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— রবীন্দ্রনাথ ঠাকুর

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— সুভাষচন্দ্র বসু

Any system of education which ignores Indian conditions, requirements, history and sociology is too unscientific to commend itself to any rational support.

— Subhas Chandra Bose

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Published by : Netaji Subhas Open University, DD-26, Sector-1, Salt Lake City, Kolkata-700 064 and
Printed at : Royal Hlaftone Co., 209/C, Bidhan Saranee, Kolkata-700 006

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NETAJI SUBHAS OPEN UNIVERSITY

Choice Based Credit System (CBCS)

SELF LEARNING MATERIAL

HZO

ZOOLOGY

Developmental Biology

CC-ZO-10

Under Graduate Degree Programme

PREFACE

In a bid to standardise higher education in the country, the University Grants Commission (UGC) has introduced Choice Based Credit System (CBCS) based on five types of courses viz. *core*, *discipline specific*, *generic elective*, *ability* and *skill enhancement* for graduate students of all programmes at Honours level. This brings in the semester pattern, which finds efficacy in sync with credit system, credit transfer, comprehensive continuous assessments and a graded pattern of evaluation. The objective is to offer learners ample flexibility to choose from a wide gamut of courses, as also to provide them lateral mobility between various educational institutions in the country where they can carry acquired credits. I am happy to note that the University has been accredited by NAAC with grade 'A'.

UGC (Open and Distance Learning Programmes and Online Learning Programmes) Regulations, 2020 have mandated compliance with CBCS for U.G. programmes for all the HEIs in this mode. Welcoming this paradigm shift in higher education, Netaji Subhas Open University (NSOU) has resolved to adopt CBCS from the academic session 2021-22 at the Under Graduate Degree Programme level. The present syllabus, framed in the spirit of syllabi recommended by UGC, lays due stress on all aspects envisaged in the curricular framework of the apex body on higher education. It will be imparted to learners over the *six* semesters of the Programme.

Self Learning Materials (SLMs) are the mainstay of Student Support Services (SSS) of an Open University. From a logistic point of view, NSOU has embarked upon CBCS presently with SLMs in English / Bengali. Eventually, the English version SLMs will be translated into Bengali too, for the benefit of learners. As always, all of our teaching faculties contributed in this process. In addition to this we have also requisitioned the services of best academics in each domain in preparation of the new SLMs. I am sure they will be of commendable academic support. We look forward to proactive feedback from all stakeholders who will participate in the teaching-learning based on these study materials. It has been a very challenging task well executed, and I congratulate all concerned in the preparation of these SLMs.

I wish the venture a grand success.

Professor (Dr.) Subha Sankar Sarkar
Vice-Chancellor

Netaji Subhas Open University

Undergraduate Degree Programme

Choice Based Credit System (CBCS)

Subject : Honours in Zoology (HZO)

Course : Developmental Biology

Course Code : CC - ZO - 10

First Print : August, 2022

Printed in accordance with the regulations of the
Distance Education Bureau of the University Grants Commission.

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UG : Zoology
(HZO)

Course : Developmental Biology
Course Code : CC - ZO - 10

Unit - 1 □ Introduction To Development Biology

Structure

1.1 Objective

From this unit you will learn—

- the mechanism of development in multicallular organism
- how the cells behave during the developmental process
- difference between growth and development
- why there are asymmetric cell division
- how the genes are expressed during development
- role of cytoplasmic determinants during developmental process

1.2 Introduction

Life and death have been fascinating subjects and have remained mysteries from time immemorial. Tragmentary knowledge was available during different stages of development of animals and birth of a baby from very ancient times.

Aristotle (384-322 B.C), a Greek philosopher studied embryos of animals and many of his observations were accurate. Anaximander (600 B.C) was perhaps the first to conceive the idea that living creatures arose from moist element and man was like fish in the beginning.

The Greek philosopher Empedocles was aware of the fact that foetus arose partly from male and partly from females. He also had knowledge regarding the different stages of development and that heart formed first and nails later.

The credit of establishing Embryology as a separate and independent branch of Biology goes to Aristotle. He believed that the soul or form of the embryo was derived from the father and mother supplies the matter or soil in which the embryo grows. Aristotle's views were acceptable through most of the medieval period, and his views appeared in the writings of Fabricius (1537-1619) and Harvey (1578-1657).

1.2 Objective

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- role of cytoplasmic determinants during developmental process

1.3 Basic concepts

These scientists stated that the soul or the determining cause for development existed in the blood of animals. With the invention of the microscope by Robert Hooke in 1665 the sperm and ovum were discovered but their importance in development was not established.

1. **Theory of epigenesis:** The epigenetic concept was proposed by C.F. Wolff (1759). According to this theory, there is no preformation of the individual in the sperm or ovum but the embryo develops by progressive growth and differentiation. The material from which the embryo formed already existed in the ovum and during development: this material rearranges itself into layers

which fold in various ways to form the embryo. This process of progressive development of the embryo from simple to more complex form is called as epigenesis.

2. **Baer's law:** Karl Ernst Von Baer (1792-1876) considered to be father of modem embryology, was the first to present embryological data in a coherent form. In 1828 he proposed his famous law known as Baer's las based on the cutensive research work on developing embryos.

   According to this law during the development of an animal from ovum, the more eneral features of the group to which it belongs develop tirse and the special features of the species appear later. For example, during development of chrich, the generalized chordate characters such as notochord, dorsal tubular nerve cord and gill slits develop on the tirst and second days followed by the specialized characters of birds, like feathers, claws and beak on the titth day.

3. **Biogenetic law:** Fritz Muller, a German scientist (180+) atter studying the process of evolution of animals stated that during the development of animals, the characteristic features of ancestral animals appear in earlier stages than the characters of more recent origin. In other words, during the ontogenic development of an animal, the entire racial history or phylogenetic relationship is repeated- ontogeny repeats phylogeny:

   During the development of chick, for example, the characteristic features of fish such as gill slits appear before the appearance of lungs and claws which are characters of amphibia and reptiles. Feathers and beak characteristic features of the birds appear in the last. Thus, developmental stages of the chick encompass all the evolutionary stages in the history of birds. Ernst Haeckel (1866) another noted German biologist coined the term ***Biogenetic theory or Recapitulation theory***.

   According to the recapitulation theory, chick during its development, should first resemble a fish, later an amphibian followed by a reptile. However, this does not occur. The chick embryo resembles a fish embryo, an amphibian embryo and a reptilian embryo in the last. Therefore, the recapitulation theory in its modern modified form can be stated as "ontogeny of an animal repeats the fundamental steps of the ontogeny of ancestral forms".

   Until the time of Wilhelm Roux (1888), embryology remained a descriptive subject, based on observations only. Wilhelm Roux, a German scientist, performed experiments on frog's eggs and introduced the modem era of experimental embryology.

4. **Germplasm theory of Weismann:** The germplasm theory proposed by A. Weismann in 1883 can be considered as a major landmark in developmental biology because he was the first to distinguish germplasm as a separate entity from somatoplasm. According to him, the germplasm is a self-perpetuating cellular entity which continues its existence through ages.

   The child inherits characters of the parents through the sex cells of the parents and not from other parts of the body. The germ cells acquire the characters from preexisting germ cells. In each new generation, a temporary soma or body is built around the germplasm that descended from the parents.

   According to Weismann, the embryo remains already organized in the chromosomes of the nucleus and during early development these characters are unpacked in an orderly manner. He recognized ***units of heredity as "determinants"*** and these are segregated during the early cell divisions of the embryo making these divisions differential.

   In the first cell division, the determinants are distributed into right and left sides of the future embryo and later divisions into anterior and posterior parts and so on. Later, interactions between them enable the possibility of epigenetic development.

5. **Mosaic theory of Roux:** In 1888, Wilhelm Roux performed an experiment on frog's egg. He killed the nucleus of frog's blastula at two-celled stage, by touching one of the two blastomeres with a red hot needle. He later observed that the embryo developed with one half partly defective. Thus it was concluded that certain areas of the egg were predetermined to develop into specific parts of the body.

   The animal pole is predetermined to develop into anterior side of the embryo and the vegetal pole into the posterior part. The grey crescent develops into the blastopore. During cleavages, these predestined regions are segregated into the blastomeres that develop into specific tissues and organs. The blastula thus is a mosaic of blastomeres with potencies to develop into different parts of the body, and as development proceeds, these are differentiated and segregated into specific cells.

6. **Regulative theory of Driesh:** The regulative theory is in contradiction to the mosaic theory. Hans Driesh (1891), a German scientist, observed that the blastomeres of sea urchin at twocelled stage, when separated from each other, grow to develop into two young embryos of comparatively smaller

size. At four cell stage, separation of the blastomeres led to the formation of four distinct embryos of even still smaller size. If intact, all the four blastomeres formed only one embryo.

However, this pattern continued only for a few cleavages, later cleavages could not produce separate fully formed embryos upon isolation of the blastomeres. Therefore, Driesh concluded that the early cleavages of the egg were all equal. The homogenous material of the ovum was quantitatively equally distributed into the blastomeres. In carly cleavages all the blastomeres have equal potencies to develop into a whole embryo

However, if not separated, their fate in the whole is determined by the position occupied by them in the whole blastula This type of development is known as regulative development. Each cleaving cgg, according to Duesh. was a harmonious and equipotent entity Each blastomere has potentially the properties of the whole. At present, it is known that every egg is both regulative and mosaic in development. The extent of regulation is dependent upon the mRNA content of the cytoplasm

7. **Gradient theory of Child:** Boveri (1901) noted that the vegetal pole region of sea urchin egg controlled the activities of the regions situated towards animal pole. In fact, the vegetal pole region had a dominant effect over the animal pole areas. No reason was given by him. Child (1940) proposed the metabolic axial gradient theory.

   According to this theory, the single physiological gradient namely metabolic axial gradient controlled the process of morphogenesis during the development of sea urchin. He observed that there was vast difference in the rate of general oxidative metabolism in the eggs of sea urchin between the vegetal pole and animal pole. It was greater in the animal pole region and gradually decreased towards the vegetal pole in a graded fashion. Similar gradient existed in the developing eggs of starfish. The animal pole region being more active metabolically, divides at a higher frequency as compared to the less active vegetal pole region.

   At the animal pole, the power to form structures characteristic of this region are most pronounced. Similarly, at the vegetal pole, the power to form structures like archenteron. mesenchyme, etc. is greatest. As both the gradients extend along the animal-vegetal axis, they overlap each other and differ quantitatively.

The animal gradient is believed to be cortical in location while the vegetal is located more internally. Later work has shown that the gradients are due to the distribution of certain chemical substances like tryptophan and nucleotides. The animal gradient promotes protein synthesis while the vegetal gradient inhibits this process so that protein synthesis may occur at different times along the animal-vegetal axis.

8. **Organizer theory of Spemann:** Spemann performed experiments on amphibian eggs during 1901-1912 and observed that the formation of the lens of eye in grass frog embryo is"dependent upon the presence of the optic vesicle. When the optic vesicle was transplanted into the belly region, the overlying ectoderm differentiated into a lens. Removal of the presumptive eye rudiment resulted in the failure of lens formation. In 1918 Spemann transplanted the dorsal Tip of the blast pore of one frog embryo into another.

   In the new site, the dorsal lip induced the formation of a second embryo. The dorsal lip induced the tissues surrounding it to differentiate into a second embryo, thus some influence was passed on from the dorsal lip to other tissues determining the development of the ectoderm of the gastrula. It was the chorda mesoderm situated in the roof of the archenteron which had an organizing effect on the over-lying tissues.

   Transplantation of skin ectoderm over the chorda mesoderm, the neural plate area resulted in the differentiation of the skin ectoderm into neural tissue. On the other hand, if a piece of neural ectoderm is transplanted into the skin area, it lost its identity and differentiated into skin ectoderm.

   After this breakthrough, many workers subsequently reported the organizing influence of chorda mesoderm of many other animals and efforts were made to identify and isolate the chemical responsible for the inducing effect. Different workers reported different substances. Waddington and his coworkers noted that even a chemical (dye) could function as an inducer.

   For his landmark discovery of dorsal lip of amphibian blastula acting as a ***primary organizer,*** Spemann received Nobel Prize in 1935. Organizers at present are recognized as embryonic tissues that influence and organize other tissues to differentiate and produce a tissue or structure that in normal course should not have been formed. This process is known as induction and the tissue producing this effect as the inductor or organizer.

1.4 Phases of Development in Animals

Developmental biology or embryology is that branch of Biology which deals with the development of animals. So long as the developing animal is inside the egg, it is called as embryo. The development of all organisms is a slow and continuous process in which changes from simple condition lead to more complicated and organized embryo. There are several important phases in the development of animals like: Gametogenesis, Fertilization, Cleavage or segmentation, Gastrulation, Organogenesis, Growth and Differentiation.

1. **Gametogenesis:** It includes the formation of male gametes or sperms by spermatogenesis and female gametes on ova bycogenesis By spermatogenesis, motile, small. haploid spermatozoa with a head, middle piece and long tail or Magellum are produced The head consists of a large nucleus with very little cytoplasm but with a pointed anterior acrosome or formed of Golgi complex. The acrosome is useful in penetration into the ovum at the time of the fertilization. In contrast, the ova are larger in size, contain nutrient rich yolk in large volume of cytoplasm and non-motile Gametogenesis involves meiotic division where the diploid number of chromosomes is reduced to half or haploid condition,

2. **Fertilization:** The union of male and female gametes to restore the diploid number of chromosomes in the zygote is called as fertilization.

   The prerequisite for fertilization to occur is the encounter of the male and female gametes. The ultimate fate of fertilization is the transformation of the Sperm nucleus into a w pronucleus and the male and female pronuclei migrate to the site of Umphimixis to fuse so that a zygote nucleus is formed. The act of fertilization is a very important stage in sexual reproduction as the diploid number of chromosomes is restored.

3. **Cleavage or segmentation:** Repeated mitotic divisions of the fertilized egg to form more and more daughter cell, called blastomeres is known as cleavage or segmentation.

   During this process, the single called zygote is converted into a compact spherical structure called as morula. The blastomeres soon get organized themselves into a hollow, sperical structure known as blastula enclosing a central cavity or the blastocoel. The single layer of cells surrounding the blastocoel is the blastoderm. The formation of the blastula is process of

blastulation during which no fresh synthesis of the chemical substances other than DNA and proteins occurs. Cell divisions proceed at the expense of the reserve food materials like glycogen and lipids in yolk. Cleavage and blastulation is important as the presumptive organ forming areas which are closely packed in ovum are isolated and distributed in the blastomeres.

4. **Gastrulation:** Gastrulation is the process in which the blastula is converted into a three layered embryo, the gastrula. This stage is an intense phase of cellular movement also known as morphogenetic movement (like epiboly, emboly, divergence, involution, convergence, delamination etc.) through the blastopore to establish the three pri germ layers namely ectoderm, endoderm and mesoderm. During gastrulation, the presumptive organ forming areas are all transferred to their final destination, through mass movement of cells. Cell divisions during this stage become reduced but the process of the transcription to synthesize fresh RNA required for synthesis of proteins and other chemical substances needed for further development of the gastrula.

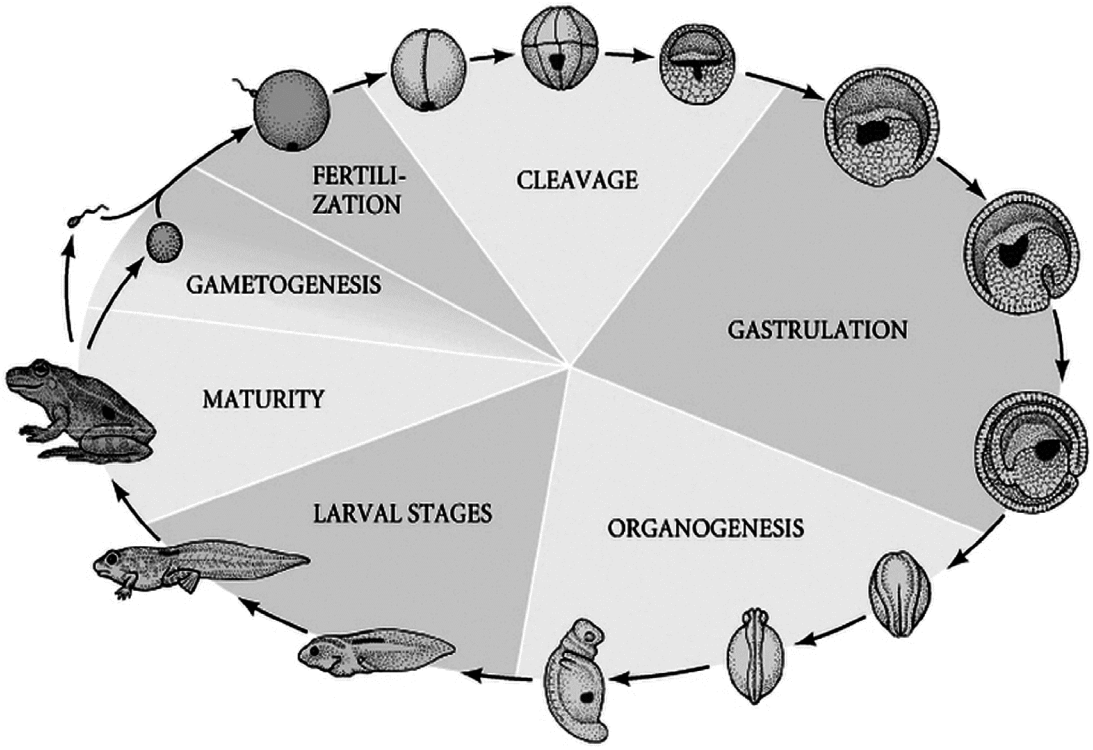


Fig. 1.1 : Different growth phases of animal development

5. **Organogenesis:** The primitive germ layers formed during gastrulation spilt into groups of cells called as primary organ rudiments and the process of formation of organs from the three germ layers is known as organogenesis. The primary organ rudiments further subdivide into secondary organ rudiments which are the initial stages in the formation of organs and their parts. At this stage the embryo acquires resemblances with the adult or a larva.

6. **Growth:** The embryo till the formation of organ rudiments is very small in size. Growth is the increase in size. This is achieved by increase in the rate of cell divisions involving fresh synthesis of nuclear material and protein synthesis. All the organ rudiments increase in mass and the embryo attains the size and the shape of the adult.

7. **Differentiation:** Differentiation occurs simultaneously with growth and the two processes cannot be separated. Differentiation is the process by which cells and tissues acquire certain characteristic features and become different from each other. Development always involves growth and differentiation. Differentiation is of several types: Morphological differentiation, Physiological differentiation and Chemo differentiation.

8. **Metamorphosis:** Metamorphosis is the last and final stage of embryonic development. We observe in nature that the young ones produced from the eggs resemble the adults in all respects expect for their small size. In other cases, there is no resemblance between the young ones that hatch out from the eggs and the parents. In fact, it becomes very difficult to identify that the young ones belong to a particular parent.

   In such cases the young ones do not grow directly to resemble the adults but some intermediate stages intervene between the egg stage and young ones. There are drastic differences between the young and adult. For instance, in butterflies and houseflies the eggs hatch out into elongated worm like structures called as larvae.

   The larval stage is followed by a pupa stage and finally a young one resembling the parent emerges from the puparium. In some cases, the larva does not grow into a pupa but undergoes a series of moultings (casting of outer skin) to become a young one. This is the case in crustanceans like prawn, crab, etc.

The process by which the larve is converted into the young is known as metamorphosis It includes a series of drastic changes in which many morphological

and physiological changes occur and the process is controlled by hormones contain structures and organs are added while other organs undergo degeneration in frog tadpole, tail and external gills are: lost while limbs are added when the tadpole metamorphoses into young frog.

1.5 Cell-cell interaction

In multicellular eukaryotes, the mechanisms that control cell division and cell growth are even more complex because a new dimension intercellular communication must play a major role. Each tissue within an organ and cach organ within the body of an organism must grow to the proper size for that particular species.

The growth of bones, muscles, the liver, the pancreas and so on must all be correctly coordinated during the growth and development of a mouse, a rabbit, or a human. Clearly, for this to occur, cell divi-sion must be under very precise control within each tissue and must be subject to different regulatory signals in different tissues and organs.

Because of the intricate interrelations that exist between the different tissues of a multicellular plant or animal, intercellular communication must play an essential central role in the growth and differentiation of higher plants and animals. How does this intercellular communication occur? What are the mechanisms by which cellular differentiation and cell growth and division are regulated?

At present, we know that there are a host of "factors" that stimulate or inhibit the growth and division of specific types of cells. However, we do not understand how any of these factors influence cell division at the molecular level. Enough information has accumulated to indicate that the total picture will be complex, but at present we are just beginning to understand a few of the pieces of this complex picture.

Cell division, like all other biological processes, is under genetic control. Certain genes must regulate the process of cell division in response to intracellular, intercellular, and Environmental signals. These regulatory genes are undoubtedly subject to mutation, like all othe enes.

Mutations that abolish the function of these regulatory genes would be expected to lea abnormal cell division in the extreme, cither the inability to divide at all or the inability dividing.

To date, we do not know the details of how cell division is controlled for any cell

of any higher animal, nor have we identified all of the genes that regulate this process in any higher eukaryote. However, recent studies of viral genes called oncogenes (from the Greek onkos, meaning tumor"), which can cause a loss of the normal control of cell division, have led to the identitication of a set of homologous genes called proto-oncogenes in the genomes of normal animals, including humans.

These normal cellular proto-oncogenes can be converted into tumor-causing cellular oncogenes by mutation or by becoming associated with new regulatory sequences through recombination processes. These and related observations indicate that the normal cellular functions of the proto-oncogenes involve specific aspects of the control of cell division.

1.6 Pattern formation

The process by which during development cells become organized in the embryo is called pattern formation. All embryos of a given species have a similar structure or stiro body plan. How does this occur? During development each cell must differentiate according to its position in the embryo, so that the "correct" cell types arise in the correct place. In other words, cells must know where they are in relation to other cells in the embryo. This is achieved by giving each cell a positional value in relation to the principal embryonic axes. Regional specification describes any mechanism that tells a cell where it is in relation to other cells in the embryo, so that it can behave in a manner appropriate for its position. Regional specification is essential for pattern formation.

Several model systems indicate that cells may acquire positional values on the basis of their distance from a source of a morphogen.

Morphogen gradient- A morphogen is a substance that can influence cell fate having different effects at different concentrations. In its simplest model, the positional information along an axis can be generated by the synthesis of a morphogen at a source at one end of the axis, and diffusion away from the source would set up a morphogen gradient. Cells at different position along the axis would receive different concentrations of the morphogen and this would induce different patterns of gene expression at different concentration thresholds. Such concentrationdependent patterns of gene expression would represent the "address" or positional identity of the cell.

Compartments and segmentation-During development the establishment of an axis is often followed by segmentation Text divided into repetitive series of similar but

independent y developmental cons Sopments occued in many species, from the obvious segmentation in the body of insects to the chombomeren und somites of vertebrale embryos. These segments can be considered as developmental compartments, in which the clonal expansion of a particular cell Ime is constrund Ilow can a cell and its clonal descendants be confined to a specific compartment. This may occur simply because there is a physical barrier to cell mixing, or compartments may be defined by patterns of gene expression in the absence of any obvious boundary. Homeotic genes give cells their positional identity. Different combinations of homcotic genes are expressed in response to different morphogen concentrations. The homeotic genes encode transcription factors that regulate downstream effector genes controlling dillerentiation and morphogenesis. Homeotic mutations cause cells to be assigned incorrect positional identities, resulting in the development of regionally inappropriate structures.

In developmental biology, pattern formation describes the mechanism by which initially equivalent cells in a developing tissue in an embryo assume complex forms and functions. Embryogenesis, such as of the fruit fly Drosophila, involves coordinated control of cell fates. Pattern formation is genetically controlled, and often involves cach cell in a field sensing and responding to its position along a morphogen gradient, followed by short distance cell-to-cell communication through cell signaling pathways to refine the initial pattern. In this context, a field of cells is the group of cells whose fates are affected by responding to the same set positional information cues. This conceptual model was first described as the French flag model in the 1960s. More generally, the morphology of organisms is patterned by the mechanisms of evolutionary developmental biology, such as changing the timing and positioning of specific developmental events in the embryo.

1.7 Differentiation

The development of either a multicellular or a unicellular organism involves two inter related processes; differentiation and growth. Cellular differentiation involves the change of a cell from an embryonic to a specialized type. Growth refers to an increase in protoplasmic mass.

What is differentiation?

The term differentiation refers to the events by which cells and other parts of an organism become different from one another and also different from the previous condition. It is a broad expression that encloses a host of operations responsible for

increasing diversification of form and function. Among dividing cells, there are multiple levels of cell potency, the cell's ability to differentiate into other cell types. A greater potency indicates a larger number of cell types that can be derived A cell that can differentiate into all cell types, including the placental tissue, is known as ***totipotent***. A cell that can differentiate into all cell types of the adult organism is known as ***pluripotent***. Such cells are called meristematic cells in higher plants and embryonic stem cells in animals. A ***multipotent cell*** is one that can differentiate into multiple different, but closely related cell types. ***Oligopotent cells*** are more restricted than multipotent, but can still differentiate into a few closely related cell types. Finally, ***unipotent cells*** can differentiate into only one cell type, but are capable of self-renewal.

Types of Differentiation Differentiation may broadly be classified into three types as follows:

a. **Morphological differentiation:** During the course of multiplication individual cells and groups of cells become structurally different from other cells and groups of cells. Example: from a common starting point in generalized ectoderm, nerve cells and epidermal cells acquire distinguishing features of size, shape and internal architecture. Morphological differentiation occurs in various ways, viz. (i) cell aggregation, (ii) localized growth, (iii) enlargement, (iv) cell migration, (v) constriction, (vi) fusion, (vii) splitting, (viii) folding, (ix) evagination, (x) invagination etc. Like, the notochord is formed by cell migration and aggregation, optic cup by invagination and metanephric duct is formed as an outgrowth of the hind end of the mesonephric duct.

b. **Physiological or Histological differentiation:** Histological differentiation is the process as a result of which the parts (cells or groups of cells) of the organism acquire the ability to perform their special functions. The special functions are however different from the basic functions of the life processes including metabolism, respiration and synthesis etc. These functions are found if differentiated as well as undifferentiated cells. Differentiation helps the cells to perform some specialized function which other cells cannot perform.

   For example, the nerve cells are capable of conducting nerve impulses at high speed and they are excitable by generating an action potential which helps conduction. The melenophores produce granules of the pigment in their cytoplasm. The liver cells secrete the bile pigment which is a very specialized

function. The existence of specialized functions is dependent on specific mechanisms within the differentiated cells. These mechanisms are observed in the organoids of the cells like myofibrils of the muscle cells, cilia of epithelial cells of trachea and the long processes of the nerve cells.

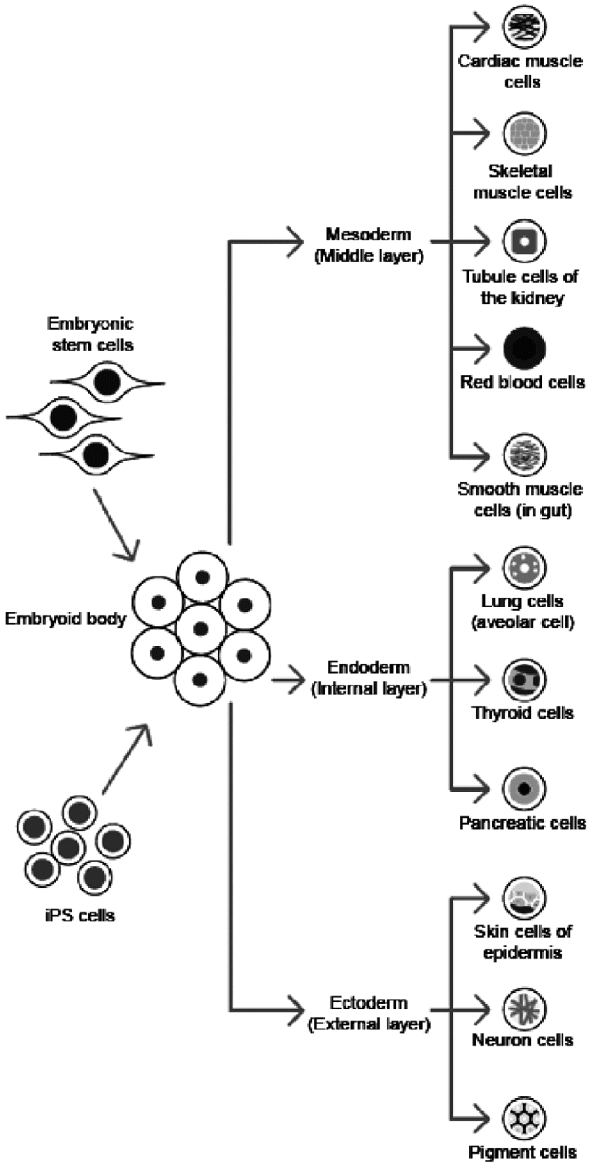


Fig. 1.2 : Cellular differentiation

Histological differentiation usually involves the changes in the cytoplasm. The nuclei of the cells are the conservative part that maintain the number of

chromosomes and the genes constant even in the differentiated conditions. The volume of cytoplasm increases in the differentiated cells and hence the ratio of the mass of cytoplasm/nucleus increases. Since cytoplasmic structures are built up on the proteins, therefore the quantification of expressed protein and enzymes provide a direct correlation to the increase cytoplasmic mass.

c. **Chemical differentiation:** During the process of development the different areas of a cell become biochemically different from one another leading to chemical differentiation or Chemodifferentiation. The purpose of chemical differentiation is a subject to the physiological changes acquired by the cell. The morphological and physiological differentiation of cells is dependent upon the chemical substances contained in them or produced by them. The process, by which cells become different due to their chemical characteristic features is known as chemo differentiation. Thus chemical differentiation can be defined as a series of events by which materials become chemically determined and then assume distinctive form and function. Chemical differentiation includes changes in the patterns of proteins and other biomolecules (viz. carbohydrates and lipids). The entire repertoire of the enzymes and the level of gene expression of the nucleic acids are altered. All these together lead to a state where the cell is in an activated condition.

1.8 Growth

The embryo upto the formation of organ rudiments is very small in size. Growth is the increase in size. This is achieved by increase in the rate of cell divisions involving fresh synthesis of nuclear material and protein synthesis. All the organ rudiments increase in mass and the embryo ttains the size and the shape of the adult. Thus Growth is the irreversible change in size of cells ue to increase in cellular mass. Growth is the result of excessive anabolic (synthetic) processes over the catabolic (breakdown) processes in an organism. If synthesis and decomposition go on at the same rate then there is no increase in the bulk of an organismie, there is no growth.

Growth can be ***determinate*** when an organ or part or whole organism reaches a certain size and then stops growing or indeterminate when cells continue to divide indefinitely Plants in general have indeterminate growth.

Characteristics of plant growth

i. Growth takes place for delinite periods before maturity.

ii. Here it does not involve increase in the number of parts.

iii. Each species has a distinct season for growth.

iv. Growing pattern is absent.

v. They have no such defined growing regions.

vi. The young one are identical to adults except in the body size and sexual maturity.

vii. A juvenile stage with different morphology does not occur in higher animal.

viii. Growth is diffused by all round increases in different organs of the body

1.9 Cytoplasmic Determinants and gene expression

There are two fundamental developmental mechanisms by which cells in embryos obtain information' (become specified) for differentiation and pattern formation. Cell fate specification and cell determination can be influenced by materials stored in the egg, so-called cytoplasmic determinants, which are molecules often localized in particular regions of the egg. Cytoplasmic determinants are special molecules which play a very important role during oocyte maturation in 8 the female's ovary. During this period of time, some regions of the cytoplasm accumulate some of these cytoplasmic determinants, whose distribution is thus very heterogenic. They play a major role in the development of the embryoic organs. Each type of cell is determined by a particular determinant or group of determinants. The action of the determinants on the blastomeres is one of the most important ones. During segmentation, cytoplasmic determinants are distributed among the blastomeres, at different times depending on the species and on the type of determinant. Therefore, the daughter cells resulting from the first divisions are totipotent: they can, independently, lead to a complete individual. That is not possible after the cytoplasmic determinants have been distributed in the differentiated blastomeres.

Mosaic development is often a term used to refer to development of embryos whose eggs contained localized cytoplasmic determinants. Mosaic in this sense is just like that of a mosaic tile or rug: The embryo is built of a patchwork of individual, rather self-differentiating parts. Each part differentiates according to instructional molecules localized in different regions of the eggs, the cytoplasmic determinants.

a. ***The orange crescent of the tunicate egg*** - An orange or yellow colored cytoplasm, initially uniformly distributed in egg, becomes localized to a crescent when the cortex rearranges right after fertilization. Cells that obtain the pigmented material become muscle, as indicated by the fate map of this

embryo. Thus this material is referred to as "myoplasm." It is possible, however, to alter early cleavage planes of the embryo so that blastomeres that normally do not get the orange myoplasm, now incorporate it. As a result, the fate of those cells is changed, or respecified, and they become muscle. The identity of the molecular determinant responsible for muscle differentiation has not been identified, but a non-protein coding RNA molecule, named YC RNA, is localized to the orange crescent.

b. Germ line specification in C. elegans by P granules (germ line granules): The germ cells, and germ cell precursors, in the nematode Caenorhabditis elegans contain distinctive granules called P granules (P-granules are ribonucleoprotein complexes). During early embryogenesis, P granules are segregated asymmetrically into those blastomeres that eventually produce the germ line, Because of the correlation between P granule distribution and the development of the germ linef P granules are widely thought to function in some aspect of germ line specification or differentiation. These are distributed only to the future germ cells. Using fluorescent antibodies to a component of the P-granules, Strome and Wood, discovered that shortly after fertilization, the randomly scattered P-granules move toward the posterior end of the zygotes of that they enter only the blastomere (P1) formed from the posterior cytoplasm.

The P-granules of the P1 cell remain in the posterior of the P1 cell and are thereby passed to the P2 cell when Pl divides. During the division of P2 and P3, however, the P-granules become associated with the nucleus that enters the P3 cytoplasm. Eventually, the P-granules will reside in the P4 cell, whose progeny become the sperm and eggs of the adult. The localization of the P-granules requires microfilaments, but can occur in the absence of microtubules. Treating the zygote with cytochalasin D (a microfilament inhibitor) prevents the segregation of these granules to the posterior of the cell, whereas demecolcine (a colchicine-like microtubule inhibitor) fails to stop this movement (Strome and Wood 1983). The partitioning of the Pgranules and the orientation of the mitotic spindles are both deficient in those embryos whose mothers were deficient in any of the par (partition detective) genes. The proteins cncoded by these genes are found in the contex of the embryo and appear to interact with the actin cytoskeleton. Mutations in partition defective or par genes par-1, par-2, par-3, results in no segregation of P granules. PAR-1 is a serine/threonine kinase that phosphorylates microtubule associated proteins.

c. **Drosophila germ cells:** At the future posterior of the fly egg pole plasm is associated with polar granules. If pole plasm is transplanted to the anterior specifies germ cells at the new position. Transplants rescue sterility of irradiated, pole-cell deficient embryos.

   At least four components make up pole plasm: germ cell-less mRNA; nanos mRNA; mitochondrial large ribosomal RNA (mt|RNA); Polar granule component (a non-translated RNA).

d. **Drosophila bicoid and nanos:** Egg cytoplasmic determinants that affect anterior-posterior (A-P) pattern: Drosophila bicoid mRNA is localized during oogenesis to anterior pole of egg. It encodes a transcription factor that forms gradient from anterior (high) to posterior (low) and regulates position-specific gene expression by nuclear responses to the BICOID protein gradient. nanos mRNA is localized to the posterior end of the egg, and NANOS protein is present in a gradient from posterior to anterior. NANOS negatively regulates hunchback (hb) mRNA translation. Hunchback protein is a transcription factor that affects gene activity.

e. **Localized RNAs in the eggs of the vertebrate *Xenopus laevis*:** Some examples are

Animal hemisphere mRNAs:

- An-1 (RNA helicase): thought to cause 'unwinding' of RNAs that are annealed to each other in the egg.
- An-2 (Mitochondrial ATPase subunit): Required for mitochondrial activity, mito' are enriched in animal pole
- An-3 (ubiquitin-zinc finger protein): Function not known. Hypothesis is that An-3 protein might get attached to other proteins to modify their function.

Vegetal hemisphere RNAs:

- Vgl: mRNA encodes a TGFB growth factor with mesoderm-inducing activity.
- Xwnt 11: mRNA encodes a wnt growth factor that may be involved in axis formation.
- Xsets it structural RNA, not protein encoding. Helps anchor Vg1 mRNA to vegetal cortex.
- BRAT or VgT, encodes a transcription factor that activates mesodermal and endodermal genes.

f. **VGI mRNA localization in frog eggs:** Specific mRNAs stored in the cytoplasm become localized to certain regions of the oocyte. While the precise mechanisms for establishing these s srulients remain unknown, grudies using inhibitors have shown that the cytoskeleton is critically important in localizing specitic RNAs and morphogenetic factors. There seem to be two pathways for getting mRNAs into the vegetal cortex. The first pathway moves messages such as those encoding the Vg! protein, which are initially present throughout the oocyte, into the vegetal cortex in a two-step process (Yisraeli et al. 1990). In the first phase, microtubules are needed to bring Vgl mRNA into the vegetal hemisphere. In the second phase, microfilaments are responsible for anchoring the Vgl message to the cortex. The portion of the Vgl mRNA that binds to these cytoskeletal elements resides in its 3' untranslated region called the **Vgl Localization Element, or VLE.** Mutagenesis of the 340 nucleotide element revealed that its ends are critical for localization to occur, which binds to a protein named **VERA** associated with the Endoplasmic Reticulum, which is highly enriched in the egg cortex.

g. **Metro (message transport organizer) pathway:** Other mRNAs, including germ plasm mRNAS such as **Xisirt and Xcat2**, leave the germinal vesicle and join the mitochondrial "cloud" located at the vegetal pole of the nucleus. These messages are compartmentalized into clusters associated with the germplasm and transported to the vegetal cortex in a manner that appears to be independent of the cytoskeleton. This mechanism is known as the Metro (message transport organizer) pathway.

1.10 Asymmetric Cell division

Cell division is commonly thought to involve the equal distribution of cellular components into the two daughter cells. During many cell divisions, however, proteins, membrane compartments, organelles, or even DNA are asymmetrically distributed between the two daughter cells. There are two mechanisms by which distinct properties may be conferred on the daughters of a dividing cell. In one, the daughter cells are initially equivalent but a difference is induced by signaling between the cells, from surrounding cells, or from the precursor cell. This mechanism is known as ***extrinsic asymmetric cell division***. In the second mechanism, the prospective daughter cells are inherently different at the time of division of the mother cell and this kind is known as ***intrinsic asymmetric cell division***. The term asymmetric cell division usually refers to such intrinsic asymmetric divisions. The mechanisms of asymmetric cell division

have been derived from studies of invertebrates specifically, I melanogaster and C elegans.

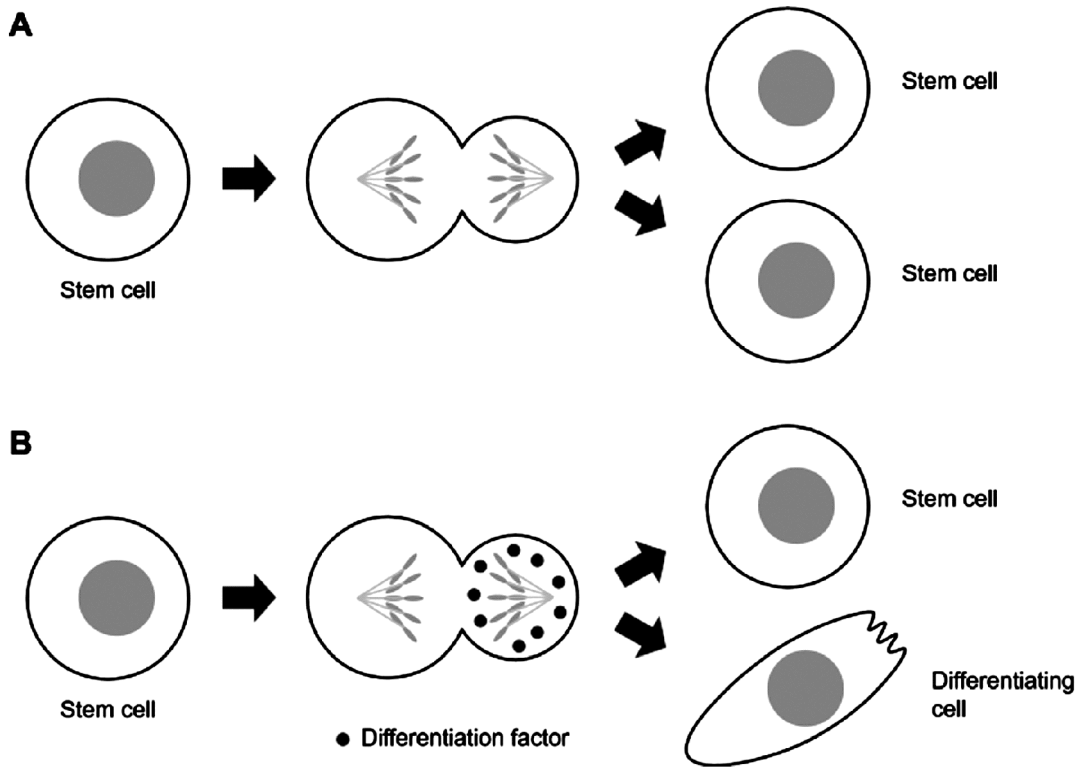


Fig. 1.3 : Asymmetric cell division

In *C. elegans*, a series of asymmetric cell divisions in the early embryo are critical in setting up the anterior posterior, dorsal/ventral, and left/right axes. After fertilization, events are already occurring in the zygote to allow for the first asymmetric cell division. This first division produces two distinctly different blastomeres, termed AB and Pl. The size and fate of these two daughter cells are different. When the sperm cell fertilizes the egg cell, the sperm pronucleus and centrosomes are deposited within the egg, which causes a cytoplasmic flux resulting in the movement of the pronucleus and centrosomes towards one pole. The centrosomes deposited by the sperm are responsible for the establishment of the posterior pole within the zygote. Sperm with mutant or absent centrosomes fail to establish a posterior pole. The establishment of this polarity initiates the polarized distribution of a group of proteins present in the zygote called the **PAR- proteins** (partitioning defective), which are a

conserved group of proteins that function in establishing cell polarity during development. These proteins are initially distributed uniformly throughout the zygote and then become polarized with the creation of the posterior pole. This series of events allows the single celled zygote to obtain polarity through an unequal distribution of multiple proteins (viz. CCCH-Zn finger proteins muscle excess 1 (meX-1), meX-5, meX-6, posterior segregation protein 1 (POS-1) and pharynx and intestine in excess protein 1 (Ple-1) etc.)

