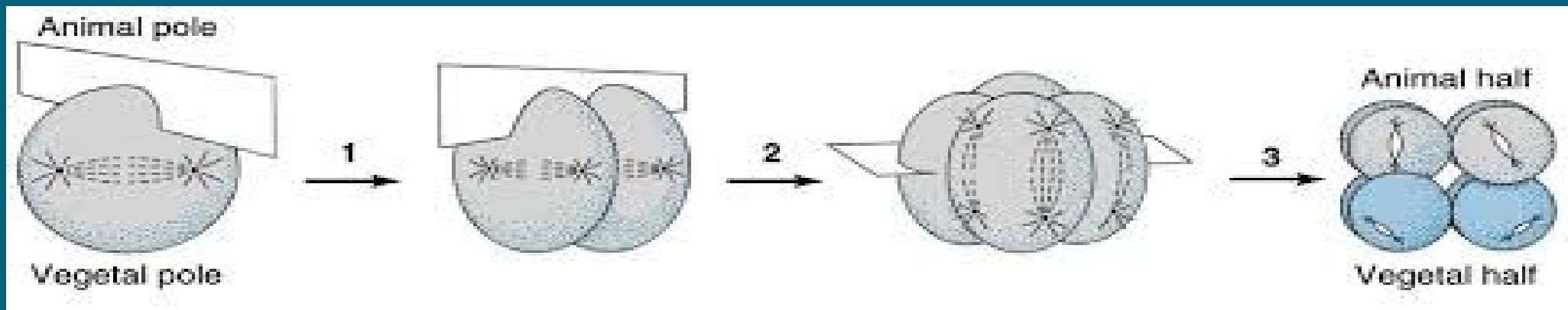
■ **karyokinesis**

- The mitotic spindle involved in nuclear division is mainly composed of protein tubulin A & B.
- These are present in egg and blastomeres (as subunit form) even when mitotic spindle is not present.
- At proper time of spindle formation, these subunits come together, forming visible mitotic apparatus.

The planes of cleavage

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. The cleaved cells may be unequal in size.

■ 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. It is also called as transverse or horizontal cleavage.

Cleavage Patterns

The amount of the yolk and its distribution affect the process of cleavage.

A. Holoblastic or 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:

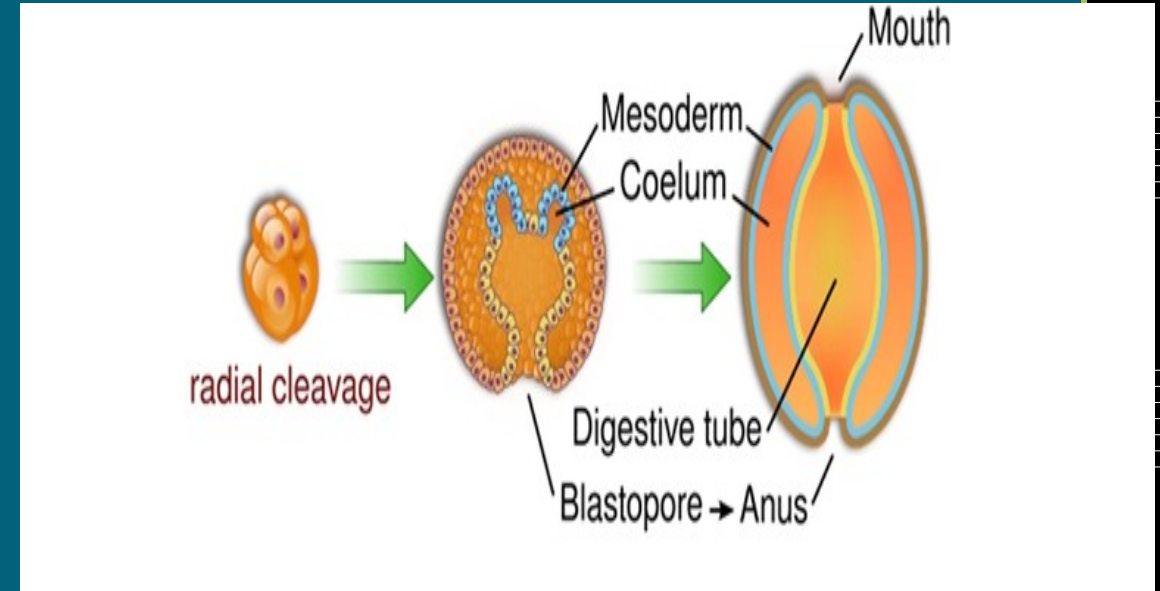
- When the resultant blastomeres become unequal in size

Types of Holoblastic Cleavage

- Holoblastic Cleavage is of four types:

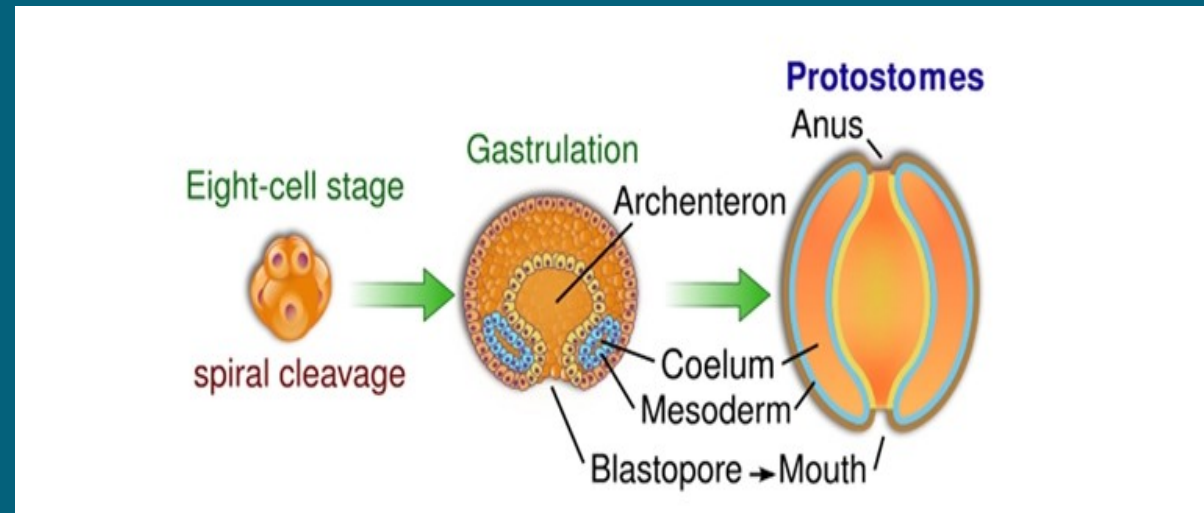
1. Radial Cleavage

- Type of cleavage that is present in deuterostomes, which is characterized by the arrangement of the blastomeres. They are arranged in a position that blastomeres of each upper tier are directly over those of the next lower tier.



2. Spiral Cleavage

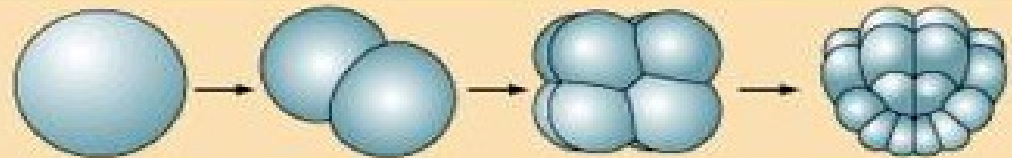
- It is mainly the arrangement of the blastomeres of each upper tier over the cell junctions that are present in the lower tier, result in making the blastomeres arranged spirally around the pole to pole axis of the embryo. It is typically present in protostomes.



3. Bilateral cleavage

- In bilateral holoblastic cleavage, the divisions of the blastomeres are complete and separate.
- The first cleavage results in bisection of the zygote into left and right halves. The following cleavage planes are centered on this axis and result in the two halves being mirror images of one another

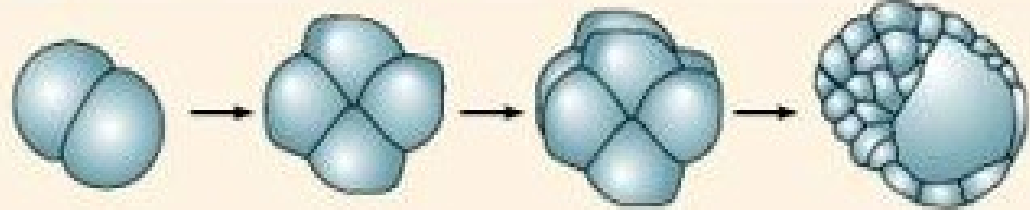
3. Bilateral
Tunicates



4. Rotational cleavage

- Rotational cleavage involves a normal first division along the meridional axis, giving rise to two daughter cells. The way in which this cleavage differs is that one of the daughter cells divides meridionally, whilst the other divides equatorially.

4. Rotational
Mammals, nematodes



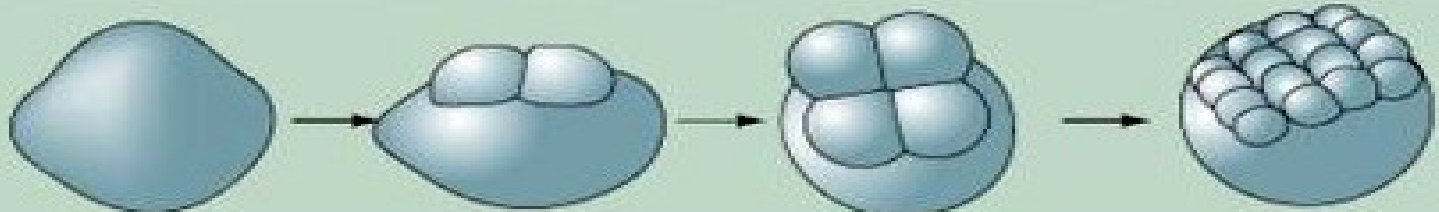
■ B. Meroblastic cleavage

- This occurs in polylecithal eggs the large yolky portion beneath the germinal disc remains unsegmented, e.g., teleosts, reptiles, birds and egg-laying mammals.
- Meroblastic cleavage may be of two types.
- 1. Discoidal cleavage
- 2. Superficial cleavage

1. Discoidal cleavage

- Since the macrolecithal eggs contain plenty of yolk
- the cytoplasm is restricted to the narrow region in the animal pole
- cleavage furrows can be formed only in the disc-like animal pole region. Such a cleavage is called discoidal meroblastic cleavage.
- Eg: birds and reptiles.

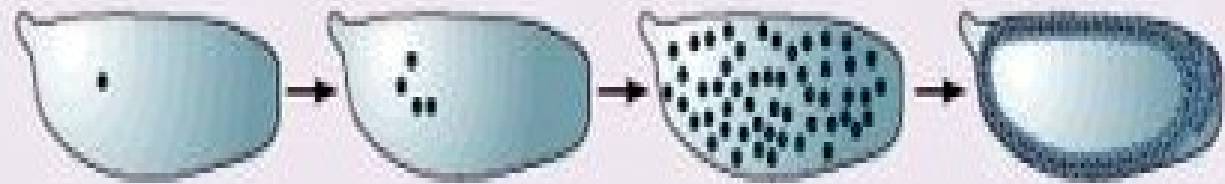
Discoidal
Fish, reptiles, birds



2. Superficial cleavage

- found in centrolecithal eggs, e.g., insects and many arthropods.
- The nucleus lying in the centre of the egg yolk surrounded by an island of cytoplasm undergoes cleavage.
- Each nuclei is surrounded by small amount of cytoplasm.
- They later move towards the periphery in the peripheral cytoplasm.
- cytoplasm fuses with the peripheral cytoplasm. Later the peripheral cytoplasm becomes subdivided by furrows extending inward from the surface
- A layer of peripheral or superficial cells is formed which surrounds the central undivided yolk.