In *Drosophila melanogaster*, asymmetric cell division plays an important role in neural development. Neuroblasts are the progenitor cells which divide asymmetrically to give rise to another neuroblast and a ganglion mother cell (GMC). The neuroblast repeatedly undergoes this asymmetric cell division while the GMC continues on to produce a pair of neurons. Two proteins play an important role in setting up this asymmetry in the neuroblast, **Prospero** and **Numb**. These proteins are both synthesized in the neuroblast and segregate into only the GMC during divisions. Numb is a suppressor of Notch, therefore the asymmetric segregation of Numb to the basal cortex biases the response of the daughter cells to Notch signaling, resulting in two distinct cell fates. Prospero is required for gene regulation in GMCs. It is equally distributed throughout the neuroblast cytoplasm, but becomes localized at the basal cortex when the neuroblast starts to undergo mitosis. Once the GMC buds off from the basal cortex, Prospero becomes translocated into the GMC nucleus to act as a transcription factor. Other proteins present in the neuroblast mediate the asymmetric localization of **Numb** and **Prospero**. Miranda is an anchoring protein that binds to Prospero and keeps in the basal cortex. Following the generation of the GMC, **Miranda** releases Prospero and then becomes degraded. The segregation of Numb is mediated by Pon (the partner of Numb protein). **Pon** binds to Numb and colocalizes with it during neuroblast cell division. The mitotic spindle must also align parallel to the asymmetrically distributed cell fate determinants to allow them to become segregated into one daughter cell and not the other. The mitotic spindle orientation is mediated by **Inscuteable**, which is segregated to the apical cortex of the neuroblast.

1.11 Summary

Cellular interactions played an important role in the developmental process of multicellular animals. Similarly the process of organisation of cell during development or the pattern formation has also enormus role in the developmental process. Positional value or every cells also have important role. There are several factors which are

present in cytoplasm also have a profound role. The differential genetic expression also played a key role in the developmental process.

1.12 Questions

1. What is Recapitulation theory?
2. What is Germplasm theory of Weismann?
3. What is Organizer concept?
4. What is Amphimixis? Discuss the phases of animal development briefly.
5. What is metamorphosis?
6. What is differentiation? Write about the different types of differentiation?
7. What is indeterminate and determinate growth? State the characteristics of plant growth?
8. What is mosaic development? Give example.
9. What are P-granules in Caenorhabditis elegans? 10. Describe the different types of cytoplasmic determinants citing examples?
11. What is cell division? Describe asymmetric cell division?
12. Define gametogenesis?
13. What is fertilization? What is the purpose of fertilization?
14. What is primary organizer?
15. What is cleavage?

Unit - 2 □ Early Embryonic Development

Structure

2.1 Objective

From this unit we will learn the following process of the developmental biology—

- the process of gametogenesis i.e the formation of spermatozoa (male gamet) and ovum (female gamet)
- classification of eggs or ovum in different animals.
- the mechanism of unification of spermatozoa and ovum i.e. the process of fertilization.
- how the division of the zygotes starts ie the mechanism of clearage.
- the process of fate mapping
- the mechanism of formation of 3 germinal layers i.e. the gastrulation.
- the process of embryonic induction and the role of organizers.

2.2 Introduction

The early embryonic development means the mechanism by which embryo is formed and the meterials needed for this purpose. This unit refers to the mechanism by which male and female gamets are formed and also how they are united together of means by the process of fertiligation. This unit also elaborates the fate of the newly formed embryo-wheather they are presumptive or not. Similarly this unit also includes the behavioural change of the cells which are participating in the developmental process.

2.3 Gametogenesis

The ongin and development of gametes is called gametogenesis. This divided into spermatogenesis and Oogenesis. Spermatogenesis deals with the development ule sex cells called sperms in the male gonad or testis. Oogenesis is the development of female -cells called ova or eggs in the female gonad or ovary,

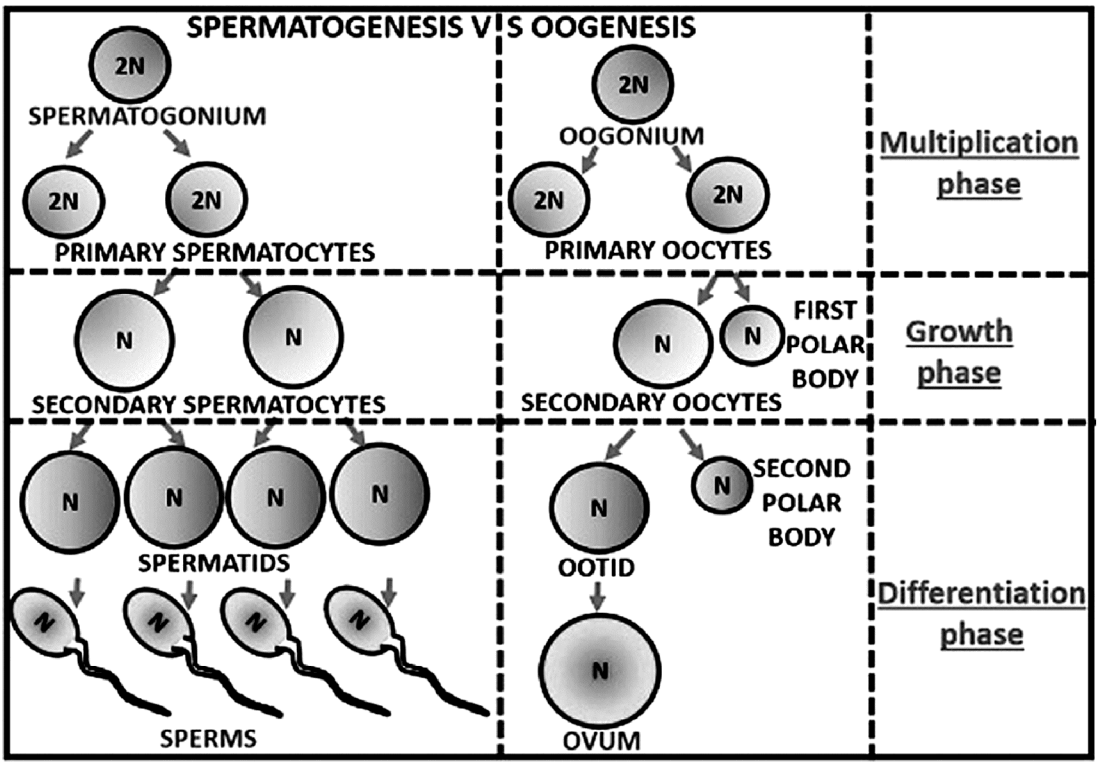


Fig. 2.1 : Stages of Gametogenesis (Spermatogenesis and Oogenesis)

2.4 Spermatogenesis

Mammalian spermatogenesis is a highly synchronized, regular, long and extremely complex process of cellular differentiation by which a spermatozonial "stem cell" is gradually transformed into a highly dillerentiated haploid cell Spermatozoon. This differentiation involves three distinct classes of germinal cells the spermatogonia, the spermatocytes, and the spermatids, which usually are arranged in concentric layers in the seminiferous tubules. In the adult mammal's spermatogenesis is a continuous process, which can be divided into two distinct phases and each characterized by specific morphological and biochemical changes of nuclear and cytoplasmic components. The two phases include:

(i) Formation of spermatids (mitosis and meiosis) and

(ii) Spermiogenesis.

(i) Formation of spermatids: The male gonad known as testis is the site of spermatogenesis. In each vertebrate a pair of testes remains attached to dorsal body wall by a connective tissue called mesorchium. Each testis is formed of thousands of minute elongated and coiled tubules called seminiferous tubule. The inner lining of semini?erous tubules is called as germinal epithelium and is made of primordial germ cells (Primary germ cells) as well as some supporting nutritive cells. The primordial germ cells give rise to spermatids through the following steps:

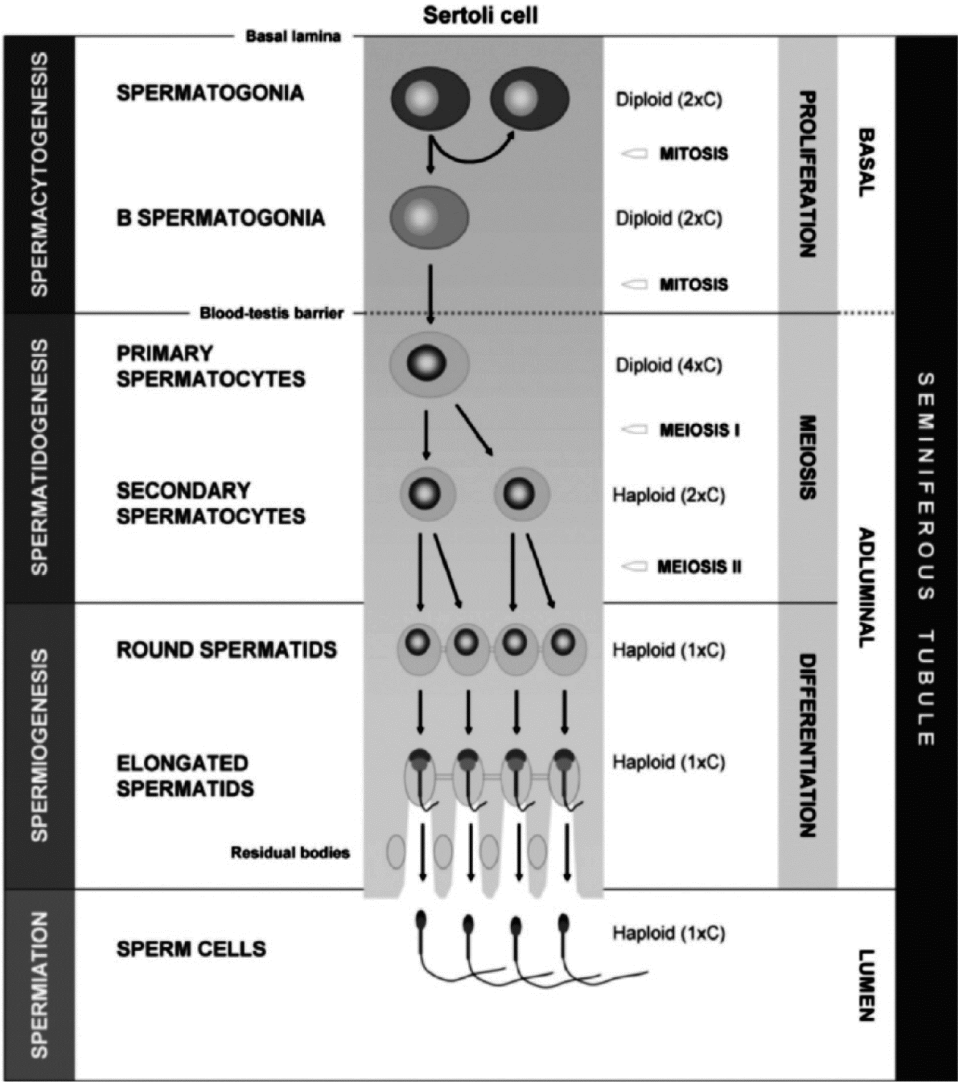


Fig. 2.2 : Step of spermatogenesis

a. Multiplication phase:

This phase is also known as proliferation and renewal of spermatogonia. During this phase the diploid spermatogonia which are situated at the periphery of the seminiferous tubule, multiply mitotically to form spermatocytes and also to give rise to new spermatogonial stem cells and enter the phase of growth.

b. Growth phase:

During this phase, a limited growth of spermatogonia takes place; their volume becomes double and they are now called primary spermatocytes which are still diploid in nature. Now these primary spermatocytes enter into the next phase namely, maturation phase.

c. Maturation phase:

The primary spermatocyte enter into the prophase of meiotic or maturation division. Meiotic prophase is a very complex process characterized by an ordered series of chromosal rearrangements which are accompanied by molecular changes. During meiosis, first nuclear DNA duplicates, each homologous chromosome starts pairing (synapsis) and longitudinally spilts up into two chromatids, both of which remain joined by a common centromere. By chiasma formation mutual exchange of some chromosome material between two non-sister chromatids of each homologous pair (tetrad) occurs (crossing over) to provide an almost indefinite variety of combinations of paternal and maternal genes in any gamete. Lastly, two chromosomes of each homologous pair (tetrad) migrate towards opposite poles of the primary spermatocyte. Now each pole of primary spermatocyte has haploid set of chromosomes. Each set of chromosome is surrounded by the nuclear membrane developed from the endoplasmic reticulum. The first meiotic division, as a rule, is followed by the division of cytoplasm (cytokinesis) which divides each primary spermatocyte into two haploid, secondary spermatocyte. Each secondary spermatocyte undergoes second meiotic or maturation division which is a simple mitosis and produces four haploid spermatids. These are non-functional male gametes. To become functional spermatozoa, they have to undergo a complex process of cytological and chemical transformations; a process usually referred to as ***spermiogenesis***.

(ii) Spermiogenesis: The changes in the spermatids leading to the formation of spermatozoa constitute the process of spermiogenesis. Because a spermatozoon

is a very active and mobile cell, in order to provide real mobility to it, all the superfluous materials of the developing spermatozoa are to be discarded and a high degree of specialization takes place in the sperm cell through a number of steps.

During spermiogenesis two major parts of the sperm, the head and tail are formed by the following process.

1. Formation of head:

The two major parts of sperm head i.e. the nucleus and acrosome, undergo the following changes to form a sperm head.

(a) Changes in the nucleus: During spermiogenesis, the nucleus of spermatid shrinks by losing much of its water from the nuclear cap and the chromosomes become closely packed into a small volume. Whole of ribonucleic acid is eliminated, leaving only the genetic material, the deoxyribonucleo-protein. Thus the material, which is not directly concerned with the transmission of hereditary characters, is removed from the nucleus.

(b) Golgi phase: The young spermatid is round with a spherical nucleus. The Golgi apparatus secretes glycoprotein rich granules which are stained with the periodic acid-Schiff technique. These granules referred to as proacrosomic granules, fuse to form a single large acrosomal granule attached to the nuclear membrane.

(c) Cap phase: The acrosomal granule flattens on the nucleus of the spermatid to form the head cap. The Golgi apparatus which secretes the acrosome separates from the head cap and move towards the opposite pole. The Controles which are close to the nucleus, on the side opposite the acrosonic cap develop a flagellum.

(d) Acrosome phase: The spherical shape of the nucleus also becomes elongated and narrow This shape is an obvious adaptation for the propulsion in any fluid medium, as well as penetrating the ovum In different animals, it assumes different shapes which ultimately determine their prospective shapes. The definitive morphological contours of the acrosome become clearly defined. The remaining part of the Golgi apparatus is gradually reduced and ultimately discarded from the sperm as "Golgi -rest" along with some cytoplasm.

In the spermatozoon, the axial body or acrosomal core appears in between the acrosomal granule and nucleus, producing itself from behind into the

acrosomal granule. This body develops during its approach to the egg. It contains a few enzymes which are used to dissolve the egg membranes during fertilization process (Colwin and Colwin, 1961).

2. *Formation of the tail of the spermatozoon:*

The Centrosome of a spermatid after the second meiotic division consists of two Centrioles which have the structure of two cylindrical bodies, lying at right angle to each other. During early stages of sperm metamorphosis, the two Centrioles move to a position just behind the sperm nucleus in the future neck region. A depression is formed in the posterior surface of the nucleus and one of the two Centrioles becomes placed in the depression with its axis approximately at right angles to the main axis of the spermatozoon.

This is the proximal Centriole and the other centriole i.e. the distal Centriole takes up a position behind the proximal one with its axis coinciding with the longitudinal axis of the spermatozoon. The distal Centriole now gives rise to the axis filament of the flagellum of the sk spermatozoon for which it serves as basal granule.

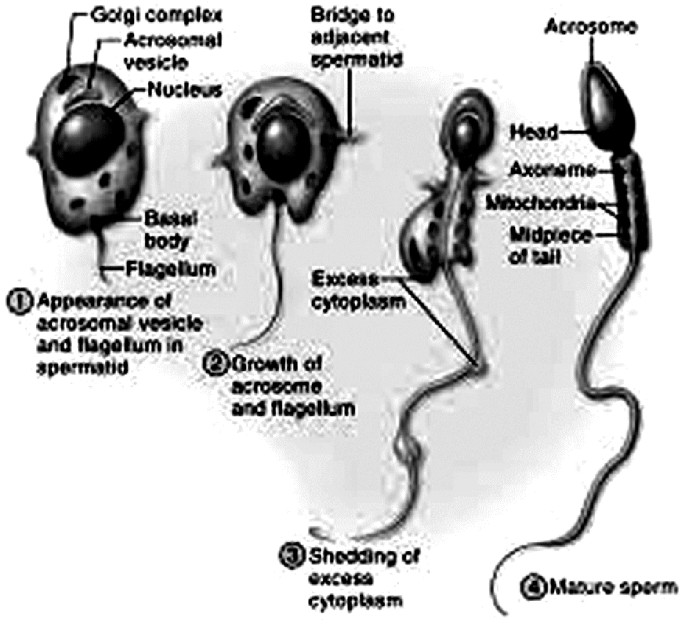


Fig. 2.2 : Spermiogenesis

Most of the mitochondria of spermatids concentrate around the distal Centriole and proximal (upper) part of the axial filament and form the neck and middle piece of the tail of spermatozoon. In the middle piece the sperm the mitochondria lose

their individuality by fusing to a greater or lesser extent. In mammals, the mitochondria join in one continuous body which becomes twisted spirally around the proximal part of the axial filament and the proximal Centriole.

In other animals, however, no spiral arrangement of mitochondria occurs, but instead, mitochondria fuse together to form massive clumps called mitochondrial bodies. The cytoplasm forms a condensed layer called **manchette** around the periphery of the middle piece. The manchette also surrounds the posterior part of the head of the spermatozoa, where it is not covered by the cap.

A dark ring called **'Ring Centriole'** of unknown function is sometimes seen at the posterior end of the middle piece. It forms the boundary between the middle piece and the principal piece of sperm tail. In most animals, except mammals, the principal piece and tail piece of the sperm are composed of the axial filament only. In mammals, the axial filament of the principal piece is accompanied on the outer side by much thicker fibres which are wedge-shaped in cross section.

These fibres start in the middle piece but do not reach upto the end piece of the spermatozoon tail. The fibres of axial filament of a mammalian sperm tail are also surrounded by flattened bands which occur as semi-circular ribs articulating with each other on the opposite sides of the sperm tail (Telkka, Fawcett and Christensen, 1961). The end piece has only axial filament which remains covered with cytoplasm and plasma membrane.

Biochemical Changes in Spermatogenesis:

A number of biochemical events occur during spermatogenesis (Monesi, 1970). These are:

(1) The RNA synthesized during meiosis is eliminated from the nucleus during the two meiotic divisions and remains in the cytoplasm. The fully formed spermatozoon does not contain any detectable amounts of RNA. The meiotic RNA is probably associated with the synthesis of acrosomal proteins and flagellum.

(2) Nuclear protein synthesis is arrested in the middle of spermiogenesis.

(3) All of the non-histone proteins in the nucleus are eliminated during spermiogenesis.

(4) All the synthetic events occuring during spermiogenesis are probably regulated by stable RNA produced during the meiotic stages.

(5) The suppression of genetic activity in spermatids and spermatozoa may depend on a regulatory mechanism which causes disappearance of activating proteins and RNA from the chromosomes.

(6) The histone molecules associated with DNA may play a protective role by stabilizing the DNA against changes occurring in their transport through the male and female reproductive tracts (Taiwan et al, 1989).

Control of Spermatogenesis:

Spermatogenesis is either controlled environmentally or physiologically. Temperature, light, hormones and psychological state play an important role depending upon the organism. Anterior Pituitary gland plays an important role in regulating spermatogenesis by secreting certain gonadotrophin hormones mainly the Follicle stimulating Hormone (FSH). But the pituitary itself and the gonadal activities of birds, rodents, and many other vertebrates are affected by the temperature, light and the length of the day.

2.4.1 *Questions*

1. State the site of spermatogenesis?
2. What is mesorchium?
3. What is spermatogenesis?
4. What is ring centriole?
5. What is manchette?
6. What is a spermatid?
7. Describe in details the events of spermatogenesis?
8. Describe the process of Spermiogenesis?
9. State the role of Golgi body in spermatogenesis?
10. What are the biochemical changes in spermatogenesis?
11. How is spermatogenesis controlled?

2.5 Oogenesis

Eggs: The egg in all animals is a larger cell in comparison to other type of cells. All contain reserve material of various kinds such as yolk, glycogen, nucleic acids (DNA, RNA, etc.) and various species of proteins. Iggs are produced as large as

possible, compatible with the number needed to ensure adequate variation, to cope of with the general enormous mortality rate of eggs, embryos and juveniles, and to distribute developing progeny beyond the terrestrial limits of the parental populations.

The number of eggs produced becomes greatly reduced and individual egg size correspondingly increased only in those cases, where special means are evolved to ensure survival whether by hiding, securing or otherwise protecting eggs, or above all by retaining developing eggs, within the maternal body as in mammals.

The egg cell or ovum has three basic functions: (i) to supply a nucleus containing half of the chromosomal component of the future embryo, (ii) to supply almost all the cytoplasm to the zygote, and (iii) to supply food reserves that will enable the embryo to develop up to a stage where it can begin to feed upon exogenous materials.

Therefore, for these future needs, an egg cell becomes immotile, large-sized, wellspecialized, packaged and programmed during the process of oogenesis. Packaging i.e. the growth of the primary oocyte, and the accumulation of condensed food reserve, yolk, glycogen etc. within, it relates mainly to the number of cells that the developing egg can become, while, programming of egg relates to the determinative or directional information that egg may possess as a specialized type of reproductive cell.

Oogenesis: The process of oogenesis is somewhat more complicated and different than spermatogenesis. Besides, the production of four unequal sized haploid cells, there is acquisition of food reserves in the egg cytoplasm for the development of embryo. Further, before the occurrence of meiosis, enormous amount of growth and differentiation of egg-cytoplasm takes place. The oogenesis is more or less similar in all vertebrate groups.

During oogenesis, the cells of germinal epithelium detach from the surface epithelium and ente the cortex of the ovary. These germinal cells are diploid and are called primordial germ cells.

They pass through the three stages to form a fully formed egy which are

(1) Phase of multiplication

(2) Phase of growth

(3) Phase of maturation

Oogenesis in insects: In insects, eggs are produced in the ovarioles of an ovary.

The thinner part of an ovariole is called germarium, which contains oogonia enveloped by mesodermal cells. The stouter part of the ovariole is called the vitellarium, which contains oocytes in a longitudinal row. Each oocyte is surrounded by a single layer of cuboidal follicle cells.

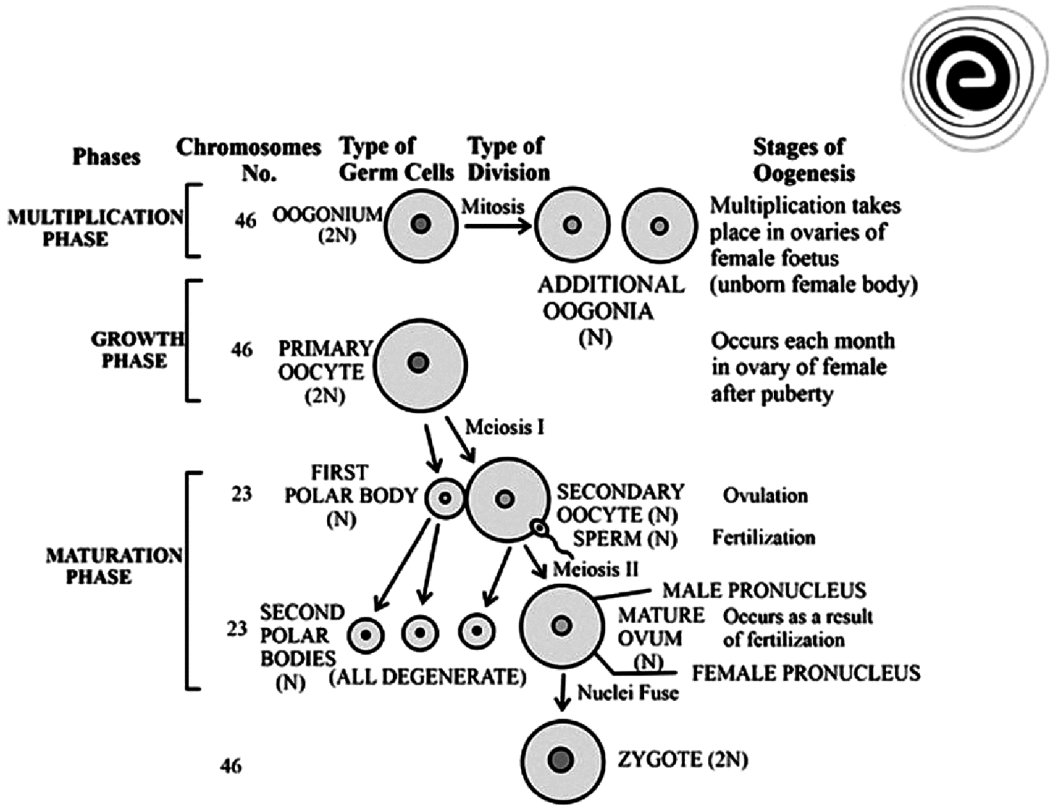


Fig. 2.4 : Oogenesis in the female Gonad

In the germarium, oogonia produce oocytes by mitosis. One oogonium divides into two cells- one primary oogonium and a cytoblast. The blast divides by four consecutive mitosis into 16 cells (cytocysts). One of these becomes an oocyte and the other 15 becomes nurse cells (trophocytes). All the 16 cells are surrounded by a single layer of flattened follicle cells.

The oocyte and the nurse cells, with their follicular covering, come to the vitellarium, where the oocyte gets yolk granules from the haemolymph and the nurse cells. As deposition of yolk continues, the oocyte grows. Then by meiosis the oocyte forms two cells, one big cell-the ovum and the other very small cell - the first polar

body. The latter degenerates after sometime. In this way, the ovum gets half the number of chromosomes of the species. When the ovum is ready for the transfer to the oviduct, the follicle cells secrete the chorion or outer shell around it. In cockroach, the nurse cells are absent.

Oogenesis in mammals: In mammals the process of oogenesis shows the following three phases:

1. **Phase of multiplication:** The primordial germ cells become the oogonia - the egg mother cell The oogonial cells eventually undergo proliferation by repeated mitotic divisions, giving rise to the eggs and become primary oocytes when cell division ccases. Now they enter into a period of growth
2. **Phase of growth:** Owing to the fact that the egg contributes the greater part of the substance used in the development, growth plays a much greater role in oogenesis than in spermatogenesis. The period of growth in the female gametes is very prolonged and tremendous growth of oocyte occurs during this phase. Most of the primordial germ cells are approx., 10 μ.m. (0,01 mm) in diameter. The young oocyte of amphibians may be about 50 μ.m. (0.05 mm) and the mature amphibian egg is rather large about 1000 to 2000 μ.m. (1.0 to 2.0 mm) in diameter. In birds the diameter of ovum is as large as 40,000 μ.m. and in mammals it is only 200 μ.m.

The rate of growth of oocytes also varies; it may be slow or fast. The young oocyte starts growing after the tadpole metamorphoses into the young frog and by the third year the eggs mature and the frog spawn for the first time. In other animals, the growth of oocyte may proceed at a much higher rate and takes shorter time for completion. In hen, the last rapid growth of oocyte occurs in 10 to 14 days preceding ovulation, and during this time the volume of the oocyte increases 200 fold. The progressive growth nuclear as well as cytoplasmic substances of oocytes may be divided into two stages - (a) Previtellogenesis - growth period and (b) Vitellogenesis - Yolk appears in the second period of growth period.

(a) Previtellogenesis growth period:

During this phase, no synthesis and accumulation of food reserve material, the yolk, takes place, but tremendous increase in the volume of nucleus and cytoplasm of primary oocyte occurs. There is qualitative and quantitative increase in the amount of cytoplasm. The mitochondria increase in number, the network of endoplasmic reticulum with ribosomes becomes more complicated, the Golgi bodies manufacture cortical granules, besides performing their normal function.

(i) Growth of nuclear substances: During this phase due to the production of the large amount of nuclear sap, the nucleus of the growing oocyte increases in size. A dark body appears at one place outside the nucleus, usually near the Golgi complex and is known as yolk nucleus of Balbiani. This large sized oocyte inflated with the fluid is now called germinal vesicle.

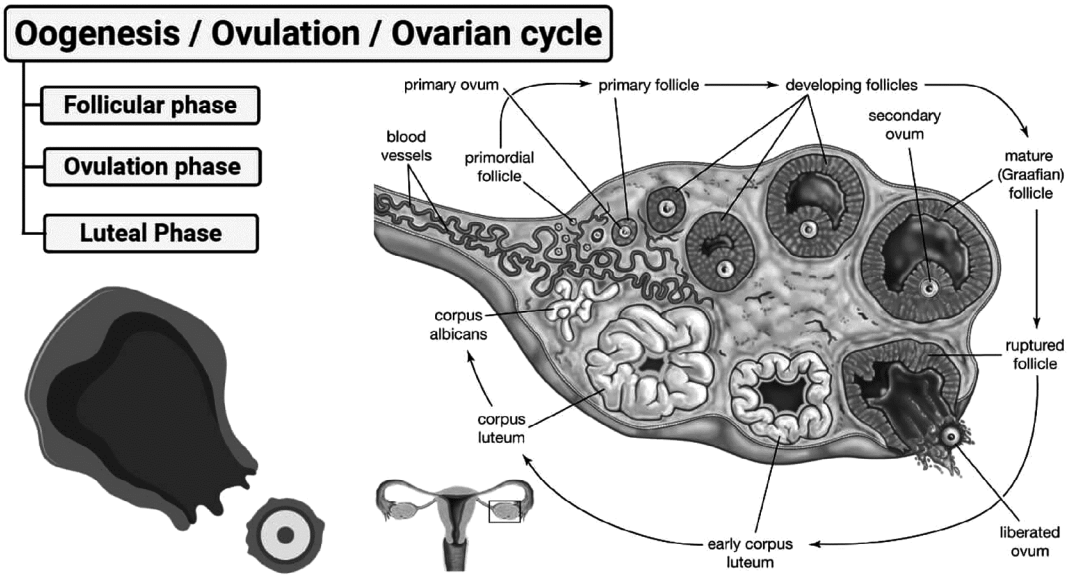


Fig. 2.5 : Oogenesis and Ovulation in Ovarian-cycle

The nucleus of the oocyte enters the prophase of meiotic division. between homologous chromosomes but the subsequent stages of meiosis are postponed and each chromosomes increases in its length, but the amount of DNA in cach chromosomes does not increase in proportion to the enlargement of the nucleus. The increased chromosomes look like bottle brush; bence they are called the lamp-brush chromosomes.

It is believed that the loop of chromosomes represent actual site for the main activity of the genes, i.e. transcription of mRNA, which in turn controls translation process in which synthesis of proteins in the cell cytoplasm takes place.

During the growth period of oocyte, all mRNA molecules are not utilized during translation but some are inactivated by the wrapping of proteins around them and stored as informosomes to be used during early cleavage of egg, when chromosomal DNA remains more actively engaged in its

own transcription of mRNA (messenger RNA) r- RNA (ribosomal RNA) or t-RNA (transfer RNA).

The RNAs are transcribed by r-DNA of 'nucleolar organizer region' of chromosomes. The nucleolus has a significant role in the storage and maturation of the ribosomal RNAs. It also synthesizes all the proteins required for the biogenesis of ribosomes.

Therefore, during growth period of primary oocytes, the nucleolus increases greatly in size and becomes very conspicuous. In many animals, particularly in amphibians, instead of one large nucleous, numerous small sized nucleoli are formed in their germinal vesicles. Most of them are localized on the periphery of the nucleus, immediately underneath the nuclear membrane.

The increased transcriptional activity (i.e. RNA synthesis) of chromosomal genes during growth period of oocytes, is called gene amplification (Ephel, 1973) or redundancy (De Robertis et al, 1982). When mRNA molecules are transcribed from DNA then it is known as transcriptional amplification. Each mRNA molecule in turn can be translocated several times into the corresponding proteins known as translational amplification. This high rate of gene amplification or gene activity is correlated with the fact that gene reduction (meiosis) does not take place until after the growth of the oocytes has been completed. As a result the oocytes remain tetraploid for a long time.

(ii) *Growth of cytoplasmic substances:* The amount of cytoplasm of oocyte increases both quantitatively and qualitatively during the Previtellogenesis growth period of oocyte. Young oocytes, in many animals, show a very simple organisation due to poor cytoplasmic inclusions and possess none of the specialized structures found in the adult oocyte and mature egg. The cytoplasm is finely granular having granules of nibonucleo protein and DNA.

Mitochondria, the carriers of oxidative enzymes are fairly scarce in young oocytes but may increase in number very considerably during the growth of primary oocyte because overall oxygen consumption increases during this time in amphibians and birds, the mitochondria become aggregated in the form of large 'Mitochondrial clouds" Mitochondria possess its own circular DNA. So in a growing oocyte, the amount of mitochondrial DNA far exceeds the amount of nuclear DNA.

The young oocytes have the granular endoplasmic reticulum in the form of numerous, small vesicles. Annulated lamellae are also found in the cytoplasm of growing oocyte. These membranous structures appear in the form of stakes of cisternae, either in parallel or in spiral arrangement. Sometimes, annulated lamellae are associated with ribosomes and RNA in high concentrations, and there is also an ATPase activity in the pore complexes of these lamellae. The lamella, thus serves as a storage site of RNA in cytoplasm and they are found to break down and disappear during late oogenesis.

In young oocyte the Golgi bodies are found around the Centrosome. In mature oocytes they form a large spherical mass in some mammals, or become located in the sub-cortical cytoplasm of frog and chick, or sometimes may disappear completely. The Golgi complex of oocyte is believed to synthesize cortical granules besides performing its normal function.

In the cortical region, cortical granules are present. These are membrane bound spherical bodies of diameter from 0.8 microns (sea urchin) to 2.0 microns (frog) and contain acid mucopolysaccharides. These mucopolysaccharides are used during fertilization, in the formation of fertilization membrane.

They are present in bivalves, some annelids, fishes, frogs and some mammals By (rabbit and man), but are absent in some insects, gastropoda, urodela, birds and some mammals (rat and guineapig). These granules are synthesized' by cisternae of Golgi complex in the interior of the oocyte and later they move to the periphery where they are arranged in a layer close to the plasma membrane of oocyte.

(b) Vitellogenesis growth period:

The process of formation and deposition of yolk is called vitellogenesis. In amphibians and fishes its synthesis takes place inside the modified mitochondria. In some insects yolk formation occurs in fat bodies whereas in most of the vertebrates yolk is formed in the liver of the mother. From the liver it is carried by blood to the ovary.

In the ovary, this material 'percolates to the follicle cells and from there to the cytoplasm of the oocyte. However, in vertebrates, a very small quantity of the material for the formation of volk (hardly 1%) is synthesized by the

oocyte cytoplasm itsell.

Thus a major part of the material forming the yolk is exogenous (formed outside the oocyte). Yolk is a general term that covers the major storage material such as glycogen, certain other carbohydrates, proteins and lipids. Hence, vitellogenesis is the period of rapid growth.

3. **Maturation phase:** The primary oocyte undergoes two successive divisions by meiosis. The first division is meiosis-1 and two unequal daughter cells are produced. The large cell is called secondary oocyte containing haploid (n) set of chromosomes (due to reductional or disjunctional division) and entire amount of cytoplasm. The smaller cell is called first polar body or polocyte containing 'n' number of chromosomes and practically no cytoplasm.

   The secondary oocyte and first polar body then undergo second maturation division by meiosis-II which is an equational division. As a result of this division one large ovum is formed containing entire amount of cytoplasm and 'n' number of chromosomes and a second polar body like the first polar body.

   Simultaneously, the first polar body may divide into two polar bodies or may not divide at all. Thus only one functional ovum is formed and the two or three polar bodies soon degenerate. In vertebrates the first polar body is formed after the primary oocyte is released from ovary and has entered into the oviduct. The second polar body is formed only when the sperm enters into ovum during fertilization.

(c) Ripening of Egg:

Oogenesis is followed by the formation of protective coverings called egg membranes. Primary membrane is formed surrounding the plasma membrane of ovum and is secreted by the ovum itself. It is called vitelline membrane in frog and zona pellucida in rabbit. The secondary membrane called chorion is formed from ovarian follicle cells. The tertiary membranes are secreted in oviduct when the ovum passes from ovary to outside. The egg white (albumin), calcareous shell etc. come under this category. The ripe ovum is spherical or oval and non-motile. Depending upon the amount of yolk, it may be as small as 0.15 mm as in mammals (microlecithal); it may be 2 mm as in frog (mesolecithal) or it may be as large as 30 mm as in hen (megalecithal). In a ripe ovum, the polarity is fixed. The top-most point is animal pole and the bottom point is vegetal pole. The density of yolky cytoplasm increases from the animal pole towards the vegetal pole. In frog, the animal hemisphere

is highly pigmented and appears black while the vegetal hemisphere is highly pigmented and appears white.

Physio-chemical Nature of Yolk

Yolk or deutoplasm is a complex of variably assembled components, rather than a definite chemical substance. This stored food is utilized by the embryo for its early development. The process of formation of yolk is known as Vitellogenesis. The yolk is a complex material consisting of proteins, fats, carbohydrates, inorganic salts, vitamins, enzymes, pigments and water The principal components are proteins, phospholipids and fats in different combinations. Depending on which of these components that predominate, the yolk is distinguished as "protein yolk" or "fatty yolk". These two kinds of yolk are present side by side in the yolks of many animals. The avian yolk as a whole contains 48.74% water, 16.6% proteins, 32.6% of phospholipids and fats and 1% carbohydrates. The fatty portion of avian yolk is mainly neutral fat (50% of the dry weight), the remaining being phospholipids and cholesterol. It is usually produced within egg cell but in platyhelminthes it is produced by vitelline glands. The amount of yolk tends to obstruct the cleavage and affects the cleavage pattern of the developing embryos. It is also related to the pattern of larval development in the larva producing individuals. Bilateria, where the embryos develop from less amount of yolkcontain eggs, become planktotrophic larvae because they feed on planktons other organisms.

The larvae which develop from the heavily yolk-laden eggs, become lecithotrophic 4 larvae because they depend on the reserve yolk of the ovum (e.g., Nereis, eunicids, some bivalves, etc.). The planktonic larvae are seen in echiurans, phyllodocids, serpulids, some gastropods, etc. In animals, yolk is found in three forms as given here under:

a. **Granular yolk:** Protein yolk of many invertebrates like echinoderms and of lower chordates (amphioxus, tunicates) consists of fine yolk granules which are fairly evenly distributed in the cytoplasm of eggs.

b. **Yolk Platelets:** In amphibian eggs, the yolk is found in the form of large granules called yolk platelets. The yolk platelets are oval and flattened in one plane. They contain two main proteinaceous substances: Phosvitin and Lipovitellin. Phosvitin is a highly phosphorylated protein (phosphorus 8.4%), whereas Lipovitellin contains a considerable amount of bound lipid (17.59.). In the yolk platelets two molecules of Phosvitin are associated with one

molecule of Lipovitellin in a structural unit. Electon-micrographs of amphibian yolk reveal that these units are arranged in the platelets in a crystalline lattice wall with regular hexagonal packing (Wallace, 1963). In addition to the yolk platelets the amphibian egg contains lipids and glycogen Lipid is found in the cytoplasm in the form of Lipochondria, which consists of an internal core of lipid surrounded by a thin protein layer.

Cyclostomes, Elasmobranchs, Ganoids and Lung fishes have eggs with a distribution of food reserve much the same as the amphibians.

c. **Yolk spheres:** The yolk of birds, reptiles and bony fishes lies in a compact mass in the interior of the egg. The cytoplasm is restricted to a thin layer on the surface with a thickened gap on the upper side. Most of the yolk is liquid but about 23% is in the form in the solid yolk spheres.

Function of yolk

1. Yolk is the most usual form of food storage in the egg.
2. It influences the differentiation of the ooplasm and the patterns of cleavage.
3. The size of the egg is determined by the amount of yolk present in each.
4. Yolk exercises an important influence on the morphogenetic movement of the blastomeres during Gastrulation.
5. The nature of development whether indirect with larval forms or direct with juvenile stages is governed by the amount of yolk present in the egg.

Role of Follicle cells and Nurse calls in Oogenesis

The growing oocytes are surrounded by special nutritive cells which help their growth in various ways. There are two main types of nutritive cells viz. foll cells and nurse cells.

a. **Follicle cells:** In mammals and some other vertebrates, the oocytes are surrounded during growth and maturation phases by special cells of the ovary, the follicle cells. These are derived from the germinal epithelium of the ovary. Initially, the young oocyte is surrounded by a single layer of follicle cells but later the number of ***follicle cells*** increase and the cells become arranged in rows. In mammals, the follicle cells and the developing oocyte constitute the Graafian follicle. As the egg approaches maturity, an eccentric

cavity called ***antrum*** appears in the mass of the follicle cells. The cavity is filled with a fluid called ***liquor folliculi*** which is secreted by the follicle cells. In the beginning, there is a simple apposition of the follicle cells and the oocyte. The cytoplasmic membranes of the adjoining cells remain separated by a narrow gap. The plasma membrane of the oocyte and follicle cells are connected by desmosomes At a later stage the space widens. The surface of the young oocyte is drawn into numerous finger like projections called microvilli. The microvilli interdigitate with the cytoplasmic processes of the follicle cells. The presence of microvilli greatly increases the surface area of the oocyte The increased area facilitates metabolic turnover between oocyte and the surrounding cells. The follicle cells help in the growth of the oocyte by secreting substances which are taken up by the oocyte The zone of microvilli appears as a radially striated layer known as ***zona radiata.***

b. Nurse cells- In some invertebrates like annelids, insects and molluscs the oocyte is surrounded in addition to the follicle cells, by special nurse cells. Derived from the egg cell, the nurse cells supplement the function of the follicle cells in providing the nutrition to the growing oocyte. Nutrients form the cytoplasm of the nurse cells pass into the oocyte through gaps developed in the cell membranes of two cell types. This type of relationship is different from that found between oocyte and follicle cells because there are no microvilli or cytoplasmic projections. In some insects and annelids the nurse cells are gradually consumed during the growth of the oocyte. In molluscs e.g., the snail Helix, the entire nurse cells are engulfed in the cytoplasm of oocyte.

2.5.1 *Questions:*

1. What is Oogenesis? What are the basic functions of the ovum?
2. Write briefly about Oogenesis in insects?
3. Describe the complete phases of Oogenesis in mammals?
4. What are informosomes?
5. What is vitellogenesis? What is the composition of yolk?
6. What are the forms of yolk evidenced in animals?
7. Mention the function of yolk?
8. What are yolk platelets?

9. What are yolk spheres?
10. Name the proteins present in yolk?
11. What is Zona radiata?
12. What is liquor folliculi?
13. What are follicle cells? State its role in Oogenesis?
14. What are Nurse Cells? State its role in Oogenesis?
15. What do you mean by ripening of the egg?
16. Differentiate between Spermatogenesis and Oogenesis?

2.6 The egg

The fully developed female sexual cell is called the ovum (I. ovum, egg). When fertilized and united with the male gametic pronuclei a zygote is formed which results in the development of a new individual. The eggs of different animals vary widely in shape, size, and distribution of yolk and in their coverings. They are relatively large in size and inert when compared to the male reproductive cell. An animal egg has three basic functions: i) it supplies a haploid set of chromosomes to the future embryo, ii) it provides most of the cytoplasm to the embryo, and iii) it supplies food reserves that will enable the embryo to attain a status where it can feed on exogenous food materials.

Size- Marine invertebrates usually produce small eggs in enormous numbers. Their size ranges from 50μ (e.g., polychaete annelids) to about 150μ (e.g., Tunicates) in diameter. In vertebrates they vary in size from0.07mm in mouse to 3.5inches diameter in ostrich. The size of the egg chiefly depends on the amount of yolk reserves in it. Sharks and Rays among fishes, reptiles and birds generally lay eggs of a larger dimension. On the contrary the eggs of mammals are minute, with very little amount of yolk and measure about 100um.

Shape- Typically the mature egg is spherical, but in few animals, as in insects the eggs are elongated. Among vertebrates, the eggs of hagfish Myxine and then ganoid fishes are oval in shape. Although the eggs of birds are also oval in shape externally, but their egg cells (yellow portion) are actually spherical in shape.

Polarity- The constituents of egg are not uniformly distributed throughout the cytoplasm. These are distributed in such a way that two poles distinct can be identified in the egg. These poles are known as animal pole and vegetal pole. The

cytoplasm is concentrated in the upper portion or animal hemisphere and the yolk material is concentrated in the lower portion or vegetal hemisphere. A plane passing through these two poles constitute the polar axis. The nucleus is always located in the polar axis, more or less towards the animal pole. The yolk shows a gradation from the animal pole towards the vegetal pole. There is also a metabolic gradation along the polar axis. Metabolic processes are highest at the animal pole and progressively diminish towards the vegetal pole.

Organization of a mature Graafian follicle (mammals)

The outermost layer of the graafian follicle is called the ***theca externa***, which consists of concentrically arranged fibres and fusiform cells. Inside the theca externa the cells are large and ovoid or spindle shaped and the layer formed is called ***theca interna***. This layer is thought to secrete estrogens and its cells show the typical features associated with cells secreting steroids. There is also a nici capillary plexes in the theca intema Follicles about 0.2 mm in diameter begin to collect pools of luids. This follicular lud is called ***liquor folliculi***, is rich in mucoproteins and is secreted by the follicle cells. The Muid occupies a single cavily called the antrum. Thue Huid pushes the ovum and its neighbouring follicle cells to one side eccentrically. A peripheral shell consisting of many layers surrounds the antrum. The layer is called ***stratum granulosum***. Smaller isolated intercellular accumulations of the follicular lluid may occur in the membrane granulosa. These may form round vesicles which stain deeply. The basal row of the granulosa cells is separated from the theca interna by a ***basement or glassy membrane***.

The mass of granulosa cells enclosing the mature ovum projects into the antrum, forming a hillock, the ***cumulus oophorous*** or ***discus proligerous***. The ovum is surrounded by prominent cona pellucida. The corona radiaty is a well-defined, radially arranged layer of columnar cells immediately surrounding the ***zona pellucida***. The cells of the corona radiata are peeled off from the zona pellucida when the oocyte passes down the oviduct following ovulation.

2.6.1 Types of Eggs

The animal eggs are classified according to four criteria. They are (i) the amount of yolk, (ii) the distribution of yolk, (iii) the presence or absence of shell and (iv) the type of development.

I. According to the amount of yolk

1. **Microlecithal eggs:** The eggs containing small amount of yolk are called

microlecithal eggs. Some embryologists called them as alecithal eggs or oligolecithal eggs. Example: Amphioxus, tunicates and eutherian mammals.

2. **Mesolecithal eggs:** Some eggs acquire a moderate amount of yolk and are described as mesolecithal eggs. Examples: Amphibia, Dipnoi and Petromyzontia.
3. **Macrolecithal or Megalecithal or Polylecithal eggs:** The eggs which contain enormous amount of yolk (food reserves) are called macrolecithal or megalecithal or polylecithal eggs. Examples- Myxinoidea, Chondrichthyes, Osteichthyes, Reptiles and Birds.

II. According to the distribution of yolk

1. **Isolecithal or Homolecithal eggs:** In isolecithal eggs the amount of yolk laid down is small and it is scattered fairly evenly throughout the cytoplasm Examples. Sponges, Amphiosus, tunicates and Futherian mammals.
2. **Telolecithal eggs:** The distribution of yolk is unequal. It is collected eccentrically in the lower part of the egg (vegetal pole). Many annelids and molluses and most amphibian have eggs of this type. They may be further subdivided as-

   i. Slightly Telolecithal- This type of egg contains only a small quantity of yolk which is distributed unevenly. The vegetal pole has the highest concentration and the animal pole the lower (e.g. eggs of fishes).

   ii. Moderately Telolecithal - This type of egg contains a moderate quantity of yolk which is distributed unevenly. Due to high concenteration of yolk in the vegetal hemisphere, the nucleus is shifted more towards the animal hemisphere (eg. Amphibian egg).

   iii. Extremely Telolecithal -In this type of egg, due to the heavy deposition of yolk, the entire vegetal hemisphere and a major portion of the animal hemisphere are occupied by yolk. Due to this extremely uneven distribution of yolk, the ooplasm and nucleus are displaced towards the animal pole (eg. reptilian and avian eggs).
3. **Centrolecithal eggs-** the amount of yolk is large and it is concentrated in the centre of the egg cell. Cytoplasm is distributed as a clear layer around the outside of the yolk. A tiny mass of cytoplasm containing the egg nucleus is also present in the centre of the egg. Eggs of this type are characteristic of some of coelenterates, most arthropods and insects.

4. **Discoidal eggs-**In these eggs, the amount of yolk is so enormous that it occupies the largest portion of the egg except a small dis-shaped area of the cytoplasm called blastodisc. The lastodise is found at the top of the yolk mass. Examples- Found in squids, octopuses, fishes, ptiles, birds and prototherian mammals.

III. According to the presence or absence of shell

1. **Cleidoic eggs-**Cleidoic means boxlike. The eggs of reptiles and birds which are laid on dry become self-contained, fully laden with yolk and are surrounded by albumen and water proof shell. Such eggs which have become self-sufficient in many aspects are called cleidoio eggs. Some terrestrial arthropods have evolved cleidoic eggs.
2. **Non-Cleidoic eggs-**The non-cleidosc eggs are not protected by shells. This type of eggs are characteristic of animals wherein the development is internal

IV. According to the type of development

a. **Mosaic Egg:** In certain eggs, every portion are predetermined with respect to its potentialities for further development. If a small portion of such an egg is removed, a defective embryo is formed, This is because removal of a portion results in a permanent loss from the egg. The remaining portion of the egg cannot make compensatory development to make good the lost part. Such an egg, in which the future developmental potentialities are predetermined in the form of a mosaic, is called mosaic or determinate egg (e.g annelids, Molluscs and ascidians).

b. **Regulative Egg:** In vertebrates and most of the invertebrates, the developmental potentialities are not predetermined in the eggs. Removal of a small portion of the egg, or even one or two early blastomeres will not affect the normal development. This type of egg in which the future developmental potentialities are not predetermined is known as regulative or indeterminate eggs.

2.6.2 Egg Membranes

The eggs are well protected by egg membranes. The membranes are produced either by the egg itself or by the follicle cells of the ovary or by the genital ducts

(oviduct) of the female, mother. Accordingly, the egg membranes are classified into three types. They are: 1. Primary membranes 2. Secondary membranes and 3. Tertiary membrane.

I. **Primary membranes:** The membranes secreted by egg cytoplasm (ooplasm) constitute the primary membrane. They are closely attached to the surface of the egg. The primary membranes are named differently in the different animals. They are described as follows:

   a. **Plasma Membrane:** It is the membrane covering the egg immediately It over it. is found in all the eggs in structure, It resembles the plasma membrane of a cell.

   b. **Vitelline Membrane:** It is closely attached to the plasma membrane of egg. Commonly found in Egg of Amphioxus. Molluscs. Echinoderms, Amphibians birds etc. It is very thin and and transparent. It is formed of mucopolysacharide and fibrous protein. The space formed between it and the plasma membrane is called perivitelline space filled with a fluid called perivitelline fluid.

   c. **Chorion:** It is found in the eggs of lower chordates like fishes (styela). It is a product of surface ooplasm.

   d. **Zona Radiata:** The egg of the shark Scyllium canicula, has two primary membranes produced by the surface ooplasm. The outer membrane is the vitetlline membrane and the inner membrane has a radiating appearance and hence called zona radiata . The eggs of teleost fishes are also covered by zona radiata.

   e. **Zona Pellucida:** All mammalian eggs are surrounded by a membrane called zona pellucida is also named as zone radiata. It is so named because it gives a striated appearance under the microscope. The striations are due to the presence of macrovilli in this zone. The microvilli are produced by the surface of the egg and microvilli are produced by follicle cells. They protrude into the zona pellucida.

II. **Secondary Membranes:** The secondary membranes are produced by the follicle cells (cells found around the developing oocytes) of the ovary. These membranes are usually tough and impermeable. The secondary membranes are as follows:

   a. **Chorion:** This is a common outer covering in the eggs of insects, Ascidians and cyclostomes (Myxine). It is found outside the vitelline membrane. As the chorion is tough and impermeable. It is provided with one or more openings called micropyles through which the sperms enter the egg.

b. Corona Radiata: It is found in mammalian eggs. This membrane is formed of a layer of follicle cells. The cells are radially arranged around the zona pellucida.

III. Tertiary Membranes:

The tertiary membranes are produced by the oviduct.

a. White Albumen: It is found in the egg of hen. It is found outside the vitelline membrane. It is formed of three layers-inner less dense albumen, a middle dense albumen and an outer less dense albumen. The albumen is formed of water and protein.

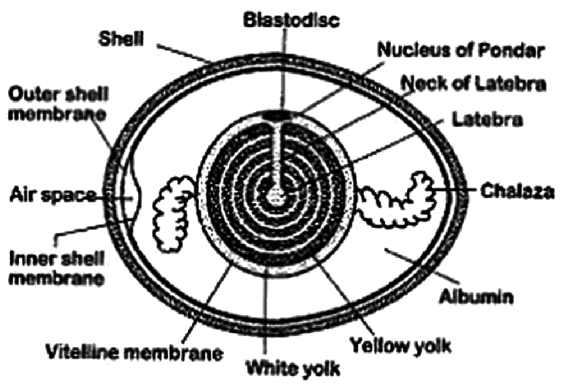


Fig. 2.6 : Egg with different structures and membranes

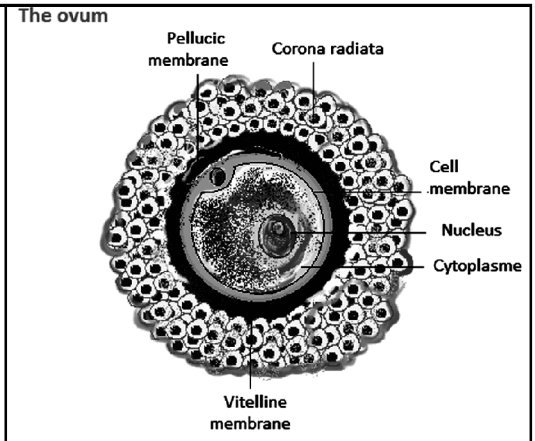


Fig. 2.7 : A Ovum with different layers

b. Shell Membrane: The shell membrane is formed around the albumen in the egg of hen. It is a double membrane. The two membranes adhere closely and are separated by an air space are the blunt end of the egg. This membrane is formed of keratin.

c. Shell: The shell is the outer covering of land animal's eggs. It is formed of calcium carbonate. It is white or brown in colour. It contains as many as 7000 minute pores. These pores are 0.04 to 0.05 mm in diameter. They are filled with a proteinous substance called collagen.

d. Jelly Coat: The amphibian eggs are surrounded by a gelatinous covering called jelly coat.

e. Mermaid's Purse: It is the egg case of some cartilaginous fishes. It is a protective hard shell secreted by the shell glands present in the oviduct. The shape of the purse varies from group to group.

2.6.3 *Questions*

1. What are the functions of an egg?
2. Describe the structure of a typical egg supported with a diagram.
3. Mention about the different types of eggs depending upon the amount of yolk and the distribution of yolk citing examples in the animal world?
4. What are cleidoic and non-cleiodoic egss? Give examples.
5. Differentiate between Regulative and Mosaic eggs?
6. What are the different types of egg membranes?
7. What is mermaid's purse?
8. What is the function of chalaza?
9. What is the function of air space in the egg?
10. What is shell membrane?
11. What are discoidal eggs?
12. What is a shell?
13. Write the characteristics of an insect egg?
14. What is macrolecithal egg?

2.7 Fertilization

Definition

The union of the cytoplasm and pronuclei of the male and female gametes is known as the fertilization (L., fertilis = to bear; frevo = to bear). The fertilization is the most commonly used method for the production of the diploid zygotes in the sexually reproducing organisms of Metazoa and Metaphyta.

In the process of fertilization, the haploid male gamete (spermatozoon or pollen), which carries the paternal genetic information of the male parent, unites with the haploid female gamete (ovum or egg), which carries the genetic information of female parent, to form a diploid zygote. The egg carries the maternal hereditary information in it. The zygote ultimately produces a diploid multicellular organism by the several repeated and organized mitotic divisions and cellular differentiation.

Site of Fertilization

Fertilization of the ova occurs either outside the body of the maternal parent (external fertilization) or inside the oviducts of the female (internal fertilization). The ***external fertilization*** is common in various invertebrates and chordates, while the

internal fertilization occurs only in those animals which possess specialized sex organs for receiving and transmitting the sperms, e.g., reptiles, birds, mammals and angiosperms, etc.

Mechanism of fertilization

The process of fertilization in animals is completed in the following steps.

(I) Encounter of spermatozoa and ova.

(II) Capacitation and contact.

(III) Acrosome reaction and penetration.

(IV) Activation of Ovum.

(V) Migration of pronuclei and amphimixis.

(I) Encounter of spermatozoa and ova- A major problem in sexual reproduction is how to bring together the spermatozoa and ova in the same locality in a fluid medium, so that individual sperms may reach the surface of ova at the right time. Previously it was thought that sperms are attracted towards the ripe eggs by chemotaxis. A chemical substance is found in the cortex of the eggs of sea urchin Companularia, fishes etc. held responsible for the attraction of sperms to ripe eggs. In general, most invertebrates, and vertebrates accomplish close approximation of spermatozoa and eggs through special devices or particular forms of behaviour. The primary needs for the encounter of spermatozoa and ova are fluid medium for the act of fertilization and delivery of large quantities of spermatozoa close to the numbers of ripe eggs at the right time, According to the place and nature of fluid media following two kinds of fertilization have been reported:

(i) External fertilization: This type of fertilization occurs in liquid medium outside the bodies of parent animals. Among fresh water animals (fishes, amphibians and fresh water invertebrates), the timing of spawning of egg by the female and shedding of sperms by the male parent are very specific. As their spermatozoa remain active usually for a few minutes, the sperms are delivered directly to the eggs of an individual female immediately after egg laying.

But marine forms shed eggs and sperms freely into the surrounding water. The time interval between the laying of eggs and the shedding of sperms may even be weeks or months, because the salty sea water serves as an important physiological medium for gametes.

During external fertilization, the movement of spermatozoa in a liquid medium is entirely at random and the spermatozoa collide with the eggs as a matter of chance which occurs regularly in nature, partly due to enormous number of spermatozoa produced by the male and partly because the eggs being relatively larger targets, they can be hit by sperms fairly well.

(ii) Internal fertilization: In oviparous forms such as reptiles and birds, the eggs are completely enclosed in impermeable egg membranes or they are retained within the maternal body throughout development in ovoviviparous and viviparous animals. In all such cases the spermatozoa are delivered internally in the body of the female by some type of copulatory mechanism or by intromittant organ of the male.

In such forms the fertilization may occur in the lower part of the oviduct (eg. Urodela), in the upper portion of oviduct (eg. Salamanders, reptiles, birds and mammals) or in the ovarian follicles in viviparous fishes (e.g. Gambusia offinis) and eutherian mammals (e.g. Ericulus).

In terrestrial animals, there is no problem of timing of the spawning of eggs and shedding of sperms because the mature sperms are commonly stored in a physiological medium capable of maintaining their life and potential activity for days or even for months, either in moisture conserving capsules or in compartments of male and female body to be picked up, transferred or utilized in one way or another.

The movement of the spermatozoa from the site of deposition to the site of fertilization usually depends on the active swimming of the spermatozoa themselves or transported passively by muscular contractions of the female tract and also by the counter currents in the cilia which propel backward flowing liquid content of the tract.

(II) Capacitation and Contact-

In some animal species like mammals, where fertilization is internal the spermatozoa undergo a process called capacitation, before they are fully capable of fertilizing an egg of the same species or the change in spermatozoa which makes it capable of fertilizing the egg has been called capacitation. The phenomenon of capacitation relates to the specificity of fertilization, and the attainment of ability to fertilize the egg.

In case of animals with external fertilization this specificity of fertilization is brought about by the help of chemo-attraction, agglutination and fertilizin antifertilizin reaction which act from a distance within the surround egg water or fluid. Frank Lillie

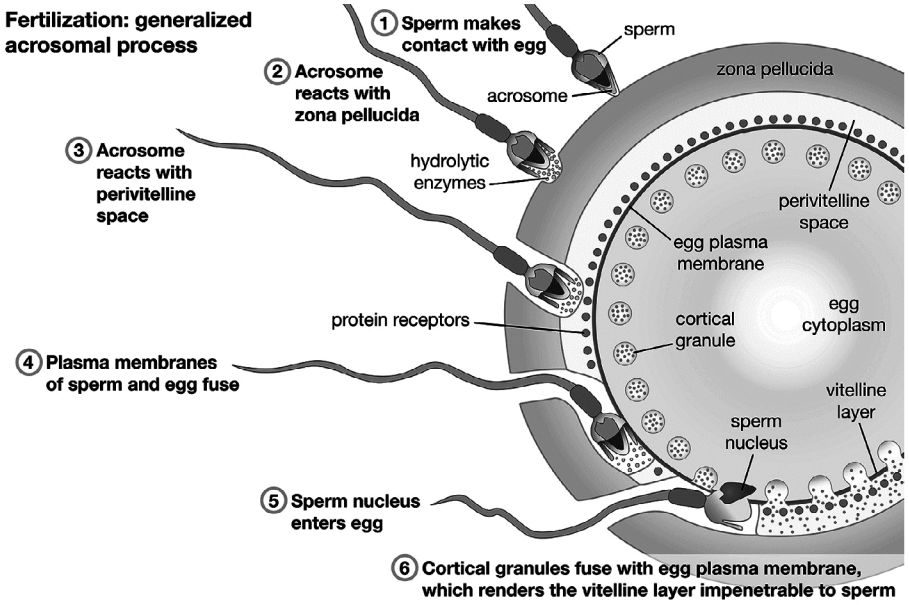


Fig. 2.8 : Fertilization

was the first to show that a chemical substance is discharged by the newly laid sea urchin egg. This substance diffuses into the sea water and causes sperm cells in the neighbourhood to become more active and attracted to the cell. This condition is called as chemotaxis, implying that the sperms were responding to this chemical substance. This substance has been found in the jelly which surrounds the eggs.

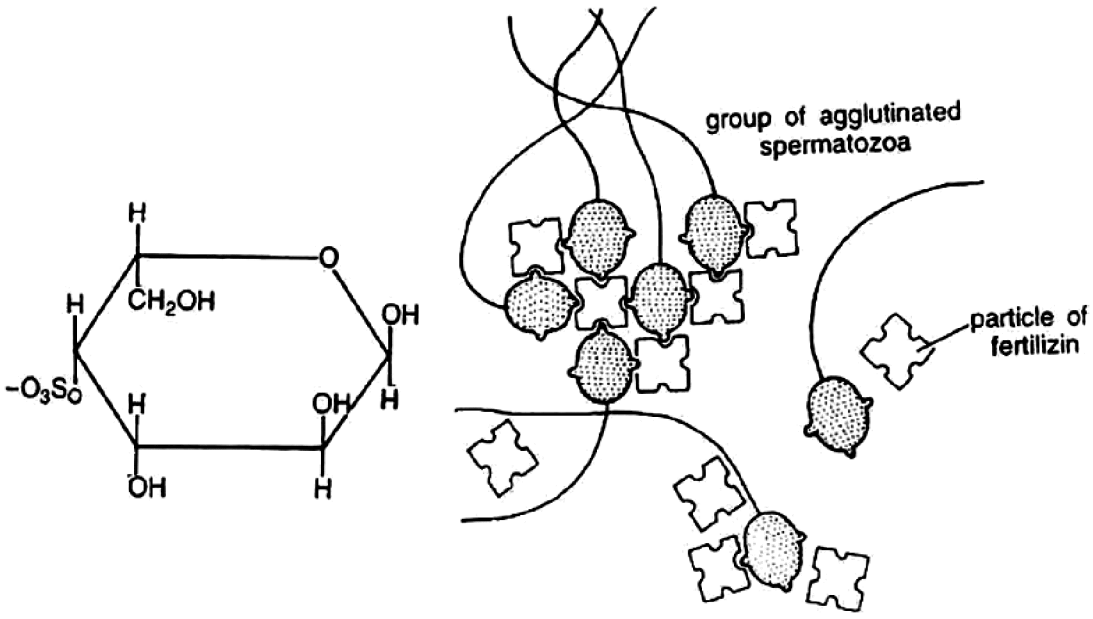
1. **Agglutination:** In most animals it has been observed that in the presence of ripe eggs or even the water in which ripe eggs of the same species have been lying for some time, the spermatozoa adhere to the surface of the egg by its lateral side and even to each other.

   This reaction is usually visible within a few seconds and the spermatozoa are seen to clump together head to head or less commonly tail to tail. This adhesion of spermatozoa results in their clumping or agglutination. It depends to a large extent on the environmental conditions.

2. **Fertilizin-antifertilizin reaction:** The cause of agglutination of spermatozoa was studied by Lillie (1919). He observed that fertilizin and antifertilizin occur in the eggs and sperms respectively. They are directly involved in the reaction between the egg and the spermatozoon.

The main source of fertilizin is the egg itself and it is located in the plasma membrane. However, in the eggs of a sea urchin and other echinoderms, it is

produced by the layer of jelly surrounding the egg, and becomes accumulated in the external gelatinous coat. The fertilizin is formed of glycoprotein or mucopolysaccharide. As a protein, it contains a number of aminoacids and as a polysaccharide it includes molecules of one or more monosaccharides. The monosaccharides glucose, fructose, fucose or galactose esterified by sulphuric as shown in the following Agglutination of Sperms are acid gel protein of formula.



Agglutination of Sperms

Fig. 2.9 : Fertilizin & antifertilizin reaction

Both the aminoacids and polysaccharides vary from one species to another, which is why each species possesses it specific type of fertilizin. The molecules of fertilizins are quite largethe molecular weight is about 300,000—and each molecule may have more than one “active group", so that one fertilizin particle may become attached to two or more spermatozoa thus binding them together. The surface layer of cytoplasm of spermatozoa (i.e. sperm plasma membrane) contains another species specific acid protein known as antifertilizin (or antifertilizins). The antifertilizins can be extracted from the spermatozoa by heating, freezing and thawing, or acidifying the water. The remarkable peculiarity of the fertilizins and antifertilizins is that they combine in a specific manner; that is, the egg fertilizin of a particular species reacts best with the sperm antifertilizin of the same species.

Reactions bet en species which are nearly related to each other are very much weaker. The reaction between fertilizin and antifertilizin is very much similar to the reaction between antigen and antibody. In both cases, a "chemical lock" is formed between two complementary substances.

Thus, during capacitation and contact stage of fertilization when spermatozoa and eggs same species come in physical contact of each other, a chemical lock is established between the antifertilizin molecules of spermatozoa and fertilizin molecules of unfertilized egg and due to the fact many spermatozoa adhere to the surface of an unfertilized egg.

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.

Sperm capacitation- Sperm capacitation refers to the physiological changes spermatozoa must undergo in order to have the ability to penetrate and fertilize an egg. This term was first coined in 1952 by Colin Russell Austin. Recognition of the phenomenon was quite important to early in vitro fertilization experiments as well as to the fields of embryology and reproductive biology.

It is very difficult to study the interactions that might be occurring between mammalian gametes prior to sperm-egg contact. One obvious reason for this is that mammalian fertilization occurs inside the oviducts of the female. A second reason for this difficulty is that the sperm population ejaculated into the female is probably very heterogeneous, containing spermatozoa at different stages of maturation. Of the 280 $\times$ 10^6 human sperm normally ejaculated into the vagina, only about 200 reach the ampullary region of the oviduct, where fertilization takes place (Ralt et al. 1991). Since fewer than 1 in 10,000 sperm get close to the egg, it is difficult to assay those molecules that might enable the sperm to swim toward the egg and become activated.

The reproductive tract of female mammals plays a very active role in the mammalian fertilization process. While sperm motility is required for mouse sperm to encounter the egg once it is in the oviduct, sperm motility is probably a minor factor in getting the sperm into the oviduct in the first place. By whatever means, mammalian sperm pass through the uterus and oviduct, interacting with the cells and secretions of the female reproductive tract as they do so. These interactions are critical for their ability to interact with the egg. Newly ejaculated mammalian sperm are unable to undergo the acrosomal reaction without residing for some time in the female reproductive tract. ***The set of physiological changes that allow the sperm to be competent to fertilize the egg is called capacitation.*** The requirement for capacitation varies from species to species. Eisenbach (1995) has proposed a hypothesis wherein capacitation is a transient event, and sperm are given a relatively brief window of competence in which they can successfully fertilize the egg. As the sperm reach the ampulla, they acquire competence, but if they stay around too long,

they lose it. Sperm may also have different survival rates depending on their location within the reproductive tract, and this may allow some sperm to arrive late but with better chance of success than those that have arrived days earlier.

The molecular changes for capacitation include four sets of molecular

- The fluidity of the sperm plasma membrane is altered by the removal of cholesterol by albumin proteins found in the female reproductive tract.
- Particular proteins carbohydrates on the sperm surface lost during capacitation. It is possible that these compounds block the recognition sites for the proteins that bind to the zona pellucida.
- The membrane potential of the sperm becomes more negative as potassium ions leave the sperm. This change in membrane potential allows calcium channels to be opened and permit calcium to enter the sperm. Calcium and bicarbonate ions activate cAMP production and facilitates the membrane fusion events of the acrosomal reaction.
- Protein phosphorylation occurs.

(III) Acrosome reaction and penetration:

As mentioned earlier, except poriferans and coelenterates, where the surface of the ripe egg is seldom naked, most animal eggs are enveloped by one or more egg membranes or gelatinous layers or follicle cells or both, outside the plasma membrane. These layers constitute barriers for the penetration by spermatozoa and serve in preventing fertilization by more than one spermatozoon or by sperm of other species.

When a spermatozoon is attached to surface of the egg, it becomes motionless. Its penetration through egg membranes and also through the plasma membrane of the egg is achieved by some physiochemical activity of the sperm acrosome. Certain enzymatic proteins called sperm lysins are produced presumably by the sperm acrosome.

The **sperm lysins** differ from one animal group to another. In some cases the dissolution of the egg envelopes may be brought about by simpler means. Thus it is believed that the jelly coat of echinoderm eggs may be dissolved as a result of acidification of seawater by carbondioxide produced by the spermatozoa in the course of their respiration.

In the case of eggs with very thick and resistant envelopes, such as the egg envelopes of fishes and insects, the sperm cannot reach the egg at all points but must penetrate through a special canal, the micropyle, left in the egg envelope, the **chorion**.

In mammals, when the eggs are released from the ovary, they are commonly encased in a layer of follicular cells, called corona radiata. These cells are held together by an adhesive cementing substance called hyaluronic acid, a mucopolysaccharide. The corona radiata, thus, acts as a barrier through which the spermatozoon must first penetrate to reach the plasma membrane of the egg.

For this purpose, the sperm produce an enzyme, hyaluronidase, which serves to dissolve the adhesive cells of corona radiata. The breaking of the membranous barriers is not only mediated by lytic agents provided by the acrosome of the spermatozoon, but the acrosome itself undergoes morphological changes and forms acrosomal filament which help the sperm penetration into the egg interior. The entire acrosome reaction has been well illustrated in echinoderms, annelids and Saccoglossus.

Acrosome reaction and penetration in Saccoglossus:

Penetration and acrosomal reaction of spermatozoa of Saccoglossus has been best described by Calwin and Colwin (1967). A spermatozoon of Saccoglossus has spherical nucleus, a flat tail and an acrosomal vesicle at the forwarding end of the sperm head. The acrosomal vesicle is bounded by an acrosomal membrane and

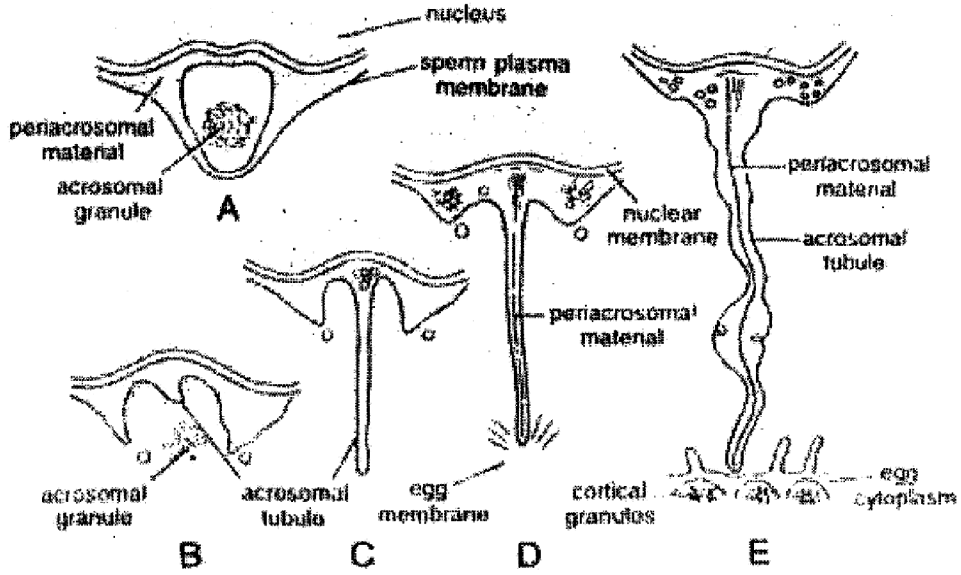


Fig. 2.10 : Acrosome Reaction in Saccoglossus: A-Acrosome of inactivated sperm; B-Extrusion of acrosomal vesicles; C-Formation of acrosomal tubule; D-Acrosomal tubule reaches egg membrane; E-Acrosomal filament reaches the surface of egg.

contains a large, dense acrosomal granule. The granule is surrounded in large part by fine, grainy material except at the apex where an apical space lies between the granule and membrane. The space between the acrosomal membrane and sperm plasma membrane and also, the space between acrosomal membrane, and nuclear membrane, filled by A Some called periacrosomal material,

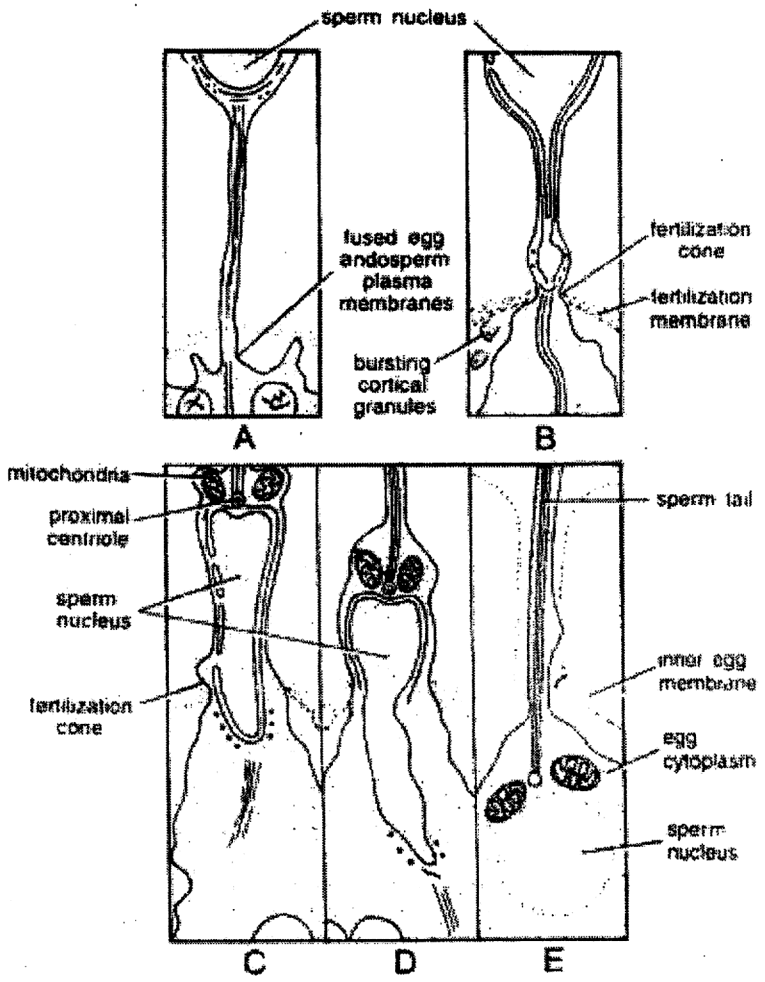


Fig. 2.11 : Spermatozoon of Saccoglossus penetrating into the egg cytoplasm.

As the spermatozzon of Saccoglossus makes its initial contact with the egg envelope, the following events occur.

(a) Bursting of Acrosome: Apex of the acrosome bursts so that the membrane of sperm and acrosome open apically and consequently expose the interior of

the acrosomal vesicle to the outside. The two membranes join around the margin of the opening.

(b) **Release of lytic enzymes:** Acrosomal granule is released and comes in contact with the egg envelope. It contains lytic enzymes which make passage through the egg envelopes. Therefore, shortly after its release, the acrosomal granule disintegrates and disappears.

(c) **Formation of acrosomal tubule:** Shallow depression of the acrosomal membrane close to the nucleus now deepens and soon lengthens into a long slender acrosomal tubule. The tubule becomes twice as long as the sperm nucleus.

(d) **Eversion of the acrosomal membrane:** Rest of the acrosomal membrane averts and is added to the ecrosomal tubule at its base. This is simply an unfolding of the already continuous membrane

(e) **Fusion of acrosomal tubule with egg membrane:** Acrosomal tubule gradually enters through the passage of egg envelope, which has been made previously by acrosomal lytic enzymes, and ultimately touches and fuses with the egg plasma membrane.

(f) **Passage of sperm contents:** Fertilization cone protrudes from the egg and engulfs acrosomal tubule. The nucleus of spermatozoon is drawn out towards fertilization cone. cytoplasm.

The acrosomal tubule dissolves. elongated nucleus along with middle piece of spermatozoon is engulfed into the egg cytoplasm.

In other animals similar types of events have been observed during the penetration of sperm into the egg envelope, i.e. before penetrating the egg contents and before activating the egg for further developmental events, the spermatozoa of many animals themselves get activated like the spermatozoa of saccoglossus.

In them sperm activation includes rupturing of acrosome and formation of acrosomal filament or acrosomal tubule. However, the number and size of acrosomal tubules may vary from species to species, as some annelids (eg. Hidroids heragonus) have several acrosomal tubules.

The mammalian spermatozoa though possess acrosome do not develop acrosomal filaments. The spermatozoon appears to contact the surface of the egg by its lateral aspect. Following this action, the plasma membrane of the egg and the spermatozoon dissolve at the point of contact, and the spermatozoon is drawn into the interior of the egg.

(IV) Activation of ovum:

Activation of ovum is that aspect of fertilization by which an egg is released from its inactive state and begins to develop. As soon as the apex of acrosomal tubule of a spermatozoon touches the surface of egg plasma membrane fusion of both membranes (i.e. plasma membranes of sperm and egg) over this limited area of contact takes place and a single continuous mosaic membrane is formed.

Thus, the plasma membrane of both gametes (sperm and ovum) becomes continuous and forms a single cell, called zygote. At this very time, certain very important changes occur in the cytoplasm of egg:

(1) Fertilization cone formation.

(2) Cortical reactions and formation of fertilization membrane.

(3) Metabolic activation.

1. Fertilization cone formation:

Immediately after the acrosomal filament of spermatozoon touches the surface of the egg, the cytoplasm of the egg bulges forward at the point of contact, producing a process of hyaline cytoplasm called the fertilization cone.

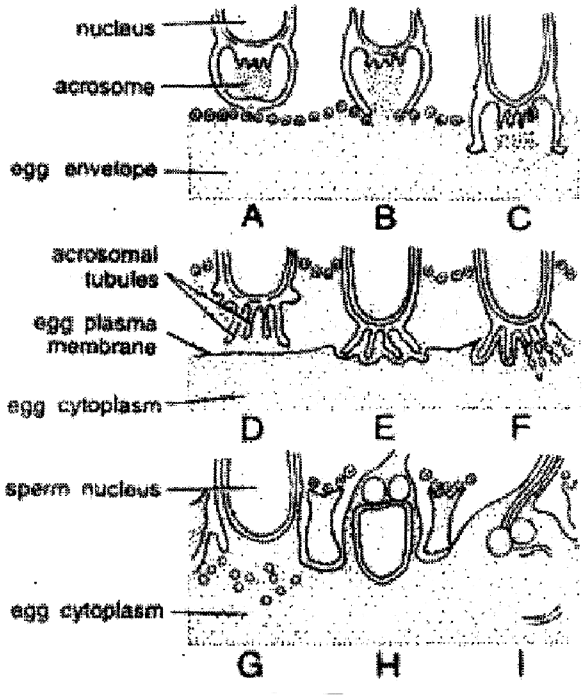


Fig. 2.12: Formation of fertilization cone

Fertilization cone develops or appears in many forms. It may be in the form of a more or less simple conical protrusion or it may consist of several irregular pseudopodium like processes, or in some cases it may take the form of a cytoplasmic cylinder stretching forward along the acrosomal filament or tubule whatever its shape, the fertilization cone gradually engulfs the spermatozoon and then begins to retract.

Here one point should be clear that normally, the spermatozoon does not enter the city cytoplasi intact. nor 1 is swallowed but the sperm nucleus and other sperm structures (renacrosomal matenal,

proximal Controle and mutochondria of mid piece of spermatozoon) pass to the fertilization cone of the egg The plasma membrane of sperm becomes one entity of plasma membrane of the egg.

Further during the whole penetration process of sperms into the che, acrosomal granule never makes its entry into the egg, but only the pericrosomal material is injected into the egg cytoplasm along with other contents of the sperm Some workers suggest that this periacrosomal material is responsible for the activation of egg.

There exists some variation in different animals, as to how much of the spermatozoon is taken into the interior of egg during fertilization. In mammals, complete structures of spermatozoon (viz., nucleus, mid piece, tail etc.) penetrate into the egg cytoplasm.

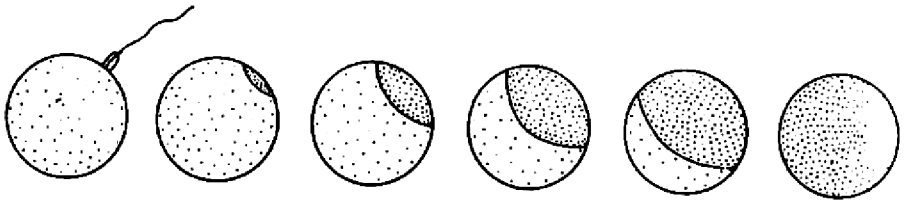


Fig. 2.13: Spreading of cortical change (black) in a sea-urchin egg after fertilization. The figure at the left shows the moment when spermatozoon contacts the egg surface.

In echinoderms, sperm tail remains exterior to the vitelline membrane; in Nereis only sperm head and proximal Centriole enter the egg cytoplasm. In most animals, however, the sperm nucleus and mid piece make their entry in the egg as a rule.

2. Cortical reactions and formation of fertilization membrane (*The slow block to polyspermy*):

Even before the fertilization cone is formed and the spermatozoon penetrates into the interior of egg, a chain of physio-chemical reactions is set in the egg cortex. All these reactions are collectively called cortical reactions.

These reactions may differ from one group of animals to another, but in most groups, the cortical reactions lead to the formation of fertilization membrane outside the egg plasma membrane. This membrane blocks the entry of the late arriving spermatozoa in the egg interior, and thus avoids polyspermy and is known as the slow block to polyspermy. The process of cortical reactions and fertilization membrane formation in different groups of animals is as under:

(a) Sea urchins: In sea urchins, as soon as the apical end of acrosomal tubule touches the surface of egg, from the site of contact, a wavelike color change from

yellow to white (under dark field microscopy) travels rapidly around the egg cortex and is shortly followed by the elevation of fertilization cone from the egg surface and the formation of fertilization membrane around the egg plasma membrane.

Electron micrographs of sea urchins unfertilized eggs show that the egg cortex is bounded by two membranes (i) an outer 30 Å thik, vitelline membrane, and (ii) an inner, 60 Å thick plasma membrane. Beneath the plasma membrane occurs a layer or cortical granules. A fertilization membrane is formed through stages different. The outer vitelline membrane separated from the plasma membrane, undergoes expansion and becomes the outer layer of the fertilization membrane. The cortical granules explode and release the following three components:

(1) Dark, denser, lamellar and folded parts of the granules—these lamellar bodies unfold and fuse with the inner side of the already elevating membrane, the vitelline membrane.

(2) Globules, which fuse together and build up a new surface of the viscous hyaline layer, just at the outer side of the egg plasma membrane. The hyaline layer adheres closely to the surface of the egg and during cleavage; it helps to keep the blastomeres together.

(3) The liquified component of the cortical granules fills the perivitelline space between the new egg surface and now the completed and elevated fertilization membrane. It contains mucopolysaccharides and abundant water.

All these structures, namely, vitelline membrane and contents of cortical granules, thus form a fertilization membrane, which is much thicker (upto 900 Å) and stronger. Among other invertebrates, fertilization membrane formation has been observed only in certain annelids (e.g., *Nereis*). In others no structural but metabolic changes have been observed in egg cortex.

(b) Vertebrates: In vertebrates the changes which occur in the cortex are similar to sea urchins. In bony fishes and frog, the cortical granules are broken down immediately after sperm's penetration into the egg cytoplasm.

Their contents become liquified and extruded on the surface of the plasma membrane of the egg and fill the perivitelline space occurring in between the chorion and plasma membrane and in between the vitelline membrane and egg plasma membrane in the frog.

In both cases, the vitelline membrane or chorion itself does not transform into the fertilization membrane as in sea urchins. The chorion in fishes becomes hardened or

"tanned' after fertilization and no new membrane is formed in either of the two animals.