Centrolecithal
Superficial
Most insects



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Types of Blastula

- blastula of various groups of animals differs in form and structure

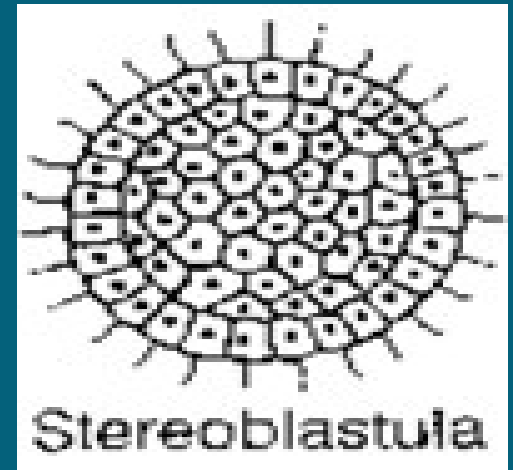
categories of blastulae

1.Coeloblastula

- It is a hollow blastula containing a large spacious blastocoel. Usually, the blastocoel is filled with a fluid containing mucopolysaccharides. The blastula resulting from holoblastic equal cleavage, is called equal coeloblastula

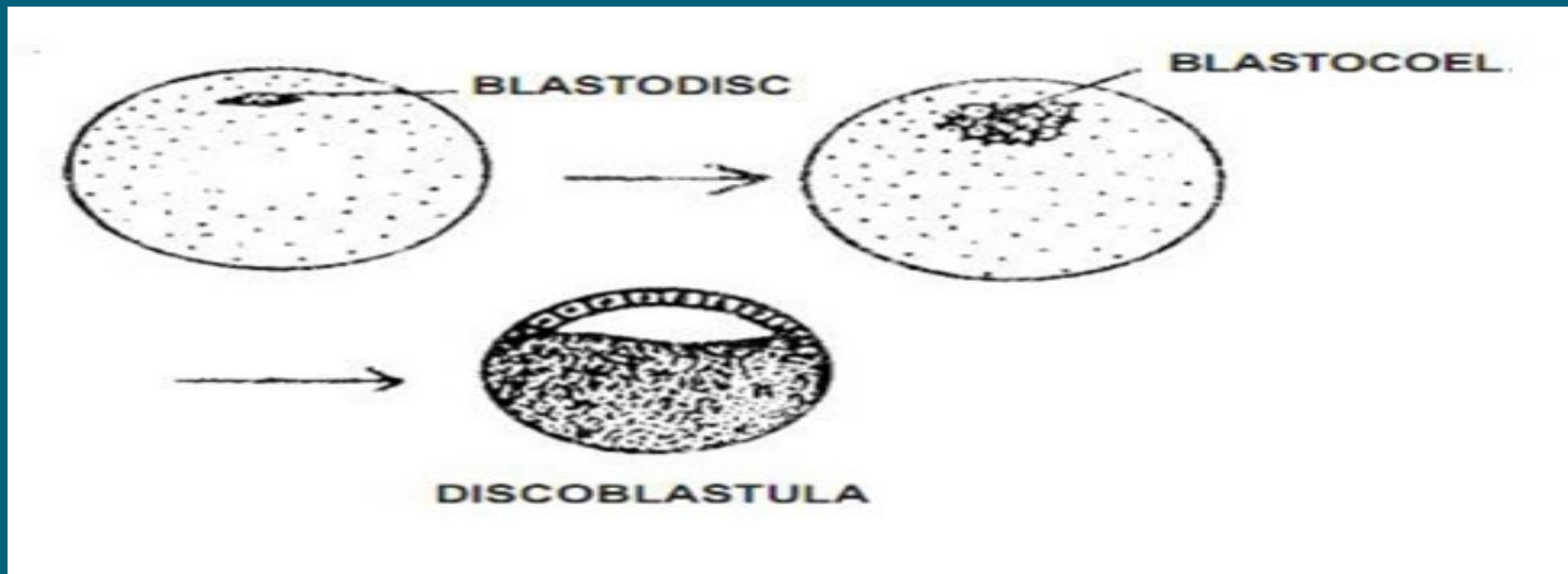
2. Stereobla

- This type of blastula is composed of an aggregate of larger sized and relatively lesser number of cells without or with extremely small blastocoelic space in the centre. Stereoblastula occurs in a variety of animals such as insects, some worms like Nereis, mollusks like Crepidula, gymnophionan



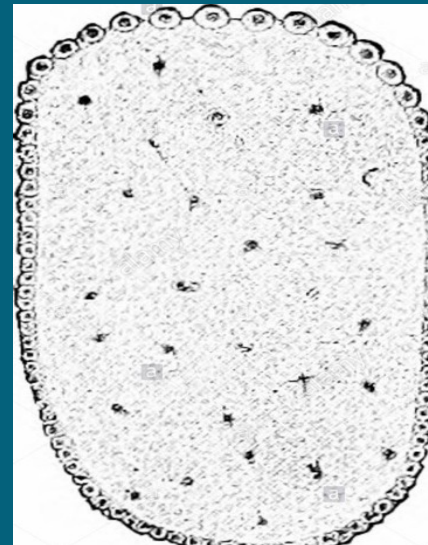
3. Discoblastula

- Discoblastula consists of a disc - shaped mass of blastomeres overlying. This blastula is the result of meroblastic discoidal cleavage as in most fishes, reptiles and birds



4. Periblastula

- The periblastula is a vesicle whose wall consists of one layer of cells and whose cavity is filled with unbroken yolk. It forms as a result of the superficial segmentation of the egg.



GASTRULATION

GASTRULATION

- Gastrulation is a phase early in the embryonic development of most animals, during which the single-layered blastula is reorganized into a multilayered structure known as the **gastrula**

Before gastrulation

- the embryo is a continuous epithelial sheet of cells

By the end of gastrulation

- the embryo has begun differentiation to establish distinct cell lineages
- set up the basic axes of the body (e.g. dorsal-ventral, anterior-posterior)
- internalized one or more cell types including the prospective gut

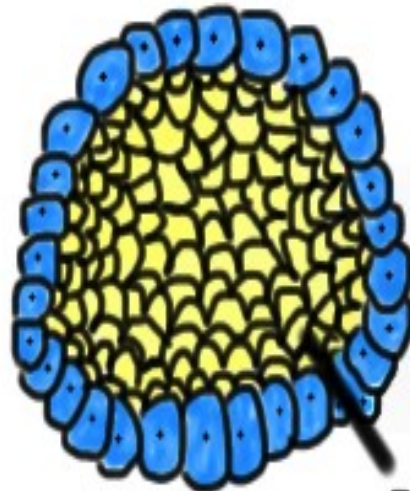


- In **triploblastic organisms** the gastrula is trilaminar ("three-layered"). These three germ layers are known as the ectoderm, mesoderm, and endoderm.
- In **diploblastic organisms**, such as Cnidaria and Ctenophora, the gastrula has only ectoderm and endoderm.
- The two layers are also sometimes referred to as the **hypoblast** and **epiblast**.

Features of gastrulation

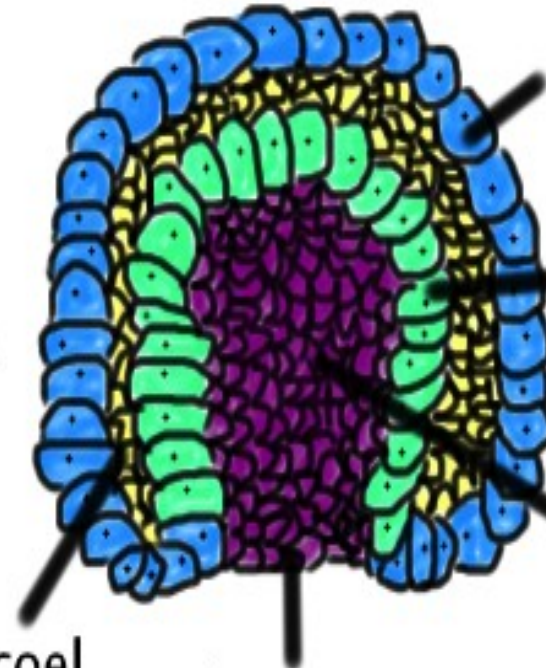
- The molecular mechanism and timing of gastrulation is different in different organisms. However, some common features of gastrulation across triploblastic organisms include:
 1. A change in the topological structure of the embryo, from a simply connected surface (sphere-like), to a non-simply connected surface (torus-like);
 2. the differentiation of cells into one of three types (endodermal, mesodermal, and ectodermal)
 3. the digestive function of a large number of endodermal cells.

Blastula



Blastocoel

Gastrula



Ectoderm

Endoderm

Archenteron


Blastopore

Gastrulation in Mammals

- the gastrulation movements of reptilian and avian embryos, which evolved as an adaptation to yolky eggs, are retained even in the absence of large amounts of yolk in the mammalian embryo.
- The mammalian embryo obtains nutrients directly from its **mother** and **does not** rely on **stored yolk**.
- This **adaptation** has entailed a dramatic restructuring of the maternal anatomy .
 1. such as expansion of the oviduct to form the uterus
 2. the development of a fetal organ capable of absorbing maternal nutrients.

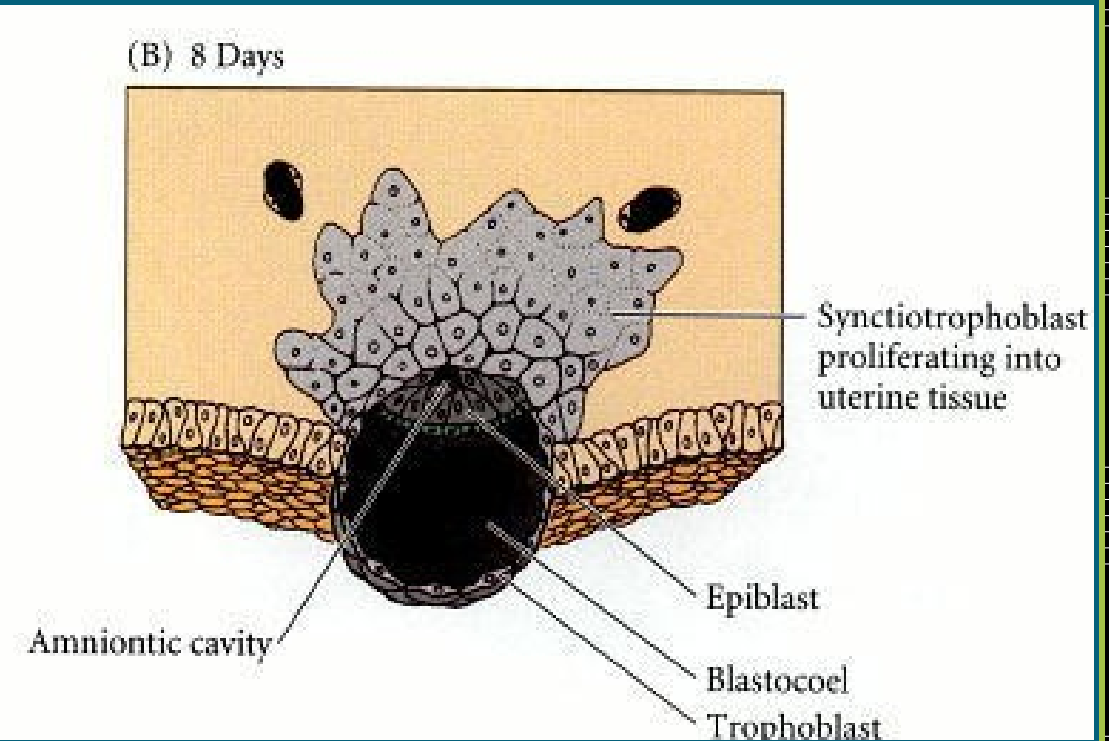
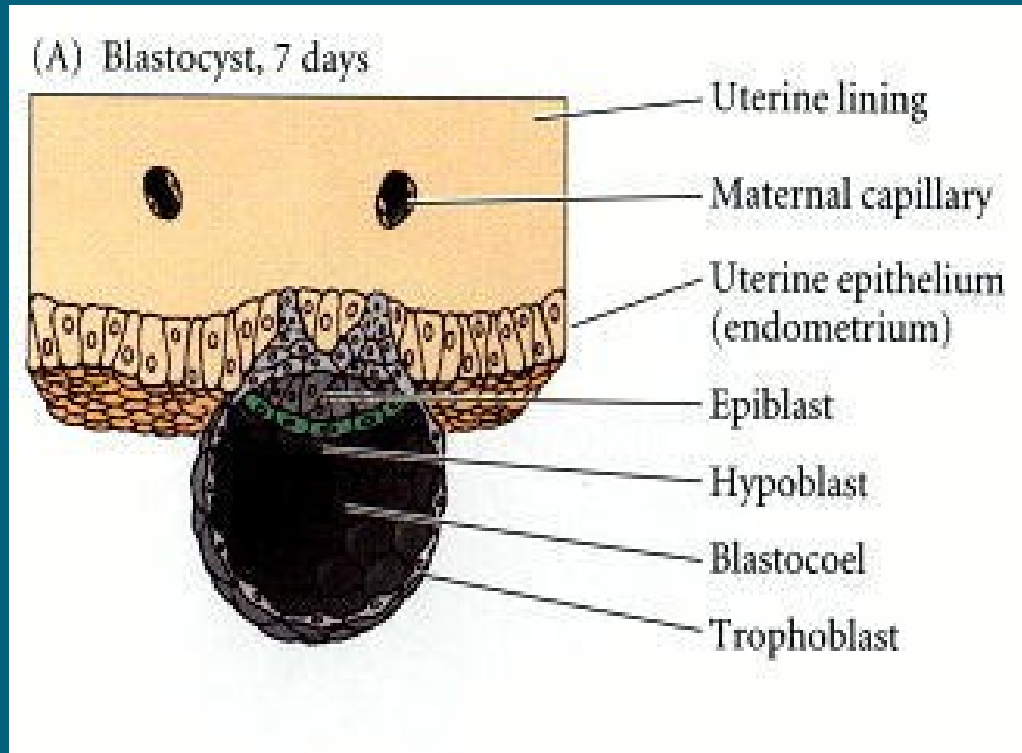
Fetal organ- chorion

- This fetal organ—the chorion—is derived primarily from **embryonic trophoblast cells**
- It is supplemented with mesodermal cells derived from the inner cell mass.
- The chorion forms the fetal portion of the placenta.
- It will induce the uterine cells to form the maternal portion of the placenta, the **decidua**.
- The decidua becomes rich in the blood vessels that will provide oxygen and nutrients to the embryo.