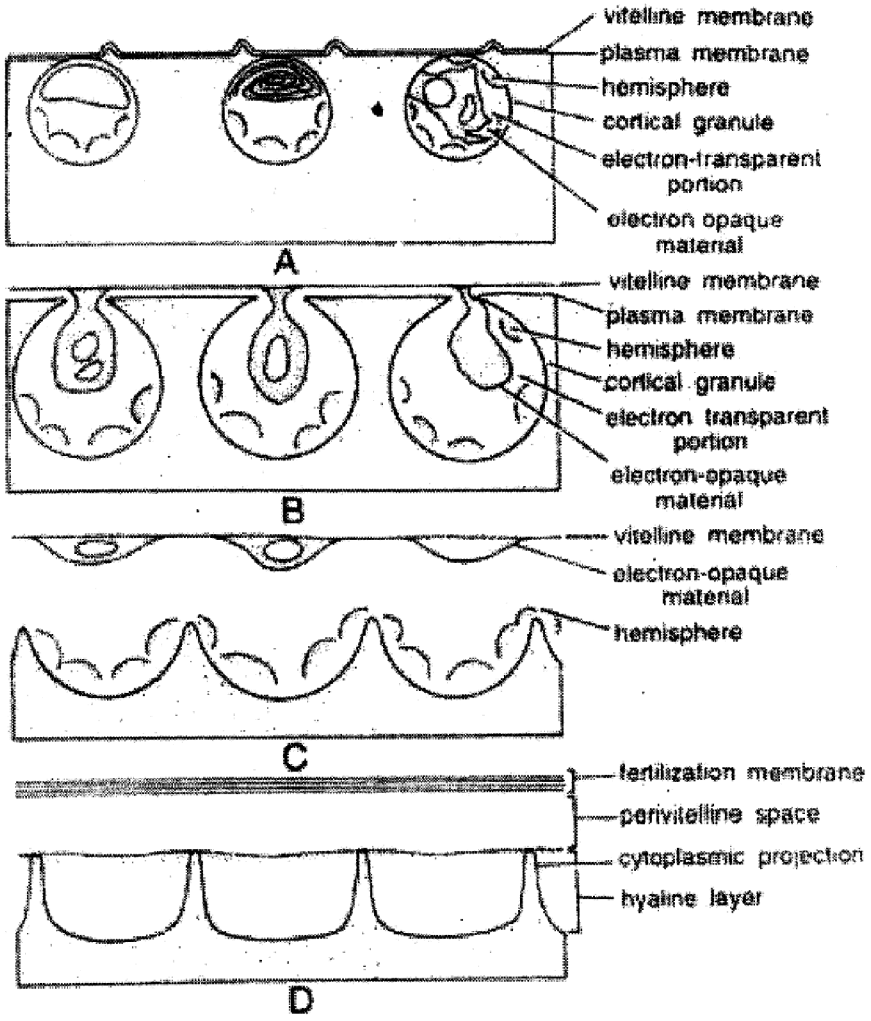


Fig. 2.14: Cortical reaction and fertilization membrane formation-A : Unfertilised egg; B: Fusion of egg plasma membrane and membrane of cortical granules; C: Adhesion of opaque material to Vitelline membrane; D: Fertilization membrane.

In some mammals (man, rabbit, etc.) the cortical granules burst and release their contents into the perivitelline space created between the egg plasmalemma and the zona pellucida.

In amphibians (Urodela) and some mammals, which lack cortical granules, neither any cortical reaction nor fertilization membrane formation occurs.

The fast block to polyspermy- The fast block to poly-spermy is achieved by changing the electric potential of the egg plasma membrane. This membrane provides a selective barrier between the egg cytoplasm and the outside environment and the ionic concentration of the egg differs greatly from that of its surroundings. This concentration difference is especially significant for sodium and potassium ions. Seawater has a particularly high sodium ion concentration, whereas the egg cytoplasm contains relatively little sodium. The reverse is the case with potassium ions. This condition is maintained by the plasma membrane, which steadfastly inhibits the entry of sodium ions into the oocyte and prevents potassium ions from leaking out into the environment. This resting membrane potential is generally about 70 mV, usually expressed as -70 mV because the inside of the cell is negatively charged with respect to the exterior.

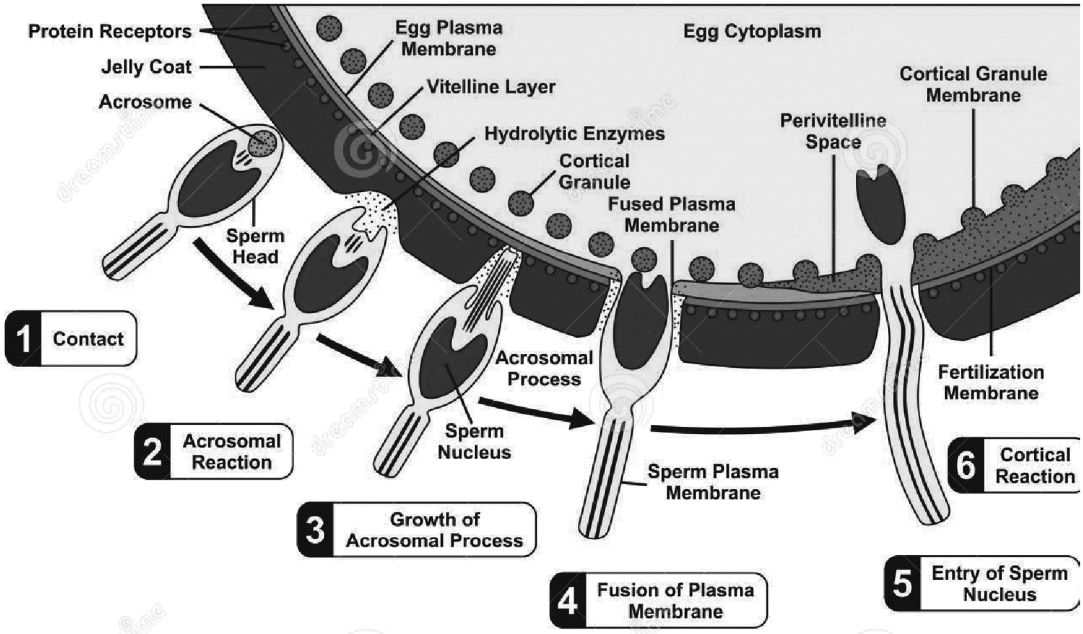


Fig. 2.15 : Fertilization and Cortical reaction steps in Sea Urchin egg

Within 1-3 seconds after the binding of the first sperm, the membrane potential shifts to a positive level, about +20 mV (Longo et al. 1986). This change is caused by a small influx of sodium ions into the egg. Although sperm can fuse with membranes having a resting potential of 70 mV, they cannot fuse with membranes having a positive resting potential, so no more sperm can fuse to the egg. It is not known whether the increased sodium permeability is due to the binding of the first sperm or to the fusion of the first sperm with the egg.

3. Metabolic activation: After the sperm penetrates the unfertilized egg a series of diverse cytoplasmic reactions are initiated. Following metabolic changes occur in the egg at fertilization.

(a) ***Changes in plasma membrane:*** The permeability of plasma membrane increases for the molecules of water and certain other chemicals like ethylene glycol, phosphate, K+ etc. At fertilization, the electrical potentiality of plasma membrane becomes more positive and it gradually becomes more negative. The change in the potentiality of the membrane is governed by the unequal distribution of chloride ions. Besides this a plasma membrane enzyme, adenyl cyclase becomes activated at the time of fertilization and it starts the formation of a chemical molecule 3 5' cyclic AMP, which is supposed to activate most of the metabolic reactions in a fertilized egg

(b) ***Ionic changes:*** Certain intracellular changes occur in the concentration of cations, especially those of sodium, potassium and calcium. The change in calcium ion concentration in a fertilized egg has great significance in the metabolic activation of the egg.

(c) ***Changes in the rate of respiration:*** In a fertilized egg, the rate of respiration either increases (e.g. sea urchins) or decreases (e.g. Chaetopterus and Molluscs). There appears to be a relation between the post-fertilization oxygen consumption and the stage of maturation of the egg at fertilization.

Because at the time of fertilization the sea urchin egg has completed maturation, the egg of Bufo is at second maturation division stage and the egg of Chaetopterus is at the first maturation division stage. The increased oxygen consumption is related with the oxidation of glycogen and other food stuffs of the egg and synthesis of numerous ATP molecules.

(d) ***Co-enzyme changes:*** The primary action of the spermatozoon consists of the release or activation of the oxidative enzymes of the egg and that the ensuing increase in oxidation provides the energy necessary for the performance of other changes in the egg and for the development of the egg in general.

In a fertilized egg inter conversion of pyridine coenzyme, NAD into another co-enzyme NADP and also NADPH due to phosphorylation of the NAD in the presence of a enzyme NAD Kinase takes place. NAD + ATP $\rightarrow$ NAD Kinase NADP + ADP

There are ample evidences that NAD kinase enzyme, though present in the unfertilized egg, it exists in an activated state. It is activated only at the time of fertilization. The increased NADP and NADPH contents may initiate many synthetic pathways of fertilized egg.

(e) Changes in the rate of protein synthesis: The cytoplasm of a mature unfertilized egg, though contains complete machinery for protein synthesis, such as DNA molecules, IRNA, MRNA, ribosomes and proteolytic enzymes required during protein synthesis, none or very little protein synthesis occurs because the mRNA of unfertilized egg remains "masked".

There are evidences that during later phases of oogenesis some inhibitor or repressor proteins are manufactured in sea urchins egg which inactivate chromosomal genes, mRNA molecules, ribosomes etc.

During fertilization there is an increase in proteolytic activity of the egg immediately following the penetration of spermatozoon which removes these inhibitor proteins from them and unmasks the mRNA and active protein synthesis is started. In the egg of frog, however, the rate of protein synthesis is increased quite early at the stage of ovulation itself.

(f) Initiation of mitosis: The initiation of mitosis for cleavage is the most significant aspect of egg activation. The initiation of mitosis occurs because (i) The rate of DNA synthesis increases with great pace immediately after fertilization; (ii) the unfertilized egg cytoplasm although possesses a Centriole, yet this Centriole is incapable of division and also to form a mitotic spindle. Thus sperm stimulates the first mitotic division (cleavage) of fertilized egg by contributing its Centriole to the egg.

(V) Migration of pronuclei and amphimixis:

At the time of penetration of spermatozoon inside the egg cytoplasm, the sperm nucleus remains compact and its mitochondria and Centriole remain situated behind it. To perform the act of amphimixis, the sperm nucleus has to undergo two activities (i) it has to become pronucleus, and (ii) it has to migrate from the site of amphimixis.

As the sperm nucleus moves inwards from the site of fertilization cone, it soon rotates through an angle of 180°C, so that its mitochondria and Centriole assume the leading position. Besides this rotation, the sperm nucleus starts swelling and its chromatin, which is very closely packed, becomes finely granular. It ultimately becomes vesicular and has an appearance like the interphase nucleus and is called male pronucleus.

At the same time, the sperm aster forms around the proximal Centriole of the sperm in the egg cytoplasm. As the male pronucleus develops and migrates towards the site of amphimixis, the sperm aster seems to lead it.

The site of amphimixis lies either near the centre of microlecithal and mesolecithal eggs or in the centre of the active cytoplasm at the animal pole of macrolecithal and Teleolecithal eggs. As the sperm pronucleus and Centriole move inward, it may be accompanied by some cortical and subcortical cytoplasm.

If the latter is heavily pigmented, as in amphibian eggs, the trajectory of the sperm pronucleus may be marked by pigmented granules trailing along its path. This is called penetration path.

This movement of the sperm appears to be directed and some investigators feel that it due to a chemotonic effect of chemicals liberated by the female pronucleus. During this movement toward the female pronucleus, the sperm may have to deviate from its penetration path. If it does the new pathway is taken. This is referred to as copulation pathway. In some cases the sperm need not alter its direction. In these cases the penetration and copulation paths would B be identical. The point of entrance. Possible sperm paths during fertilization penetration path and copulation path are all believed to be responsible for establishing the primary plane of bilateral symmetry in the embryo.

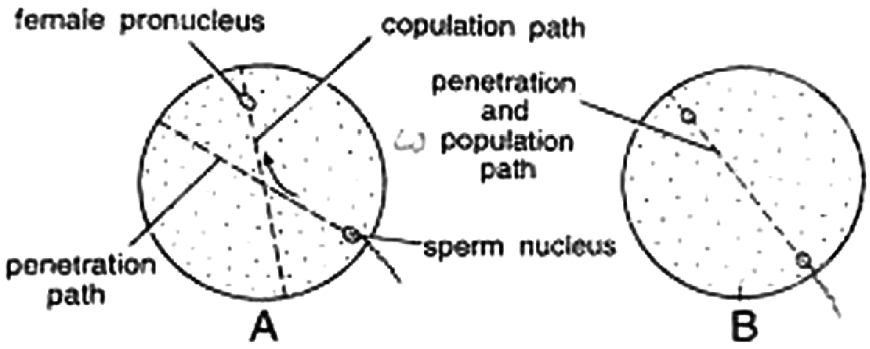


Fig. 2.16 : Possible sperm paths during fertilization

Before amphimixis the nucleus of the egg also undergoes certain changes like the sperm nucleus. After the completion of the second meiotic division, the haploid nucleus of the egg is located near the surface of the egg in the form of several vesicles, known as karyomeres In a fertilizing egg, these karyomeres fuse together to form a female pronucleus which swells increase in volume and becomes vesicular. It also migrates towards the site of Amphimixis.

Amphimixis: The fusion of male and female pronuclei is called as amphimixis. The actual fusion of pronuclei may differ in different animal.

(1) In sea urchins and vertebrates, the nuclear membranes of both pronuclei are broken down at the point of contact and their content unite in one mass surrounded by a common nuclear membrane. At the approach of first cleavage of fertilized egg, the nuclear membrane dissolves, chromosomes of maternal and paternal origin become arranged on the equator of the achromatic spindle.

(2) In Ascaris, some molluscs and annelids, the male and female pronuclei don't fuse but the nuclear membranes in both dissolve and the chromosomes become released. In the meantime, the Centrosome of the spermatozoon has divided into two and the Centrosome derived from male and female pronuclei become attached to the spindle.

Only after the completion of the first division of the fertilized egg, the paternal and maternal chromosomes become enclosed by common nuclear membrane to form the nuclei of two blastomeres into which the egg has been divided in both these types (1 and 2), the chromosomes of maternal and paternal set retain, of course, their individuality.

(3) In the Copepod Cyclops, the paternal and maternal nuclear components remain separate for some time even after cleavage has started, so that each blastomere has a double nucleus consisting of two parts lying side by side and each surrounded by its own nuclear membrane.

Post-fertilization changes in the egg cytoplasm:

The penetration of spermatozoa into the egg causes far-reaching displacements of the cytoplasmic constituents. As a result, the distribution of various cytoplasmic substances and inclusions in a fertilized egg may be very considerably different from that in the unfertilized egg and even quantitatively new areas may appear.

As a result of the extrusion of cortical granules, a large part of the original outer egg cell surface is replaced by the inner surface which surrounds the cortical granules and now are averted on the exterior. Most spectacular post-fertilization displacements in the egg cytoplasm have been observed in Ascidian, Styela partita and in frog. In both these animals, there establishes a bilateral symmetry in the cytoplasm of fertilized eggs.

The mature egg is covered by a layer of cortical cytoplasm containing yellow

granules. The moment spermatozoon enters the egg near the vegetal pole, the yellow cortical cytoplasm falls into violent commotion.

The yellow cytoplasm begins to stream down along the surface of the egg toward vegetal pole, and accumulated as a cap. As the male pronucleus penetrates deeper into the cytoplasm and moves toward female pronucleus, the yellow cytoplasm, reverses its movements and streams upward only on the side where the spermatozoon entered the egg. Just before the first cleavage, the yellow cytoplasm forms a cresentic arca (mesodermal crescent) just below the equator of the egg.

Simultaneously, a crescent of light gray cytoplasm (notochordal crescent) appears subequatorially on the opposite side of the egg. Thus, in a fertilized egg, four different kinds of cytoplasmic regions are now present-(i) The yellow cytoplasm on one side, and (ii) the light cytoplasm on the other side. These two together form a belt surrounding the egg just below the equator. Below this zone toward the vegetal pole, (iii) the cytoplasm in salty grey colour contains abundant yolk granules and in the sub cortical layer, there are a large number of mitochondria. But the cytoplasm in (iv) the animal hemisphere contains less yolk and a few mitochondria and appears more transparent. Thus, the cytoplasmic displacements following fertilization not only bring some kind of cytoplasm to more restricted areas, but also give the egg a distinct bilateral structure.

Significance of fertilization: The process of fertilization has following significances:

(1) The fertilization ensures the usual specific diploidy of the organisms by the fusion of the male and female pronuclei.

(2) It introduces genetic variations in the species.

(3) It activates the egg to start cleavage.

2.7.1 *Questions*

1. What is fertilization?
2. What is acrosomal tubule?
3. What is bindin?
4. What is chemotaxis?
5. What is agglutination?
6. State the role of hyaluronidase in fertilization?

7. What is the site of fertilization?
8. Give the biochemical nature of fertilizing along with its chemical structure?
9. State the significance of fertilization?
10. Differentiate between external and internal fertilization?
11. State the steps in fertilization?
12. What is Acrososme reaction? Describe the process aided with diagram.
13. What is Fertilizin and Anti-Fertilizin reaction?
14. What is capacitation? Describe the biochemical and molecular basis of the process?
15. What is block to polyspermy? Why is it essential?
16. Describe the types of block to polyspermy?
17. What is cortical reaction?
18. Describe the formation of fertilization membrane aided with diagram?
19. Describe the slow block to polyspermy?
20. Describe the fast block to polyspermy? In which animals does it occur?
21. Enumerate the metabolic changes that occur in the egg/ovum at fertilization?
22. What is penetration path? How does the pronuclei migration occur during fertilization?
23. What is Amphimixis? Describe the process briefly in different animals?

2.8 Planes and Patterns of Clavage

1. Meaning of Cleavage :

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

At first, the cells remain closely asso-ciated, but later on they form the lining of a hollow sphere called blastula. The blastula contains a cavity named blastocoel and its outer covering is designated as blastoderm. The formation of blastula culminates the cleavage period.

The process of segmentation prepares the groundwork for the future design of the embryo by producing adequate number of cells. The cleavage also establishes the fundamental conditions for the initiation of next developmental stage Gastrulation.

2. Planes of Cleavage :

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

The planes of division are:

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

b. ***Vertical plane of cleavage:*** When a furrow passes in any direction (does not pass through the median axis) from the animal pole towards the opposite pole.

c. ***Equatorial plane of cleavage:*** This type of cleavage plane divides the egg halfway between the animal and vegetal poles and the line of division mums 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 planc.

3. Types of Cleavage:

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

Different types of cleavage encountered in different eggs are given below:

1. **Holoblastic or total cleavage:** When the cleavage furrows divide the entire egg. When the cleavage furrow cuts the egg into two equal cells, it is called ***equal holoblastic*** (e.g. Amphioxus, marsupials and placental mammals). When the resultant blastomeres become unequal in size it is called unequal holoblastic (lower fishes and amphibians). It may be radially symmetrical, bilaterally symme-trical, spirally symmetrical or irregular. In the absence of a large concentration of yolk, four major cleavage types can be observed in isolecithal cells (cells with a small even distribution of yolk) or in mesolecithal

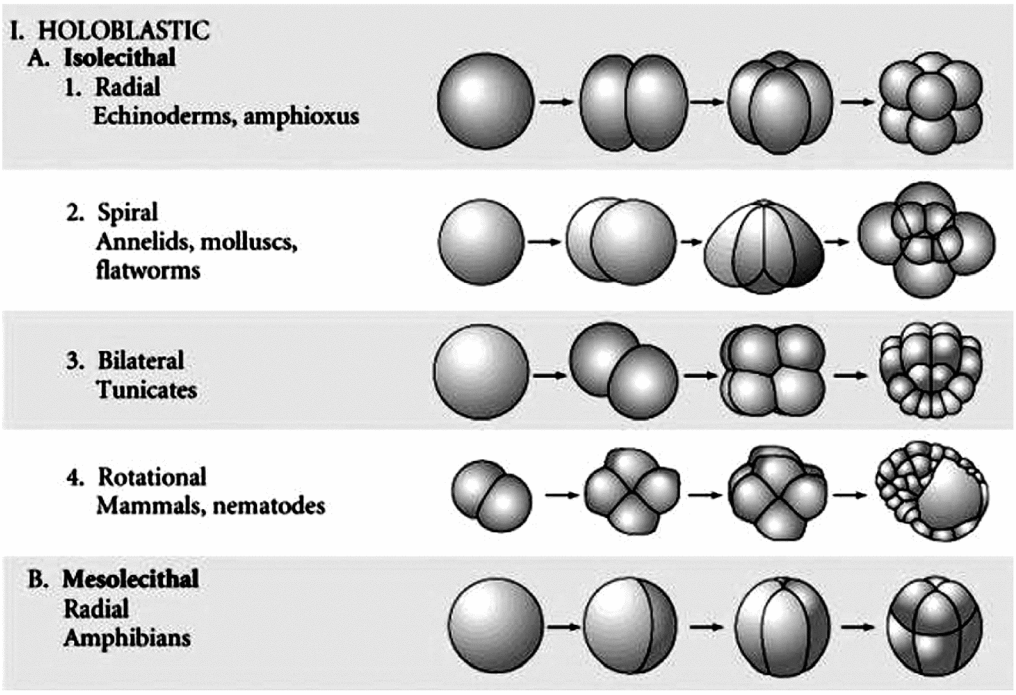


Fig. 2.17(a) : Types of Holoblastic Cleavage

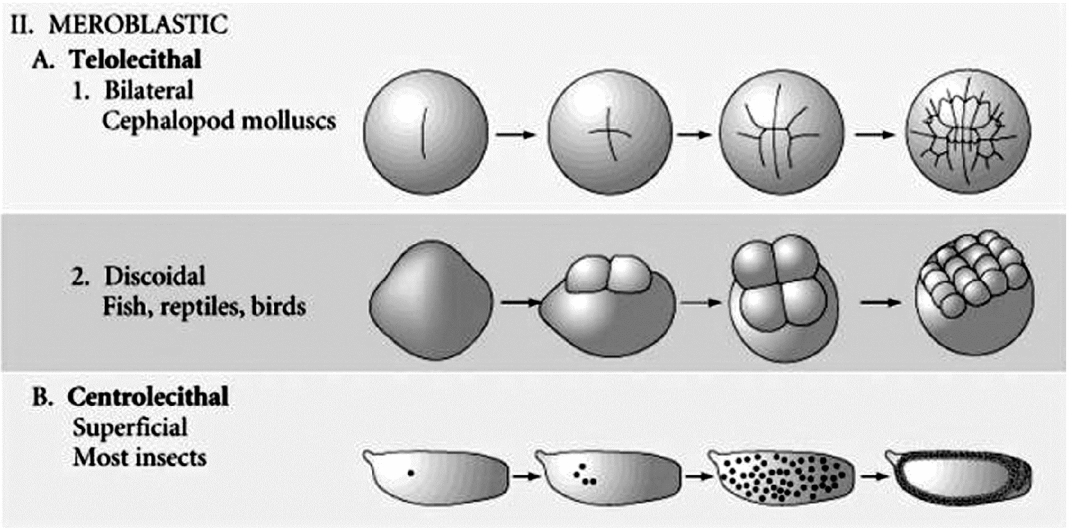


Fig. 2.17 (b) : Types of Meroblastic Cleavage

cells or microlecithal cells (moderate amount of yolk in a gradient) ***bilateral holoblastic, radial holoblastic, rotational holoblastic, and spiral holoblastic cleavage.***

i. ***Bilateral***-In this pattern of cleavage, the blastomeres are so arranged that the right and left halves become distinct. The cleavage pattern is dependent upon differences in the size of the blastomeres. In bilateral holoblastic cleavage, the divisions of the blastomeres are complete and separate; compared with bilateral meroblastic cleavage, in which the blastomeres stay partially connected. Examples-Nematodes, cephalopod molluscs, some echinoderms and tunicates.

ii. ***Radial***-In this cleavage pattern. divisions take place in such a manner that all the blastomeres are placed in radially symmetrical fashion around the polar axis. When such an egg is viewed from the poles, the blastomeres seem to be arranged in a radially symmetrical form. Example-Sponges, coelenterates, sea-urchin, sea cucmber, Amphioxus.

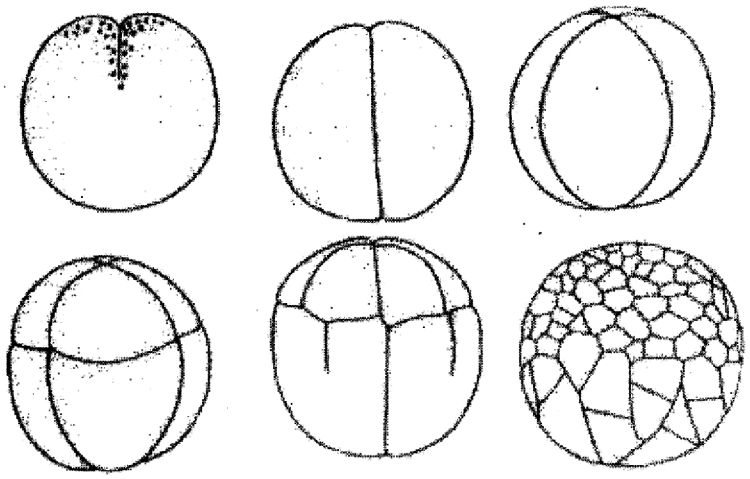


Fig. 2.18: Unequal holoblastic cleavage of frog

iii. ***Rotational***- Rotational cleavage involves anormal first division along the meridional axis. 6 giving rise to two daughter cells. In this cleavage one of the daughter cells divides meridionally, whilst the other divides equatorially. Mammals display rotational cleavage, and an isolecithal distribution of yolk (sparsely and evenly distributed). Because the cells have only a small amount of yolk, they require immediate implantation onto the uterine wall in order to receive nutrients. The nematode C. elegans, a popular developmental model organism, undergoes holoblastic rotational cell cleavage.

iv. ***Spiral-*** The spiral cleavage is diagonal to the polar axis. In this type the spindles for the third cleavage, instead of being erect, are oriented diagonally so that the resulting upper tier of cells is displaced sidewise. The upper 4 cells are placed over the junctions between the 4 lower cells. The upper smaller cells are called the macromeres. The spiral cleavage results due to oblique positions of the mitotic spindles. This type of cleavage is called spiral because the four spindles during the third cleavage are arranged in a sort of spiral. Examples-Eggs of annelids, molluscs, nemerteans and some Planarians.

2. **Meroblastic cleavage:** When segmentation takes place only in a small portion of the egg resulting in the formation of blastoderin, it is called meroblastic cleavage. Usually the blastoderm is present in the animal pole and the vegetal pole becomes laden with yolk which remains in an uncleaved state, i.e., the plane of division does not reach the periphery of blastoderm or blastodisc. In the presence of a large amount of yolk in the fertilized egg cell, the cell can undergo partial, or meroblastic, cleavage. Two major types of meroblastic cleavage are ***discoidal and superficial***.

i. ***Discoidal-***In discoidal cleavage, the cleavage furrows do not penetrate the yolk. The embryo forms a disc of cells, called a blastodisc, on top of the yolk. Discoidal cleavage is commonly found in monotremes, birds, reptiles, and fish that have telolecithal egg cells (egg cells with the yolk concentrated at one end). The layer of cells that have incompletely divided and are in contact with the yolk are called the "syncytial layer".

ii. ***Superficial-*** In superficial cleavage, mitosis occurs but not cytokinesis, resulting in a polynuclear cell. With the yolk positioned in the center of the egg cell, the nuclei migrate to the periphery of the egg, and the plasma membrane grows inward, partitioning the nuclei into individual cells. Superficial cleavage occurs in arthropods that have centrolecithal egg cells (egg cells with the yolk located in the center of the cell).

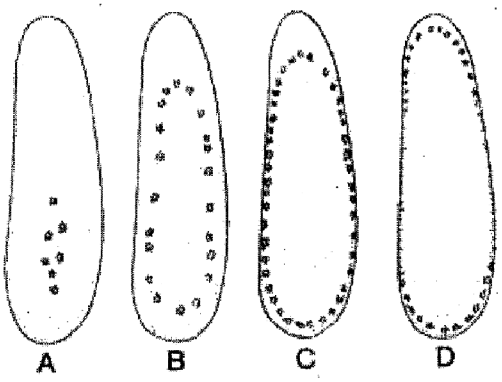


Fig. 2.19 : Superficial Cleavage of a beetle

3. Transitional cleavage : In many eggs, the cleavage is atypical which is neither typically holoblastic meroblastic, but transitional stage between the two.

4. Determinate: 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. Example- bilateral cleavage in Ascidia.

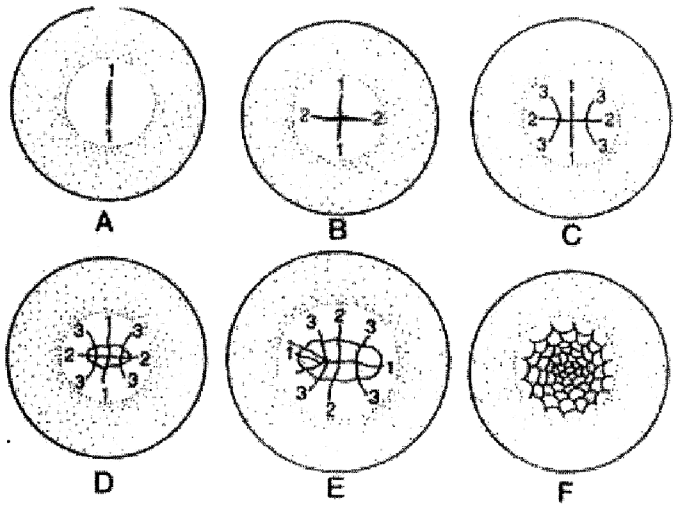


Fig. 2.20: Discoidal meroblastic cleavage of a telolecithal egg of a hen

5. Indeterminate Regulative : A cell can only be indeterminate (also called regulative) if it has a complete set of undisturbed animal vegetal cytoarchitectural features. It is characteristic of deuterostomes - when the original cell in a deuterostome embryo divides, the two resulting cells can be separated, and each one can individually develop into a whole organism. ExamplesEchinoderms, coelenterates, and Amphibians.

Mechanism of Cleavage: The incidence of cleavage provides unique opportunity to study the mechanism of cell division and specially the role of different cell organelles during division. Opinions differ regarding the accumulation of force for the initiation of cleavage and following factors are believed to be responsible for controlling the cleavages:

(a) Localized expansion of cortex.

(b) Increased stiffness of the cortical cytoplasm.

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

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

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

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

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

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

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

Balfour's law: The rate of cleavage is inversely proportional to the amount of yolk material present in the egg.

Chemical Changes during Cleavage: Significant chemical changes go on in the fertilized egg during cleavage. They are:

- *Increase of nuclear material:* During cleavage a steady increase in nuclear material (predominantly DNA) is obser-ved. Cytoplasm of the egg is the source of such nuclear material. Cytoplasmic DNA contained in mitochondria and yolk platelets are available.
- *RNA synthesis:* During cleavage messenger RNA (mRNA) and transfer RNA (tNA) are synthesised during cleavage, especially in late stages.
- *Synthesis of proteins:* Throughout the period of cleavage there is steady and spectacular increase in protein synthesis.

2.8.1 Types of Blastula

At the end of the cleavage, the embryo consists of a hollow sphere of cells in holoblastic types and a layer of cells over yolk in meroblastic types. This developmental stage is called a blastula. The development of blastula is called Blastulation. The layer of cells is known as the blastoderm and the cavity is the blastocoel. At first, blastocoel

may be represented just by a narrow crevice between the blastomeres, but it gradually increases as the cleavage goes on. The blastocoel is identified in the 8-cell stage as the space enclosed by the two quarters of blastomeres. The types of blastulae greatly varies on various factors like egg size, the amount and distribution of yolk and the pattern of cleavage. The various types of blastula are classified into four types

I. Holoblastically formed blastulae: In holoblastic embryos, the blastula is either solid (stereoblastula) or hollow (coeloblastula).

i) Stereoblastula-This type of blastula is composed of densely packed cells but large sized and relatively smaller in number of cells. blastocoelic space in the centre is very small or virtually absent. The balstomeres in a stereoblastula may reach from the surface to the centre; or separate blastomeres may occupy the interior. Examples-Stereoblastula occurs in a variety of animals such as insects , some worms like *Nereis*, mollusks like *Crepidula*, gymnophionan amphibians and certain in fishes.

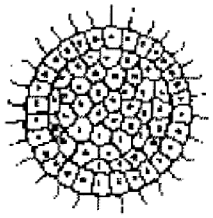


Fig. 2.21(a) : Stereoblastula

ii) Coeloblastula- It is a hollow blastulacontaining a large spacious blastocoel. Usually the blastocoel is filled with a fluid mucopolysaccharides. The bklastula resulting from holoblastic equal as in the case of echinoderms and Amphipoxus, is called equal coeloblastula.

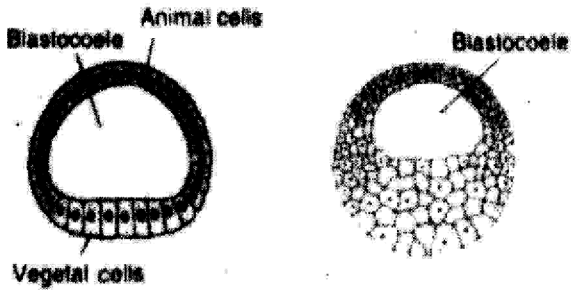


Fig. 2.21 (b) : Microlecithal egg Amphioxus Holobiastic cleavage — Mesolecithal egg Amphibians Holobiastic cleavage

In case the blastoderm is single layered. Holoblastic unequal cleavage, as in frog, results in unbequal coeloblastula. It has a blastocoel displaced

towards the animal pole and a multilayered blastoderm. It is of two types:

a. Adequal- The cells of the blastoderm are of about the same size throughout. The blastocoel is placed. E.g. Echinoderms.

*b. Inequal-*The wall of the vegetal half is thicker than that of the animal half. The blastocoel is volume and distinctly eccentric, i.e., displaced towards the animal pole, e.g., us, amphibians.

II. Meroblastically formed blastulae

The blastula stage has a layer of cells over yolk.

i) Periblastula-This type of blastula is found in insects and some arthropods having thal eggs with a superficial cleavage. In such blastulae, there is no cavity comparable to coel. It consists a single layer of epithelial cells, the blastoderm which envelopes the cleaved yolk.

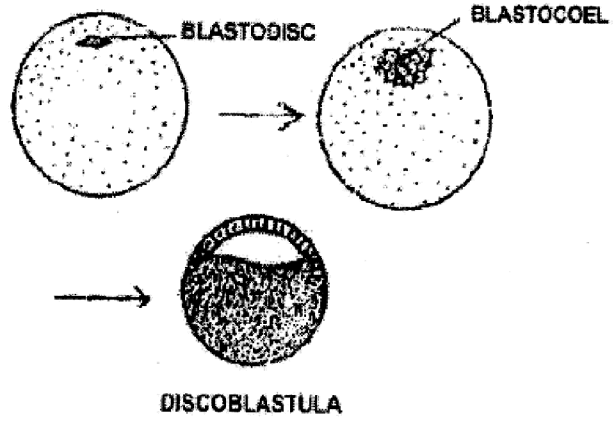


Fig. 2.21 (c) : The blastula stage has a layer of cells over yolk

ii) Discoblastula- Discoblastula consists of a ped mass of blastomeres overlying a mass called blastodisc. This blastula alt of meroblastic discoidal cleavage as ishes, reptiles and birds. There is no , instead a slit like cavity called mal cavity appears in between the m and the yolk mass.

2.8.2 *Questions*

1. What is cleavage?
2. What are the different planes of cleavage? examples?

3. State the significance of cleavage?
4. What is holoblastic cleavage? Describe the types of holoblastic cleavage with examples?
5. What is meroblastic cleavage? Describe the types of meroblastic cleavage with examples?
6. Differentiate between determinate and indeterminate type of cleavage?
7. What is transitional cleavage?
8. Describe in brief the mechanism of cleavage?
9. Define blastulation?
10. Classify the different types of blastula?
11. Write short notes on: Discoblastula, Stereoblastula. Coeloblastula and Periblastula.
12. Describe the role of yolk on cleavage?

2.9 Fate map construction

Meaning of Fate Map:

A fate map is a diagram of an egg or blastula, indicating the fate of each cell or region, at a later stage of development. Fate maps are essential tool in most embryological experiments. They provide researchers with information on which portions of the embryo will normally become which larval or adult structure. The analysis of the fate of each blastomere after first and second cleavage is called cytogeny or cell lineage study.

Construction of Fate Map:

Fate map of different types of animals have been constructed by the following methods:

i. ***Observing Living Embryos:*** In some invertebrates, the embryos being transparent and having relatively few daughter cells that remain close to one another, it has been possible to look through the microscope and trace the descendants of a particular cell to the organ they subsequently formed.

   This type of study was performed by Edwin G. Conklin (1905) in the tunicate. *Styela* partita, where the different cells contain different pigments. As for example, the muscle-forming cells always have a yellow colour.

ii. ***Vital Di Marking:*** Most embryos, however, do not have the facilities

(transparent, low cells, Ninterent colours cto las described above in sala parnta II was in 1929 that Vogi was able to ce the date of different areas of amplubian eggs by applying vital dyes. These vital dyes stain the cells without killing them.

iii. ***Radioacriw labelling and Fluorescent Dues:*** A variation of the dye marking technique is to make one area of the embryo radioactive. I donor embryo is taken and grown in a solution contammg radiode thymidine. Thus thymidine base is subsequently incorporated into the Il lor the dividing embryo.

A second embryo, acting as the host embryo, is grown under normal conditions. The region of interest is cut off from the host embryo and is replaced by a radioactive graft from the donor embryo. The cells that are radioactive will be the descendants of the cells of the graft, and are distinguished by autoradiography

iv. ***Genetic Marking:*** Radioactive and vital dye marking have their own drawbacks such as dilution over many cell divisions and the laborious preparation of slides. Onc permanent way of cell marking is to create mosaic embryos having different genetic constitutions. The best cuample of such a marking is to grali quail cells inside a chick embryo. By doing so, finestructure maps of the chick brain and skeletal system can be made.

Fate Map of Vertebrates

i. Fate Map of Amphioxus: The fate map of epidermal ectoderm Amphionus can be traced at an early stage prior to the onset of cleavage. The presumptive neural plate organ forming areas in the untcleaved egg is material notochord given in Fig. 5.32. The future endodermal cells substance the vegetal pole and would subsequently the floor or hypoblast of the blastula. The at the animal pole would form the endoderm mesoderm presumptive ectodermal cells. The ventral grey kin Fate Map of Amphioxus area at the future posterior end of the, in between the future ectoderm and endoderm, forms the future mesoderm. the dorsal crescent, in

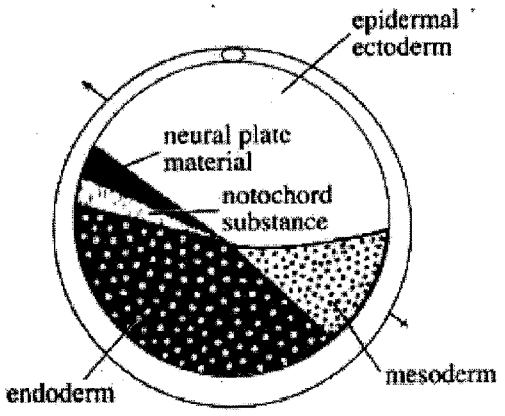


Fig. 2.22 : Fate Map of Amphioxus

between the ectoderm and endoderm on the anterior side, gives rise to the notochord and neural cells. The presumptive ectodermal, mesodertmal, notochordal and neural cells would subsequently form the epiblast of the blastula.

ii. **Fate Map of Frog:** The blastula od Xenopus at the 32 cell stage gives no indication as to how the different regions will develop. However, by following the late of individual cell, or group of cells, the fate map of the blastula can be made. One way of making the late map is by staining the varitous parts of the carly embryo with a lipophilic dye such as dil and observe where the labelled regions end up.

Another sophisticated way of labelling the blastomeres is by injection of high molecular weight molecules such as rhodamine-labelled dextran, which cannot pass through cell membrane and are, therefore, restricted to the injected cell and its progeny. These cells can be easily detected later, under a UV microscope.

The fate map of the Xenopus shows the presence of yolky macromeres at the vegetal pole which gives rise to the endoderm. Depending upon the position of the blastopore, the endodermal area can be divided into the sub-blastoporal and supra-blastoporal endoderm.

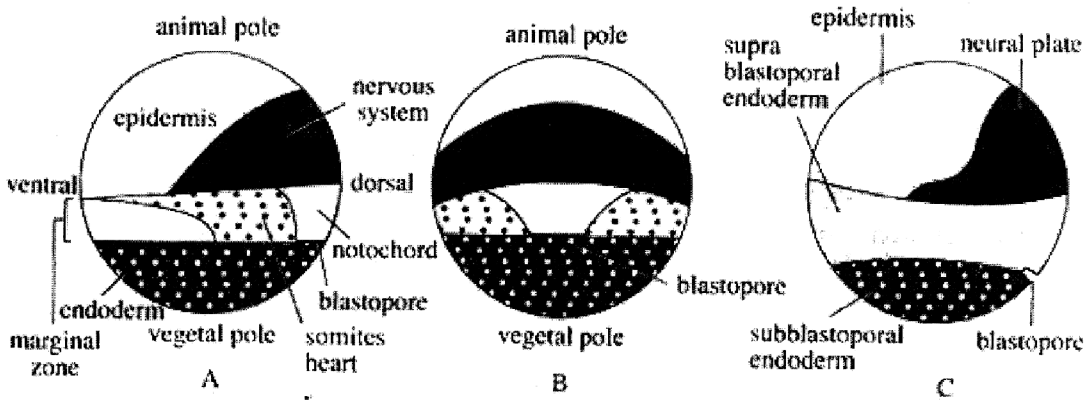


Fig. 2.23 : Fate map of Xenopus late blastula; A: Lateral view; B: Dorsal view; C: Exterior view

The cells toward the animal pole gives rise to the cctoderm, which becomes further subdivided into epidermis and the future nervous tissue. The epidermal ectoderm forms at the ventral side of the animal hemisphere, while the neutral

ectoderm forms at the dorsal side. The mesoderm forms a beltlike region, known as the marginal zone, around the equator of the blastula.

The mesoderm becomes subdivided along the dorsoventral axis of the blastula. The most dorsal mesoderm gives rise to the notochord. From this ventrally, the mesoderm is differentiated by the somites (which gives rise to muscle tissue), lateral plate (which contains heart and kidney mesoderm) and blood islands. In Xenopus, a thin outer layer of prof sumptive endoderm overlies the presumptive mesoderm in the marginal zone.

iii. **Fate Map of Chick:** Before going through the fate map of chick one should go through the formation of area pellucida and area opaca, and also through the formatiod of hypoblast and epiblast From the study of the above formations, it becomes clear that the hypoblast does not contribute any cells to the formation of the embryo proper, rather they contribute to the formation of portion of the external membranes.

Recent studies with cell adhesion molecules (CAMs), it has become possible to construct the fate map of chick. All the three germ layers of the embryo proper is formed by the epiblastic cells. The epiblast also forms a considerable amount of extrafembryonic (mesoderm) knobflike structure facing membrane. The fate map of chick reveals that the cells of the epiblast are organised around the notochord and nervous system. The neural ectoderm is present as a towards the anterior side. The cells at the anterior part of the epiblast form the ectoderm, while the cells at the posterior side gives rise to mesoderm (body proper), endoderm and extraembryonic mesoderm.

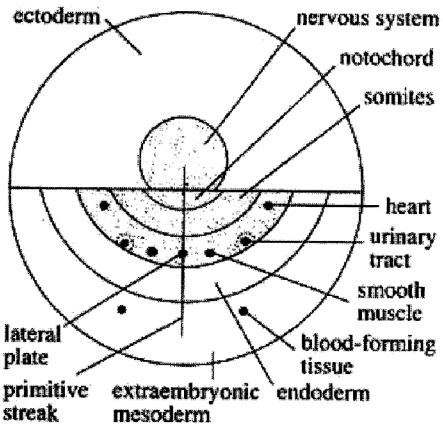


Fig. 2.24 : Fate map of Chick Embryo

Usefulness of Fate Map: The fate map of organisms is helpful in tracing the morphogenetic movements of the cells and the ultimate positions they take up. However, they tell us nothing about the tissue developmental potentialities during morphogenesis.

2.9.1 ***Questions :***

1. What is a fate map?
2. State the usefulness of a fate map?
3. What is vital dye?
4. Write a brief note on the techniques used to construct a fate map?
5. Write short notes on: fate map of frog, chick and Amphioxus aided with a diagram of each.

2.10 Early Developmnt of Frog (Up to Gastrulation)

Frogs lay their eggs in water in early spring. In pseudocopulation or mating, the male frog firmly clasps the body of the female frog by his forelegs and enlarged thumb pads (nuptial pads). These nuptial pads help in clasping the body of lemale. This sexual embrace is called amplexus. As the eggs are extruded through the cloaca of female (oviposition), the male deposits sperm cells over them (insemination). Thus, fertilisation is external, taking place in water.

Spawning: The mesolecithal eggs of frog enclosed in a protective gelatinous albumen are laid in water. The cluster or masses of eggs which remain stick together is called spawn. A spawn of Rana tigrina may have 3000 to 4000 ova. The spawn is laid during pseudocopulation or amplexus.

Fertilization: In frog, fertilization is external and occurs at once in water outside the body of the oviparous female. The entire sperm penetrates the ovum anywhere around the animal hemisphere. If fertilization is delayed, the albumen layers around the ovum become too thick for the sperm to pass through them and the ovum also starts to show degeneration. In the fertilization process, vesicular sperm nucleus and vesicular female nucleus (or pronuclei) fuse together to form zygote nucleus.

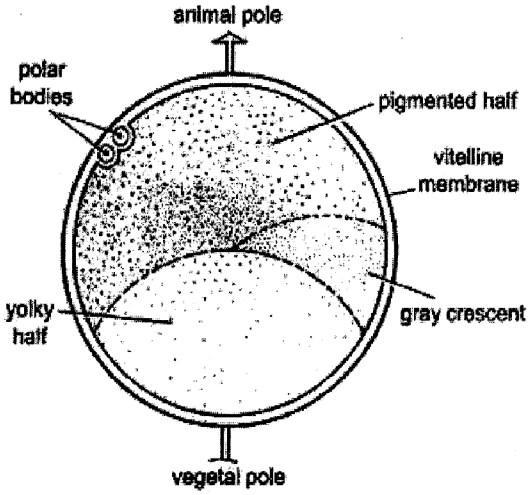


Fig. 2.25 : Fertilised egg of Frog

The fusion of both male and female pronuclei is called amphimixis. The fertilißed egg or zygote is about 1.6 mm in diameter; it rotates within the vitelline membrane so that the animal pole becomes dorsal. The upper half of the zygote or animal hemisphere is pigmented black and it contains the cytoplasm and a nucleus the lower vegetal hemisphere is white and full polar of yolk.

On one side between the black and white areas is a gray crescent region which marks the future dorsal side. At this region cortex becomes thin and this area is crescent shaped. The plane passing through the centre of grey crescent and the animal pole defines the median plane of bilateral symmetry. It coincides with the embryonic axis and is the only plane which separates the egg into two equivalent parts, each containing half the crescent material.

Cleavage and Blastulation:

Cleavage or segmentation is holoblastic and unequal.

- A vertical furrow from the animal to the vegetal pole divides the zygote completely into two equal sized cells.
- A second vertical furrow at right angles to the first divides the zygote into four cells. Further cleavages divide the micromeres more.
- The third cleavage is horizontal and above the equator which segments the zygote into upper four smaller, black-coloured cells, and lower four larger,

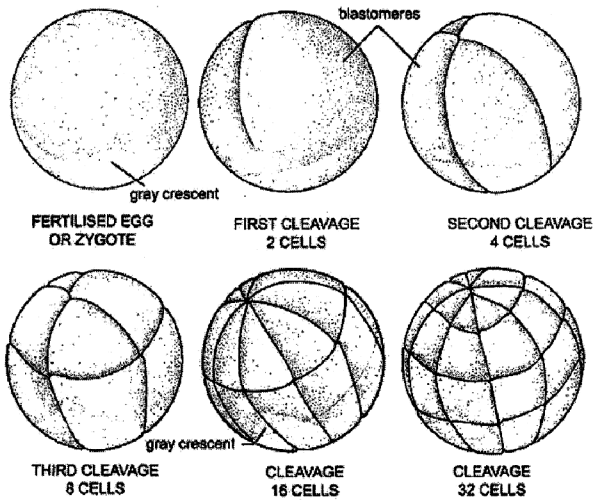


Fig. 2.26 : Cleavage Pattern of Frog

white-coloured cells. The cells formed by cleavage are blastomeres, the upper black blastomeres are called micromeres, and lower white ones are macromeres.

- Further cleavages divide the micromeres more rapidly than the lower macromeres whose division is hindered by yolk. The blastomeres mutual pressure flattens their surfaces in contact with each other but free surfaces of each blastomere remain spherical. At this stage the whole embryo acquires a characteristic appearance reminiscent of a mulbery and so it is called morula.
- About fourth and fifth cleavage stages a small space, the blastocoel appears between the blastomeres of morula. In the beginning it is like narrow crevices between blastomeres of morula, which gradually increases as the cleavage goes on.

Blastulation :

At the end of cleavage the solid ball of cells give rise to blastula which consists of a number blastomeres. The characteristic features of the blastula stage are the presence of a wella defined cavity called the blastocoel. This is the beginning of the primary body cavity. The process of the formation of blastula is called **blastulation**. The blastula offroy is called amphiblastian as the cavity is contined 10 only the animal pole The vegetal pole however is composed of a solid mass of non pigmented yolky cells.

In the **thirty two cell stage, the blastuia consists of single layer of cells and is called the early** blastula The pigmented cells (micromeres) are found in the anterior hall while the yolky megameres are present in the posterior hall As has been already pointed out the blastocoel lies entirely in the anterior hall The blastula of Troy is hollow and has a very well developed blastocoel. It is said to be a coeloblastula.

The blastocoel shills more and more towards the animal pole due to more rapid multiplication of the micromeres, and infiltrated by water and albuminous Muid secreted by the surrounding cells. The blastocoel also enlarges due to uptake of more water. As cleavage proceeds, the blastomeres arrange themselves into a true epithelium called **blastoderm**.

As segmentation proceeds, the number of cells in the blastula increase; so also the blastocoel. The floor of the blastocoel is that while its top portion is arched. The roof is made up of three to four layers of pigmented micromeres while the floor is formed by yolky megameres. Between the micromeres and the megameres and along the equator is found a group of cells which are intermediate in size (between megameres and micromeres). These cells constitute the germ ring. The germ ring is formed in the region of the grey crescent.

Presumptive Areas from the fate map (diagram of fate map given before): In the blastula, the blastomeres which have to form different germinal layers and different organs of the adult""frog can be observed by artificial vital staining methods of Vogt (1925) and prospective organ region maps or fate maps have been prepared.

According to the fate map studies, the whole surface of blastula can be divided into the following three areas:

(i) Prospective ectoderm area is present on and around the animal pole and it is pigmented black. The neural ectoderm occurs largely on the future dorsal side of blastula, while the epidermal ectoderm occupies the antero-ventral side of the blastula. Inside the neural ectoderm occurs a small sub-area that develops into the eye of the embryo. The sub-area of nose, sucker, ears and mouth are present inside the epidermal ectoderm.

(ii) Prospective notochord and mesoderm area is present behind the pigmented animal hemisphere. It is crescentic gray area, the marginal zone along the equator of blastula. It has blastomeres for the formation of notochord and mesoderm of the embryo. The large area of dorsal side of the gray crescent is occupied by notochordal cells.""Beneatli the notochordal area, toward the vegetal pole lies a narrow strip of cells which form the pre-chordal plate of the embryo. On either sides of notochordal area, the part of grey crescent forms the segmental muscles (somites) and tail mesoderm is a narrow strip of cells on the dorsal side, toward animal hemisphere. Lateral and ventral parts of grey crescent give rise to ventro-lateral mesoderm.

(iii) Prospective endodermal area is the entire nonfpigmented area of the vegetal hemisphere, which give rise to endodermal lining of the mouth, gill region, pharynx, midgut and hindgut, and other organs such as liver, pancreas, urinary bladder and certain endocrine glands.

Gastrulation

Gastrulation is a process of migration and rearrangement of prospective organ forming cells already present in the blastula. It is brought about by several types of morphogenetic movements taking place at the same time.

i. ***Invagination of Endoderm:*** Certain prospective endodermal cells just beneath the midf dorsal point of gray crescent of blastula assume the elongate shape of a bottle and move toward the interior of the blastula. Their steadily elongating necks remain attached to the surface of the blastula with the outermost cementing layer.

Thus, as the bulky-cell bodies move inward, a pull exerted along their attenuated necks and creates an indentation at the surface. With continued multiplication and attenuation of bottle cells, the invagination deepens, and expands internally to form the archenteron or gastrocoel and its outer

opening (original indentation) is called the blastopore lying at the future posterior end. The area immediately above the blastopore is the dorsal lip of blastopore. Gradually, the blastoporal invagination extends circulo laterally, so the blastopore becomes crescentic, then horse-shoe-shaped and finally circular. Thus, lateral lips and ventral lip of blastopore are also formed and fused with each other along with dorsal lip, forming circular lip of blastopore. The endoderm of foregut involute over the dorsal lip along chorda-mesoderm. The rest of prospective endoderm of vegetal region passes into the interior of the embryo passively and come to lie in the floor of gastrocoel.

ii. ***Involution of Pharyngeal Endoderm and ChordaMesoderm:*** The endodermal cells bordering the dorsal lip of blastopore form the prospective pharyngeal endoderm, which is followed by pre-cherdal plate. notochord and tail mesoderm. When dorsal lip is formed, and the pharyngeal endoderm cells involute over the dorsal blastoporal lip. These cells move to the interior and their place take the converging prechordal plate cells and they also involute.

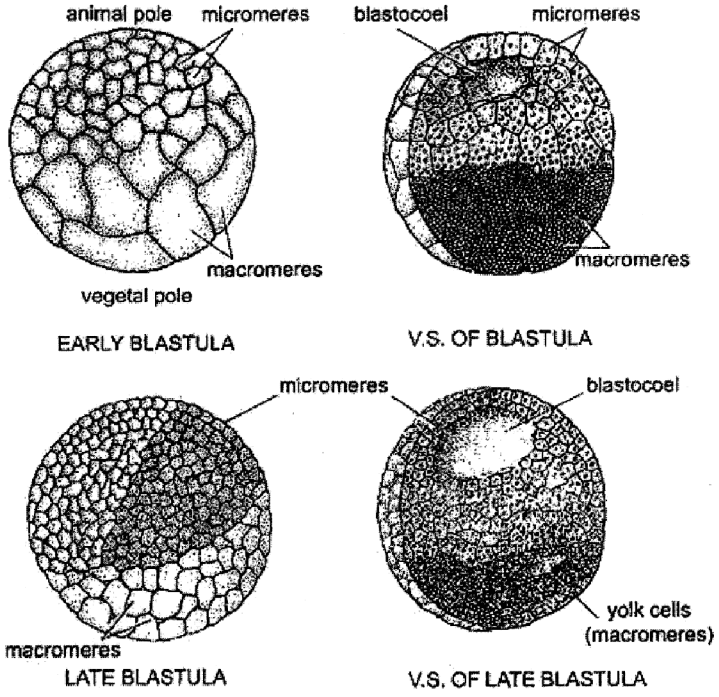


Fig. 2.27 : Early and late blastula of frog

Behind these cells are present notochordal cells and tail mesoderm cells, which also involute and move to the interior. As these materials move inward

around the dorsal lip they become considerably narrowed and elongated. The prospective pharyngeal endoderm in later stages of gastrulation forms the foregut whose lateral, ventral and anterior walls consist of a thin layer of endoderm. The dorsal wall of foregut consists of prechordal plate and anterior tip of notochord.

The notochord cells of the posterior region also involute and move anteriorly over the dorso lateral lips of blastopore. Thus, the notochord forms the mid-dorsal wall of the archenteron, which is in the form of strip. The prechordal plate also forms the dorsal wall of the archenteron in front of notochord. The tail mesoderm remains near the blastopore, and marks the posterior end of the embryo.

The mesoderm (i.e., trunk somites and ventro-lateral mesoderm) rolls over the lateral and ventral lips of blastopore and then invaginates. After rolling inside the entire mesoderm (i.e., notochord, prechordal plate mesoderm, somites and ventro-lateral mesoderm) move from posterior side (blastoporal end) towards the anterior side as a single unit called chordamesodermal mantle in between outer ectoderm and inner endoderm. Thus, it occupies the entire space between ectoderm and endoderm except a small space at the anterior end of embryo where mouth will be formed in late stage.

iii. ***Epiboly of Ectoderm:*** Throughout gastrulation the embryo retains its spherical shape and a uniform size. Alter involution of gastral endoderm and entire mesoderm, this space is taken up by the ectoderm (epidermal and neural). The expansion of cctoderm from animal hemisphere over the vegetal hemisphere is an active process. The presumptive epidermal ectoderm expands in all directions, but the presumptive neural ectoderm expands mainly in the longitudinal direction, i.e., from anterior end towards the blastopore and also contracts transversely.

Thus, the ectoderm expands up to the circular lip of blastopore through which unpigmented endodermal cells is visible, which form the yolk plug. Due to contraction of circular lip of blastopore, yolk plug slightly comes outside. Thus, in the end of gastrulation a new cavity gastrocoel is formed and the blastocoel is obliterated.

Due to accumulation of endodermal mass on the future ventral side, the gravity is shifted and embryo rotates within fertilization membrane so as to bring its dorsal side uppermost. The protruding yolk plug gradually withdraws to the interior, and the blastopore steadily contracts to all form a slit-like opening at the end of gastrulation.

iv. Gastrula: Thus, gastrulation changes the radially symmetrical single layered blastula into a o spherical, bilaterally symmetrical, triploblastic gastula having a head-to-tail axis. It is externally covered by ectoderm and endoderm, and mesoderm lies in the interior. Gastrocoel forms the lumen of the forming gut. Its lateral walls and floor is formed by the endoderm and its roof is formed of chorda-mesodermal cells.

Neurulation: By the time gastrulation is being completed, the ectoderm along the mid-dorsal side of the embryo thickens to form a pear-shaped medullary or neural plate. The neural plate cells change in shape and become elongated and arranged themselves into a columnar epithelium. The epidermal cells remain more or less flat and arranged as a stratified epithelium usually two cells thick. The edges of the neural plate become

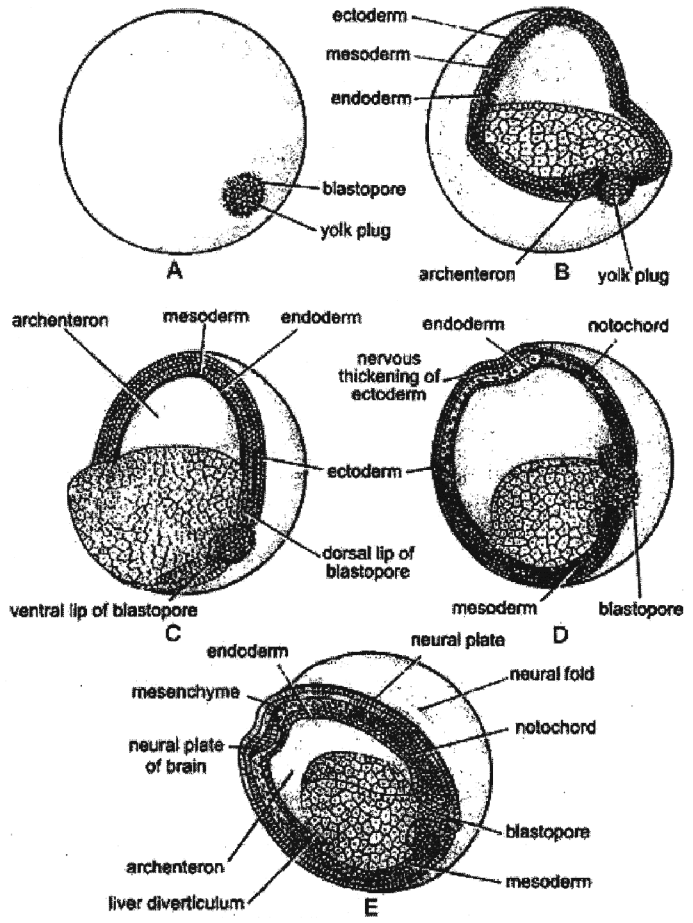


Fig. 2.28 : Frog: A: External appearance of gastrula; B, C: Partial sections of early gastrula (delaminations of endoderm and mesoderm); D, E: Sections of late gastrula (germ layers)

thickened and slightly raised above the general level as ridges called neural folds. The neural plate narrows transversely especially in its posterior parts and the neural folds raised higher due to which a neural groove is formed along its length. The neural folds grow and fuse with each other in the mid-dorsal line to form a neural tube.

The lateral epidermal ectoderm of either side also meet and fuse at the mid-dorsal line above the neural tube, thus, enclosing it. The neural tube remains open in front for a time as a neuropore, posteriorly the neural folds cover and fuse over the blastopore so that the cavity of the neural tube communicates with the archenteron by a neurenteric canal which is the narrow canallike opening of blastopore.

The anterior broad part of the neural tube forms the brain and the remaining narrow posterior part becomes the spinal cord. The neural tube also forms neuroglia cells of the nervous Sistem.

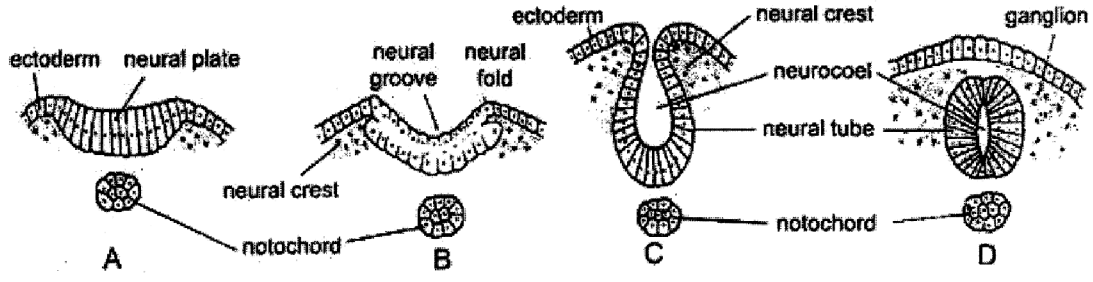


Fig. 2.29 : Stages in the formation of neural tube in amphibians.

Neural Crest Cells: The cells from the neural folds that come to lie between the dorsal epidermis and the dorsal part of the neural tube are the neural crest cells. These lie along the dorso-lateral sides of the neural tube. The neural crests give rise to melanocytes, dorsal root ganglia of spinal nerves, parts of the autonomic nervous system and adrenal glands, and to some mesenchyme cells which form the visceral arches.

Notogenesis: The notochord cells separates off from the prechordal plate of mesoderm as a narrow rod of cells. This notochordal rudiment also separates off from the rest of the chordamesodermal mantle and notochordal cells transform into colligocytes.

These cells secrete a collagenous sheath around them. Soon fluidfcontaining vacuoles appear in the notochordal cells which push the nucleus and cytoplasm toward the periphery. Thus, the notochord becomes round, turgid and elongated in antero-posterior axis. **Mesoderm Differentiation and Coelom:** The chordamesodermal

mantle at the time of closure of blastopore, separates off from the endoderm and mesoderm, thus, lies in between ectoderm and endoderm. The notochordal cells also splits off from the prechordal plate at the anterior end.

Simultaneously the tip of mesoderm at each side of notochord thickens and subdivides transversely, beginning at the anterior end, into a series of cell masses or somites. Each somite remains separated from its neighbours but remains joined to the lateral and ventral parts of the mesodermal mantle on each side by strands of cells.

These lateral and ventral mesodermal mantles are called lateral plates and these strands of cells in between somites and lateral plates are called mesomeres or nephrotomes, which later on forms the kidney tissue.

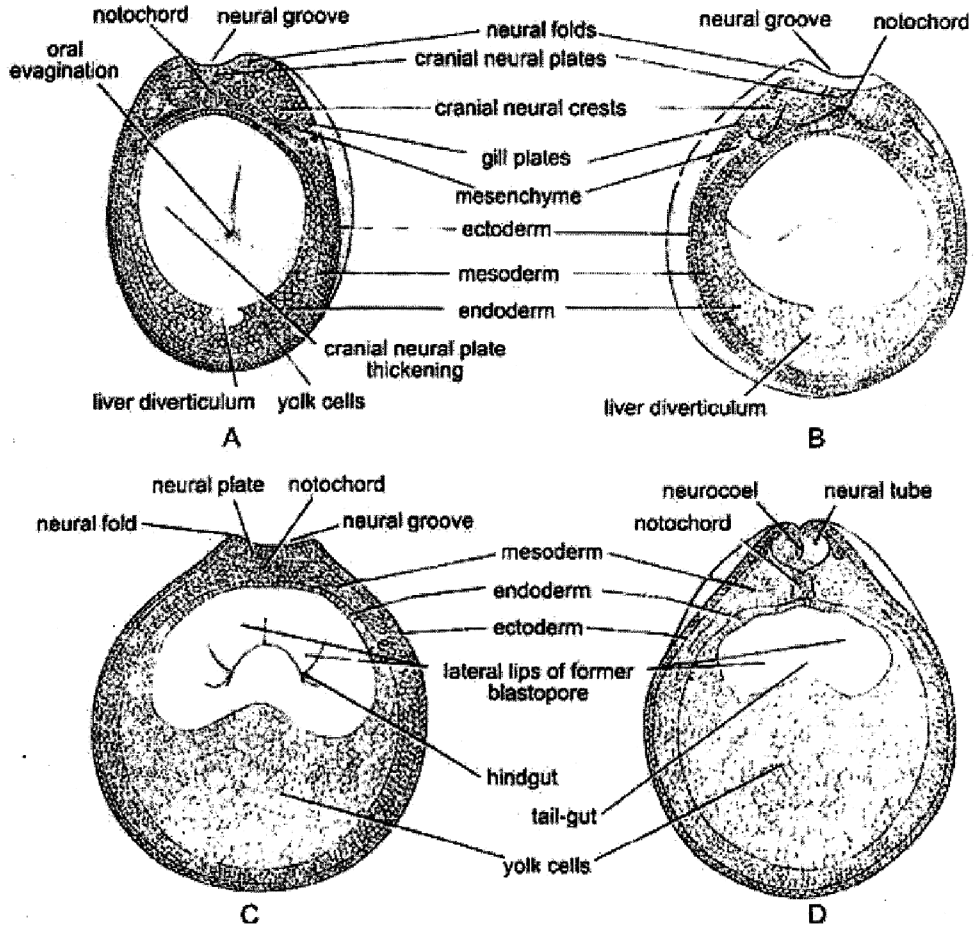


Fig. 2.30 : Frog. A, B: Anterior sections of early Neurula; C, D: Posterior sections of late Neurula

Each somite or epimere (dorsal part of mesoderm) on either side of notochord splits into three layers:

(i) Inner is the sclerotome or skeleton forming tissue around the notochord,

(ii) Middle is the myotome whose cells differentiate to form the striated muscle nfforming somatic muscles, and

(iii) Outermost narrow strip is the dermatome which forms the dermis of skin.

The hypomere or lateral plate of mesoderm of each side is divided by a split which passes downwards on each side to separate the hypomere into an outer somatic or parietal layer, and an inner splanchnic or visceral layer and the space between these two layers is a splanchnocoel or perivisceral coelom.

The inner visceral layer gives rise to smooth muscles of the intestine and to the blood and blood vessels, and outer somatic layer with the ectoderm forms the somatopleure. Anteriorly the coelom is restricted to the ventral side only as a pericardial cavity below the pharynx which gets separated from the splanchnocoel by a transverse septum.

Primitive Gut: The nerve cord endoderm differentiates slowly and eventually forms the enteron or primitive gut. At the end of gastrulation it is an open trough. After the formation of notochord and separation of mesoderm, the free margins of

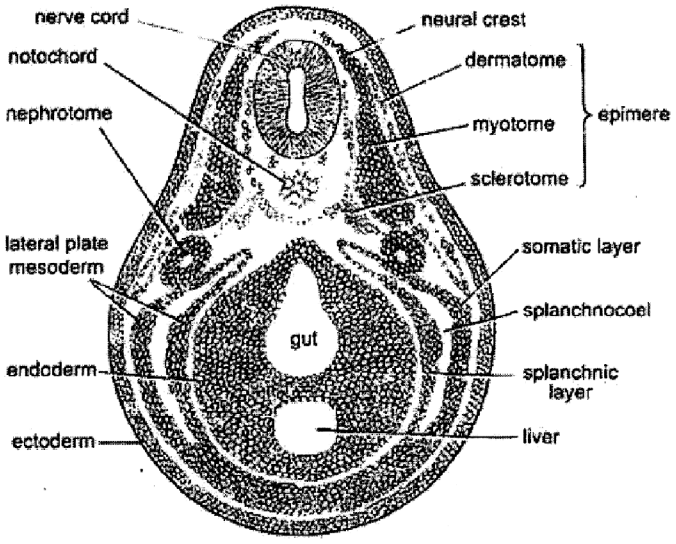


Fig. 2.31 : T.S. of frog embryo through trunk showing mesoderm differentiation

endoderm fuse in the mid-dorsal line beneath the notochord to form the definitive gut or enteron. The floor of enteron persists as thick layer of large yolk-ladden cells. At

the antero-ventral end the enteron makes contact with the ectoderm, where later on mouth invagination occurs which communicates with the enteron. Later, the lungs, liver, pancreas develop as evaginations of the gut. The pharyngeal pouches develop as lateral out-pushings of the foregut, which later forms the gill-clefts. During neurulation, embryo elongates in antero-posterior axis, it also flattens laterally. The part of the embryo above the blastopore elongates beyond the blastopore forming the tail bud or rudiment of tail. The neurula at this stage is called tail bud embryo.

2.10.1 ***Questions***

1. What is grey crescent?
2. What is the primary organizer of the frog embryo?
3. Describe the cleavage in Frog aided with diagram?
4. What type of blastula does a frog have? Describe the presumptive areas from the fate map of frog?
5. How does blastulation occur in frog? Explain with suitable illustration?
6. What is the role of the dorsal lip of the blastopore?
7. Describe the role of different morphogenetic movements in the gastrulation of frog?
8. What is neurulation? Describe the stages of formation of neural tube in frog?
9. What is Notogenesis? State the role of neural crest cells in gastrulation of frog?

2.11 Early development of Chick (upto gastrulation)

The embryology of chick has been worked out more extensively only because of the following reasons :

1. Tiggs of fowl are large or of convenient size, casily available throughout the year and can be incubated artificially
2. The various developmental stages can be easily available in laboratory conditions for experimental purposes.
3. Moreover, the process of development has been most thoroughly worked out in fowl. The embryology of chick bears many resemblances with those of reptiles and mammals.
4. The comparative study of embryology of different birds exhibits that it is essentially similar in all the birds with only minor unimportant differences. Despite these reasons, the embryology of chick is important due to phylogenetic significance. In chick, development is direct without a larval stage.

Fertilization: The ova are released from the ovary in the form of primary oocytes. The second maturation division occurs after ovulation in the oviduct. The released primary oocyte in the body coelom is grasped and swallowed by the ostium of the oviduct. The secondary oocyte or ovum after second maturation division is surrounded by several (5 or 6) sperms which enter in it (polyspermy), but only one sperm succeeds in the fertilization process. The nucleus of one sperm fuses with the female nucleus (Amphimixis) and the nuclei of other sperms degenerate.

As the fertilized egg passes downward inside the oviduct, it rotates, undergoes cleavage and various accessory egg membranes are laid down over the developing egg. Thus, fertilization is internal. The various egg membranes secreted around egg are albumen, shell membranes and shell.

Structure of Egg of Hen: The egg is about 3.0 cm in diameter and is polylecithal. It is entirely filled up by the yolk and over it, i.e., in animal pole lies a small cytoplasmic disc with a nucleus. This disc is called blastodisc. The yolk has a central mass of white yolk around which are alternate concentric layers of yellow and white yolk. The central flask-shaped white yolk called latebra runs from the centre to the lower side of the blastodisc and there it spreads to form the nucleus of Pander. The yolk and blastodisc are bounded by a plasma membrane and outer vitelline membrane, which is a converted form of zona radiata. The vitelline membrane is of double origin. The inner layer of this membrane is produced in the ovary between the oocyte and follicle cells and it is composed of very tough libres. The outer layer of it is formed in the upper part of fallopian tube.

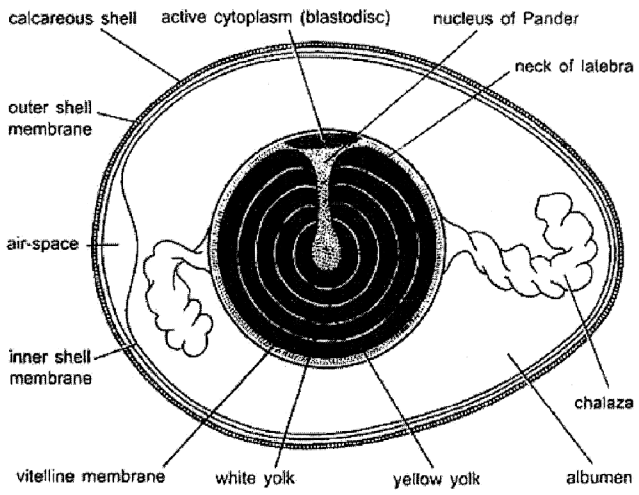


Fig. 2.32 : Longitudinal section of a hen's egg

The yolk contains 48.70 water, 32 6. phospholipids and fats, 16 proteins 1% carbohydrates and 11° other chemical molecules. Its proteins are in the form of phosvitin and Tipovitelline. The fat is predominantly neutral fat (50%), rest is phosphatids, cerebrosides and cholesterol.

The fertilized egg or zygote is covered by a laver of dense viscous albumen which forms a thin chalaziferous layer around the vitelline membrane. This dense albumen

forms two twisted cords or chalazae, one at each end of the movement zygote. They are formed by rotation of the egg during its through the oviduct.

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

The albumen and yolk also contains a variety of enzymes, vitamins, pigments and phosphorus. The isthmus part of the oviduct secretes two shell membranes made of tough keratin fibres matted together. The two shell membranes are closely applied except at the blunt end of the egg where they are separated by an air space formed after the egg is laid. The nidamental glands of the oviduct secrete a porous, calcareous shell which soon hardens.

It is pierced by a large number (about 7,000) of fine pores filled with a protein related to collagen. The diameter of the pores varies from 0.04 to 0.05 mm. These pores allow exchange of gases (oxygen and carbon dioxide) during respiration of the developing embryo. The egg is laid 24 hours aller fertilization, and its further development takes place, when the egg is incubated by the female. Incubation must continue steadily tor 21 days at a temperature of 103° F.

Cleavage and Blastulation:

Cleavage is meroblastic or discoidal blastodise and is contined only to germinal dise or . It does not segment the yolk surrounded by the growing tissues of the embryo. First two cleavages are at night angles to each other in the centre of blastodisc. These cleavage furrows do not cut the germinal disc completely through in the vertical plane.

The third set of cleavage furrows is vertical, cutting across the second set of vertical furrows. The fourth cleavage furrow is also vertical and circular cutting across all the cleavage

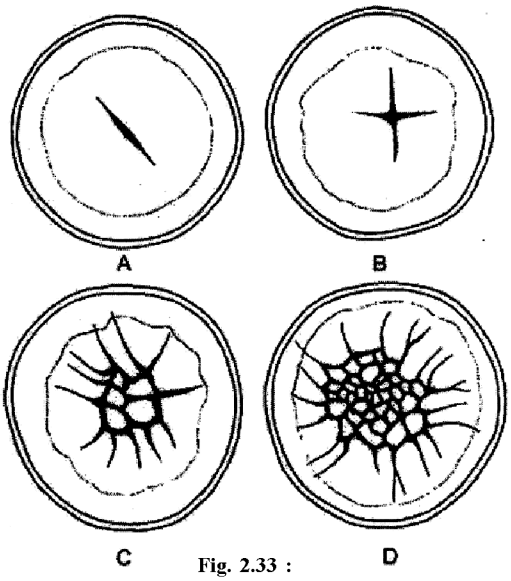


Fig. 2.33 : Cleavage pattern of chick. A: two cell stage; B: Four cell stage; C: Twenty cell stage; D: A late cleavage stage

furrows, forming eight central blastomeres which are surrounded by eight marginal blastomeres. Thus, these cleavage furrows separate the daughter central blastomeres from each other, but not from the yolk. The central blastomeres are continuous with the underlying yolk at their lower ends.

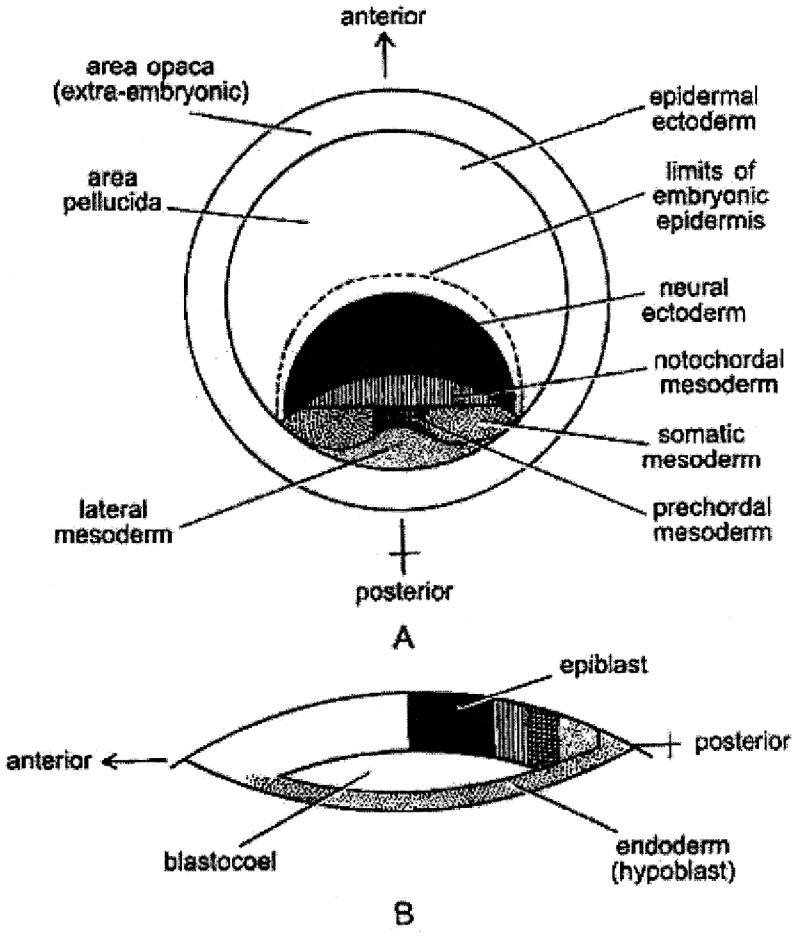


Fig. 2.34 : Fate map of chick blastoderm

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

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

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

The appearance of subgerminal cavity separates the blastoderm from the underlying yolk, but the marginal cells remain overlapping the yolk. The embryo is now called the blastula stage. At blastula stage the embryo reaches in the uterus. It may be compared to the blastula of Amphioxus and frog, its sub-germinal cavity is equivalent to the blastocoel, the blastoderm is the animal pole, and the yolk is the vegetal pole.

During later part of cleavage, about 12 to 14 hours after the egg reaches the uterus or 6 to 8 hours before the egg is laid, some cells on the inner or under side of the blastoderm become detached or delaminated from the blastoderm and fall on the floor of subgerminal cavity due to presence of relatively more yolk. The delamination of these yolky cells from the blastoderm starts at the posterior edge and spreads forward until whole blastoderm becomes free from yolky cells. As a result, the epithelial layer in the central region of blastoderm becomes thinner (few layers of cells) and transparent. Thus, this region is called the area pellucida because it seems to be transparent when viewed from the upper side. The peripheral part of blastoderm, the yolky cells is not delaminated (shed), so this part of the blastoderm seems to be opaque, because beneath these cells blastocoel is not present. This region of blastoderm is, thus, called area opaca. These delaminated cells at the posterior edge of area pellucida gradually link up with each other, forming a, continuous layer of flattened cells, which extends anteriorly. This layer is called the hypoblast and the upper layer is the epiblast containing ectoderm and mesoderm cells. Hypoblast is exclusively composed of endoderm cells.

The egg is laid by the female about the time the blastula is formed or even a little later.

Presumptive Fate Maps of Blastula:

Fate maps of the blastula of chick have been prepared by using the vital stains such as carmine or carbon (charcoal) particles or radioactive thymidine. It shows that blastomeres of area opaca do not form any part of the embryo proper, they form only extra-embryonic membranes.

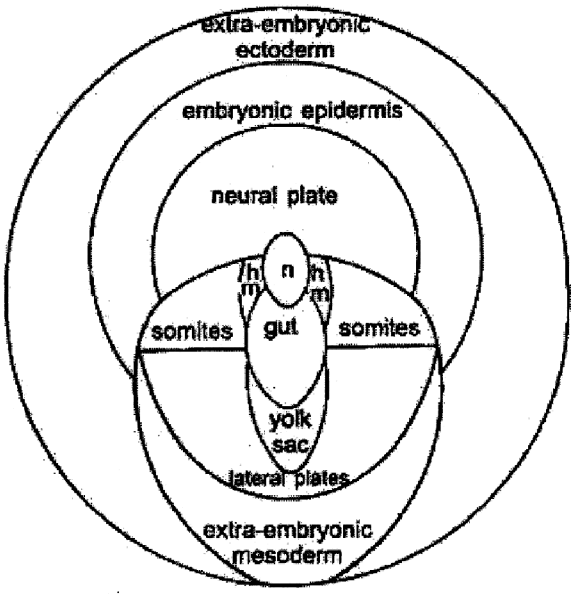


Fig. 2.35 : Fate map of epiblast of a bird prepared by thymidine marking: hm, presumptive head mesoderm; n, notochord

The epiblast and hypoblast of area pellucida have different fates in the course of embryonic development. The fate maps prepared by the use of tritiated thymidine have shown the following structures in epiblast - In the centre of area pellucida lies a small area destined to produce the notochord. Posterior to it, in the median plane is found an elongated oval area of presumptive endoderm which will form the gut.

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

The anterior half of epiblast is the presumptive ectoderm containing central

presumptive epidermis and outer to it in the form of complete ring is the extra-embryonic ectoderm.

Gastrulation: Gastrulation includes the following two types of morphogenetic movements:

a. Emboly: It involves only the epiblast which contains cells of ectoderm, mesoderm and notochordal cells. It includes convergence, invagination and involution. Formation of primitive streak and head process is due to emboly.

b. Epiboly: It includes the overgrowth of ectoderm epiblast and also of hypoblast.

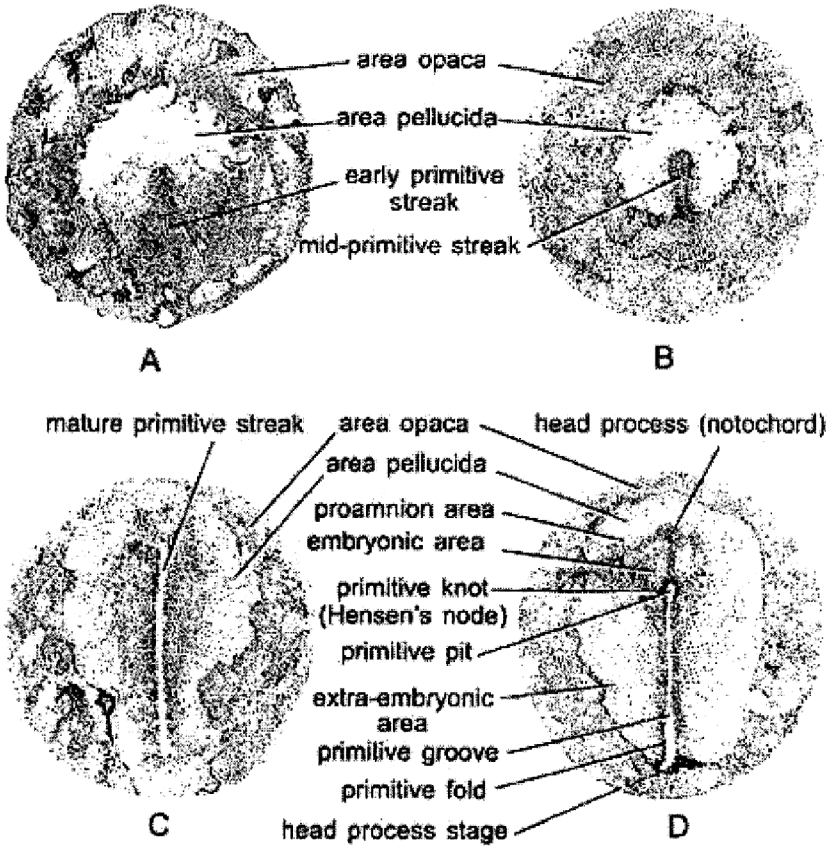


Fig. 2.36 : Chick blastoderm showing the development of primitive streak and head process (neurulation) A: initial streak; B: Intermediate streak; C: definitive streak; D: Head process

Formation of Primitive Streak:

Various prospective mesodermal and endodermal cells forming notochord of the epiblast converge toward the posterior edge of the area pellucida and form a conical thickening in the midline, called the initial primitive streak. It appears after 6 to 7 hours of incubation.

The primitive streak grows anteriorly because of proliferation of its own cells as well as of the addition of cells that migrate to it from anterior and lateral parts of area pellucida. The elongated axis of the primitive streak marks the antero-posterior axis of the future embryo. It, thus, eventually extends to, about three fifths of the entire length of area pellucida This is the fully developed definitive primitive streak and it is usually completed after 18 10 19 hours of incubation. The arca pellucida also becomes pear shaped. Along the middle of the primitive streak, when it is fully developed runs a narrow furrow, the primitive groove. Al the anterior end of the primitive streak there is a thickening, the primitive knot or llensen's node. The centre of Hensen's node is excavated to form a funnel-shaped depression.

The movements in the blastoderm leading to the final placement of cells in the hypoblast and to the formation of the primitive streak in the epiblast may be called pregastrular movements.

Invagination and Involution: At the stage of short primitive streak, the cells of the blastoderm already begin to migrate (invaginate and involute) into the blastocoel cavity between epiblast and hypoblast. Immigrating cells are replaced by more epiblast cells converging toward the streak area. The inward migrating cells also spread out sideways and forward from the anterior end of primitive streak. The notochordal cells immigrate through primitive pit. Endodermal cells invaginate through that part of the streak which lies just behind primitive pit.

The mesodermal cells of somites just follow the path of endodermal cells. Whereas the lateral plate mesoderm cells invaginate through the middle section of primitive streak, but only after the disappearance of endoderm from the area pellucida. The extra-exbryonic mesoderm (of the yolk sac) immigrates through the posterior part of primitive streak.