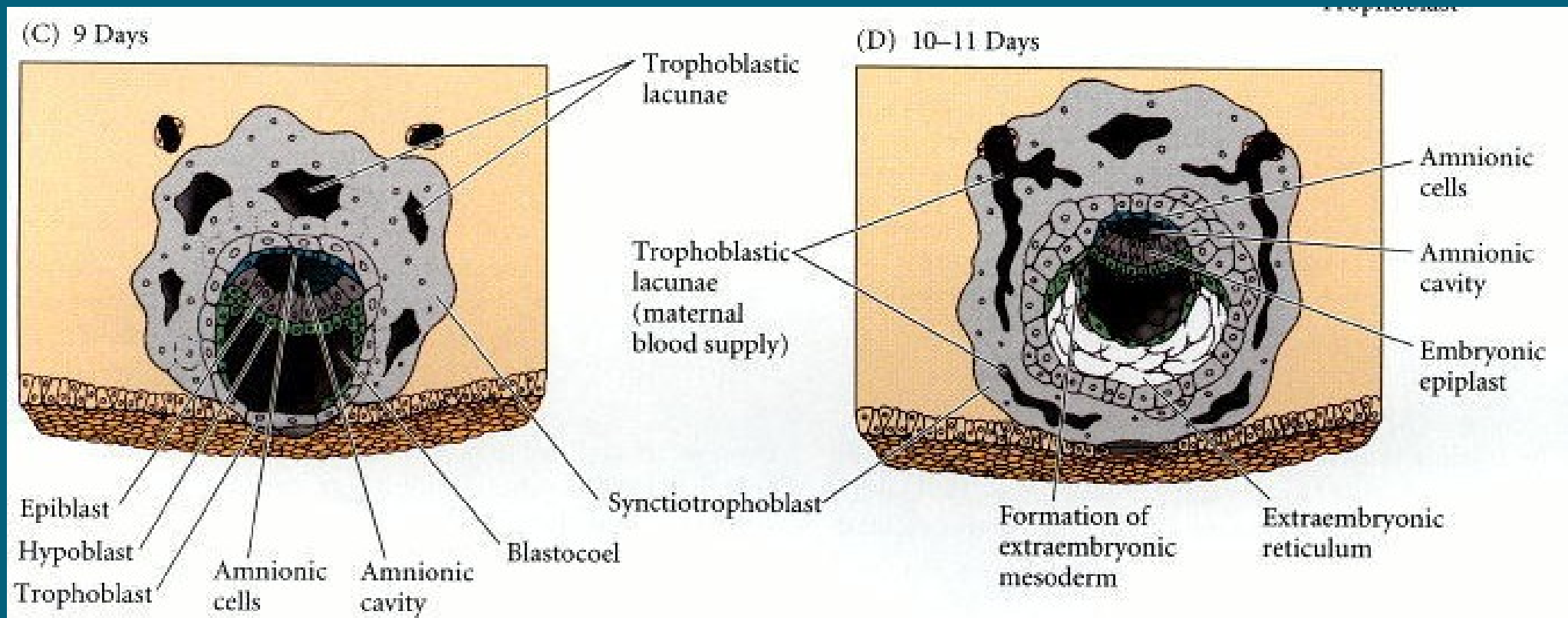
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- The first segregation of cells within the inner cell mass results in the formation of the **hypoblast** (sometimes called the primitive endoderm) layer
 - The hypoblast cells delaminate from the inner cell mass to line the blastocoel cavity, where they give rise to the extra embryonic endoderm, which forms the **yolk sac**.
 - The remaining inner cell mass tissue above the hypoblast is now referred to as **the epiblast**.
 - The epiblast cell layer is split by small clefts that eventually coalesce to separate the embryonic epiblast from the other epiblast cells, which form the **amnionic cavity**.



- Once the lining of the amnion is completed, it fills with a secretion called **amniotic** (amniotic) fluid, which serves as a shock absorber for the developing embryo while preventing its desiccation.
- The embryonic epiblast is believed to contain all the cells that will generate the actual embryo.
- Gastrulation begins at the posterior end of the embryo, and this is where the **node** forms.
- Like the **chick epiblast cells**, the mammalian mesoderm and endoderm migrate through a primitive streak
- like their **avian counterparts**, the migrating cells of the mammalian epiblast lose E-cadherin, detach from their neighbors, and migrate through the streak as individual cells.
- Those cells migrating through the node give rise to the **notochord**.




Tissue formation in the human embryo between days 7 and 11. (A, B)



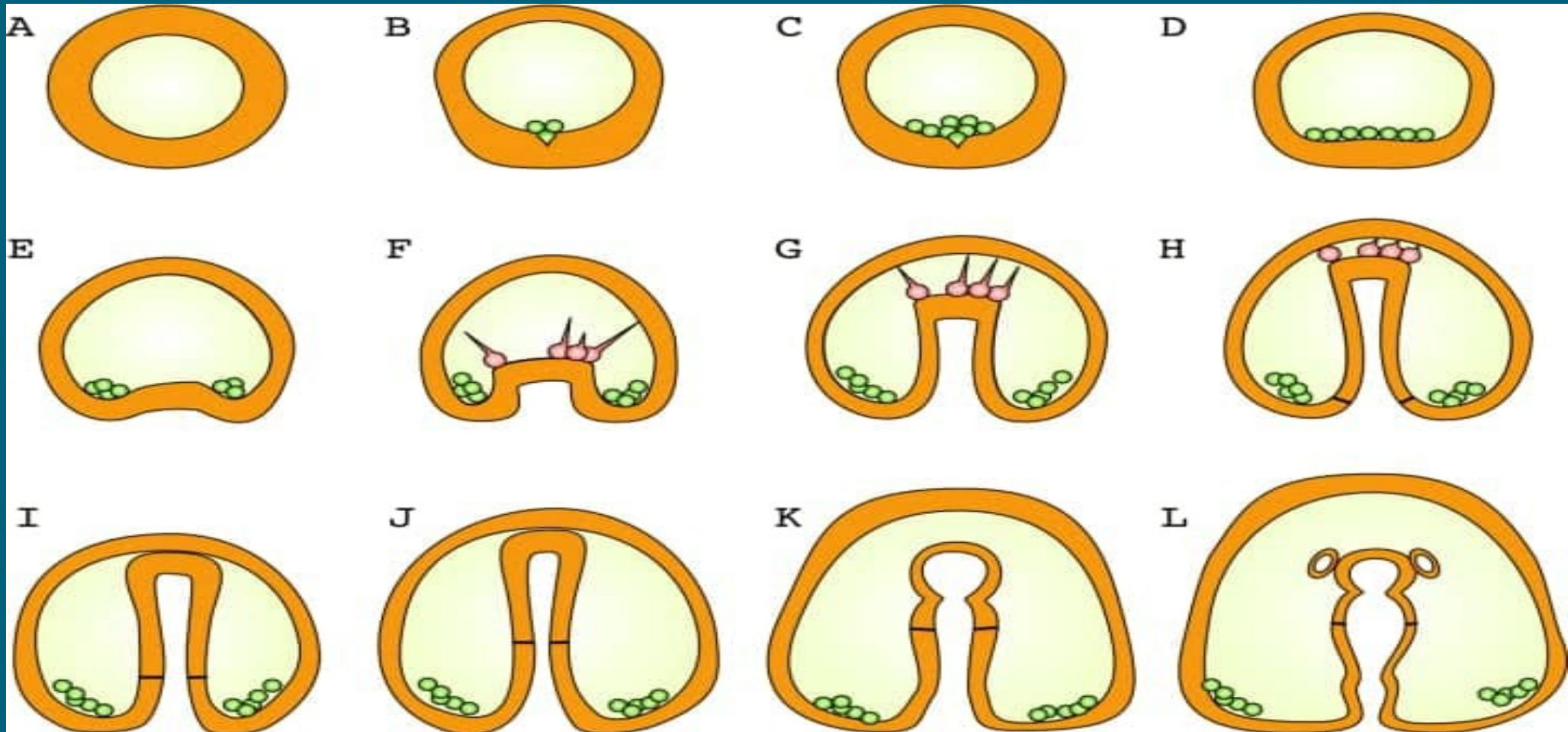
Human blastocyst immediately prior to gastrulation. The inner cell mass delaminates hypoblast cells that line the blastocoel, forming the extraembryonic endoderm of the primitive yolk. (C) Meanwhile, the epiblast splits into the amnionic ectoderm (which encircles the amnionic cavity) and the embryonic epiblast. The adult mammal forms from the cells of the embryonic epiblast. (D) The extraembryonic endoderm forms the yolk sac.

GASTRULATION IN SEA URCHIN

- Processes of sea urchin gastrulation have been conventionally divided into **two** distinct phases, primary and secondary invagination.
- In this review, the processes are divided into four steps for convenience.
- Preceding the occurrence of invagination, the cells around the vegetal pole become elongated and give rise to a thickened vegetal plate (**Step 1**).
- Then the thickened vegetal plate bends inwardly and gives rise to a short stub-like gut rudiment (**Step 2, primary invagination**).

- 
- During a couple of hours after primary invagination, the gut rudiment scarcely elongates. Meanwhile, another population of mesodermal cells, secondary mesenchyme cells (SMC), appears at the archenteron tip (**Step 3**).
 - After such a pause of archenteron elongation, the gut rudiment rapidly elongates until its tip reaches the inner surface of the apical plate (**Step 4, secondary invagination**).

Step 1: ingression of primary mesenchyme cells and formation of the vegetal plate.





- (A) **Hatching-blastula stage.** Embryos are composed of a monolayered epithelium and spherical in shape.
- (B) **Early mesenchyme blastula stage.** The vegetal plate thickens. A small number of primary mesenchyme cells (PMC) appear in the blastocoel.
- (C) **Middle mesenchyme blastula stage.** Ingression of PMC culminates.
- (D) **Late mesenchyme blastula stage.** Most PMC have entered the blastocoel. The vegetal plate becomes somewhat thinner at this stage than the preceded stages.
- (E) **Beginning of primary invagination.** The vegetal plate bends inwardly.
- (F) **Early gastrula stage.** Primary invagination completed, and a stub-like gut rudiment forms. Secondary mesenchyme cells (SMC) appear at the tip of the gut rudiment.
- (G) **Mid to late gastrula stage.** The gut rudiment is stretched along the animal vegetal axis by contraction of SMC filopodia.



(H) **Late gastrula stage.** The archenteron cells are rearranged, and slender archenteron forms.

(I) **Very early prism stage.** The ectodermal layer begins to expand, and the cells near the blastopore are pulled into the base of the archenteron.

(J) **Early prism stage.** Recruitment of the archenteron cells continues as the ectodermal layer expands.

(K) **Mid prism stage.** A constriction appears between the esophagus and stomach.

(L) **Late prism stage.** Late recruitment of endodermal cells completed. The cells that had invaginated by the end of secondary invagination occupy the esophagus and the anterior half of the stomach. PMC and SMC are colored in green and pink, respectively. The short lines in (H–L) indicate the boundary of the cells that have invaginated by the end of secondary invagination.

Step 2: primary invagination

- Unlike in amphibian embryos, bottle cells are arranged in a circle at the central region of the vegetal plate in *Strongylocentrotus purpuratus*.
- Kimberly & Hardin (1998) showed that elimination of a 90–180 degree arc of bottle cells markedly retards invagination, while ablation of other types of cells does not cause the significant delay.
- Thus, it is clear that bottle cells trigger the initial inward bending of the vegetal plate.