Meanwhile, some hypoblast cells expand into the area opaca to become extra-embryonic endoderm (the lining of yolk sac), while other hypoblast cells attach to mesodermal and notochordal cells are carried along by the latter's migration.

Formation of Head Process:

Prospective notochoral cells converge on the node, sinks through it and then passes directly forward as a tongue of tissue known as head process or notochord process.

Disappearance of Primitive Streak: With the gradual disappearance of endodermal, notochordal and mesodermal cells from the primitive streak, it begins to shrink from anterior towards posterior side and its remains are partly included in the tail bud and partly into the cloacal region of the embryo.

Development of Head Process: The midline area of notochordal tissue develops into a rigid rod, anterior to the receding primitive streak As the streak regresses posteriorly, the embryo develops anterior to it. The head process consists of a thick central mass of cells and more diffuse lateral it is also blended in the midline with the hypoblast wings. In the beginning

The thicker central portion forms the definitive notochord, whereas the lateral wings form the paraxial (somitic) mesoderm. With its differentiation, the notochord becomes detached from the hypoblast below, except at the extreme end. Thus, the head ocess stage is completed at about 20 to 25 hours of incubation. Gastrulation is also completed at this stage.

Completion of Endoderm: The first cells that migrate through the anterior part of streak form the endoderm. As the Hensen's node recedes backward, and the notochordal process elongates. the presumptive endoderm of the middle and posterior part of the gut, located just behind the node, migrate inside as an endodermal strip beneath the notochord.

The original hypoblast at the floor of the blastocoel contribute a very less amount to the gut, the upper migrated endodermal cells form the major part of the gut. In chick no archenteron is formed during gastrulation.

Fully Formed Gastrula: Gastrula is fully formed when primitive streak completely disappears. The fully formed gastrula consists of three germ_layers-ectoderm, chorda-mesoderm and endoderm. The ectoderm and chorda-mesoderm remain in continuity along the axis of primitive streak. The endoderm is also united with the mesoderm and ectoderm at the anterior and posterior end of streak.

Formation of Neural Tube (Neurogenesis): The ectoderm, anterior and lateral to the head process, becomes thickened to form the neural plate while the gastrulation process is going on. The neural plate appears in the brain region. As Hensen's node recedes farther and farther, parts of the neural plate become differentiated, and the anterior parts of the neural plate proceed to close into a tube, the neural tube.

The formation of neural tube occurs due to sinking in of the neural plate due to which a neural groove is formed along its longitudinal axis. Its elevated margins are called neural folds, which rise up and grow toward the midline and fuse to forin the neural tube.

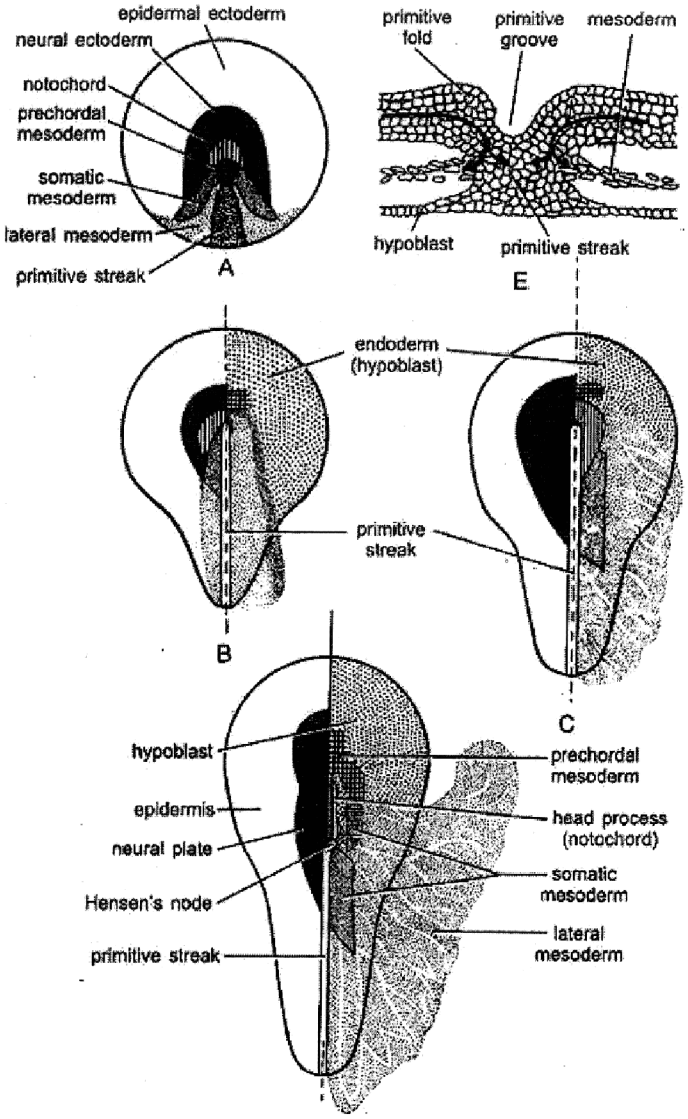


Fig. 2.37 :

Primitive streak and germ layer formation in chick embryo. A: surface view of epiblast; B, C and D: the left side shows the surface and the right side the pattern of mesodermal areas as they spread over the hypoblast; E: cross-section showing the involution of mesoderm through primitive streak.

Formation of Notochord and Mesoderm: While the neural plate is folding into the neural tube, the chorda-mesoderm is also differentiating. Its most anterior part, the prechordal plate mesoderm gives rise to the mesenchyme of head, and behind it the notochordal cells become separated from the rest of the adjoining sheets of mesoderm and differentiated into notchord. On either side of notochord are three longitudinal strands or sheets of mesoderm.

1. Epimere, axial or somatic mesodermis are the thicker dorsal medial strands, which merge anteriorly into the mesenchyme of head.
2. Mesomeres or intermediate mesodermal strands are located lateral to the axial mesoderm. They are thin in an early stage
3. Hypomeres or lateral plate mesoderm lies beneath the mesomere.

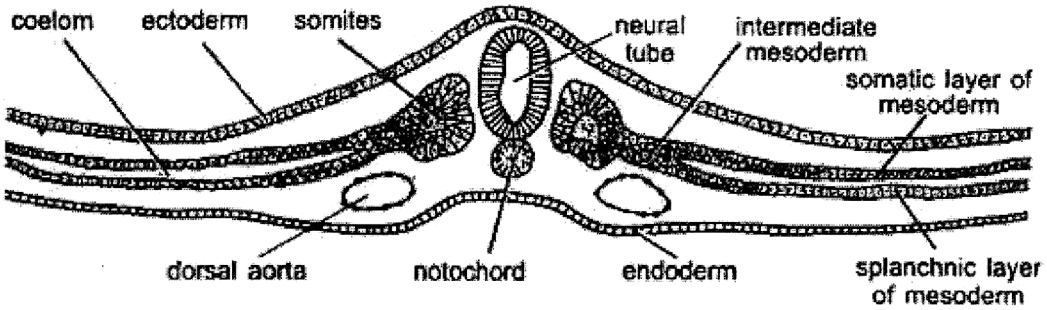


Fig. 2.38 : Cross-section of an early showing the relationship of the germ layers.

Formation of Somites: The axial or somatic mesoderm begins to differentiate after at about bageros 21 hours of incubation. It becomes a thick band on either side of notochord and nerve cord. A short distance anterior to the primitive knot a transverse cleft appears across each band, marking the division between first and second somites.

About one hour later a second cleft appears posterior to first cleft and, thus, second somite is formed. This process goes on approximately after each hour up to 20 hours. After that somites formation become somewhat slower.

The first three pairs of mesodermal somites are formed in the paraxial mesoderm in the presumptive hind brain region, and other mesodermal somites appear as the primitive knot regresses. Somites, when first formed, are masses of mesodermal cells with a small cavity in the centre. The cells are arranged radially around the central cavity.

With further development, they become extended in the dorso-ventral direction and flattened medio-laterally. The cavity (myocoel) of cach somite also changes, from spherical to a narrow vertical slit. An inner wall and an outer wall become clearly distinguishable, corresponding to the parietal and visceral layers of the lateral plates. The inner wall of the somites becomes very much thicker and produces skeletogenous tissue and the voluntary striated muscles of the body.

The outer of the somites are thinner and form the connective tissue layer of the skin and are therefore, called the dermatomes Furthermore, the dorsal part of the inner wall of the somite s which is the source of somatic muscles of the body is called myotome, whereas the lower edge of the inner wall is called sclerotome which is responsible for skeletogenous tissue. The sclerotome breaks up into a mass of mesenchymal cells.

Formation of Coelom: After the somites and the lateral plates have been formed, the mesoderm of lateral plate splits into two layers- the external or parietal layer and the internal or visceral layer. The cavity between the two is the coelom. Thus, the origin of coelom is schizocoelic.

The cells migrate into the spaces surrounding the notochord and spinal cord, envelop these organs and later differentiate into cartilage, thus, forming the bodies and neural arches of the vertebrae. Haemal arches and the ribs are also formed by these cells.

Folding of Embryo: The entire blastoderm does not form the embryo. Its central portion, called the area pellucida, only forms the embryo, and its outer portion, the area opaca forms the extraembryonic membranes, such as yolk sac, amnion, chorion and allantois.

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

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

The posterior fold undercuts the tail end and the posterior part of trunk which also project freely over the surface of yolk sac. These body folds gradually contract underneath the embryo and eventually the body of embryo is connected with the yolk sac and other extra-embryonic membranes by a narrow stalk, the umbilical cord.

2.11.1 *Questions*

1. Draw and describe the structure of a typical egg of hen?
2. Describe the pattern of cleavage of chick blastoderm?
3. How is blastula formed in chick embryo?
4. What is the primary organizer in chick embryo?
5. Describe the formation of primitive streak in chick gastrulation?
6. Describe the formation of hed process?
7. State the role of epiboly, emboly, invagination and Involution in the chick gastrulation?
8. How is neural tube formed in chick?
9. Describe the formation of germ layers in chick in details?
10. How is coelom formed in chick embryo?

2.12 Embryonic Induction and Organizers

Introduction:

The organizer is an embryonic tissue, which organizes the surrounding tissues to develop an embryo. The existence of the organizer was discovered by Spemann. He was given Nobel Prize in 1935 for his landmark discovery. Transplantation is a process in which small piece of a blastula, gastrula or even an embryo is cut off and inserted into suitably prepared incision of the same or another embryo. The embryo from which a part of tissue is taken is referred to as the donor, and the embryo to which the part is transplanted is called the host. The transplanted tissue is known as the graft.

The effect of embryonic interaction or organizer is a morphogenetic effect by which one organic tissue transmits a chemical substance that influences other embryonic part to produce a structure that otherwise could not come into existence. The embryonic tissue which exerts such an influence is called an ***inductor*** and the chemical substance secreted by an inductor is known as ***evocators***. The tissue on which evocator works and the tissue responses is known as responsive tissue. The action of the indicator through evocator is known as induction action or organizer action. This process of induction influences greatly the protein synthesis mechanism of responsive tissues as a result of which definite structure forming cells become very active.

Historical Background of Embryonic Induction:

For the discovery of neural induction, the German embryologist, Hans Spemann and his student, Hilde Mangold (1924) worked a lot and for his work Spemann received Nobel Prize in 1935. These two scientists performed certain ***heteroblastic transplantations*** between two species of newt, i.e., Triturus cristatus and Triturus taeniatus and reported that the dorsal lip of their early gastrula has the capacity of induction and organization of presumptive neural ectoderm to form a neural tube and also the capacity of evocation and organization of ectoderm, mesoderm and endoderm to form a complete secondary embryo.

They called the dorsal lip of the blastopore the primary organizer since it was first in the sequence of inductions and as it had the capacity to organize the development of a second embryo. Later on, the primary organizer was reported to exist in many animals, eg in frogs (Daloq and Pasteels, 1937); in cyclostomes (Yamada, 1938); in bony fishes (Oppenheimer, 1936), in birds (Waddington, 1933) and in rabbit (Waddington, 1934).

Types of embryonic induction: Lovtrup (1974) classified different types of embryonic induction into two basic categories-endogenous and exogenous inductions.

1. ***Endogenous induction:*** Certain embryonic cells gradually assume new diversification pattern through the inductors that are produced by them endogenously. Due to these inductors, these cells undergo either self-transformation or self-differentiation. Examples of such induction were reported in Mesenchymal cells of ventral pole of Echinoid and in small sized, yolk-laden cells of dorsal lip of amphibian blastopore.
2. ***Exogenous induction:*** When some external agent or a cell or a tissue is introduced into an embryo, they exert their influence by a process of diversification pattern upon neighbouring cells through contact induction. This phenomenon is called exogenous induction. It may be homotypic or heterotypic depending on the fact that whether the inductor provokes the formation of same or different kind of tissues respectively (Grobstein, 1964).

In homotypic induction, a differentiated cell produces an inductor. The inductor not only serves to maintain the state of the cell proper, but also induces adjacent cells to differentiate according to it, after crossing the cell boundaries. Best example of the heterotypic exogenous induction is the formation of a secondary embryonic axis by an implanted presumptive notochord in amphibians.

Spemann's Experiment and The Embryonic Induction: In these experiments, Spemann and Mangold used differently pigmented embryos from two newt species: the darkly pigmented Triturus taeniatus and the non-pigmented Triturus cristatus. So when Spemann and Mangold prepared these transplants, they were able to readily identify host and donor tissues on the basis of color. When the dorsal lip of an early *T. taeniatus* (a type of newt) gastrula was removed and implanted into the region of an early T. cristatus gastrula fated to become ventral epidermis (belly skin), the dorsal lip tissue invaginated just as it would normally have done (showing self-determination), and disappeared beneath the vegetal cells. The pigmented donor tissue then continued to selfdifferentiate into the chordamesoderm (notochond) and other mesodermal structures that normally form from the dorsal lip. As the new donor-derived mesodermal cells moved forward, host cells began to participate in the production of the new embryo, becoming organs that normally they never would have formed. In this secondary embryo, a somite could be seen containing both pigmented (donor) and unpigmented (host) tissue. Even more spectacularly, the dorsal lip cells were able to interact with the host tissues to form a complete neural plate from host ectoderm. Eventually, a secondary embryo formed, face to face with its host.

Spemann (1938) referred to the dorsal lip cells and their derivatives (notochord, prechordal mesoderm) as the organizer because (1) they induced the host's ventral tissues to change their fates to form a neural tube and dorsal mesodermal tissue (such as somites), and (2) they organized host and donor tissues into a secondary embryo with clear anterior-posterior and dorsal-ventral axes. He proposed that during normal development, these cells organize the dorsal ectoderm into a neural tube and transform the flanking mesoderm into the anterior - posterior body axis.

The process by which one embryonic region interacts with a second region to influence that second region's differentiation or behavior is called induction. Because there are numerous inductions during embryonic development, this key induction wherein the progeny of dorsal lip cells induce the dorsal axis and the neural tube is traditionally called primary embryonic induction.

Characteristics of the organizer:

- Organizer has the ability for self-differentiation and organization.
- It also has the power to induce changes within the cell and to organize surrounding cells, including the induction and early organization of neural tube.
- Primary organizer determines the main features of axiation and organization of the vertebrate embryo.

Induction is a tool-like process, utilized by this center of activity through which it affects changes in surrounding cells and as such influences organization and differentiation. These surrounding cells, changed by the process of induction, may in turn act as secondary inductor centers with abilities to organize specific sub-areas. Thus, the transformation of the late blastula into an organized condition of the late gastrula appears to be dependent upon a number of separate inductions, all integrated into one coordinated whole by the ***"formative stimulus"*** of the primary organizer located in the pre-chordal plate area of the endodermal-mesodermal cells and adjacent chorda mesodermal material of the early gastrula.

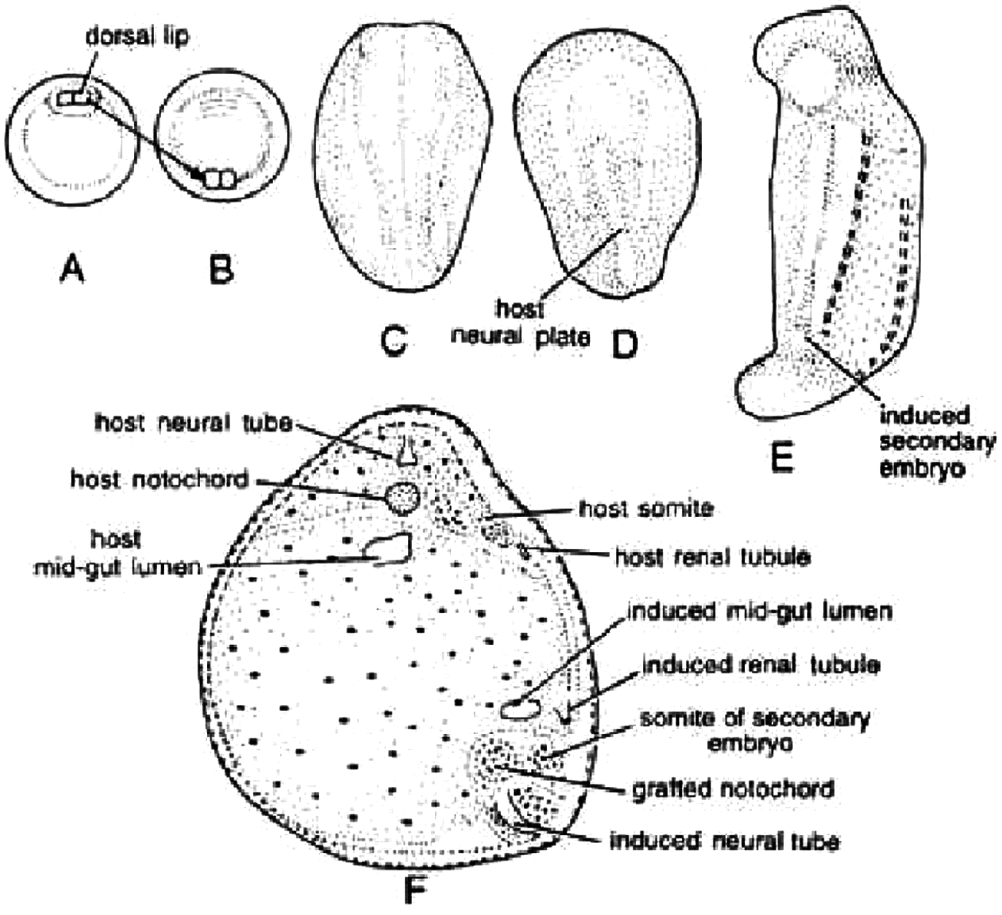


Fig. 1.1 : Induction of Secondary embryo in *Triturus sp.* by transplanting a piece of dorsal lip to the future belly region of another gastrula (A-B); C-E: Stages of resulting primary embryo embryo, with a secondary embryo attached to it; F: T, S of the same embryo

Structure of organizer : The organizer has special region. Each region has the capacity to induce the development of a specific organ. There are two very important regions

(1) Head inductor and

(2) Trunk inductor

Head inductor: The head inductor is anterior part of the chordo-mesoderm, because it induces the development of head organs. The head inductors may be additionally divided into the archencephalic inductor, which induces the prosencephalon, eyes and the nose rudiments and the duterencephalic inductor which induce the hind brain and the ear vesicles.

Trunk inductor: The later part of the chordo-mesoderm functions as the trunk inductor. It induces the development of trunk organs and the tail bud The neural tube under the induction arises from the chordomesoderm. The endoderm and the mesoderm cannot develop into the neural tube under this stimulus. Therefore, the ectoderm has the competence to develop into the neural tube but the endoderm and the mesoderm do not have the competence to develop into the neural tube.

Primary induction and gray crescent: The dorsal lip region of the blastopore at the onset of gastrulation can be traced back to the gray-crescent of the undivided fertilized amphibian egg. It was conceived by some developmental biologists that the crescent material of egg cortex initiated gastrulation and has the capacity of neural induction. A.S.G. Curtis (1963) performed a series of experiments of transplanting parts of the cortex of the fertilized egg of the clawed toad, Xenopus laevis at the beginning of cleavage.

In one experiment, the gray-crescent cortex was excised from the fertilized egg and it was observed that the cell division though proceeded undisturbed, the gastrulation failed to take place (**Fig. 2.40A**). In another experiment, the gray crescent cortex of uncleaved fertilized egg was excised and transplanted into a ventral position of a second egg, so that the egg receiving the graft had two gray crescents on opposite sides.

As a result, egg cleaved to form a blastula, which underwent two separate gastrulation movements to produce two separate primary nervous systems, notochord and associated somites (**Fig. 2.40D**). Similar experiments conducted on the eight-cell stage showed that something had happened during the short – interval represented by the first three cleavages.

Gray crescent cortex of the eight-cell stage still retained its inductive capacity when grafted to younger stages (**Fig. 2.40C**). Removal of the gray crescent at this stage no longer inhibits subsequent gastrulation and normal development, the missing crescent properties being replaced from adjacent cortical regions (**Fig. 2.40B**). According to Curtis, a change in cortical organization spreads across the surface of the egg during the second and third cleavages, starting from the gray crescent; when this change is completed, interactions, probably of a biophysical nature, can take place among various parts of the cortex.

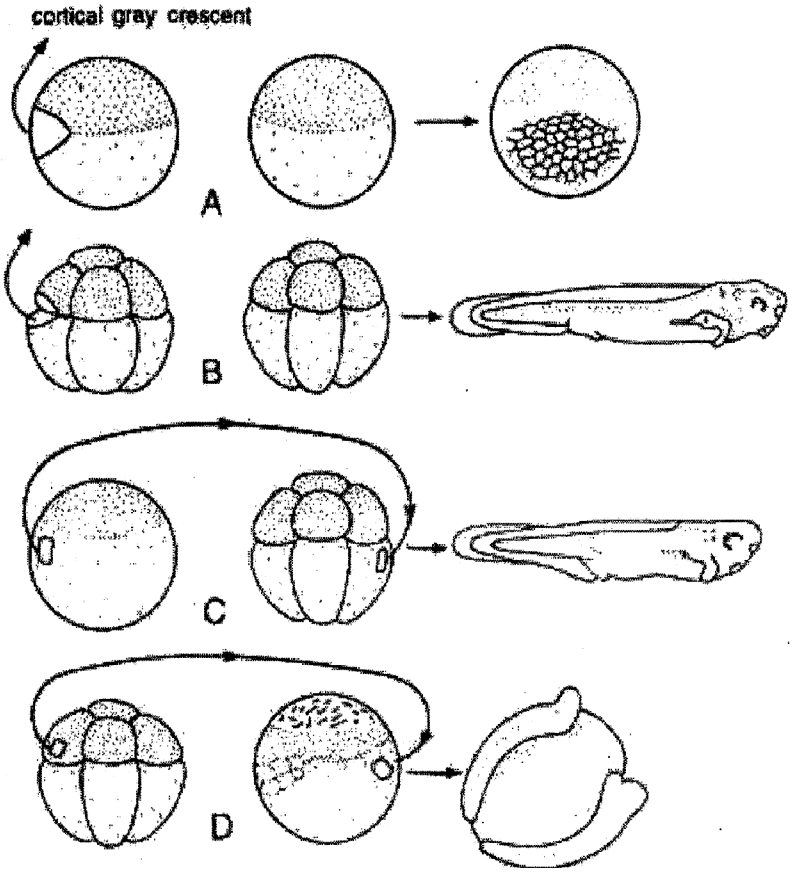


Fig. 2.40

Experimet of Curtis on *Xenopus*. A: Excision of the cortical gray crescent area at one cell stage (no gastrulation); B: Same experiment at 8-cell stage: normal embryo; C: Graft of gray crescent cortex of one cell stage to the ventral part of the 8-cell stage does not result in the induction of a secondary embryonic axis; D: Graft of gray crescent cortex from 8- cell stage to the ventral margin of one cell stage induces a secondary embryonic axis.

Secondary, Tertiary and Quaternary Organizers: As gastrulation continues, the different organ system of the embryo are laid down under the power of the primary organizer and they themselves then obtain the influence of inducing later formed structures to develop. It is thus probable to know a sequence of secondary, tertiary and quaternary organizers, which are set in a short of chain of command at whose summit is the primary organizer. These developed tissues then work together with another tissue in rotation and induce it to develop. In other words, one tissue gives the stimulus for the progressive development of the other tissue subsequently.

Competence: Waddington in 1932 introduced the concept of competence. It is always related to particular stimuli and particular corresponding responses. During induction, the reacting cells of the host must be in a particular physiological state so as to differentiate into nervous system under the influence of the inductor. This special state of reactivity is called Competence, which sums up the ability of the enzyme complement of the embryonic cell to adopt to a particular ratio of metabolites.

When the ectoderm of amphibian embryo is transplanted from various developmental stages of blastula to early neurula, it gradually loses neural competence. With aging, the ectoderm gradually loses its capacity to respond to the inductive stimulus of chordo-mesoderm. Therefore, an isolated ectoderm unexposed to neural induction, and late neurula epidermis no longer convertible into neural tissue becomes competent to respond to other inductors under the influence of eye vesicle, hindbrain and forebrain respectively. It differentiates into lens, car vesicle and nasal pits during post-neurula stage of development. Thus, competence is a timelimited phenomenon with a beginning and an ending. As the age of the embryo advances, the competence of the various structures gets gradually reduced.

Embryonic induction in different chordates: Although neural induction was first discovered in urodele amphibians, it was found that the dorsal lip of the blastopore and the roof of the archenteron of other vertebrates have the same function. The chorda-mesoderm in all vertebrates induces the nervous system and sense organs. Neural inductor has been investigated in the following chordates:

(1) In Cyclostomes, especially in lampreys, the property of neural induction lies in the presumptive chorda mesodermal cells of dorsal lip of the blastopore.

Prior to cyclostomes, in Ascidians different blastomeres of eight cell stage have the following presumptive fates-(i) the two anterior animal pole blastomeres produce head epidermis, palps and the brain with its two pigmented sensory structures, (ii) two posterior animal pole blastomeres produce epidermis, (iii) two anterior vegetal blastomeres produce notochord,

spinal cord and part of the intestine (iv) two posterior vegetal cells produce mesenchyme, muscles and part of the intestine.

From these experiments, Raverberi (1960) concluded that the formation and differentiation of brain by two anterior animal blastomeres is dependent on the induction of two anterior vegetal blastomeres, which act as neural inductors. It was further concluded that the two anterior vegetal blastomeres gave rise to diverse tissues, namely, endoderm, notochord and spinal cord.""98

(2) Wu and Tung (1962) proved the existence of the primary organizer and neural induction in Amphioxus. They transplanted pieces of tissues from the inner surface of the dorsal blastopore lip of an early gastrula of Amphioxus into the blastocoel of another embryo in the same stage and observed that secondary embryo developed in the ventral region of the host with a notochord and mesoderm produced by the graft and the neural tube from host tissue.

Thus, the chordal tissue of Amphioxus gastrula possesses the power of neural induction, while mesodermal and endodermal tissues have little such inductive power,

(3) In bony fishes, induction of secondary well developed embryos were produced by transplanting the posterior edge of the blastodisc which corresponds to the dorsal lip of the blastopore, into the blastocoel of another embryo or by transplanting the chordamesoderm and ectoderm. Neural inductions were also obtained by transplanting the dorsal lip of the blastopore in the sturgeon.

(4) In frogs, the induction of secondary embryo can be produced by the dorsal lip of the blastopore transplanted into the blastocoel of a young gastrula, in very much the same way as in newts and salamandars.

(5) In reptiles archenteron has the same inducing activity as in other vertebrates but there is no experimental proof of occurrence of neural inductor.

(6) In birds the existence of primary organizer was established by Waddington and co-workers. Anterior half of the primitive streak was the inducing part similar to the lips of the blastopore in amphibians. In the experiment whole blastoderms were removed from the egg in early gastrulation and cultivated in vitro on the blood plasma clot.

From another embryo, parts of the primitive streak were then inserted between epiblast and hypoblast, inductions of secondary embryos obtained. Primitive streak was found dependent on the underlying hypoblast for its formation.

(7) A successful neural induction was performed in a rabbit embryo by cultivating the early blastodisc on a plasma clot and implanting the primitive streak of the chick as inductor. Tissues of the mammalian gastrula were found having competence for neural induction. Anterior end of a rabbit embryo, with two pairs of somites, induced a neural plate in a chick embryo when placed under a chick blastoderm.

2.12.1 *Questions*

1. What is organizer concept?
2. Names the types of organizer?
3. What is induction and competence?
4. What is exogenous and endogenous induction?
5. What is head inductor and trunk inductor?
6. State the characteristics of an organizer?
7. Write a note on embryonic induction in different chordates?
8. Describe the Spemann's Experiment of embryonic Induction supported with a diagram?

Unit - 3 □ Late Embryonic Development

Structure

3.1 Objective

The main objective of this unit are the :

- we can learn the mechanism of the formation of three germinal layers.
- mechanism of transformation of each germinal layer into different organ system.
- we can learn how the placenta is devolped in mammalian development.
- we can also learn the basic functions of the placenta in development and their classification

3.2 Introduction

Late embryonic stage means when the cells of the embryo are destined to their fate and the embryo takes the shape of the animal. These processes are discussed in details with the fate of the cells of each germ layers. This unit also discussed the mechanism of the development of the placenta as well as the development of the extra embryonic membrane. The classification of the different types of placenta are also discussed in this unit.

3.3 Formation and fate of three germ layers

Transformation of blastula or blastocystinto gastrula is called gastrulation During gastrulati the cells of the inner cell mass of blastocyst or blastula move in small mass to their new fin location Such movement of cells is called morphogenetic movements Grastrulation results in the formation of three germ lavers ectoderm, mesoderm and endoderm Fach germ layer gives to specific tissues, organs and organ-systems.

The fate of the germ layers is the same in all triploblastic animals.

(i) Formation of Endoderm:

The blastocyst grows in size by obtaining nutrition from the uterus. Some cells separate from inner cell mass (embryonic knob) to form endoderm in blastocoel. The endoderm differentia into the primitive gut; a part of it gives rise to alimentary canal and the other portion forms y sac. After the formation of endoderm, the remaining mass of cells of the inner cell mass for embryonic dise. It has three parts: cephalic margin, embryonic disc proper and caudal margin.

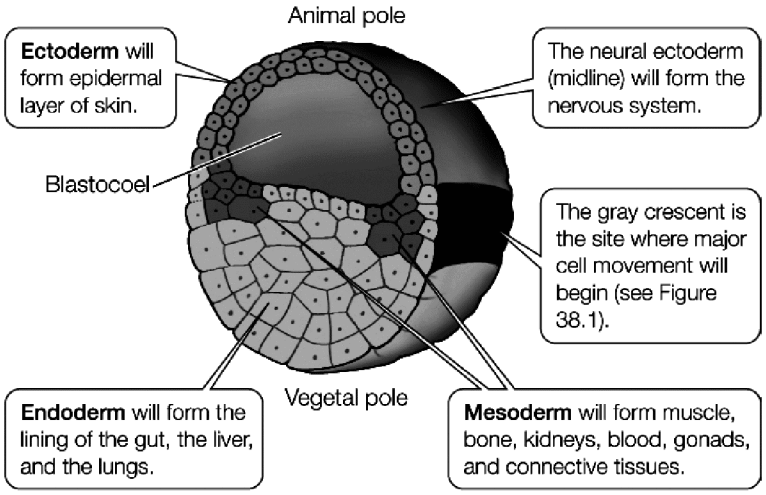


Fig. 3.1 : Three germ layers of the embryo

(ii) Formation of Mesoderm : Mesoderm is formed from the caudal margin of the embryonic disc. Prior to this the cells undergo rapid division and a mass of cells detach from the embryonic disc to mesoderm.

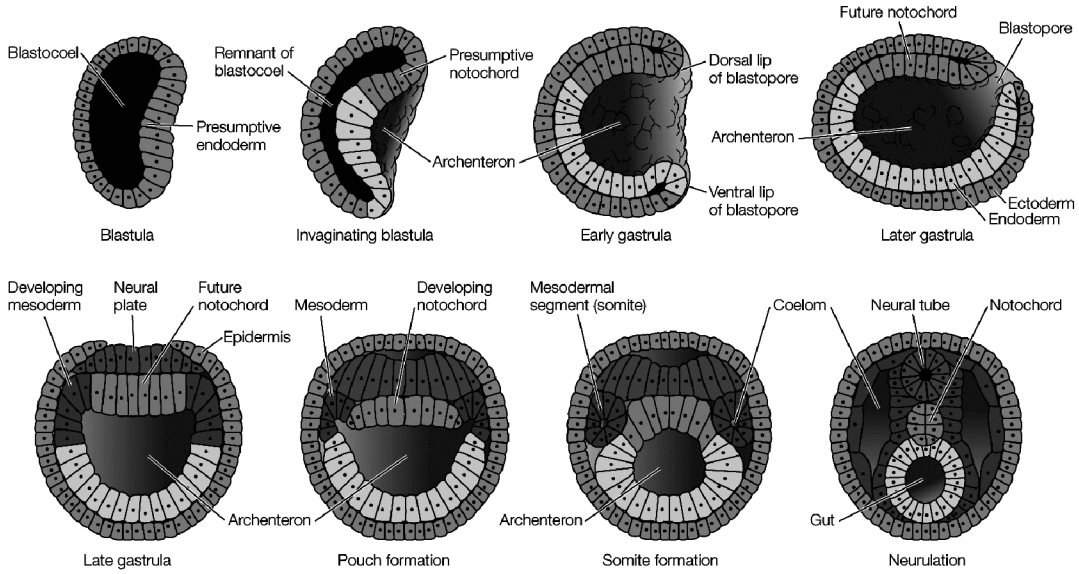


Fig. 3.2 : Positions and formation of 3 germ layers of an embryo

(iii) Formation of Ectoderm: After the separation of mesoderm, the remaining cells of the embryonic disc form the ectoder layer. In this manner the three germ layers such as ectoderm, mesoderm and endoderm a formed

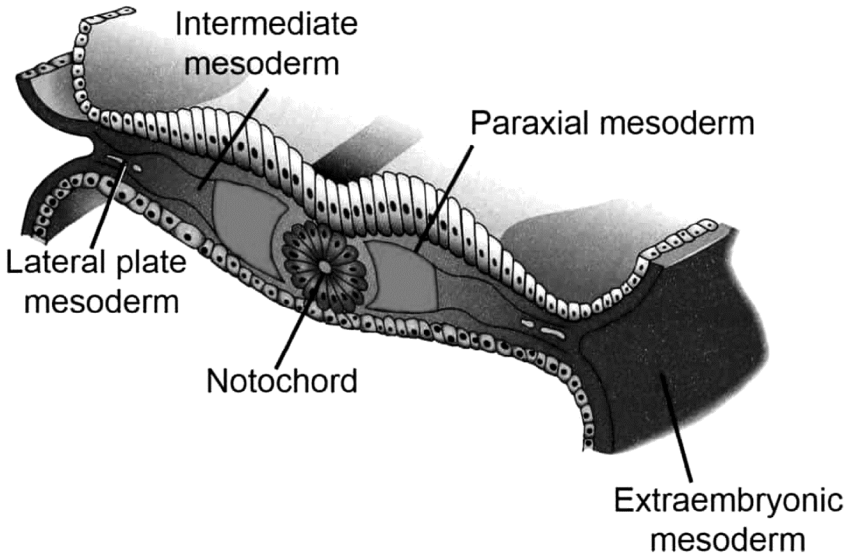


Fig. 3.3 : Formation of mesoderm

Fate of Three Germ Layers:

Each germ layer forms specific tissues, organs and organ-systems. The three germ laye produce tissues, organs and organ-system in following manner:

1. Ectoderm: It forms:

(i) Epidermis of skin, epidermal derivatives like epidermal glands, hair, nail etc.

(ii) Nervous system,

(iii) Medulla of adrenal gland, posterior and intermediate lobes of pituitary gland, pineal gland, and ciliary muscles), mammary glands, salivary glands and lacrimal glands.

(iv) Eye (conjunctiva, cornea, lens, retina, iris

(v) Internal car,

(vi) Nasal and olfactory epithelia,

(vii) Enamel of teeth,

(viii) Epithelium of fore gut and hind gut

(ix) Some glands-sweat glands, oil glands.

2. Mesoderm: It forms:

(i) Dermis of skin,

(ii) Muscles except iris and ciliary muscles,

(iii) Connective tissues,

(iv) Kidneys,

(v) Gonads,

(vi) Notochord,

(vii) Heart, blood and lymph vessels,

(viii) Urinary and reproductive ducts

(ix) Most of skeleton,

(x) Coelomic epithelium,

(xi) Pericardium and pleura,

(xii) Dentine of teeth,

(xiii) Cortex of adrenal gland,

(xiv) Mesenteries,

(xv) Sclera and choroid of eyes,

(xvi) Wall of the gut except its lining.

3. **Endoderm:** It gives rise to:

(i) Lining of gut except for gut and hind gut,

(ii) Some glands - pancreas, liver, gastric glands, intestinal glands, thyroid, parathyroid, thymus and larger part of prostate,

(iii) Inner layer of tympanic membrane,

(iv) Lining of middle ear,

(v) Trachea, bronchi and lungs,

(vi) Urinary bladder,

(vii) Urethra.

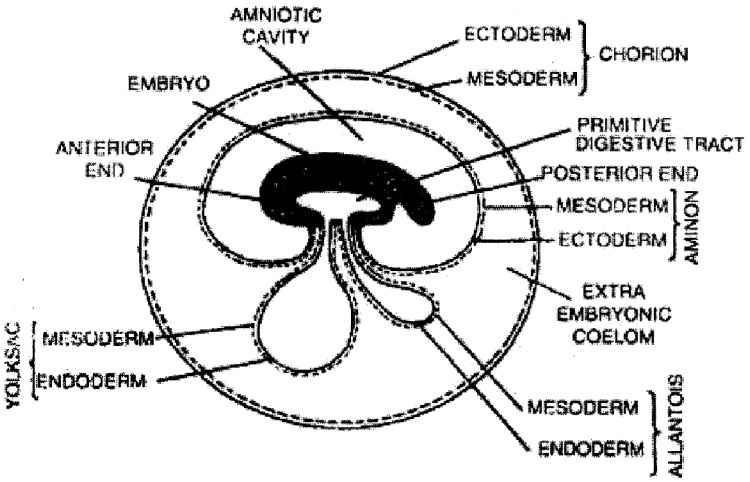


Fig. 3.4 : Diagram showing germ layers and foetal membranes

Ectoderm	Mesoderm	Endoderm
Epidermis	Bones, cartilage, tendon	Digestive System
Central Nervous System	Muscle (smooth, striated)	Respiratory System
Neural Crest Derivatives: • Peripheral Nervous System • Melanocytes • Facial cartilage • Dentin of teeth • Skull bones	Circulatory System, including heart, vessels	Pancreas
	Lymphatic system	Liver
	Gonads	Thyroid Epithelium
	Kidney	Parathyroid Epithelium
	Adipose Tissue	Thymus
	Notochord	Bladder

3.3.1 questions

1. Describe the formation of germ layers?
2. What are the fates of the three germ layers repectively?

3.4 Extra-Embryonic Membranes in Bird (Taking Chick as a model organism)

Introduction

During the development of chick and other vertebrates, certain specialized embryonic tissues or structures are produced that temporarily or permanently do not enter into the formation of the embryo themselves. These are external and devoted in one way or another to the care and maintenance of the developing embryo.

These structures are collectively termed as extra-embryonic memfbranes or foetal membranes or extra-embryonic sacs. These are not precursors of any of the organs of the adult or the larva but serve to satisfy the requirements of the embryo in connection with nutrition, gas exchange, removal or storage of waste materials and protection.

The extra-embryonic membranes have developed to make the eggs capable of developing on dry land. The eggs of reptiles, birds and prototherian mammals have a protective shell around it. In some reptiles and eutherian mammals the shell has given way to uterine development, but the basic form and function of the extra-embryonic membranes has remained the same. Extraembryonic membranes are those membranes formed of embryonic tissues, which extend out and beyond the strict confines of the embryonic body and are adapted to fulfill the care and maintenance of the developing embryo.

Kinds of Extra-Embryonic Membranes: Four sets of extra-embryonic membranes are common to the embryos of all terrestrial vertebrates including chick.

i. **Amnion:** The amnion is a thin mem #brane which eventually encloses the entire developing embryo in a fluid-filled sac. It is formed just above the embryo. It consists of ectoderm inside and mesoderm outside. The space between the embryo and the amnion is called as amniotic cavity. It is filled with a clear, watery fluid secreted by both the embryo and the membrane. Reptiles, birds and mammals possessing this amnion are often called amniotes, while fishes and amphibians, lacking it, are collectively called Anamniotes.

ii. **Yolk Sac:** It is the most primitive structure containing network of blood vessels and encloses the yolk of the egg. A yolk sac is also present in those fishes which have megalecithal eggs. Despite the lack of stored yolk in mammalian eggs (except in prototherians), the yolk sac has been preserved, as it serves many important secondary functions. It is a relatively larger sac-like structure formed below the embryo. It contains fluid, but not yolk. Its wall consists of endoderm inside and mesoderm outside. The yolk sac is non-functional in human beings. The yolk sac is responsible for forming blood cells until about oth week.

iii. **Allantois:** It is a sac-like structure which arises from the gut of the embryo near the yolk sac. Allantois is formed of endoderm inside and mesoderm outside. In man the allantois is small and forms blood vessels of placenta and blood cells. Allantois serves as an excretory and respiratory structure. It is a large sac like structure in reptiles and birds, while its role in mammals varies with the efficiency of the interchange that takes place at the foetal-maternal inter-face. In pig embryo, the allantois rivals that of the bird's in both size and functional importance? while the allantois in human has been reduced to a mere vestige which contributes only as a well-developed vascular network to the highly efficient placenta.

iv. **Chorion (Serosa):** Chorion is a very thin membrane and it covers the embryo and other extra-embryonic membranes. It completely surrounds the embryo and protects it. It consists of ectoderm outside and mesoderm inside. It is formed by the fusion of the amniotic folds over the embryo. All these extra-embryonic membranes are composite structures as they involve two germ layers. It takes part in the formation of placenta for the exchange of materials between the foetus and the mother.

The amnion and chorion are made up of extra-embryonic ectoderm and somatic layer of mesoderm, while the yolk sac and allantois are composed of extra-embryonic endoderm and splanchnic layer of mesoderm.

Development of Extra-Embryonic Membranes: During neurulation (neural tube formation) of chick, the lateral plate mesoderm splits into an outer somatic layer mesoderm of lying at the inner side of the ectoderm and an inner splanchnic layer of mesoderm lying outer to the endodermal layer. Both these mesodermal layers enclose a coelomic space between them.

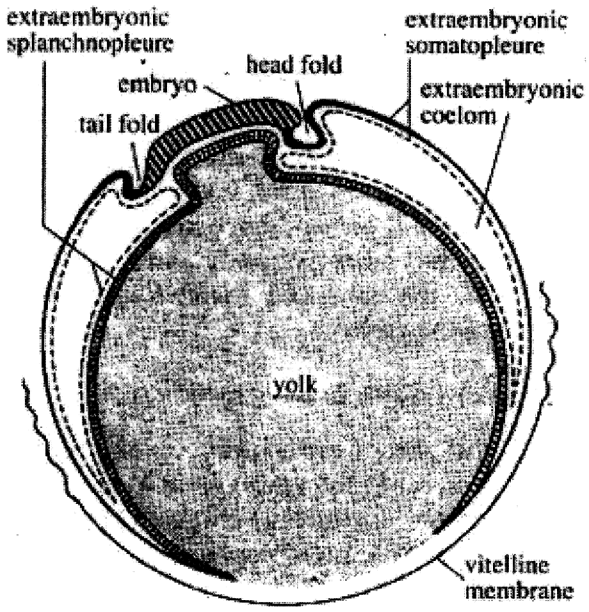


Fig. 3.6 : Early chick embryo showing body folds delimiting embryo from extraembryonic areas

The somatic layer of mesoderm and ectoderm are collectively known as somatopleure, while the splanchnic layer of mesoderm along with endoderm forms the splanchnopleure. At the time of development of the avian blastoderm, the somatopleure and splanchnopleure gradufally spread peripherally over the yolk mass, far beyond the area where the body of the embryo is taking form. Shortly, the embryo proper begins to be undercut by a series of body folds that serve to delimit the embryonic regions from the more peripheral extraembryonic somatopleure and splanchnopleure. After the formation of the body folds, the somatopleure and splanchnopleure of chick develop into the four extra-embryonic membranes.

i) **Development of Yolk Sac:** The yolk sac is the first extra-embryonic membrane to make appearance. As the early blastoderm expands, the extra-embryonic splanchnopleure continues spread over the yolk mass and eventually encloses the yolk completely to form the yolk sac.

   Coincidentally, the intra embryonic splanchnopleure is subjected to superficial body folds, which serve to establish a walled digestive tract or gut, body of the embryo. The middle of the gut (mid gut) remains connected with the yolk sac by a narrow yolk stalk , where the walls of the gut is continuous with the walls of the yolk sac. Although the yolk sac is connected with the

digestive tract by the volk stalk, the volk lood reserves are not transmitted to the embryo by this route. Rather the digestion of the volk is done by the

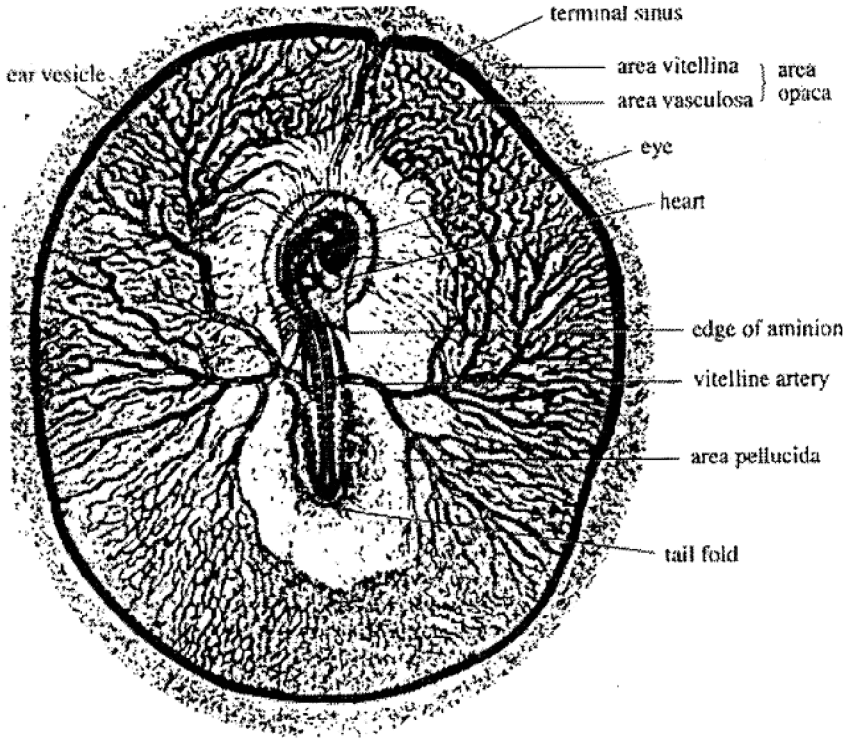


Fig. 3.7 : Chick embryo surrounded by area vasculosa

endodermal Timing of the yolk sac through the mediation of appropriate enzymes""In cluck on the 2nd and ind day of incubation, networks of blood vessels develop on the inner part of the area opaca, which becomes the area vasculosa. The outer part of arca opaca is called the area vitellina. All the blood vessels of arca vasculosa communicate with cach other and are joined together on the periphery by the terminal smus, which incidentally forms the boundary between area vasculosa and area vitellina. The network of the area vascuosa becomes prolonged into the area pellucida and eventually establishes connection with the embryo proper.

Connections with the blood system of the embryo is formed at two points:

(a) With the Venus system by means of the right and left vitelline (omphalomesenteric) veins. These join with the unpaired ductus venosus, which in turn enter the sinus venosus of the heart.

(b) With the arterial system by means of right and left vitelline (omphalomesenteric) arteries, which branch off from the dorsal aorta. At about the middle of the 2nd day of incubation, the heart of the embryo begins to beat. Between the

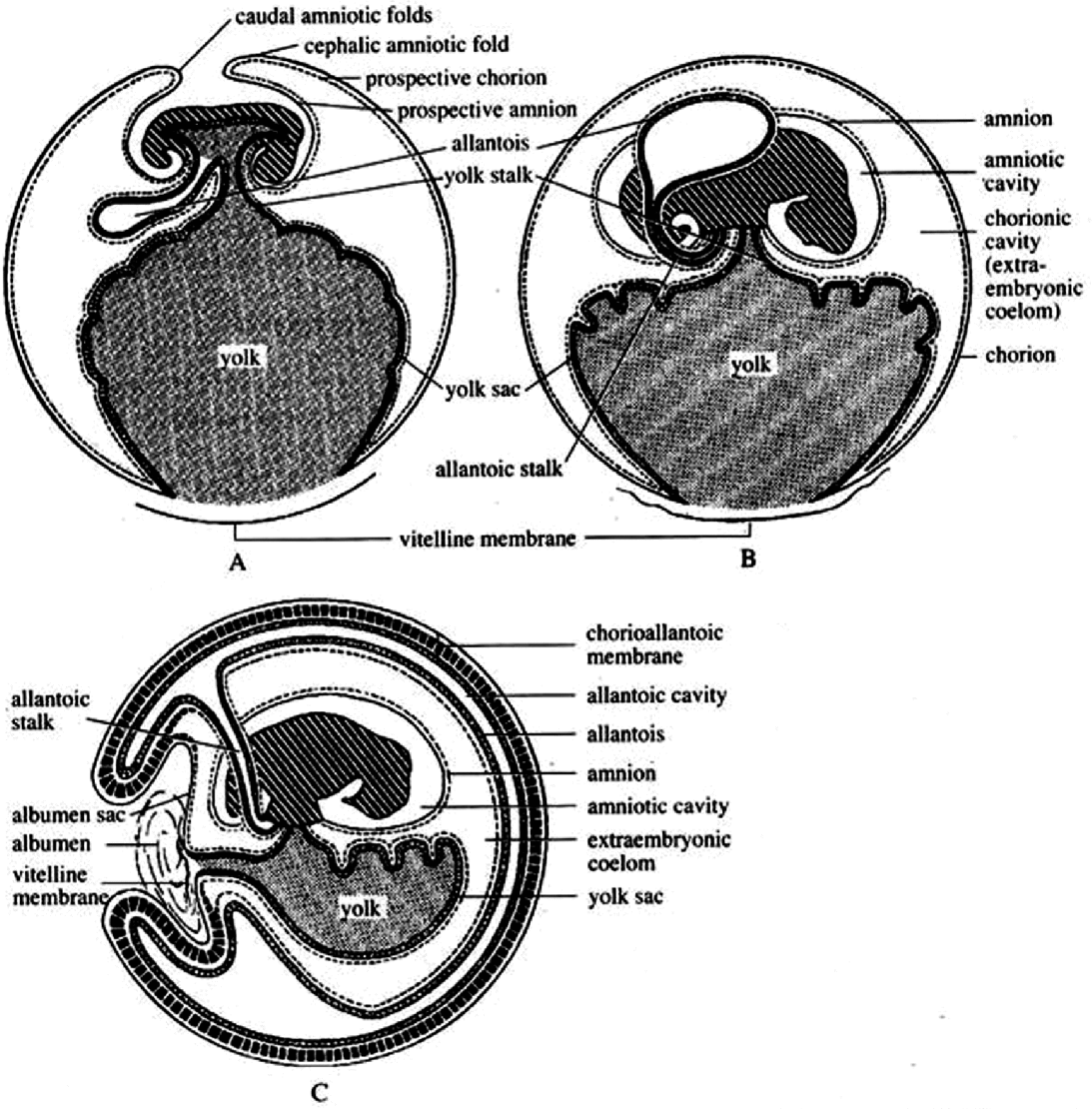


Fig. 3.8 : Development of Extra-embryonic membranes in chick. A: Early stage; B: later stage; C: Fully matured stage.

38th and 40th hours of incubation the blood starts circulating through the network of the yolk sac. The blood vessels in the arca vasculosa penetrate deep into the yolk. The endodermal surface of the yolk sac is thrown into

folds that penetrate the yolk mass. Then, through the action of appropriate digestive enzymes secreted by the endodermal cells, the yolk is digested or made soluble and is ultimately absorbed by the endodermal lining of the yolk sac. It is then passed on through the endothelial lining of the vitelline blood vestsels to the circulating blood, from where it is carried to all parts of the growing embryo. According to Young et al. (1980), the endo derm of the yolk sac in addition to its absorptive function, is also the sole site of synthesis of the serum proteins like transferfring, alpha globulins and prefalbumin. Also during the growth of the allantois, the albufmen is forced towards the distal end and gets surrounded by an extension of the yolk sac. It is absorbed along with yolk and transferred by way of the extrafembryonic circulaltion to the embryo. The entire yolk is not completely absorbed during embryonic life. On the 19th day when the period of incubation is nearing its end, the remains of the yolk sac are enclosed within the body walls of the embryo.

During the first 6 days after hatching, the resorption of the remaining part of the yolk sac and yolk gets completed. This remaining yolk reserves are vital to the newly hatched chick while it is adapting to a free-living existence and is developing its feeding behaviour.

ii. **Development of Amnion and Chorion:** The amnion and chorion are devetloped simultaneously and both are derived from the extra-embryonic somatopleure. At about the 30th hour of incubation, the head of the embryo sinks somewhat into the yolk and at the same time the cutrembryonic somatopleure is elevated over the embryo by a folding process consisting essentially of a doubling of the somatopleure upon itselt.

The initial elevation is over the head end of the embryo, producing a double somatopleuric hood, called the cephalic amniotic fold or head fold of the amnion (Fig 5.48A). From a dorsal aspect, the margin of this fold is crescentic in shape, with its concavity directed towards the head of the embryo. As the embryo increases in length, its head grows forward into the amniotic fold.

As the cephalic amniotic fold gradually extends backward, towards the tail region, its caudally extending side limbs called lateral amniotic folds arch over the embryo from each side to be joined tinally by a similar fold or elevation from the tail region called the caudal amniotic fold or tail fold.

All these amniotic folds finally converge at the midline, so as to encase the embryo by two sheets of somatopleure from all the sides except from the region of

the yolk stalk. The place where all the amniotic folds meet is called the seroamniotic connection or the amniotic raphe, which is a scar like thickening. The seroamniotic connection opens before the hatching of the embryo and admits albumen into the amniotic cavity.

The fusion of the amniotic folds results in the formation of two sac-like membranes and two cavities. The inner somatopleuric membrane becomes the amnion and the outer one, the chorion or serosa. The cavity between the amnion and the embryo is called the amniotic cavity and is lined by ectoderm. Muscle fibres differentiate within the mesoderm of the amnion and the amniotic cavity gets filled with fluid called amniotic fluid. The cavity lying between the amnion and chorion is called the chorionic cavity and is lined by the mesoderm.

This chorionic cavity is actually the extra-embryonic coelomic cavity, which is continuous with the coelomic cavity in the embryo proper. The chorion is lined on the outside by the extra embryonic ectoderm. According to Balinsky (1970) the formation of the amniotic cavity has a somewhat negative effect as it removes the embryo from the source from where it could obtain oxygen

iii. **Development of Allantois:** The allantois first appears late in the 3rd day of incubation. It bulges out as a ventral out-growth of the endodermal hindgut and corresponds exactly in nature to the urinary bladder of the amphibians. The outgrowth consists of an inner layer of endoderm and an outer layer olsplanchnic mesoderm.

The allantois cnlarpes very rapidly from the fourth day to the tenth day of incubation. It penetrates into the extra embryonic coelom, into the space between the yolk sac, the amnion and the chorion.

The base of the allantois remains connected with the hindgut of the embryo by means of a narrow allantorc stalk When the body of the embryo contracts separating the embryo from the extra embryonic parts, the allantoic stalk and the stalk of the yolk sac remain enclosed together forming an umbilical cord.

The distal part of allantois penetrates between the amnion and yolk sac on one side and the chorion on the other side. By the 4th to 10th day of incubation period, the allantois spreads rapidly and completely covers the coelomic space.

Soon, the mesodermal layer of the allantois becomes fused with the adja-cent mesodermal layer of the chorion to form a single mesodermal layer called chorioallantoic membrane. In the mean-time, the expanding chorioallantois bursts through the vitelline membrane of egg and pushes outward towards the shell membrane.

As it does so it progressively envelops the albumen and becomes a sac filled with albumen, called the albumen sac, that helps in the absorption of water and albumen. The chick embryo, through the chorioallantoic membrane and the shell, takes up about 5 liters of oxygen and gives off about 4 liters of carbon dioxide during its 21 days period before hatching. On the external surface of the allantois, a network of blood vessels develop and this network is in communication with the embryo proper by means of blood vessels running along the stalk of the allantois and through the umbilical cord.

Blood flows to the allantois through the right and left umbilical arteries, that leaves the dorsal aorta at a point which is much more caudal than the starting point of the vitelline arteries. The returning blood flows to the heart through a pair of umbilical veins that originally enter the right and left ducts of Cuvier. Soon, the right umbilical artery and the right umbilical vein disappear and the left umbilical vein develops a new connection. It joins the left hepatic vein and the connection with the duct of Cuvier gets closed. The allantoic circulation functions till the hatching of the chick and when it starts breathing the surrounding air. The umbilical vessels then close. The allantois dries up and separates from the body of the young chick.

Functions of the Extra-Embryonic Membranes:

Development of extraembryonic membranes are important for those vertebrates that lay their eggs on land. Eggs developing in water, encounter minimum external interfrerence and water provides the egg with various favourable environmental conditions. Ilowever, none of the favourable features are provided on dry land where eggs are subjected to desication and sudden changes in temperature. The extra-embryonic mem-branes have thus developed to serve the following functions:

i. Functions of Yolk Sac:

(a) The yolk sac which spreads over the large amount of yolk, serves as the digestive and absorptive organ by which the yolk is made available for the growing embryo.

(b) It functions as the first respiratory organ.

(c) It acts as a haemopoetic organ like the liver.

(d) The yolk sac also serves as the place of origin of blood cells, at later stages of development.

ii. Functions of Amnion and Chorion:

(a) The amniotic cavity contains a salty fluid surrounding the embryo. Thus, the embryo can accomplish its development in a fluid medium although it is "on dry land". Therefore, the amnion serves as a protective organ where the embryo is saved from the danger of desiccation.

(b) The amniotic fluid acts as an efficient shock absorber and thus, protects the soft, collapsible and almost skeletons early chick embryo from mechanical shocks.

(c) As the amnion isolates the embryo from the egg shell, it thus protects it from adhesion to the shell or from friction against it.

(d) The mesoderm of amnion, during later developmental stages, form muscle cells which contract rhythmically, thus rocking the embryo within the amniotic fluid. This rocking prevents the adhesion of amnion to the different embryonic membranes. It also helps in preventing the stagnation of blood in the vessels, a condition that might tend to occur on account of pressure from growing organs.

(e) The chorion at later developmental stage joins with the allantois to serve as a nutritional and respiratory organ.

iii. Functions of Allantois:

(a) Allantois acts as a reservoir for the secretions (excretory wastes, serves as a urinary bladder) coming from the developing excretory organs. During early stages of development the chick excretes mostly urea, but later it becomes chiefly uric acid. This change is significant as urea is a relatively soluble substance and would require large amount of water to keep it at nontoxic level. Uric acid is relatively insoluble and can be stored without any ill effects.

(b) The chorioallantoic membrane acts as a respiratory surface for the embryo. Thus, the yolk sac, amnion, chorion and allantois can be regarded as an adaptation for the egg and embryo to carry on its development on dry land.

(c) Together with the chorion, the allantois also surrounds the albumen to form albumen sac and thus assists in the absorption of nutritionally rich albumen.

(d) The vascular chorioallantoic membrane lies in close proximity to the inner surface of the porous shell. It acts as an extra-embryonic lung by supplying the embryo with oxygen. Gaseous exchange takes place between the blood

circulating in the chorioallantoic membrane and the external air through the porous shell. A network of blood vessels develop in this membrane and this network is in communication with the embryo proper.

3.4.1 Questions

1. What are extra-embryonic membrane? Name the types?
2. What are amniotes?
3. What are extra-embryonic membranes important?
4. Describe the formation of Amnion and yolk sac? Give proper diagram.
5. State the functions of Amnion, Chorion, Allantois and yolk sac.
6. Why Allantois is called the urinary bladder of the embryo?

3.5 Implantation of embryo in humans

Human embryogenesis is the process of cell division and cellular differentiation of the human embryo during early prenatal development. It spans from the moment of fertilization to the end of the 8th week of gestational age, where after it is called a fetus.

From One Cell to Blastocyst: A human develops from a single cell called a zygote, which results from an ovum (egg) being fertilized by a single spermatozoan (sperm). The cell is surrounded by a strong membrane of glycoproteins called the zona pellucida which the successful sperm has managed to penetrate The zygote undergoes cleavage, increasing the number of cells within the zona pellucida. Afer the 8-cell stage, embryos undergo what is called compactation, where the cells bind tightly to cach other, forming a compact sphere. After compactation, the embryo is in the morula stage (16 cells), Cavitation occurs next; where the outermost layer of cells (the trophoblast) secretes water into the morula. As a consequence of this when the number of cells reaches 40 to 150, a central, fluid-filled cavity (blastocoel) has been formed. The zona pellucida begins to degenerate, allowing the embryo to increase its volume. This stage in the developing embryo, reached after four to six days, is the blastocyst (related to the blastula stage), and lasts approximately until the implantation in the uterus.

Blastocyst Differentiation: The blastocyst is characterised by a group of cells, called the inner cell mass (also called embryoblast) and the mentioned trophoblast (the

outer cells). The inner cell mass gives rise to the embryo proper, the amnion, yolk sac and allantois, while the trophoblast will eventually form the placenta. The blastocyst can be thought of as a ball of a layer of trophoblast cells, with the inner cell mass attached to this ball-inner wall. The embryo plus its membranes is called the conceptus. By this stage the conceptus is in the uterus. The zona pellucida ultimately disappears completely, allowing the blastocyst to invade the endometrium, performing implantation.

Implantation: The trophoblast then differentiates into two distinct layers- the inner is the cytotrophoblast consisting of cuboidal cells that are the source of dividing cells, and the outer is the syncytiotrophoblast. The syncytiotrophoblast implants the blastocyst in the endometrium (innermost epithelial lining) of the uterus by forming finger-like projections called chorionic villi that make their way into the uterus, and spaces called lacunae that fill up with the mother's blood.

This is assisted by hydrolytic enzymes that erode the epithelium. The syncytiotrophoblast also produces human chorionic gonadotropin (hCG), a hormone that "notifies" the mother's body that she is pregnant, preventing menstruation by sustaining the function of the corpus luteum. The villi begin to branch, and contain blood vessels of the fetus that allow gas exchange between mother and child,

i. ***Implantation Window:*** There are many conditions that must be satisfied for a successful implantation to take place. There is only a specific period of time during which implantation is possible: this is the "implantation window". A reason for this window is that if implantation does not occur at a certain time, then it signifies that something is wrong. And when there is a risk that something is wrong, there will most likely be a miscarriage rather than the continued gestation of a malformed fetus.""The implantation window is started by preparations in the endometrium of the uterus, both structurally and in the composition of its secretions.

ii. ***Adaption of Uterus:*** To enable implantation, the uterus goes through changes in order to be able to receive the embryo.

Predecidualisation: Predecidualisation is a preparation of the endometrium of the uterus, prior to implantation, to facilitate it. The endometrium increases in thickness, becomes more vascularised and its glands grow to be tortuous and boosted in their secretions. These changes reach their maximum about 7 days after ovulation.

Furthermore, the surface of the endometrium produces a kind of rounded cells, which cover the whole area towards the uterine cavity. This happens about 9 to 10

days after ovulation. These cells are called decidual cells, which emphasises that the whole layer of them is shed off in every menstruation if no pregnancy occurs, just as leaves of deciduous trees. The uterine glands, on the other hand, decrease in activity and degenerate already 8 to 9 days after ovulation in absence of pregnancy.

The stromal cells originate from the stromal cells that are always present in the endometrium. However, the decidual cells make up a new layer, the decidua. The rest of the endometrium, in addition, expresses differences between the luminal and the basal sides. The luminal cells form the zona compacta of the endometrium, in contrast to the basalolateral zona spongiosa, which consists of the rather spongy stromal cells.

iii. ***Decidualisation:*** Decidualisation succeeds predecidualisation if pregnancy occurs. This is an expansion of it, further developing the uterine glands, the zona compacta and the epithelium of decidual cells lining it. The decidual cells become filled with lipids and glycogen and take the polyhedral shape characteristic for decidual cells.

a. Trigger: It is likely that the blastocyst itself makes the main contribution to this additional growing and sustaining of the decidua An indication of this is that decidualisation occurs at a higher degree in conception cycles than in non-conception cycles. Furthermore, similar changes are observed when giving stimuli mimicking the natural invasion of the embryo.

b. Parts of Decidua: The decidua can be organised into separate sections, although they have the same composition.

1. *Decidua Basalis:* This is the part of the decidua which is located basalolateral to the embryo after implantation.
2. *Decidua Capsularis:* Decidua capsularis grows over the embryo on the luminal side, enclosing it into the endometrium. It surrounds the embryo together with decidua basalis.
3. *Decidua Parietalis:* All other decidua on the uterine surface belongs to decidua parietalis.

4. Decidua throughout Pregnancy: After implantation the decidua remains, at least the first trimester. However, it's most prominent time is during the early stages of pregnancy, meanwhile as implantation. Its function as a surrounding tissue is replaced by the definitive placenta. However, some elements of the decidualisation remain throughout pregnancy.

The compacta and spongiosa layers are still observable beneath the decidua in pregnancy. The glands of the spongiosa layer continue to secrete during the first trimester, when they degenerate. However, before the disappearance, some glands secrete unequally much. This phenomenon of hypersecretion is called the Arias-Stella phenomenon, after the pathologist Javier Arias-Stella.

iv. **Pinopodes:** Pinopodes are small, finger-like protrusions from the endometrium. They appear between day 19 and day 21 of gestational age. This corresponds to a fertilization age of approximately 5 to 7 days, which corresponds well with the time of implantation. They only persist for 2 to 3 days. The development of them is enhanced by progesterone but inhibited bestrogens.

a. Function in Implantation : Pinopodes endocytose uterine fluid and macromolecules in it. By doing so, the volume of the uterus decreases, taking the walls closer to the embryoblast floating in it. Thus, the period of active pinocytes might also limit the implantation window.

b. Function during Implantation : Pinopodes continue to absorb fluid, and removes most of it during the early stages of implantation.

c. Adaption of Secretions Proteins, glycoproteins and peptides secreted by the endometrial glands

v. **Nourishment:** The embryoblast spends approximately 72 hours in the uterine cavity before implanting. In that time, it cannot receive nourishment directly from the blood of the mother, and must rely on secreted nutrients into the uterine cavity, e.g., iron and fat-soluble vitamins.

vi. **Growth and Implantation:** In addition to nourishment, the endometrium secretes several steroid- dependent proteins, important for growth and implantation. Cholesterol and steroids are also secreted. Implantation is further facilitated by synthesis of matrix substances, adhesion molecules and surface receptors for the matrix substances.

vii. **Mechanism:** Implantation occurs approximately 7 days after fertilization, and is initiated when the blastocyst comes into contact with the uterine wall.

*a. Zona Hatching:*To be able to perform implantation, the blastocyst first needs to get rid of its zona pellucida. This process is called "hatching".

b. Factors: Lytic factors in the uterine cavity, as well as factors from the blastocyst itself are essential for this process. Mechanisms in the latter are

indicated by that the zona pellucida remains intact if an unfertilized egg is placed in the uterus under the same conditions.

A substance probably involved is plasmin. Plasminogen, the plasmin precursor, is found in the uterine cavity, and blastocyst factors contribute to its conversion to active plasmin. Furthermore, plasmin inhibitors also inhibit the entire zona hatching in rat experiments.

viii. **Apposition:** The very first, albeit loose, connection between the blastocyst and the endometrium is called the apposition.

Location: On the endometrium, the apposition is usually made where there is a small crypt in it, perhaps because it increases the area of contact with the rather spherical blastocyst.

On the blastocyst, on the other hand, it occurs at a location where there has been enough lysis of the zona pellucida to have created a rupture to enable direct contact between the underlying trophoblast and the decidua of the endometrium. However, ultimately, the inner cell mass, inside the trophoblast layer, is aligned closest to the decidua.

Nevertheless, the apposition on the blastocyst is not dependent on if it is on the same side of the blastocyst as the inner cell mass. Rather, the inner cell mass rotates inside the trophoblast to align to the apposition. In short, the entire surface of the blastocyst has a potential to form the apposition in the decidua.

ix. **Adhesion:** Adhesion is a much stronger attachment to the endometrium than the loose apposition. The trophoblasts adhere by penetrating the endometrium, with protrusions of trophoblast cells.

x. **Invasion:** Invasion is an even further establishment of the blastocyst in the endometrium.

a. *Syncytiotrophoblasts:* The protrusions of trophoblast cells that adhere into the endometrium continue to proliferate and penetrate into the endometrium. These penetrating cells differentiate to become a new type of cells, syncytiotrophoblast. The prefix syn- refers to that the boundaries between these cells disappears, forming a single mass of a multitude of cell nuclei. The rest of the trophoblasts, surrounding the inner cell mass, are hereafter called cytotrophoblasts. Invasion continues with the syncytiotrophoblasts reaching the basal membrane beneath the decidual cells, penetrating it and further invading into the uterine stroma. Finally, the whole embryo is embedded in the endometrium. Eventually, the syncytiotrophoblasts come into contact

with maternal blood and form chorionic villi. This is the initiation of forming the placenta.

b. Secretions: The blastocyst secretes factors for a multitude of purposes during invasion. It secretes several autocrine factors, targeting it and stimulating it to further invade the endometrium. Furthermore, secretions loosen decidual cells from each other, prevent the embryo from being rejected by the mother, trigger the final decidualisation and prevent menstruation.

c. Autocrine: Human chorionic gonadotropin is an autocrine growth factor for the blastocyst. Insulin-like growth factor type 2, on the other hand, stimulates the invasiveness of it.

d. Dislodging: The syncytiotrophoblasts dislodges decidual cells in their way, both by degradation of cell adhesion molecules linking the decidual cells together as well as degradation of the extracellular matrix between them. Cell adhesion molecules are degraded by syncytiotrophoblast secretion of Tumor necrosis factor-alpha.

3.6 Placenta (Structure, Type and Functions)

Meaning of Placenta: The word placenta is derived from a Greek word meaning a "flat cake". Placenta may be defined as a temporary structure formed by the association or fusion between the extra-embryonic membranes of the foetus and the endometrium of mother for the purtpose of physiological exchange of materials.

Therefore, the placenta from its origin point of view consists of two parts a foetal placentta furnished by the extra-embryonic membranes and a maternal placenta contributed by the uterine endometrium. The method of formation and fusion of the foetal placenta to the uterine wall is called placentation.

Structure: Placenta is a structure that establishes firm connection between the foetus and the mother. From the outer surface of the chorion a number of finger like projections known as chorionic villi grow into the tissue of the uterus. These villi penetrate the tissue of the uterine wall of the mother and form placenta. The placenta is a connection between foetal membrane and the inner uterine wall. Thus, placenta is partly maternal and partly embryonic. By means of placenta the developing embryo obtains nutrients and oxygen from the mother and gives off carbon dioxide and nitrogenous waste.

In the placenta, the foetal blood comes very close to the maternal blood, and this permits the exchange of materials between the two. Food (glucose, amino acids, lipids), water, mineral salts, vitamins, hormones, antibodies and oxygen pass from the maternal blood into the foetal blood, and foetal metabolic wastes, such as carbon dioxide, urea and warn pass into the maternal blood.

The placenta, thus, serves as the nutritive, respiratory and excretory organ of the foetus. The blood of the mother and foetus do not mix at all in the placenta or at any other place. The blood of the foetus in the capillaries of the chorionic villi comes in close contact with the mother's blood in the tissue between the villi, Inn they are always separated by a membrane, through which substances must diffuse or lie transported by some active, energy requiring process.

From the maternal side only a single component, the endometrium, is involved, but from the foetal side there are four prospective elements amnion, chorion, yolk sac and allantois. The amnion, being the inner most membrane, does not directly contribute to the making of the placenta. In mammals although the fertilized ovum develops in the body of the mother, the extra embryonic membranes are formed in similar fashion like that of the birds. The extra-embryonic somatopleure contributes to the formation of amnion and chorion while the splanchnopleure forms the yolk sac and allantois.

The allantois grows out of the hindgut of the embryo and expands into the extra-embryonic coelom. It later fuses with the chorion. Although no yolk is present in the mammalian ovum, the yolk sac is established in a similar manner like that of the birds. However, with the enlargement of the allantois, the yolk sac rapidly declines and becomes a shriveled remnant. At the same time, the endometrium of the mother's uterus has nearly completed its preparation to receive the embryo. The uterine stromal cells undergo a pronounced transformation where its cytoplasm becomes filled with glycogen and lipid droplets. These transformed stromal cells are called decidual cells. The endometrium containing these cells will contribute to the formation of an entity called placenta.

Types:

I. Classification of placenta based on the type of foetal membrances involved

i. **Choriovitelline Placenta (Yolk-sac Placenta):** In some mammals, particularly in most marsupials (*Didelphys, Macropus*), the allantois remains relatively

small and never makes contact with the chorion. The yolk sac on the other hand becomes very large and fuses with the chorion. In these mammals the chorion receives its blood supply from the yolk sac (vitelline circulation) and

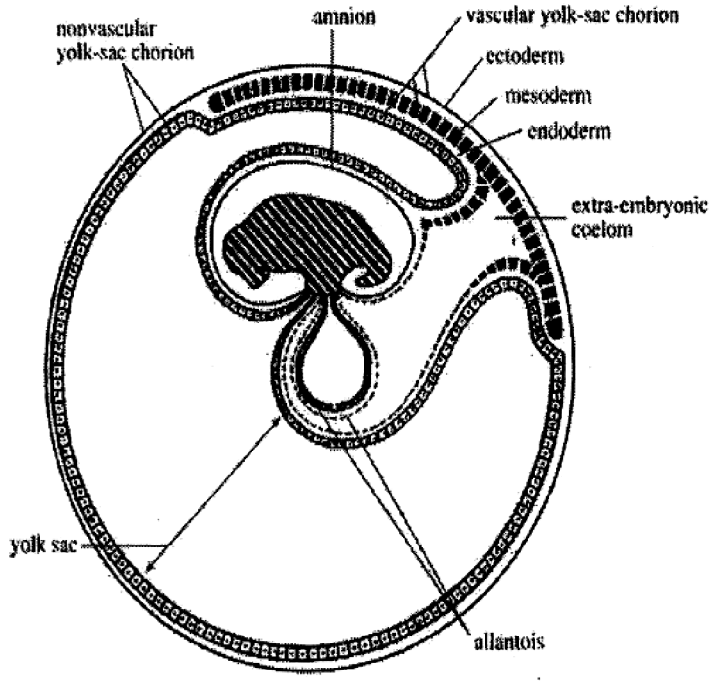


Fig. 3.9 : Choriovitelline Placenta

the placenta is thus called choriovitelline placenta. In marsupials, although only a portion of the yolk sac (and thus the chorion) is provided with vascular mesoderm, it is still referred to as yolktsac placenta. The chorion, however, never advances beyond a smooth membrane, applied closely with the endometrium. Among eutherian mammals, many carnivores, rodents and insectivores, a similar type of placenta may exist either temporanfly or permanently in those where the yolk ac placenta exists temporarily, the yolk sac provides the initial vascular supply 11 then gradually regresses, while the developing allantois reaches the chonion and vasculaluizen il in the other type the yolk sac shares with the allantors the task of vascularizing the chorion.

ii. **Chorioallantoic Placenta:** In most cutherian mammals and in some marsupials. (Parameles, Dasvurus), the yolk sac remains rudimentary, while the allantois becomes well developed, fuses with the chorion and provides the chorionic

circulation. This type of foetal placenta is called chonoallantoic placenta Tere the chorion possesses linger-like vascular processes, the villi, which grows out into the adjacent matemal tissue. In chorioallantoic placenta the surface of the blastocyst forms finger like outgrowths known as chorionic villi that penetrate into depressions or crypts in the wall of the uterus. Such outgrowths are initially formed by the trophoblast, i.e. the epithelial layer of chorion, but connective tissue and blood vessels later enter the villi. Thus a vascular chorio-allantoic membrane (allanto-chorion) is established.

iii. **Chorionic placenta-** The chorionic placenta consists of a greatly thickened layer of trophobalst or chorion containing lacunac or sinuses filled with maternal blood. Chorionic villi containing foetal connective tissue and capillaries extending to and across the trophoblastic lacunae. These villi are not homologous with the true villi observed in chorioallantoic placenta. Blood flows into the lacunac and in this way nutritive substances contained in the blood becomes available to the foetal tissue. This type of placenta is found in human beings.

II. Classification of placenta based on the Nature of contact:

It is three types, non-deciduate, deciduate and contra-deciduate placenta.

i. **Non-deciduate type placenta:** Here the implantation is superficial type. The chorianic villi are simple projections, they lie in contact with uterus. The villi are initially formed by the trophoblast but later on the blood vessels and connective tissues are extended to them. They have a loose contact. There is no fusion. At the time of birth of embryo uterus is not damaged. No bleeding occurs at the time of parturition as the chorionic villi are simply drawn out from the crypts in the wall of the uterus. This type of placenta is also called **semi-placenta** as there is a mere apposition of the fetal and the maternal components. Ex. Ungulate, Cetaceans, Sirenians. Lemurs

ii. **Deciduate type Placenta:** Here the degree of intimacy between the maternal and foetal tissues is great. The allanto-chorianic villi penetrate into uterine villi. They are intimately fused. Hence at the time of birth, the uterus is damaged because not only the foctal component is shed but a variable amount of maternal tissue is also torn away hence, bleeding occurs, as an open wound is left in the uterus. The uterine wall that enters into formation of placenta is called decidua. This type of placenta is also called placenta Vera or true placenta because chorionic villi fuse with the eroded uterine endometrium.

The bleeding normally stops by the contraction of the muscular wall of the uterus which constricts the blood vessels. Ex: Primates, Rodentia, Insectivora, chiroptera""a iii. Contra-deciduate placenta: This is a modified type of deciduate placenta. In this placenta there is a loss of maternal tissues and foetal portion of the placenta, both of them are absorbed in situ by maternal tissues. Example- *Parameles* and *Talpa*

III. Classification of placenta based on the distribution of Villi on the blastocyst:

In different types of mammals the placenta is further classified into six types according to the distribution and arrangement of the chorionic villi on the surface of the blastocyst. They are as follows:

1. **Diffuse placenta:** The villi are uniformly distributed all over the surface of the chorion except qat the extreme ends. It is found in ungulates. E.g., pig, mare.

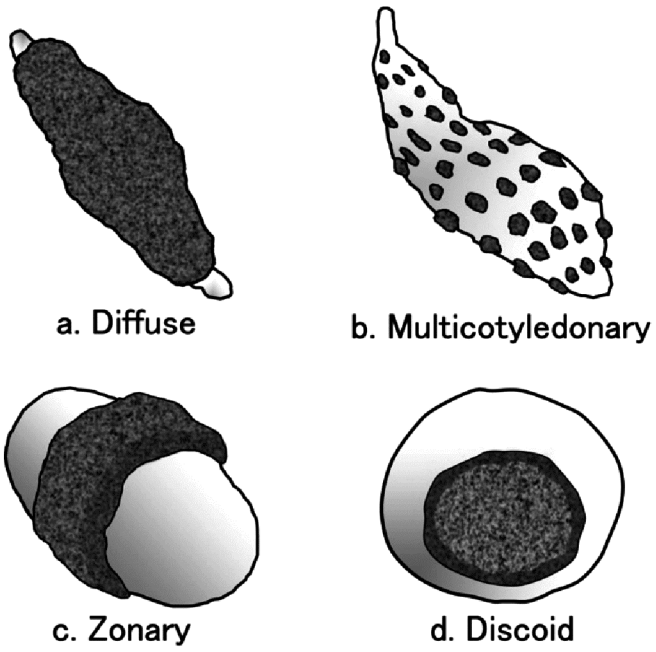


Fig. 3.10 : Placenta based on the distribution of Villi on the blastocyst

2. **Cotyledonary placenta:** In cattle, sheep and goat the villi are found in groups or patches called cotyledons while the rest of the chorion surface is smooth. The placenta of this type is known the cotyledonary placenta.

3. **Intermediate placenta:** In this type the isolated villi are scattered between the cotyledons. Thus the blastocyst is provided both with the villi and cotyledons. Eg, camel and giraffe.
4. **Zonary placenta:** In carnivores, like dog, cat, lion, tiger, fox, mongoose etc. the villi are developed in the form of a belt around the middle of their blastocyst which is generally elliptical in shape.
5. **Discoidal placenta:** The villi are restricted to a small disc shaped area of the blastocyst. The functional placenta therefore has the shape of a disc and is known as discoidal placenta. E.g. Insectivores, bat, bear, rodents (rat, guineapig, porcupine) and rabbit. In monkeys the placenta consists of two discs and is thus called the bidiscoidal placenta.
6. **Metadiscoidal placenta:** The metadiscoidal placenta is found in human beings and the anthropoid apes. The early blastocyst is at first all covered with villi but later they disappear from its free surface, persisting on a small disc-shaped area attached to the uterine wall. This secondary discoidal placenta is known as the metadiscoidal placenta.

IV. Classification of placenta based on the degree of connection and histological association between the maternal and foetal tissues:

Just prior to formation of the placenta, there are a total of six layers of tissue separating maternal and fetal blood. There are three layers of fetal extra-embryonic membranes in the chorioallantoic placenta of all mammals, all of which are components of the mature placenta:

1. Endothelium lining allantoic capillaries
2. Connective tissue in the form of chorioallantoic mesoderm
3. Chorionic epithelium, the outermost layer of fetal membranes derived from trophoblast

There are also three layers on the ***maternal side***, but the number of these layers which are retained - that is, not destroyed in the process of placentation - varies greatly among species. The three potential maternal layers in a placenta are:

1. Endothelium lining endometrial blood vessels
2. Connective tissue of the endometrium
3. Endometrial epithelial cells

According to number of layers of cells present between foetus and uterus blood supply the placenta is classified into five per:

1. **Epithelio-chorial placenta:** When the chorionic epithelium makes a contact with the uterine epithelium, the placenta is called epithelia-choual placenta. In this type of placenta six layers of tissue or barriers lie between the loctal and matemal blood streams in the followiny order
    1. The endothelial wall of the maternal blood vessel.
    2. The connective tissue around the maternal blood vessel (endometrial connective tissue).
    3. The uterine epithelium.
    4. The epithelium of Chorion.
    5. Connective tissue of the Chorion.
    6. The endothelial wall of the blood vessel in the Chorion.

This is the most primitive type of placenta from which other types have been derived and represents the apposition of foetal and maternal components and six tissue barriers between the two blood streams. Example: All marsupials, Ungulates (horses, asses, pig)

2. **Syndesmo-Chorial placenta:** In this type of placenta, the foetal and maternal components are fused so intimately due to the destruction of the uterine epithelium. As a result chorion comes in contact with the endometrial connective tissue, so only five barriers intervene between the two blood streams. Syndesmo-chorial placenta is found in ruminants, ungulates like camel, giraffe, goat, sheep, buffalo, cow and reindeer.
3. **Endothelio-chorial placenta:** If the destruction involves the uterine epithelium and the underlying connective tissue is reduced, the chorionic epithelium comes into direct contact with the endothelial walls of the maternal blood vessels. A placenta is then formed which is called an endothelio-chorial placenta. In this case the number of barriers between foetal and maternal blood streams is reduced to four. A placenta of this type is characteristic of carnivores, e.g. dog, all cats including house cat, tiger, lion etc, fox, bear, mongoose, walrus.
4. **Haemo-chorial placenta:** A reduction of the barriers to three is found in the primates(e.g. lemurs, apes), many insectivores, bats and rodents. This type of placenta is called haemo-chorial

placenta in which the endothelium of the maternal bood vessels also disappear and the chorionic bathed directly in the maternal blood. Actualy, the chorionic villi are surrounded by Y sinuses into which maternal blood enters through the arteries of the uterus and from which the villibre blood tlows into the uterine veins.

5. **Haemo-endothelial placenta:** In this type of placenta all the three maternal tissues and two foetal tissues, i.e., chorionic epithelium and chorionic connective tissues are completely eroded, so that the foetal vessels dip into the blood lacunae of the uterus. The number of barriers between the maternal and foetal blood streams is reduced to just one. This type of placenta is found in higher rodents. E.g., rat, guineapig and rabbit.

   Functions of Placenta: Histologically the placenta consists of barriers that prevent the blending of blood of the foetus and mother. From the maternal side the blood, enters into the inter villous spaces or crypts through about 30 spiral arteries and at high pressure.

The arterial blood rich in oxygen, nutrients etc. passes over the villi in small fountain like streams and then under reduced pressure settles down at the maternal base of the placental compartment from where it is removed by open-ended uterine veins. On the foetal side blood enters the villi through the branches of umbilical arteries. Although arterial, the blood is poor in oxygen and high in carbondioxide and other waste products. The foetal vessels at the terminal end of the villi form capillary network and at this region bulk of the placental exchange takes place. The blood now richer, is placental villus drained back to the foetus via the umbilical vein.

1. **Exchange of substances from one blood stream to the other, takes place by various transfer mechanisms such as:**
   (i) Diffusion,
   (ii) Active transport,
   (iii) Pinocytosis and
   (iv) Leakage (i.e., by breakage of placental membrane).
2. **Anchorage:** Placenta serves as adhesion or anchorage of the developing embryo with the uterine wall.

b. **Nutritional Role:** The foetus gets its nutrition from the maternal blood. Monosaccharide's, lipids, amino acids, vitamines and hormones pass by diffusion or active transport. Macromolecules of polysaccharides, lipids and

proteins are absorbed by the trophoblast cells by pinocytosis. Water and electrolytes such as chlorides and phosphates of sodium, potassium and magnesium pass by diffusion from mother to foetus.