▀ **Step 3:** lag phase in archenteron elongation

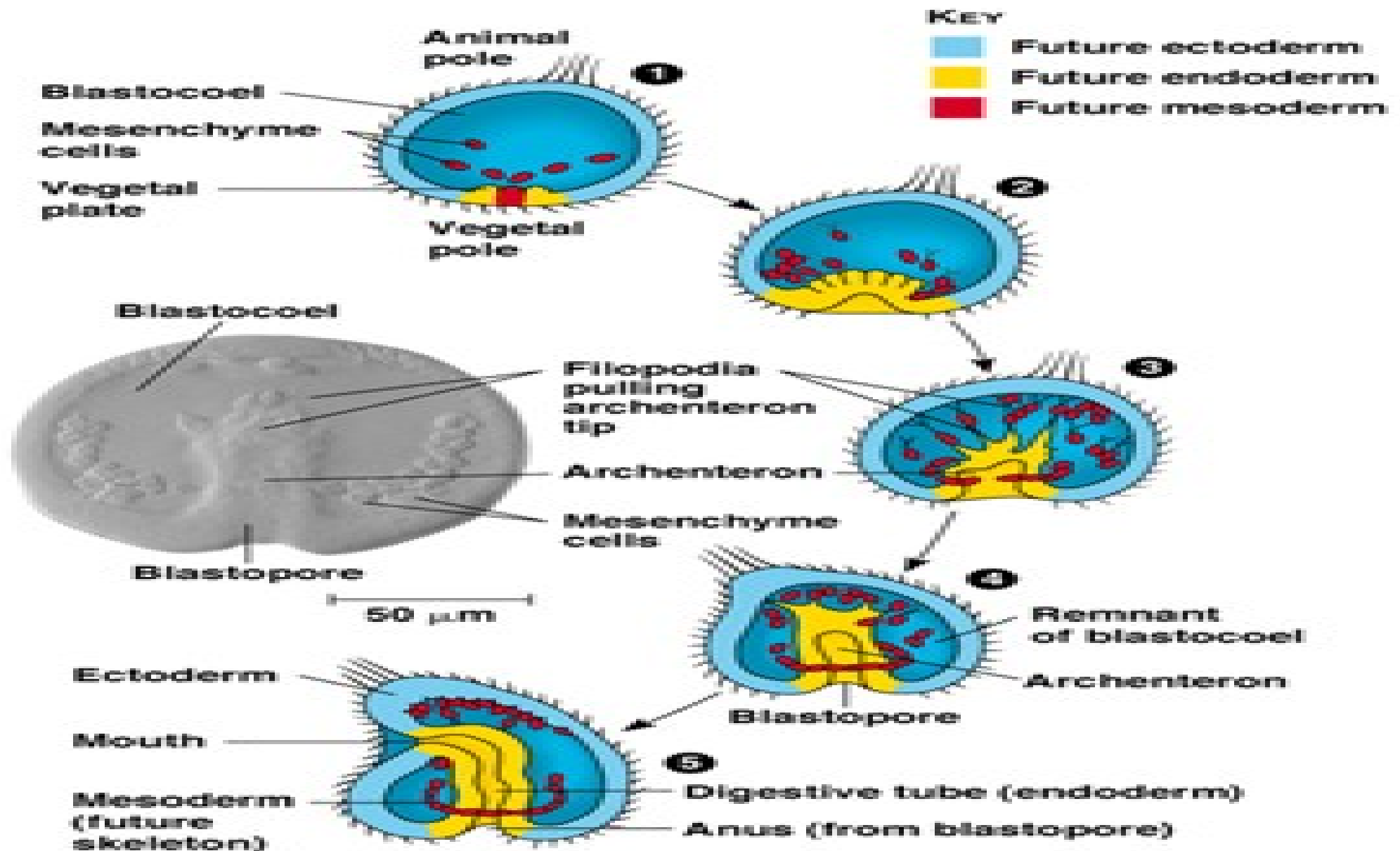
- During a couple of hours after primary invagination, the gut rudiment scarcely elongates.
- Meanwhile, another population of mesodermal cells, secondary mesenchyme cells (SMC), appears at the archenteron tip.

Step 4: Secondary invagination

- A short stub like gut rudiment is converted into a slender archenteron through a series of morphogenetic movements called **secondary invagination**.
- It has been thought that contraction of the **filopodia** connecting the archenteron tip and the apical plate pulls the gut rudiment upward.
- In general, the height (length along the animal–vegetal axis) and width of embryos become larger during gastrulation due to the expansion of the blastocoele wall.
- A part of the ectoderm layer to which SMC filopodia adhere is flattened or depressed.
- Further, elongation of the archenteron is blocked when the pseudopodia are broken by expanding the blastocoel, the length of the archenteron reaches two thirds to three quarters of the full length of the archenteron formed in normal embryos.




- Ettensohn (1985) found that a dozen cells are initially observed on a cross section of the gut rudiment, but the number decreases to 7–8 after secondary invagination completes laser beam.
- These observations and experiments clearly indicate that the pseudopodia exert contractile force for archenteron elongation.
- This explicitly indicates that the archenteron cells are rearranged during this morphogenetic process.
- Together with the force exerted by SMC filopodia, cell rearrangement leads to the formation of a slender archenteron.



GASTRULATION IN BIRDS

- It is characterised by movement and rearrangement of cells in embryo.
- During gastrulation the blastoderm splits into two layers: an upper layer of cells called **epiblast** and a lower layer of cells called **hypoblast**.
- **Epiblast** is mainly presumptive ectoderm and mesoderm.
- **Hypoblast** is mainly presumptive endoderm because hypoblast cells grow outward over the surface of yolk, then downward around it to form the endodermal lining of a yolk cell.

- 
- At this stage, the central cells of blastoderm can be separated from the yolk, under these central cells a pool of fluid develops rising them of the yolk and giving the area an translucent appearance (**area pellucidae**)
 - the peripheral part of blastoderm where the cells lie unseparated from the yolk is termed as **AREA OPACA**, the white area that transmits light.
 - The upper layer of the blastoderm consists of the presumptive mesoderm and ectoderm.

1. Formation of Primitive Streak, Primitive Groove, Primitive Ridges and Hensen's Node


- In chick, the mesodermal cells migrate medially and caudally to form a mid line thickening called **primitive streak**.
- Presumptive mesodermal cells continue migrating and the length of primitive streak grows and finally, the shape of blastoderm changes from circular to pear.
- The primitive streak elongates almost half of the length of ectoderm.

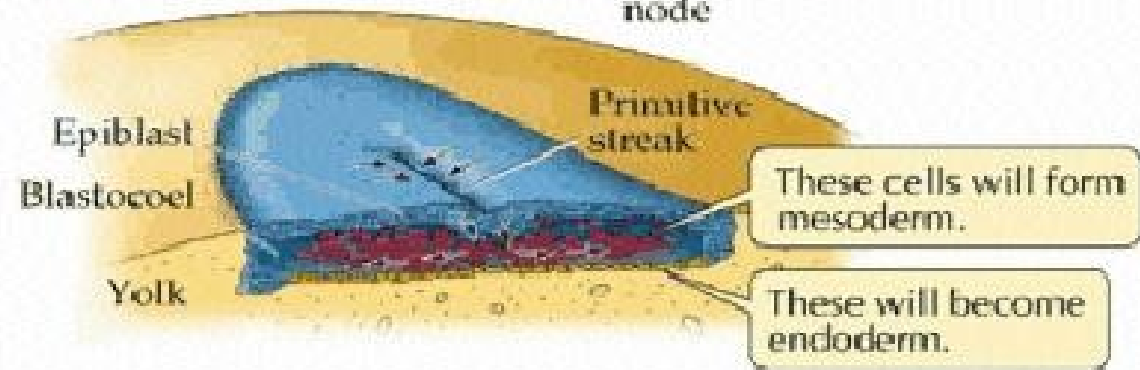
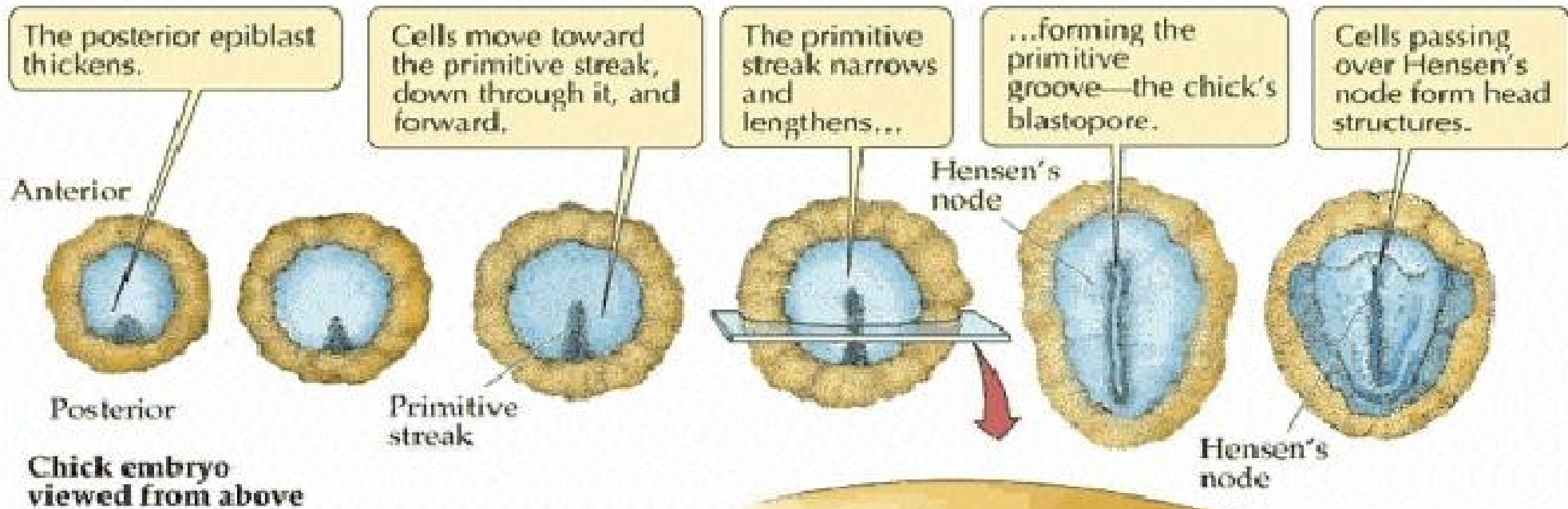


- The anterior end of the primitive streak is occupied by an aggregation, the **primitive node or notochordal** cells while the rest of the cells are **mesodermal cells**.
- The cells continue to migrate between epiblast and hypoblast and form mountain like ridges called **primitive ridges** and the groove between those ridges is called **primitive groove**.
- Primitive ridges and primitive groove are formed from primitive streak.
- Primitive node is called **Hensen's node**, in birds, which is a thickening at the top end of primitive streak.

2. Formation of Notochord, Primitive Gut and Somites

- After this, the cells start pushing in from the region of Hensen's node and form a rod like structure, beneath the ectoderm, called **notochord**.
- Notochord is a prominent feature in a chick embryo of 18 hours.
- The ectoderm becomes a coherent layer of cells merging with yolk and marginal area and forms **germ wall**, where the expanding germ layers merge with underlying yolk.
- The cavity between the yolk and the endoderm, which was previously called **gastrocoele** is now termed as **primitive gut**.

- 
- Hensen's node form the dorsal mesoderm which form the **somites**.
 - The groove between them is called **neural groove**.
 - Remember that primitive groove and ridges were in primitive streak (in almost one half) while neural groove and somites are formed above the notochord (in almost other half).
 - Mesoderm gets split in somatic mesoderm and splanchnic mesoderm and the space between them is called **coelom**.
 - Somites are seen in 25-26 hours embryo



Cross section through chick embryo

■ Gastrulation in Amphibians (Frog)

- The amphibian embryo undergoes a midblastula transition during which the cell cycle slows down (as a result of acquisition G1 and G2 phases of the cell cycle)
- cell division becomes synchronous, the cells gain the ability to move from their original positions
- the transcription of new mRNA is seen from the nucleus for the first time in the animal's life.
- In **Xenopus**, this transition occurs immediately after **the twelfth cleavage**.



- There occur three types of morphogenetic movements in amphibian gastrulation.

1. Invagination
2. Involution
3. Epiboly

1. Invagination

- In frog embryos, gastrulation is initiated at the future dorsal side of the embryo, just below the equator in the region of the grey crescent.
- Here the marginal endodermal cells sink into the embryo thus forming a slit like **blastopore**.
- These cells now change their shape and become flask shaped. These are called as **bottle cells**.



- The bottle cells maintain the contact with the outer surface with the help of cytoplasmic strands whereas their main body is displaced towards the inside of the embryo.
- Therefore in frog, gastrulation begins in the marginal zone near the equator of the blastula.
- Furthermore, this deep layer of cells appears to be responsible for the continued migration of cells into the embryo.

2. Involution

- The next phase of gastrulation involves the involution of the marginal zone cells
- while the animal cells undergo epiboly and converge at the blastopore.
- On reaching the tip of the blastopore, the marginal cells turn inward and travel along the inner surface of the outer cells sheets.
- Thus, the cells constituting the lip of blastopore are constantly changing.



- The first cells to form **the dorsal lip** are endodermal cells that invaginated to form the leading edge of the archenteron.
- These cells later become **the pharyngeal cells of foregut**.
- As these first cells pass into the interior of the embryo, the blastopore lip becomes composed of **involuting cells** that are **precursors** of the head mesoderm.
- The next cells involuting over the dorsal lip of the blastopore are called **the chorda** mesoderm cells.
- These cells will form the **Notochord**, a transient mesodermal “**back bone**” that is essential for initiating the differentiation of **nervous system**.

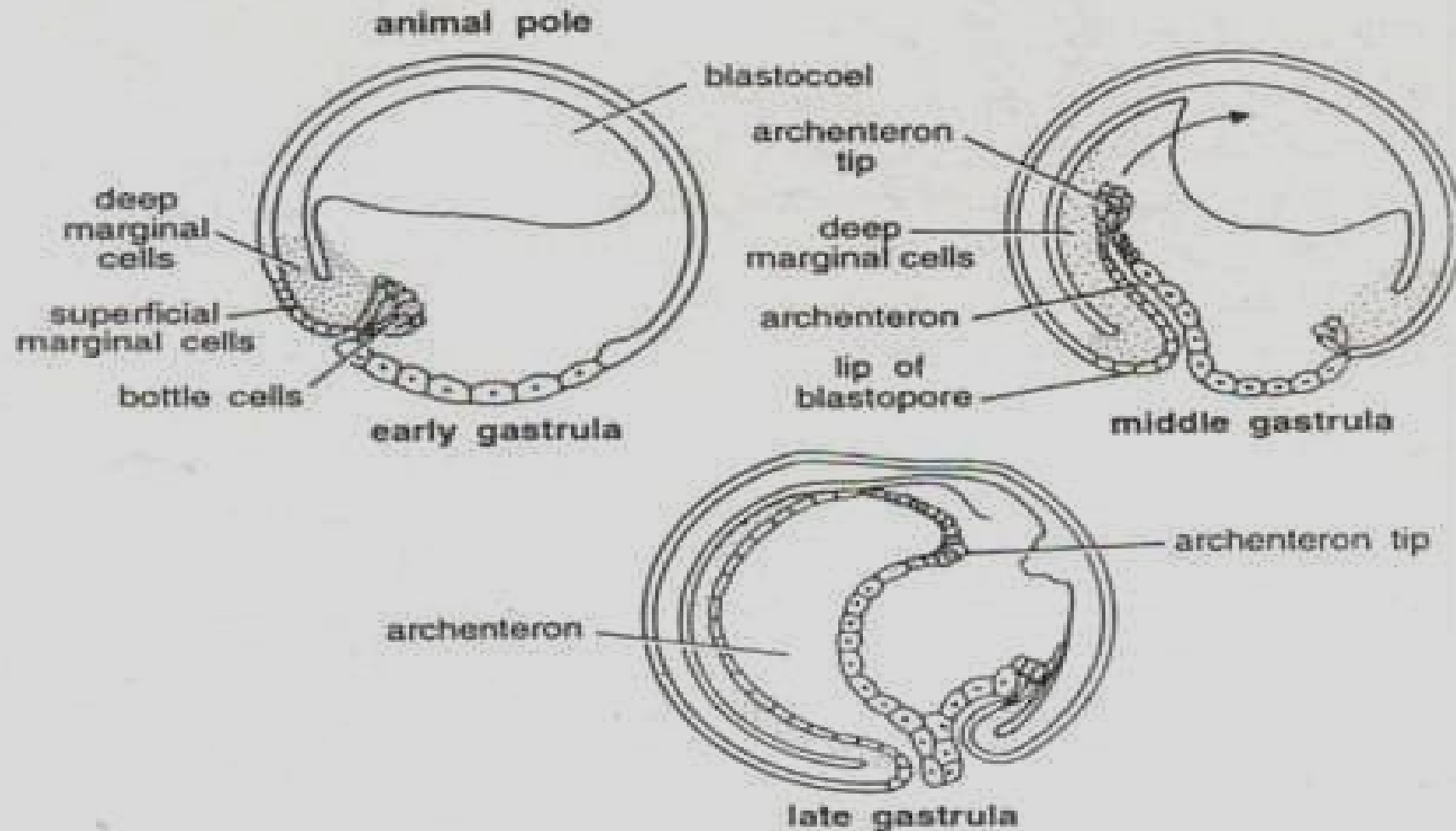


Fig. 12 Invagination of endodermal cells in an amphibian egg. The bottle cells originate from superficial marginal cells and become the archenteron tip. The involuting cells that form the mesoderm are derived from the deep marginal cells

3. Epiboly

- As the new cells enter the embryo, the blastocoel is displaced to the side opposite the dorsal blastoporal lip.
- Meanwhile, the blastopore is displaced vegetal and widens as more animal hemisphere cells **converge** at the blastopore lip.
- The widening blastopore develops **lateral lips** and finally a **ventral lip** over which the additional mesodermal and endodermal precursor cells pass.
- With the formation of the **ventral lip**, the blastopore has formed a **ring** around the large endodermal cells that remain exposed on the surface.



- The remaining patch of the endoderm is called the **yolk plug**, and it too, is eventually internalized .
- At this point, all the endodermal precursors have been brought into the interior of the embryo, the **ectoderm has encircled the surface** and the **mesoderm has been brought between them**.

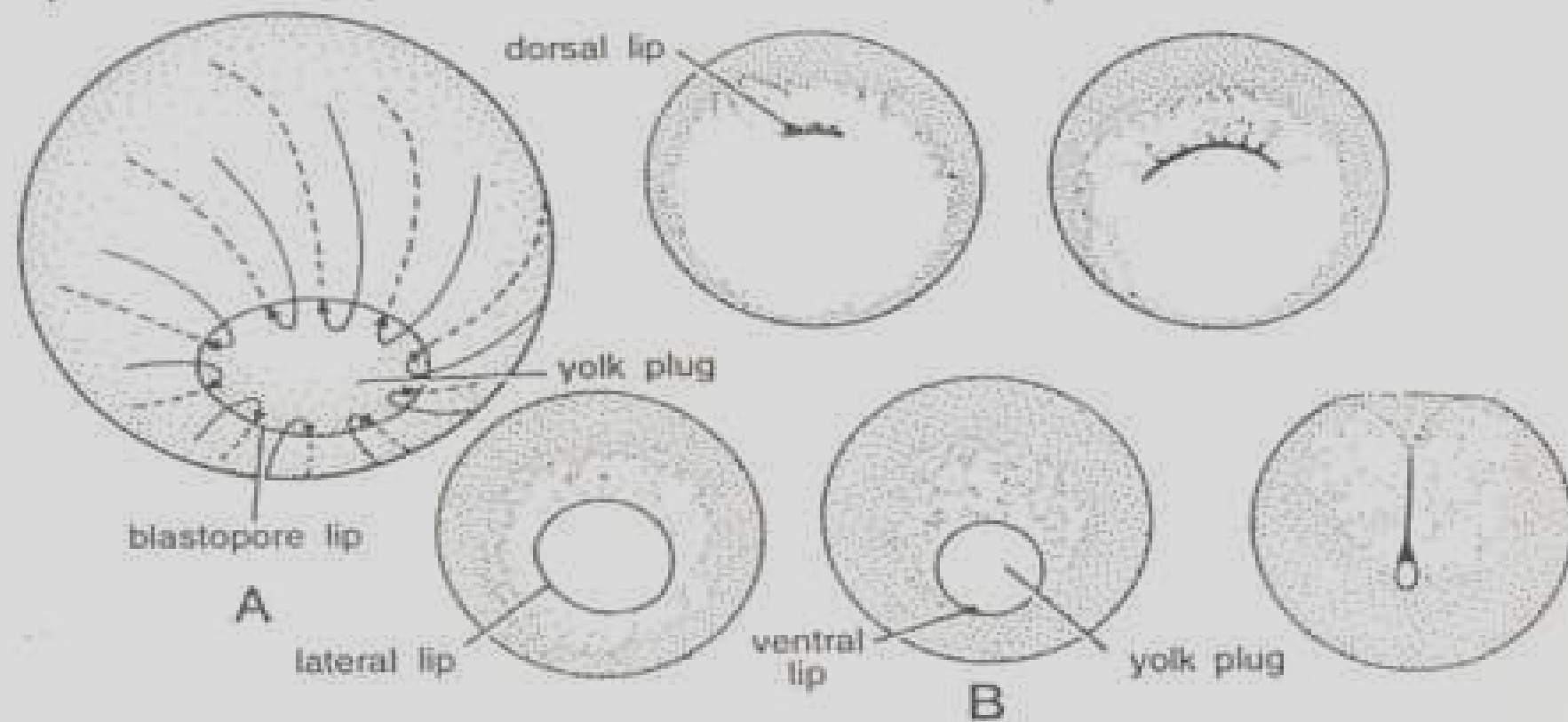


Fig. 14. Epiboly of the ectoderm (A) morphogenetic movements of the cells migrating into the blastopore and then under the surface, (B) changes in the region around the blastopore, as the dorsal, lateral and ventral lips are formed in succession when the ventral lip completes the circle, the endoderm becomes progressively internalized (After Gilbert, 1988).



CELLULAR BASIS OF MORPHOGENESIS



Two types of morphogenesis:

- **Cellular basis**
- **Molecular basis**

Cellular basis of morphogenesis:

- cell sorting
- Differential Adhesion Hypothesis
- Epithelial-mesenchymal transition
- Cell-cell adhesion
- Cell Adhesion Molecules (CAMs)
- Extracellular matrix
- Cell contractility

Cell sorting

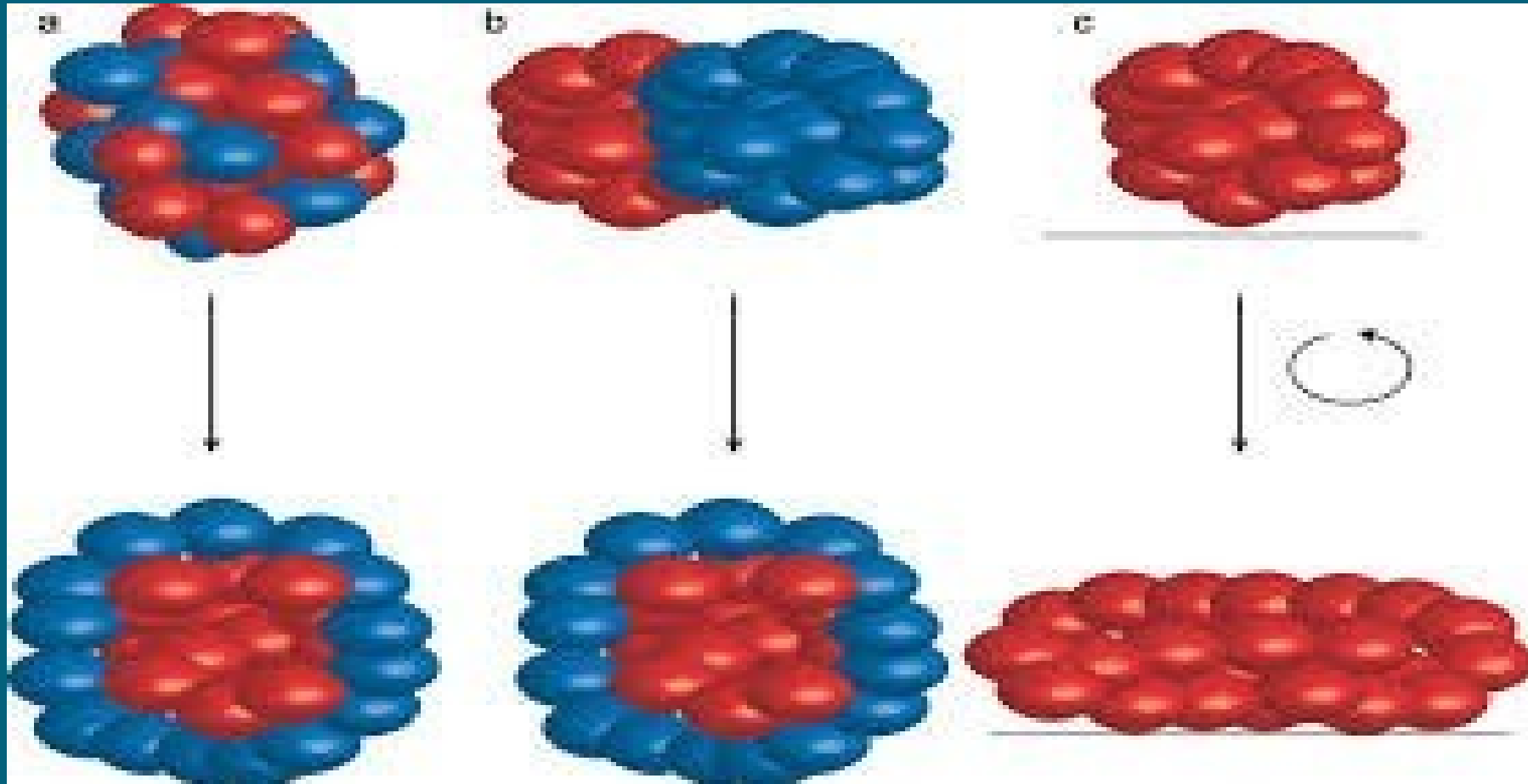
- “The changes in tissues cause the elongation, thinning, folding or separation of one tissue into distinct layers. This is often referred as cell sorting. (Cell sorting is the ability to separate cells according to their properties.)”

Morphogenesis arises because of changes in the cellular structure or how cells interact in tissues cell sorting.

Cell "sorting out" consists of cells moving so as to sort into clusters that maximize contact between cells of the same type.



Cell sorting



Differential Adhesion Hypothesis

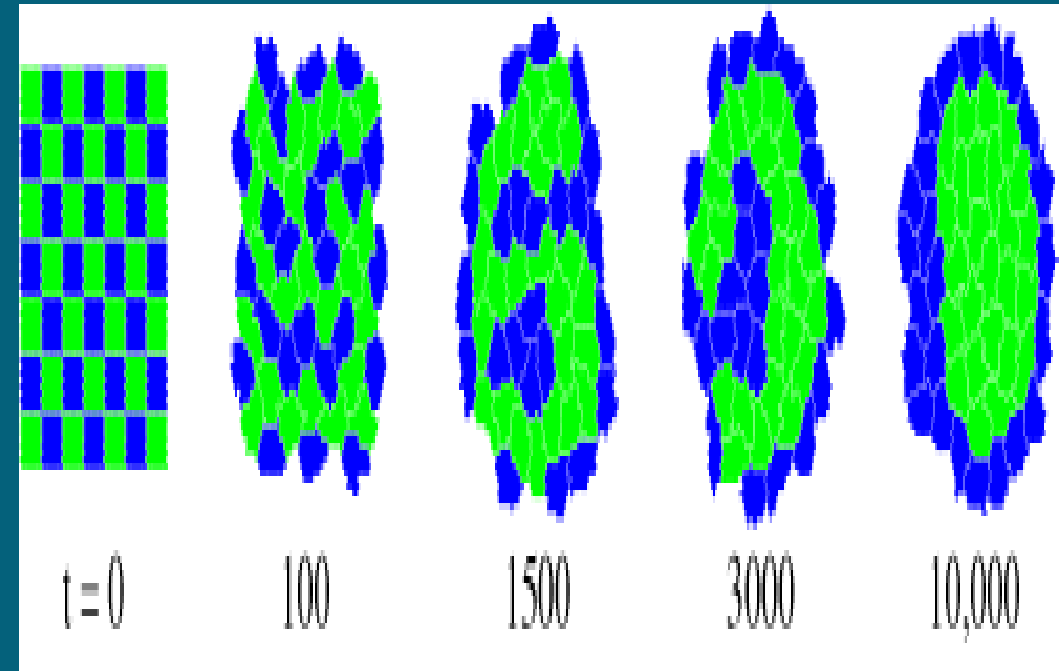
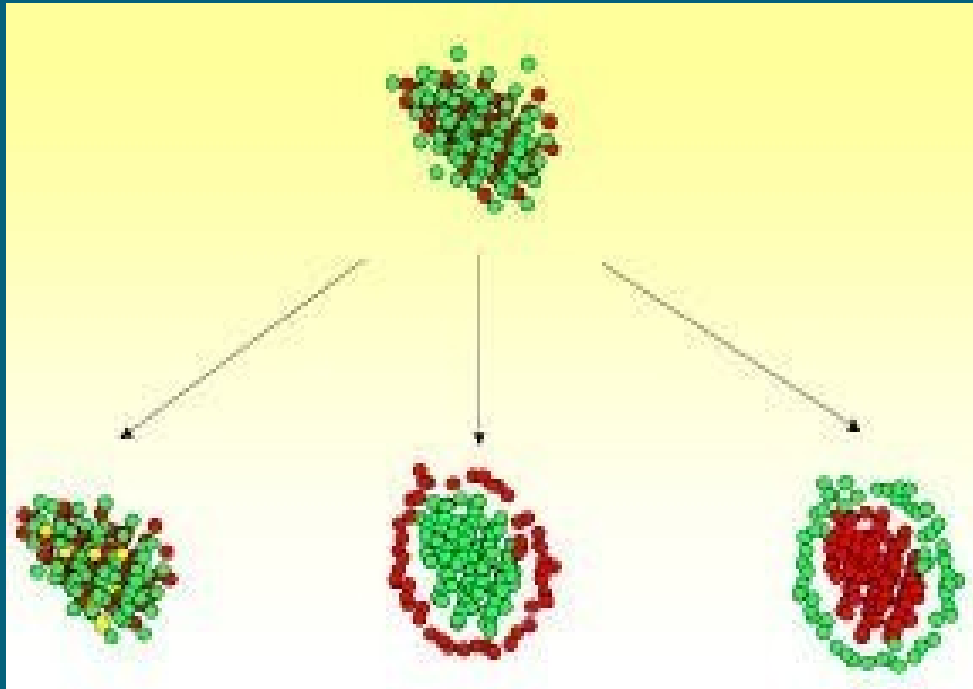
Explained by:

The ability of cells to do this has been proposed to arise from differential cell adhesion by [Malcolm Steinberg](#) through his Differential Adhesion Hypothesis.

Definition:

“According to DAH, cells move to be near other cells of similar adhesive strength in order to maximize the bonding strength between cells and produce a more thermodynamically stable structure.”

Differential Adhesion Hypothesis

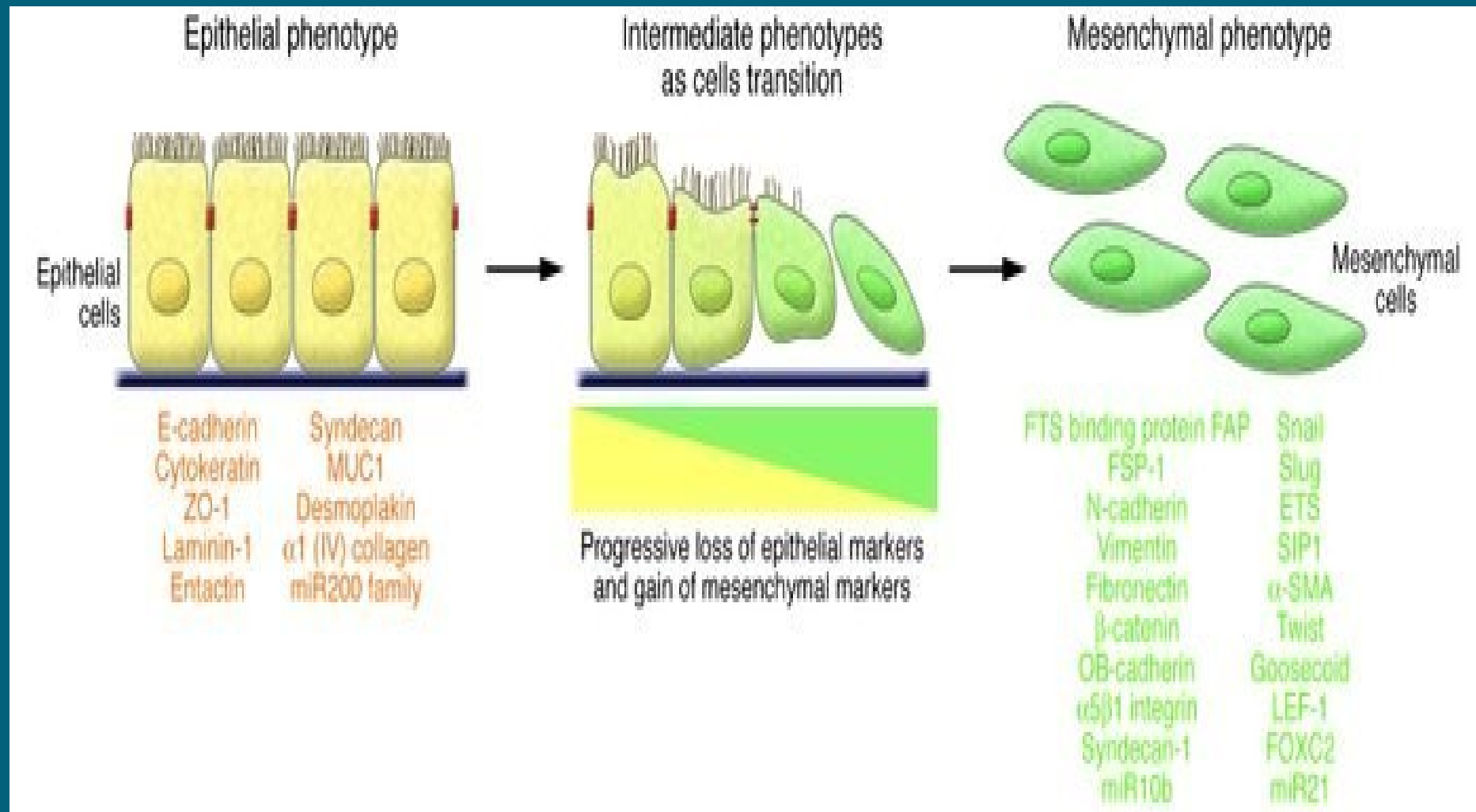


Epithelial-mesenchymal transition

“The **epithelial-mesenchymal transition (EMT)** is a process by which epithelial cells lose their **cell polarity** and **cell-cell adhesion**, and gain migratory and invasive properties to become mesenchymal stem cells”

Mesenchymal cells typically leave the epithelial tissue as a consequence of changes in cell adhesive and contractile properties.

Following **epithelial-mesenchymal** transition, cells can migrate away from an epithelium and then associate with other similar cells in a new location.

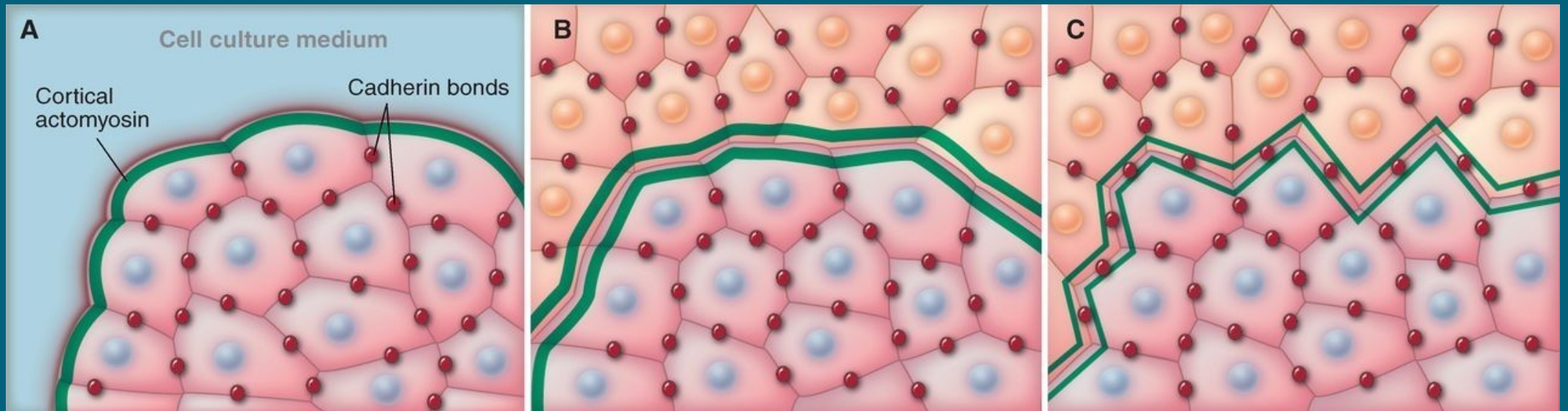
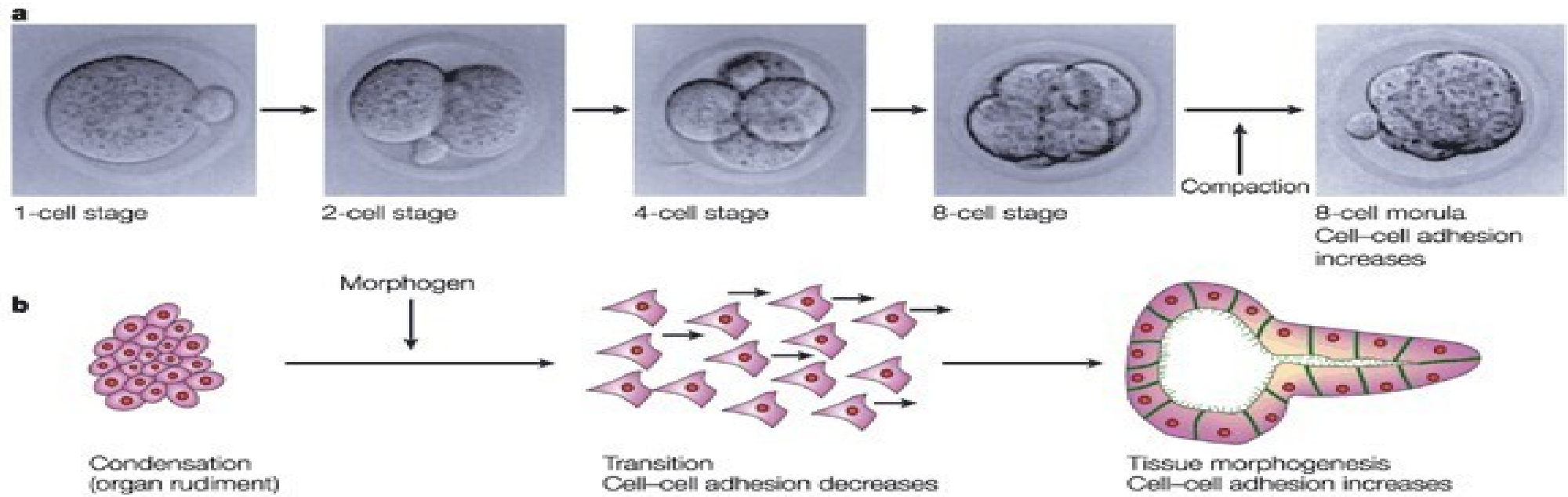


Cell-cell adhesion

During embryonic development, cells are restricted to different layers due to differential affinities. One of the ways this can occur is when cells share the same cell- to-cell adhesion molecules.

- **For instance, homotypic cell adhesion can maintain boundaries between groups of cells that have different adhesion molecules.**

Furthermore, cells can sort based upon differences in adhesion between the cells, so even two populations of cells with different levels of the same adhesion molecule can sort out.



Cell Adhesion Molecules (CAMs)

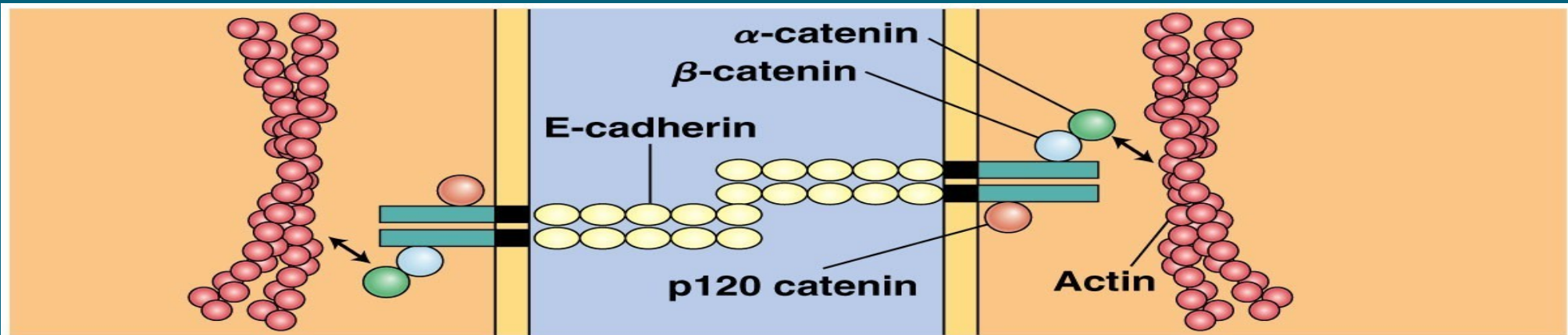
The molecules responsible for adhesion are called **cell adhesion molecules (CAMs)**.

Several types of cell adhesion molecules are known and one major class of these molecules are **cadherins**.

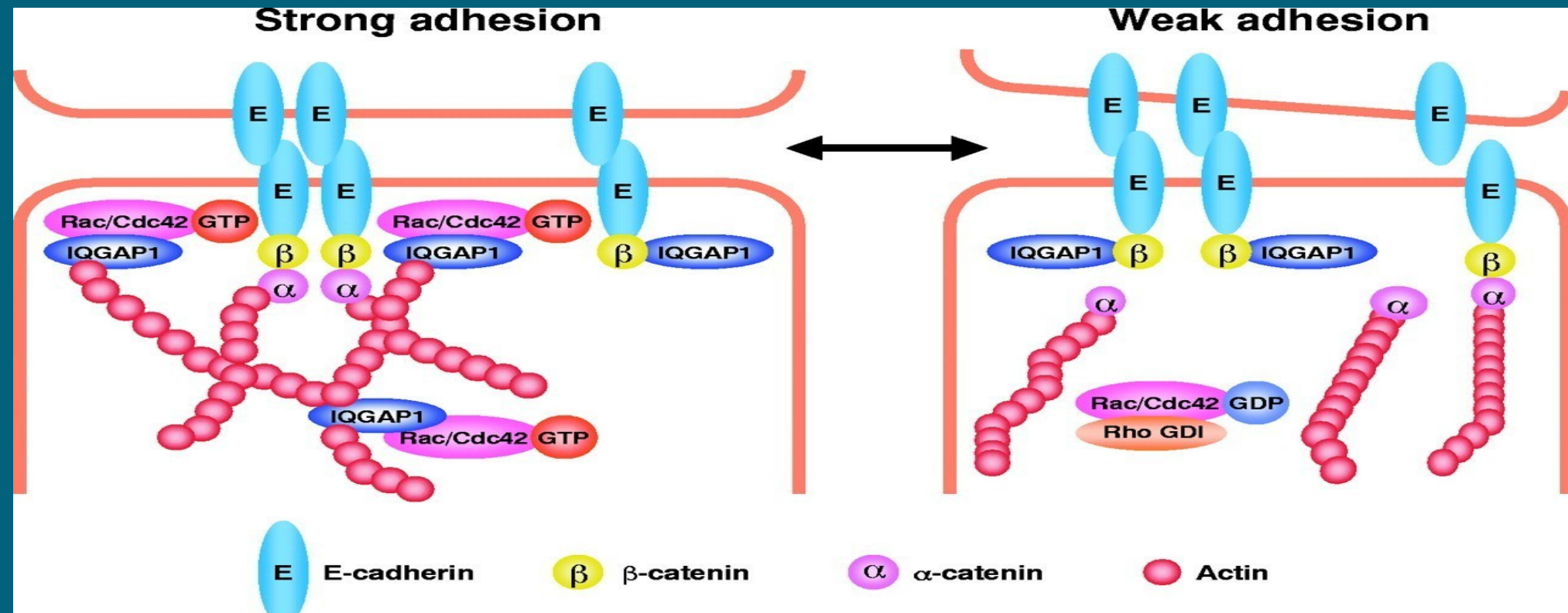
There are dozens of different cadherins that are expressed on different cell types. Cadherins bind to other cadherins in a like-to-like manner:

E-cadherin (found on many epithelial cells) binds preferentially to other E-cadherin molecules.

Mesenchymal cells usually express other cadherin types such as **N-cadherin**.



(a) Adherens junction



Role Of Extracellular Matrix

The extracellular matrix (ECM) is a collection of extracellular molecules secreted by cells that provides structural and biochemical support to the surrounding cells.

The extracellular matrix (ECM) is involved in:

- ⊕ keeping tissues separated
- ⊕ providing structural support
- ⊕ providing a structure for cells to migrate on.

■ ECM molecules

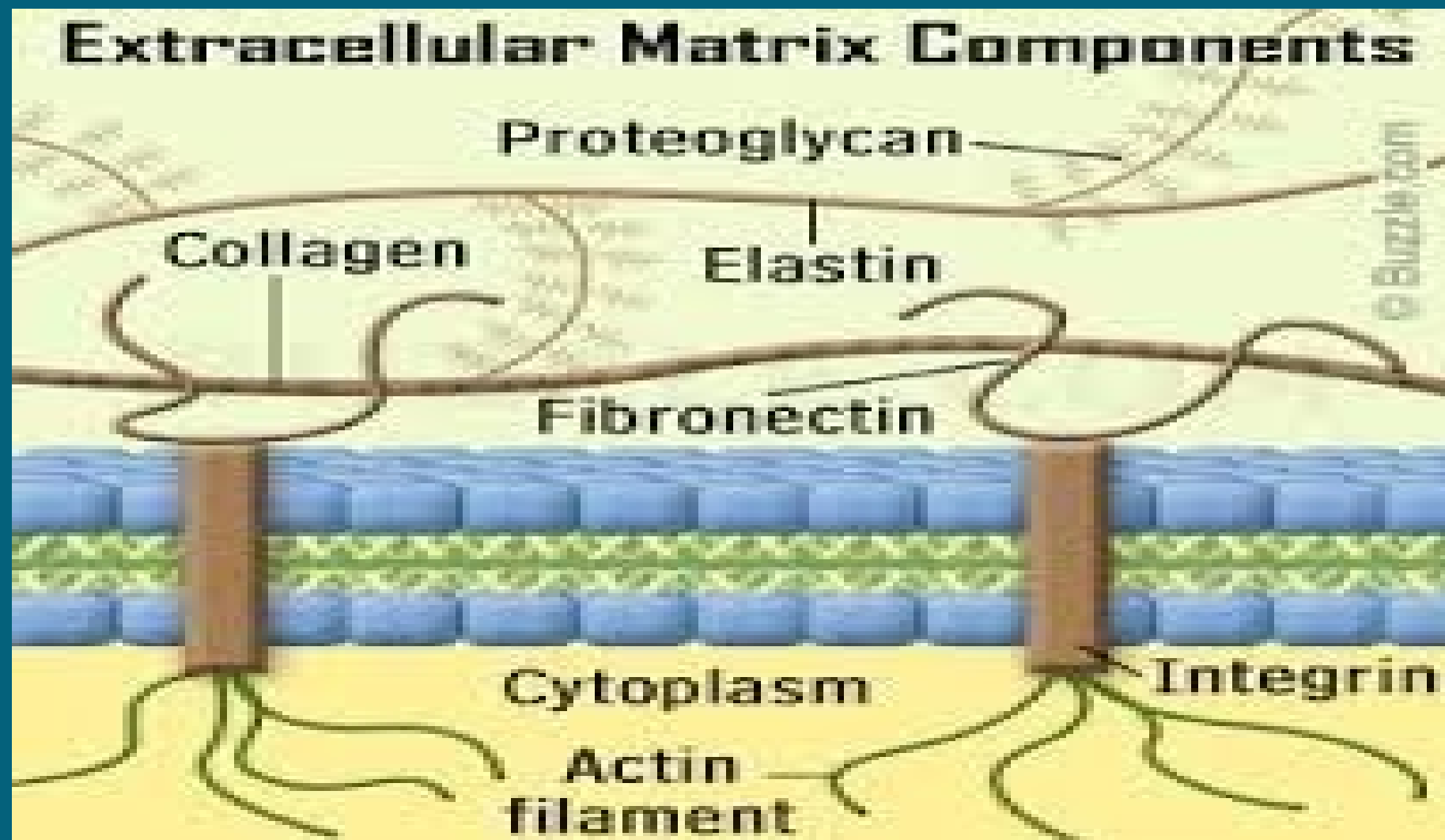
Collagen Laminin and fibronectin are major ECM molecules that are secreted and assembled into sheets, fibers, and gels.

Multisubunit transmembrane receptors called **integrins** are used to bind to the ECM.

Example:

A well-studied example of morphogenesis that involves ECM is mammary gland ductal branching.

Extracellular Matrix Components

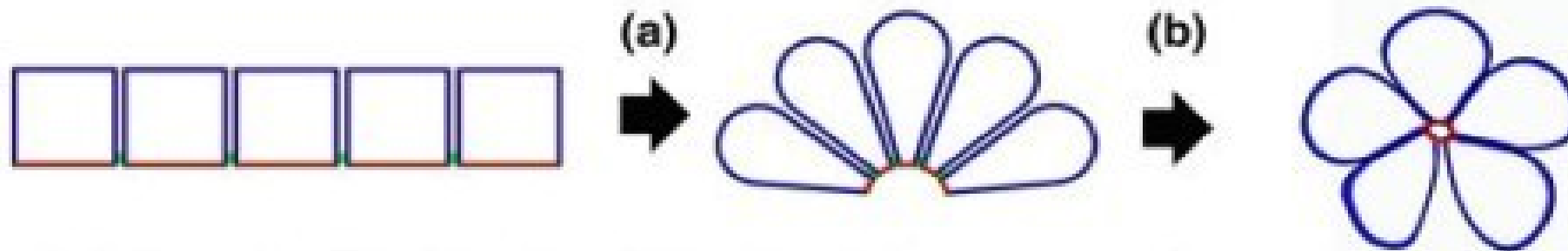


Cell contractility

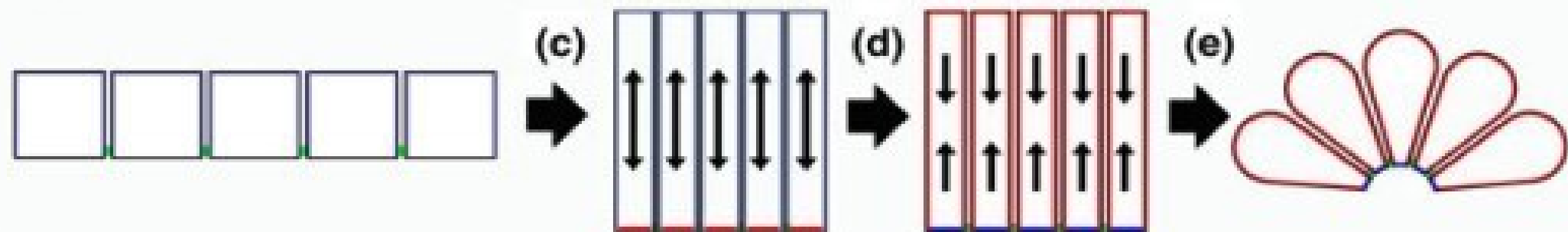
- Tissues can change their shape and separate into distinct layers via cell contractility.
 - Just like in muscle cells, myosin can contract different parts of the tissue to change its shape or structure.
 - Example:
 - Typical examples of **myosin-driven** contractility in tissue morphogenesis occur during the separation
 - of **drosophila and zebrafish germ layers**. Often, during embryonic morphogenesis, cell contractility occurs via periodic pulses of contraction.

Cell contractility

Apical contraction with high basolateral resistance to elongation



Apical contraction, low basolateral resistance, secondary apicobasal shortening.



■ Morphogenesis in mouse for lungs development

⊖ From foregut:

Like most internal organs the lungs develop from the foregut (in the early embryo a ventral longitudinal tube).

⊖ Formation of buds:

Initially, two buds extend from the foregut resulting in the left and right bronchus. In the mouse four secondary buds extend from the two initial branches (three on the right-hand side and one on the left) giving rise to four lung lobes.

⊖ Genetic control:

The genetic control of these processes is not completely understood but it has been shown that *Gli* genes are involved in the induction of the secondary buds. It is surprising that also FGF (fibroblast growth factor) is crucially involved in mammalian lung formation. Mammals possess more than one *FGF* Fibroblast growth factor gene. Currently there are more than 20 different FGFs known and many of them have overlapping functions.

- However, in mice, lacking FGF10 are born without lungs and limbs (limbs are also formed from buds!).

- **Process:**

Epithelial cells, expressing *FGF receptor*, respond to the secretion of FGF from nearby mesenchyme by bud formation and bud extension towards the **FGF10** source.

Exposure of the branch tip to high concentrations of FGF induces the expression of secondary genes in the tip such as:
bone morphogenetic protein 4 (*BMP4*), sonic
hedgehog (*Shh*) and
a mammalian sprouty ortholog (*Sprouty 2*)

- thus, turning the tips of the bronchial branches into signaling centers.
- BMP4 inhibits epithelial cell proliferation limiting branch extension.
- Shh is proposed to inhibit *FGF10* expression in the mesenchyme near the tip, which splits *FGF10* expression promoting the next round of branching and
- Sprouty2 restricts branching to the tip of the branch.

Fig. 2

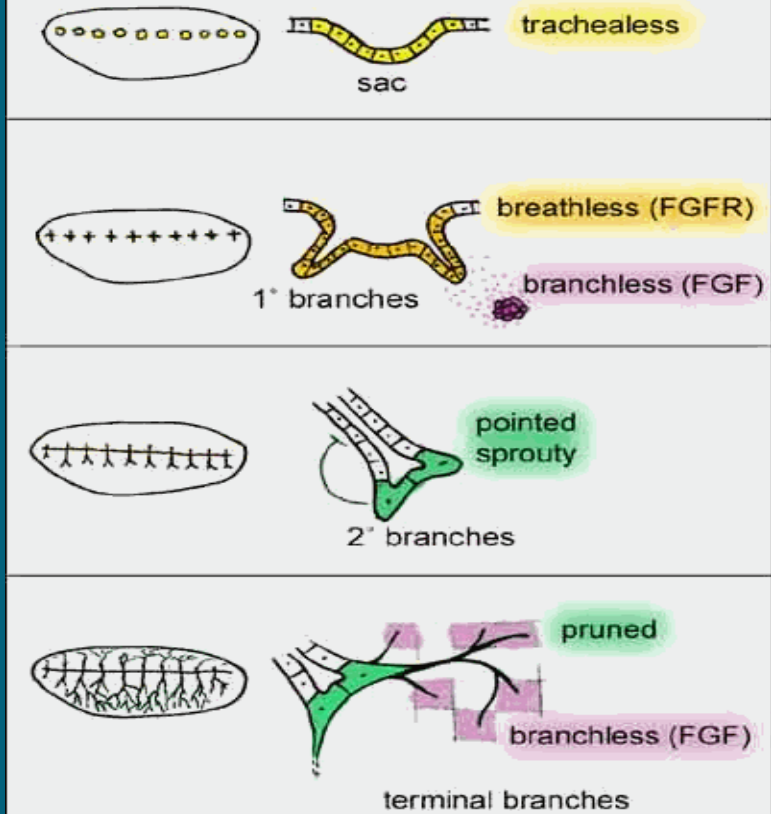
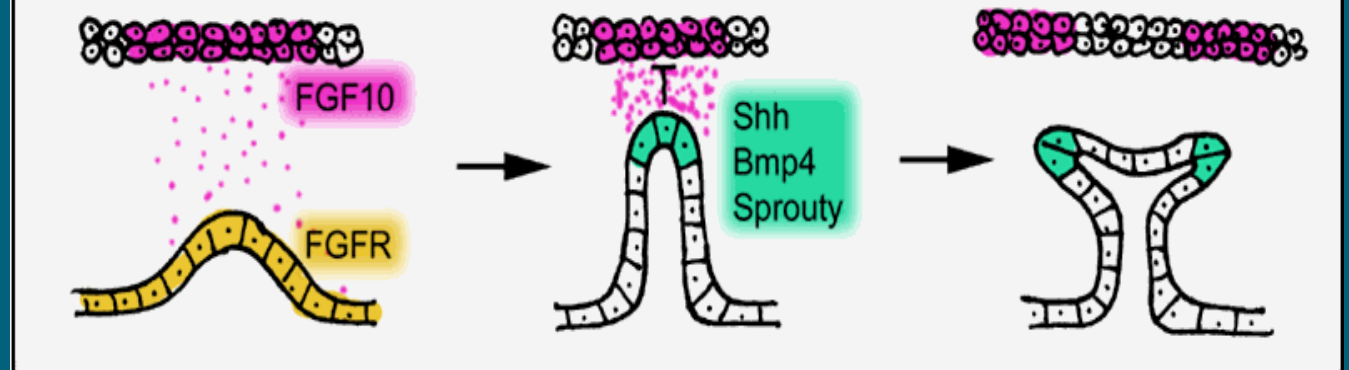


Fig. 3



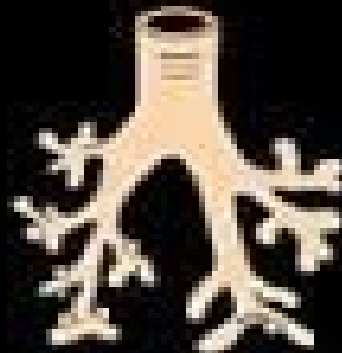
Lung Development Phases: Human & Mouse



I. Embryonic
Mouse: E 9–12
Human: Wk 3–7



II. Pseudoglandular
E 12–15
Wk 5–17



III. Canalicular
E 15–17
Wk 16–26

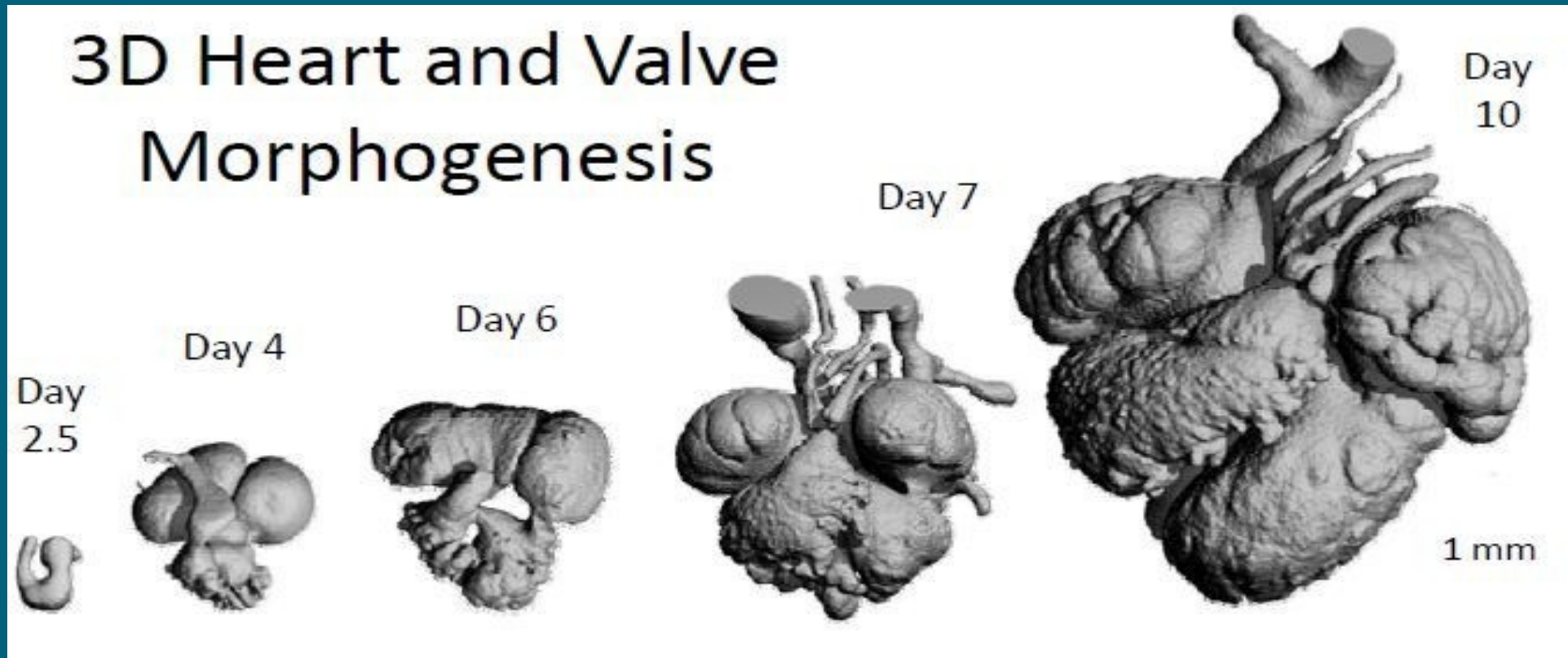


IV. Saccular
E 17–Birth
Wk 26–36



V. Alveolar
Birth–PN20
Wk 36–3 years

■ In human





NAME: -----ROLL No.-----

Department of Zoology
The Islamia University of Bahawalpur

Exam	Title Paper	Class/Subject	Time Allowed	Max. Marks
Mid-Term Spring 2020	Developmental Biology	BS Zoology 7th Semester	12 Minutes	6

Q # 1 (a). Mark each statement as True or False. Write true statement if false. (0.5x6=3 Marks)

1. Chemotaxis has been demonstrated in amphibians.

2. After 20 minutes of fertilization, the rate of protein synthesis increases three to twelve folds.

- 3.

4. .

Q # 1 (c): Choose the right word for each statement.

(0.5x6=3 Marks)

1. The ----- makes the egg impenetrable to more than one sperm.

(A)	Vitelline membrane	(B)	Follicle cells of corona radiata	(C)	Proteolytic enzymes	(D)	None of these
-----	--------------------	-----	----------------------------------	-----	---------------------	-----	---------------

2. ----- facilitate sperm entry in Sea Urchins.

(A)	Cytoplasmic bridge	(B)	Microvilli	(C)	Polymerization of actin	(D)	None of these
-----	--------------------	-----	------------	-----	-------------------------	-----	---------------

3. Meroblastic cleavage occurs in -----

(A)	Birds	(B)	Reptiles	(C)	Egg-laying mammals	(D)	All of these
-----	-------	-----	----------	-----	--------------------	-----	--------------

4. Tick the statement/statements which is/are WRONG.

(A)	Superficial cleavage occurs in Telelecithal eggs.
(B)	Many proteins required for cleavage are synthesized in the presence of Puromycin.
(C)	blastocoel is filled with a fluid containing lipopolysaccharides.
(D)	The attachment between the acrosomal process and the vitelline envelope is not a species specific process

Exam	Title Paper	Class/Subject	Time Allowed	Max. Marks
Mid-Term Spring 2020	Dev. Biology	BS Zoology 7 th Semester	50 Minutes	6

Q. 2: Answer the following short questions.

(0.5x10=5 Marks)

1. Which two processes are implicated to regulate spermatogenesis?
2. What is the temperature required for spermatogenesis? What is the effect of athletic support strap on sperms?
3. Differentiate between Spermatogenesis and Spermiogenesis?
4. What is Gastrulation? Why it is important?
5. Define Holoblastic Cleavage.
6. .
7. .
8. .
9. .
10. .

Q. 3: Write a note on Slow and Permanent block to polyspermy?

1