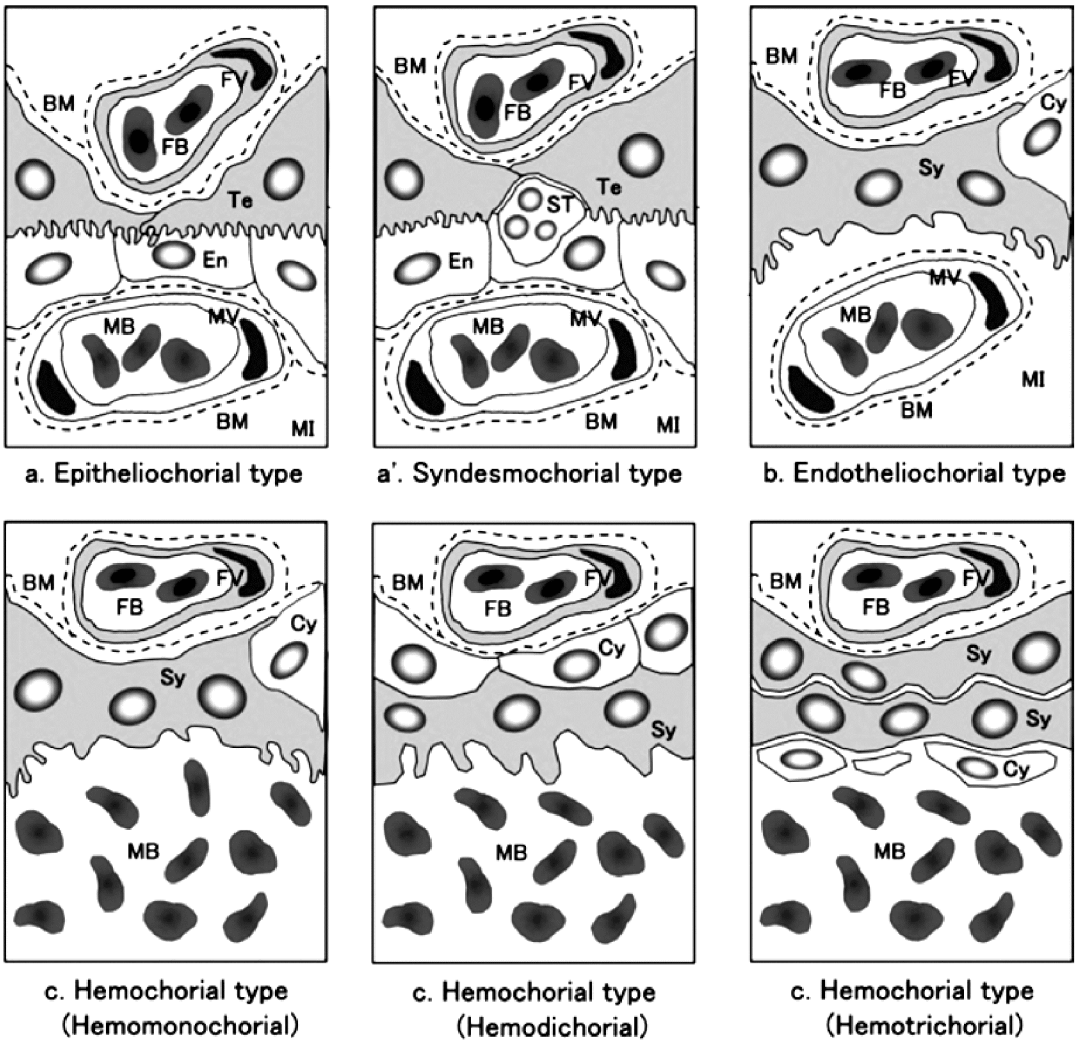


Fig. 3.11 : Classification by relationship between the chorion and uterine wall. BM = basement membrane, Te = trophectoderm, Cy = cytotrophoblast, En = endometrium, FB = fetal blood, FV = fetal vessel, MB = maternal blood, MI = maternal interstitium, MV = maternal vessel, ST = specific tropholast, Sy = syncytiotropho blast.

c. Respiratory Role: Gaseous exchange takes place by diffusion across the foetal membrane. Oxygen diffuses from maternal blood into the foetal blood, while reverse dif-fusion takes place in case of carbon dioxide.

d. **Excretory Role:** Waste products like urea, uric acid and creatinine are eliminated via placenta, from the embryonic blood to the maternal blood stream by diffusion. The kid-ney of mother removes these wastes of foetal metabolism along with her own waste products.

e. **Storage Function:** Glycogen, fats and some inorganic salts are stored in the placenta to be utilized when diet of the foetus is inadequate

f. **Enzymatic Function:** Placenta produces various enzymes such as diamine oxidase, oxytocinase and phospholipase-A2, which protects the foetus.

g. **Endocrine Function:** Placenta acts temporarily as an endocrine organ. It secretes many hormones such as estradiol, progesterone, chorionic gonadotropin in most mammals and also placental lactogen in human female. In some animals, such as rabbit, human females etc., the placenta is a significant source of relaxin, that relaxes the pelvic liga-ments to facilitate child birth.

h. **Immunological Role:** Placenta acts as a barrier against the transportation of microbes into the embryo. However, antibodies which have developed in the blood of a mother who has acquired immunity against certain diseases like diphtheria, scarlet fever, small pox and measles are passed on to the foetus, who become passively immunized to these illness in the first period after birth.

i. **Destructive Function:** Certain pathogenic organisms can penetrate through the placental barrier and infect the foetus. This occurs if the mother is infected by those pathogens causing syphilis, small pox, chicken pox, measles and rubella. Similarly any drug used during pregnan-cy can cross the placental barrier and cause disastrous effect on the foetus.

Thus, the drug thalidomide, taken as a sedative by ladies during early pregnancy, is found to be a ter-atogen (i.e., it causes deformities in limb development, perforation of anus and deve-lopment of a defective heart). Children born to such mothers have flipper-like limbs and are called thalidomide babies.

V. Classification Based on the Types of Implantation:

Implantation is the process by which the embryo becomes attached to a nutritional substance. The term in case of placental mammals is referred to the process by which

the embryo remains associated intimately with the uterine wall. Generally three types of implantation are seen which are as follows:

1. **Central or Superficial Implantation:** The chorionic sac of the embryo grows and makes superficial attachment with the uterine mucosa. This type of implantation is called central or superficial implantation and the embryo remains within the lumen of the uterus. It is seen in all cases of implantation in lower vertebrates. It is also present in Preambles and Dasyurus among the marsupials, while among eutherians it is seen in pig, cow, rabbit, sheep, dog, cat etc
2. **Eccentric Implantation:** In mouse, rat, beaver, squirrel etc the blastocyst in its early stage comes to lie between the uterine epithelial folds or pocket, and this type of implantation is called eccentuic implantation. The epithelial folds at a later stage, encloses the blastocyst almost completely
3. **Interstitial Implantation:** In interstitial implantation the embryo burrows into the uterine mucosa below the epithelium and becomes surrounded completely by the endometrial tissue of the uterus. This type of implantation is seen in hedge-hog, guinea-pig, some bats, chimpanzee, man etc.

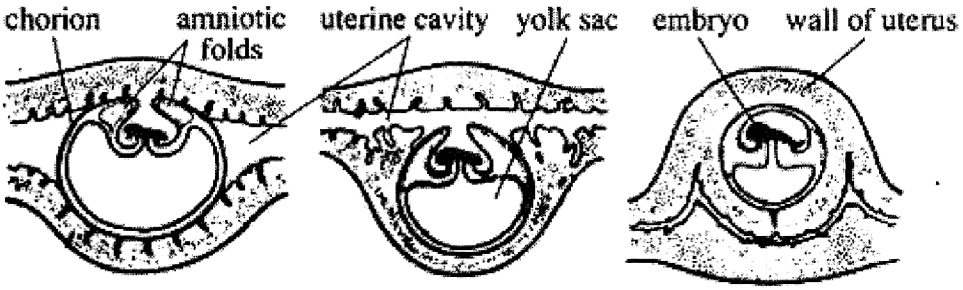


Fig. 3.12 : Classification of placenta based on depth of implantation among mammals. A. Superficial (dog), B. Eccentric (ground squirrel); C. Interstitial (hedgehog).

3.6.1 *Questions:*

1. Classify placenta giving suitable examples on the basis of Implantation?
2. Write a short note on the formation of placenta?

3. What are the six histological barriers present in the classical primitive placenta?
4. Classify placenta giving suitable examples on the basis of distribution of chorionic villi? Give proper diagram.
5. Classify placenta giving suitable examples on the basis of histological association between the maternal and foetal tissues? Give proper diagram.
6. Enumerate the functions of placenta?

Unit - 4 □ Post embryonic development

Structure

4.1 Objective

The primery objective of this unit are—

- to learn the mechanism of post-embryonic development and how it affects the development of adult animals.
- to learn the mechanism of metamorphosis i.e. how an animal change its one form to other form like tadpole to toad.
- to learn the mechanism of regeneration in animals particularly in *Hydra*.
- to learn the effects of different hormones and heow it will affects the post-embryonic development process in Insects.

4.2 Introduction

Post embryomic development means development after hatching/or birth of the animal concern. Post embryonic development means the changes or development occurs after hatching/or birth of the animal. For example in toad birth means the production tadpole and the post embryonic development means the mechanism of transformation of tadpole into toad. This unit also elaborates the mechanism by which several organs/tissues are regenerated/degenerated in several animals development. This unit also elaborates the mechanism of metamorphosis in animals.

4.3 Phases of Development in Animals

4.3.1 Metamorphosis of Amphibians

Metamorphosis may be defined as "a rapid differentiation of adult characters after a relatively prolonged period of slow or arrested differentiation in a larva". According to Duellman and Trueb (1986) Metamorphosis can be delined as "a radical transformation from larval life to the adult stage involving structural, physiological, biochemical and behavioural changes".

4.3.2 Types of Amphibian Metamorphosis

A. Progressive metamorphosis

During metamorphosis if the animal progresses in the evolutionary grades, the metamorphosis is considered as a progressive metamorphosis; e.g., in most anurans of Amphibia.

B. Retrogressive metamorphosis

When metamorphosis takes place in lower direction, i.e., by metamorphosis the animal retrogresses or shows indication of degeneration in the scale of evolution, called retrogressive metamorphosis; e.g., Ascidia of urochordates or in neotenic forms like salamanders.

4.3.3 Metamorphic changes of amphibians

Etkin (1968) have divided three stages

a. **Premetamorphic stage:** The stage is characterized by the considerable growth and development of larval structures but metamorphosis does not occur.
b. **Prometamorphosis:** The stage is characterised by the continuous growth specially the development of limbs and initiation of metamorphic changes.
c. **Metamorphic climax:** The stage is characterised by the radical changes in the features of the larva, and climax is considered by the loss of most larval features.

4.3.4 Structure of Metamorphosis

Structure of a freshly hatched tadpole larva

i. A freshly hatched tadpole larva has a limbless body.
ii. The body is divided into an ovoid head, a short trunk and a slender tail.
iii. A small opening situated ventrally at the root of the tail is known as anus.
iv. An adhesive sucker is present on the ventral side of the head by which the tadpole larva attaches itself to the aquatic weeds.
v. The mouth is lacking and as a result it cannot take anything from outside.
vi. The yolk material provides the nutrition
vii. The respiratory organs comprise of three pairs of highly vascular and branched feathery external gills.
viii. After a few days the mouth is formed near

ix. A pair of horny jaws surrounds the mouth.

x. The tail becomes more elongated and develops a dorsal and a ventral fin.

xi. V-shaped myotomes develop on both the sides of the tail.

xii. At this time this free-swimming tadpole larva ingests aquatic weeds, as a result of which the alimentary canal becomes extremely elongated.

xiii. To accommodate such a long alimentary canal inside the cavity of the short trunk, it becomes spirally coiled like the spring of a watch.

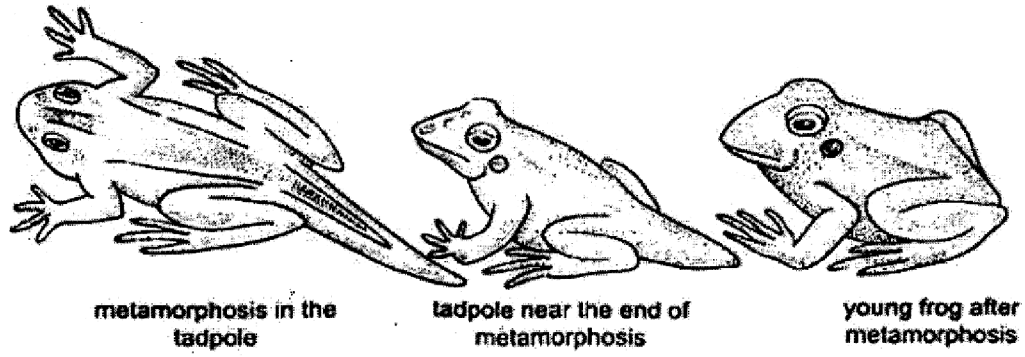


Fig. 4.1 : Frog stages in metamorphosis

4.3.5 Structure of an advanced tadpole larva

i. In the advanced stage, the pharynx of the tadpole larva becomes perforated by gill- slits.

ii. External gills disappear and the internal gills are formed between the gill slits.

iii. The gills and the gill slits are covered by the operculum (or gill-cover).

iv. Thus the tadpole larva has three pairs of external gills at the start which are subsequently replaced by three pairs of internal gills.

v. In the larval stages, the arterial arches also show modifications in terms of both external and internal gills (Fig).

vi. The operculum fuses with the trunk on all sides except a small opening, called spiracle on the left side.

vii. Water enters into the pharynx through the mouth and goes out through the spiracle.

viii. During this transit of water the internal gills are bathed with water containing oxygen dissolved in it.

ix. While the internal gills are functioning, a pair of lungs develops as outgrowths from the pharynx on the ventral surface.

x. The hind limbs appear prior to the forelimbs.

xi. The forelimbs remain first hidden under the operculum and subsequently emerge through it.

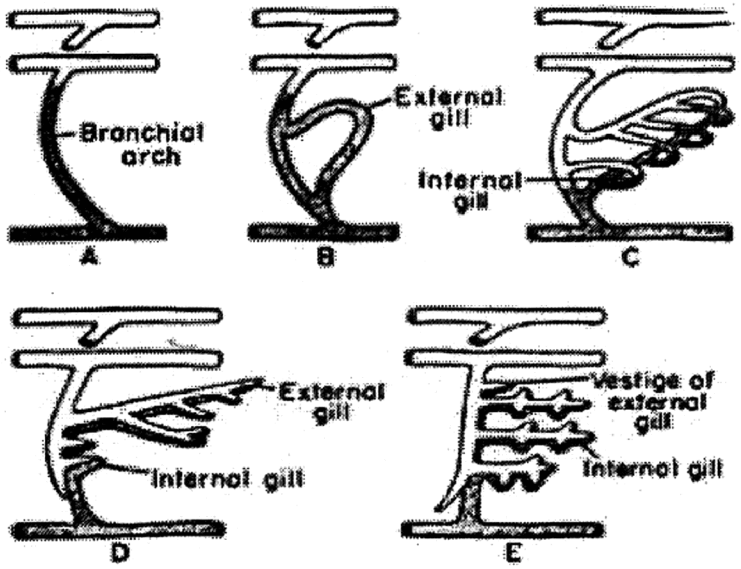


Fig. 4.2 : Showing the stages (A-E) of development of an aotic arch in relation to external and internal gills in larval phase of *Bufo* sp (= *Duttaphrynus* sp).

xii. At this stage both the internal gills as well as the newly formed lungs are functional.

xii. When the lungs become fully developed, the internal gills become degenerated.

xiv. At this stage it looks like a miniature toad except having a tail.

xv. As the limbs are developing, the animal enters into a period of starvation.

xvi. The material of the tail becomes eventually absorbed into the body.

4.3.6 Structure of a freshly formed toad

i. After the absorption of tail, the young toad leaves the primal aquatic home and comes to the land and hops.

ii. The mouth becomes wider and a pair of true bony jaws replaces the horny jaws.

iii. It now changes its food habit to become carnivorous type, as a result the alimentary canal becomes short and less coiled.

The changes that take place in the tadpole can be divided into four groups. They are:

A. Changes of tadpole in habit and habitat

i. With the metamorphosis, the metamorphosed larva leaves aquatic medium and frequently visits the land.

ii. The herbivorous tadpole larva changes into carnivorous specially consume the insects (insectivorous).

iii. The praying habits develop by the adults and the adult animals become more active and swift moving.

iv. In the first stage of adult toad, they jump into nearby pond and in other aquatic medium, and then jump on the land by their elongated hind limbs.

B. Morphological metamorphic changes

a. Regressive changes

(i) The tissues of tail and tailfin are completely absorbed into the body.

(ii) The horny jaws with teeth are shed and mouth becomes a large transverse slit.

(iii) The external gills disappear and the gill slits communicate to the pharyngeal cavity.

(iv) The length of the alimentary canal reduces much.

(v) The changes of the blood vascular system take place and ultimately some blood vessels are reduced.

(vi) The lateral line sense organ disappears.

(vii) Operculum and spiracle disappear.

b. Progressive changes

(i) The fore and hind limbs increase in size.

(ii) The tongue becomes long and more elastic which is free and bifid posteriorly.

(iii) The eyes become large and prominent and develop eye-lids and nictitating membrane.

(iv) External nostrils communicate with buccal cavity through internal nostrils.

(v) Tympanum and middle ear develop.

(vi) Liver becomes more enlarged.

(vii) Three chambered heart develops from two-chambered heart.

(viii) Pronephros is replaced by mesonephros.

C. Biochemical changes during metamorphosis

(i) The concentration of serum protein becomes about double during metamorphosis.

(ii) Biosynthesis and concentration of haemoglobin are greater in adult than in larvae.

(iii) In the liver, DNA synthesis, lipid synthesis, enzymes for ornithine urea cycle increase during adult stage.

(iv) Alkaline phosphatase and hydrolase decrease in adult stage of the anurans.

D. Change in Physiology

(i) At the beginning of metamorphosis, the pancreas starts to secret insulin and glucagon hormones. This is related to the increased role of the liver.

(ii) During the larval stage, the end product of nitrogen metabolism is ammonia. But after metamorphosis, the toads and frogs excrete most of their nitrogen in the form of urea. This is a shift from ammonotelism to ureotelism with the change of environment from aquatic medium to land.

4.3.7 Hormonal Control for Metamorphosis

Two hormones such as Triiodothyronine (T3) and Tetraiodothyronine (T4) or thyroxine are necessary for biochemical and morphological changes during anuran metamorphosis. These thyroid hormones are produced by the induction of anterior pituitary lobe or pars distalis when it reaches certain degree of differentiation.

Then it is capable to synthesize a hormone, thyrotropin (Thyroid Stimulating Hormone, TSH) which acts on the thyroid, stimulating the production and secretion of triiodothyronine (T3) and thyroxine.

In pre-metamorphic stage the prolactin level is high but levels of thyroid stimulating hormone (TSH) and thyroid hormone (T3, T4) are low. The hypothalamus – pituitary link is poorly developed. In pro-metamorphosis, the hypothalamus and pituitary link develops.

The prolactin level is low but the levels of thyroid stimulating hormone (TSH) and thyroid hormones (T3, T4) are high. In metamorphic climax, the prolactin level increases suddenly, then maintains steady low level. The TSH is high until end of climax and the thyroid hormone (T4) level becomes low.

4.4 Metamorphosis in Insects

Metamorphosis can be defined as "a rapid and complete transformation from an immature larval life a sexually adult form involving morphology, function and habitat changes". Ecdysis of moulting is the periodic shed ding off the old exoskeleton. The duration of the period between two successive moults of a developing insect is called stadium The form of the developing insect between two moults is called instar.

A larva is a motile, immature feeding stage in arthropods which is morphologically different from the adult stage. The larvae of hemimetabolous insects are called nymphs. The adult stage of holometabolous insects is called imago.

4.4.1 Types of Metamorphosis

On the basis of degree of changes there are 5 basic types of metamorphosis seen in insects. They are:

(A) Ametabolous development or Ametamorphic

(B) Gradual metamorphosis or Paurometabolous development

(C) Incomplete metamorphosis or Hemimetabolous development

(D) Complete metamorphosis or Holometabolous development and

(E) Hypermetamorphosis or Hypermetabolous development.

4.4.2 Ametabolous Development or Direct Development

Ametabolous type of development is called when the insects undergo little or no metamorphosis. Here the young's emerge from the eggs resemble the adults in all respects except in size and sexual structures. It grows only in size by replacing its old skin through a process, called moulting.

The young which emerges from the egg resembles a miniature adult, called nymph. In nymph the reproductive organs are undeveloped, and after several moults the nymph becomes an adult. This type of development is seen in apterygotan (wingless) insects (e.g., Lepisma and spring tails or Collembola etc.).

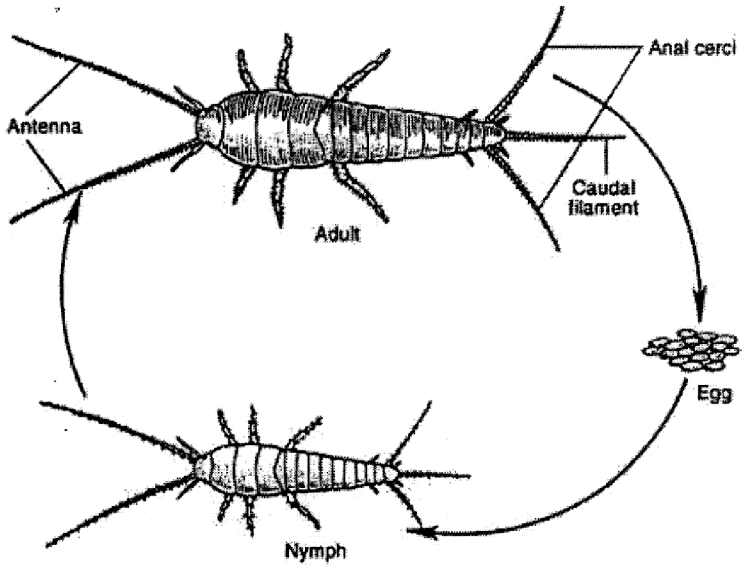


Fig. 4.3 : Ametabolous development in *Lepisma*

4.4.3 Gradual Metamorphosis or Paurometabolous Development

This type of metamorphosis is seen in less primitive forms like cockroaches, grasshoppers (Fig), mantis and white ants, etc. Here the newly young which comes out of egg closely resembles the adult in general body form, habits and habitat but many adult features, i.e., wings and reproductive organs are undeveloped and their relative proportions of the body also differ. The young's are called nymphs.

The wings develop as wing pads in the second and third thoracic segments at early age and gradually increase in size by mitosis in each moult. The external genitalia develop gradually at each moult. These nymphs lead an independent life and attain adult form through several moults.

This type of metamorphosis is called gradual metamorphosis or paurometabolous development because the young undergoes slow but steady change in each moult and attains the adult form.

Sometimes the gradual metamorphosis or paurometabolous development is included under hemimetabolous development. In each moult the proportion of the head gradually becomes smaller and the abdomen becomes longer.

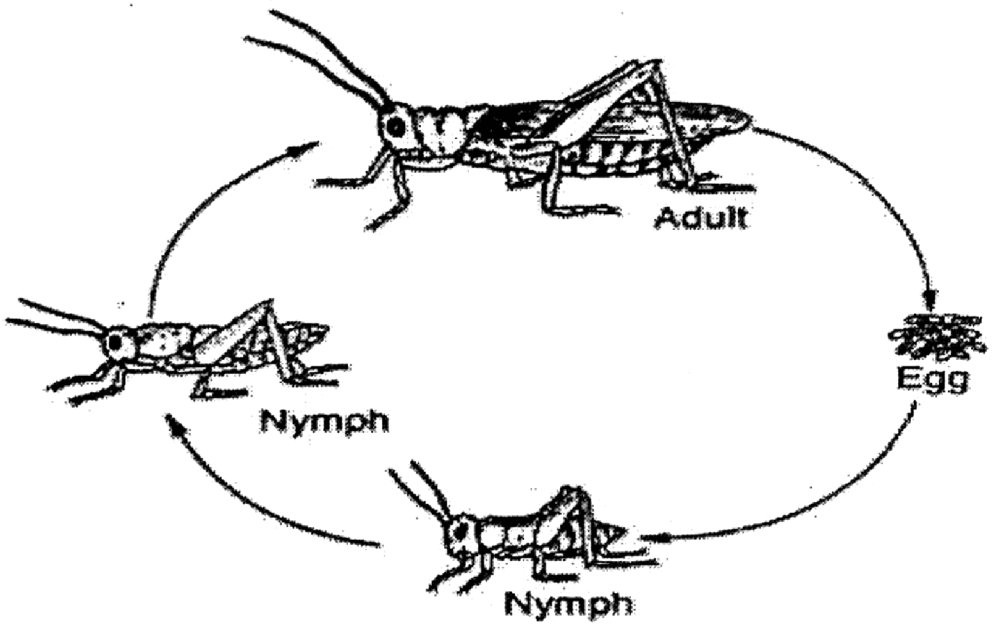


Fig. 4.4 : Paurometabolous development in grasshopper

In the life cycle of these insects there are three stages, e.g., egg nymph imago There is no pupal stage.

4.4.4 Incomplete Metamorphosis or Hemimetabolous Development

The insects which attain adult forms by gradual morphological change with successive moults are called incomplete metamorphosis or hemimetabolous development. In many insects, e.g., dragonflies (Fig), mayflies and damselflies, the different stages of the life cycle resemble to paurometabolous development except the nymphs are called naiads which are aquatic and respire by external gills but the adults are terrestrial.

When these nymphs are ready to be adult they come out of water and adult winged forms are released. The wings and genitalia develop externally but are not fully formed until adulthood. No further moulting takes place after the formation of wings, only exception in mayfly where winged form comes out of aquatic nymph and rests on a tree to undergo another moulting to become an adult.

In the naiads of hemimetabolous insects there are 3 pairs of thoracic legs, a head with compound eyes, antennae and small abdomen with posterior tracheal gills.

The naiads when attain adult stage after several moults, the head of the adult

becomes proportionately smaller but the abdomen becomes larger. The tracheal gills are lost and spiracles appear for aerial breathing. Many insects live for longer period as nymphs and the adult stage is short, the chief purpose of which is multiplication.

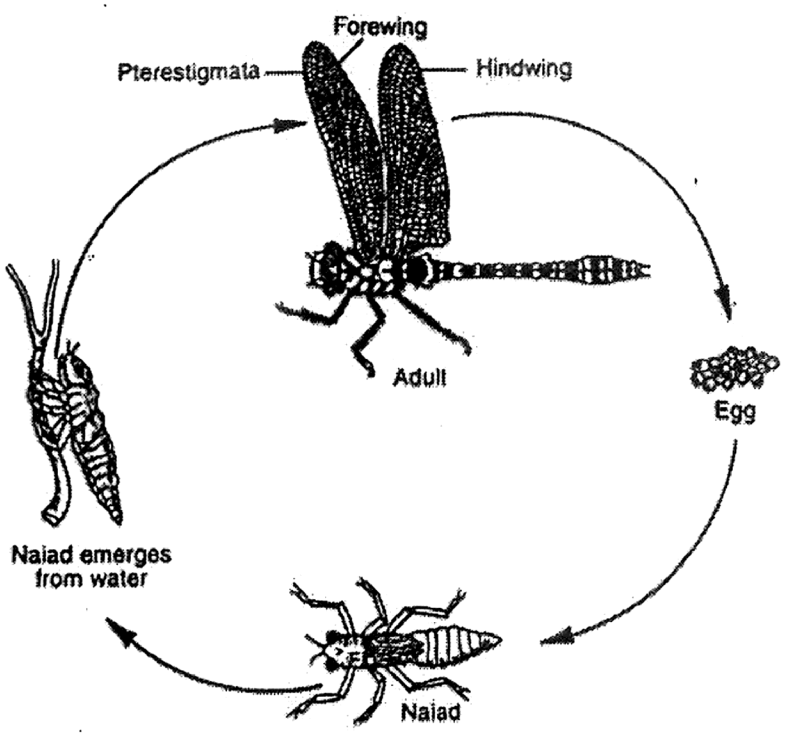


Fig. 4.5 : Hemimetabolous matemorphosis in dragon fly

The best example is the may fly where adult stage lasts only for a day but nymphs take one year to grow.

From the egg → nymph → adult cycle of incomplete metamorphosis, it is evident that insects which are advanced from the primitive Lepisma like forms, have started to explore two types of environments. But success in such attempts is achieved in forms with complete metamorphosis.

This climax of double life has been attained through a cycle of egg → larva → pupaadult; larva existing in an altogether different environment than the adult. Nearly 87% of known insects develop through this cycle which involves two changes of form—one is from egg to caterpillar and the other from caterpillar to pupa and the adult.

4.4.5 Complete Metamorphosis or Holometabolous Development

Complete metamorphosis or holometabolous development is a kind of rapid morphological change during post embryonic transformation in some forms of insects where larva has no similarity with the adult and there is always a pupal stage. Complete metamorphosis takes place in beetles, caddis- flies, butterflies, moths, mosquitoes, flies, bees and wasps (Fig).

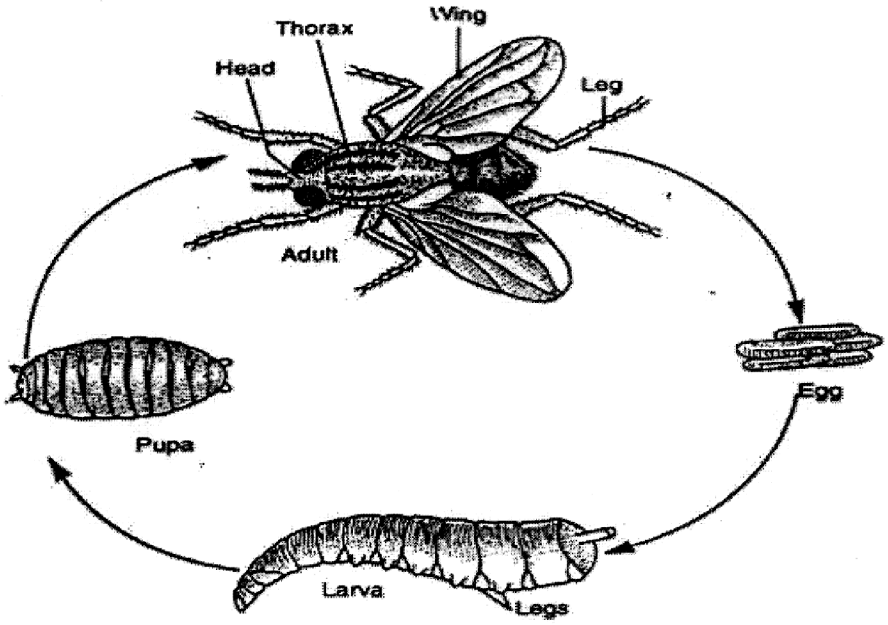


Fig. 4.6 : Illustration of holometabolous development in fly.

In the house-fly (order Diptera) the larve is worm-like and devoid of appendages. It is called maggot. The mature larva is about 12 mm long. The head is indistinct and with a pair of oral lobes and hooks.

In case of beetle (Order Coleoptera) the larva is known as grub. The body of the grub is thick and with thoracic legs and well-developed head. They are usually sluggish in nature. In the moths and butterflies (Order Lepidoptera), the larva is known as Caterpillar, which possesses a distinct head with powerful mandibles and three pairs of jointed thoracic legs.

The abdomen possesses four or five pairs of un-jointed, short abdominal legs or also called pseudo-legs or prolegs. The caterpillars are often with protective colour

or defensively shaped. These larvae eat voraciously and grow rapidly with several moultings. After sometime the larva is transformed into a stage, called pupa.

Again, these above mentioned larvae are also included under three categories, such as the maggot is called apodous larva for the absence of appendages on thorax and abdomen and segmented body with a small head with sense organs. The larvae among beetles are also called campodeiform or oligopod larvae for the resemblance to apterous Campodea (Order Thysanura).

The larvae are characterised by without abdominal appendages except cerci and the skin of the body is thick, provided with thoracic legs and sense organs. The caterpillar type larva is also called polypod or eruciform larvae (Fig.) which is characterised by a fleshy body with a thin skin and prolegs on the abdomen and six legs on the thorax.

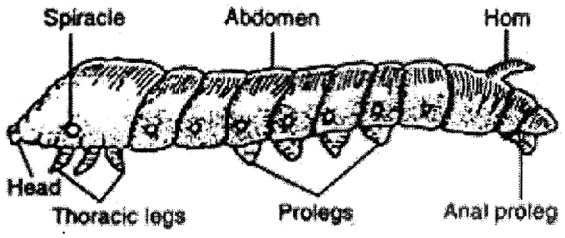


Fig. 4.7 : Eruciform larva

The pupa is the third stage in the life of holometabolous insects and usually immobile and often remain within a protective covering from predators, called cocoon. In the mosquito the pupa is very active but does not eat anything. Though the pupal stage considers as a quiescent stage but it undergoes many internal changes. There are three types of pupa among the holometabolous insects. They are:

(i) Exarate pupa: This type of pupa is very common and found in almost all holometabolous insects except Lepidoptera. The pupa is characterized by the presence of free legs and appendages and the abdomen is capable of movement. This type of pupa is called free pupa.

(ii) Obtect pupa: This type of pupa is seen among moths and butterflies and is characterized by the wings, and appendages are not moved and fixed to the body by a moulting fluid. The pupa of butterflies is called chrysalis and it possesses a slender stalk at the top by which the pupa remains attached to the twigs.

(iii) Coarctate pupa: This type of pupa is seen among dipterans (Fig.) The pupa of house-fly is enclosed by a hard barrel-shaped chitinous case, called puparium. The puparium is segmented externally and the spiracles remain projected outwardly. The pupa of mosquitoes is commashaped and contains broad anterior cephalothorax.

The dorsal side of thorax bears a pair of small respiratory trumpets; the openings are guarded by numerous hairs. The abdomen is nine segmented and a pair of paddles on the eighth segment by which the pupa swims. After a period of pupal existence, the young insect emerges out by breaking or dissolving the pupal case. The holometabolous insects include 4 stages in their life cycle.

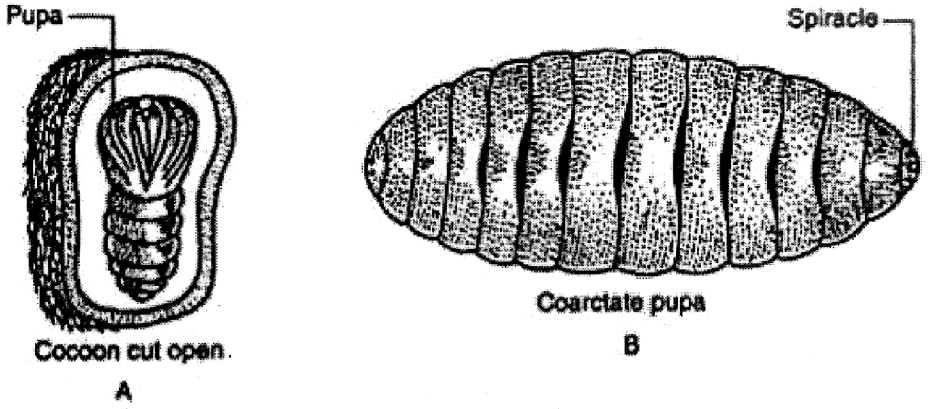


Fig. 4.8 : A. Exarate pupa, B. Coarctale pupa

Egg → Larva → Pupa → Imago (adult):

Prepupa: In the holometabolous insects, a stage is seen before the pupal stage, called prepupa. During this stage the feeding usually stops and sometimes a cocoon is produced. The prepupa resembles the larva but it is often shrunken and less pigmented.

4.4.6 Hypermetamorphosis or Hypermetabolous Development

It is a kind of metamorphosis in which there are two or three distinct types of larval instars with different habits and structures found in certain insects. This type of metamorphosis is seen in blister beetles (Fig.)

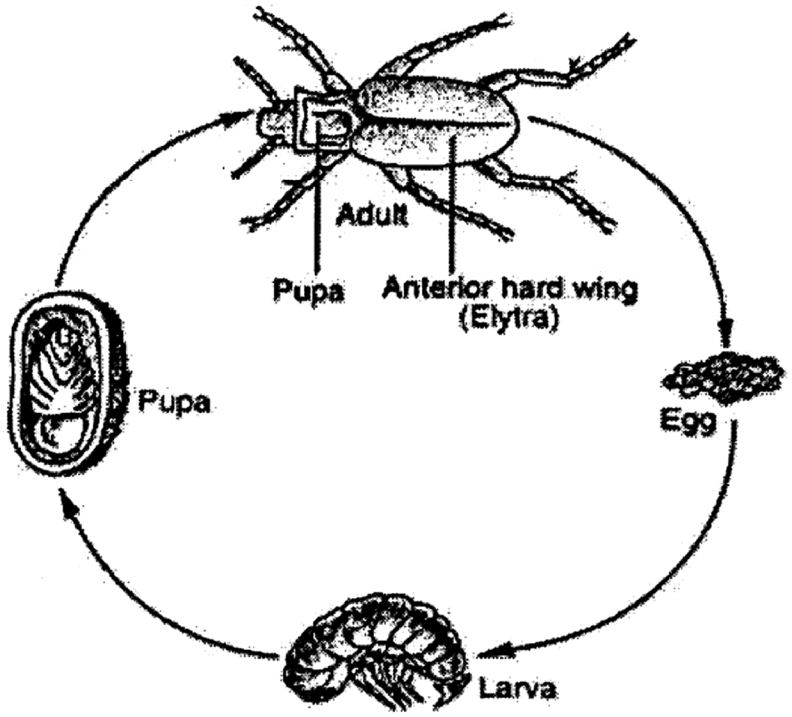


Fig. 4.9 : Illustration of hipermetabolous development in blister beetle.

4.4.7 Events of Metamorphosis

In recent years considerable amount of work has been done to understand the events of metamorphosis. It has been found that at the time of early development, in the developing egg the cells are segregated into two groups one group for working at the larval life and the second group to take charge during pupal and adult life.

In a growing larva, the larval cells increase only in size but never undergo division. The second group of cells, called imaginal buds and discs, remain inactive in the body of larva. When the larva is full-grown, second group of cells take over the charge. Within the apparently inactive pupa tremendous activities go on at cellular level. Imaginal buds grow by division.

During metamorphosis, most of the larval organs in the pupa except the central nervous system and developing reproductive organs are broken down by enzymes and the process of disintegration of the larval organs is called histolysis and these larval disintegrated cells die and is used up by the imaginal cells.

In certain insects, within larva, pupal cells become fluid in consistency and imaginal cells continue to form the adult structures. The imaginal buds are the groups

of formative cells but remain inactive in the larva but form the rudiments of future organs by mitosis.

These formative cells set aside in the pupa and reach functional organs by differentiation in the imago (adult). The process of formation of tissues and organs from the imaginal buds, called histogenesis. The wings, mouth parts, internal organs, muscles and legs develop from the imaginal buds.

4.2.8 Role of Hormones during Metamorphosis

It has also been well established that the moulting and metamorphosis in insects are controlled by hormones (Fig.//). The secretions of three organs are related to this process. These organs are:

(i) The brain (protocerebrum)

(ii) The prothoracic gland and

(iii) The corpora allata.

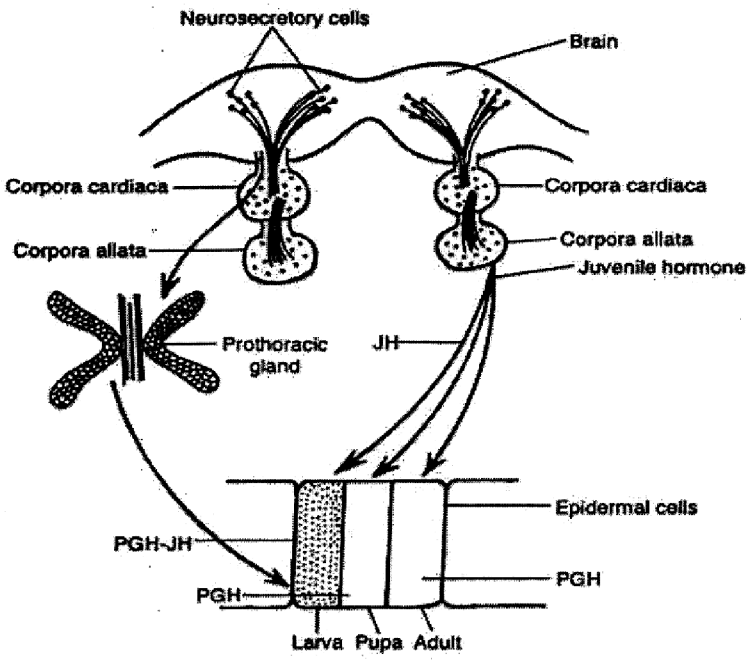


Fig. 4.10 : Endocrine glands of insects and their influence during moulting.

(i) The Brain (protocerebrum): In the brain there are four groups of neurosecretory cells. Of these two groups lie on the midline and another two group's lie on the sides-one group on each side. The neurosecretory cells

secrete a kind of protein hormone, called prothoracotropic (PTTH) or brain hormone that activates the prothoracic glands which in turn produce moulting hormone.

The protocerebrum sends the neurosecretory axons to the corpora cardiaca, a pair of small glands which lie posterior to the brain. Prothoracotropic hormone passes to the corpora cardiaca along the axons where it is released to the blood.

(ii) Prothoracic Glands (PG): There is a pair of glands located in the prothoracic region. These are also called moult gland or ecdysial gland. Each gland appears V-shaped and is a mass of glandular tissue of non- nervous origin.

It produces a hormone, known as ecdysone. It is steroid in nature. Butenandt and Karlson (1951) first isolated from the pupa of silkworm. Its empirical formula is $C_{27}H_{44}O_6$. The hormone stimu lates growth and initiates the process of moulting and shedding the old cuticle of the larva and the new cuticle is formed beneath the old cuticle.

(iii) Corpora Allata: They are paired non- nervous secretory cells and situated behind the brain posterior to corpora cardiaca. The corpora allata secrete another fat soluble hormone, called juvenile hormone (JH). Chemically it is related to ecdysone and also a steroid in nature. The juvenile hormone keeps the larval cells active and also controls the qualitative changes in the body during metamorphosis.

As long as the juvenile hormone is secreted from the corpora allata, the pupa and imago (adult) stages are not developed. After a certain period the production of ecdysone instructs to stop the flow of juvenile hormone on one hand and on the other hand triggers the imaginal buds to be active.

The absence of juvenile hormone causes the death of larval cells and they are used as nutrients for the growing imaginal buds. If the amount of juvenile hormone (JH) becomes lower in the blood, the moulting from larva to pupa takes place and absence of juvenile hormone in the blood, there occurs from pupa to adult moult. So it has been determined that the process of moulting is under hormonal control.

4.5 Regeneration with special reference to *Hydra*

Regeneration, in biology, the process by which some organisms replace or restore lost or amputated body parts. Organisms differ markedly in their ability to regenerate

parts. Some grow a new structure on the stump of the old one. By such regeneration whole organisms may dramatically replace substantial portions of themselves when they have been cut in two, or may grow organs or appendages that have been lost. Not all living things regenerate parts in this manner, however. The stump of an amputated structure may simply heal over without replacement. This wound healing is itself a kind of regeneration at the tissue level of organization: a cut surface heals over, a bone fracture knits, and cells replace themselves as the need arises.

4.5.1 Basic patterns

Not all organisms regenerate in the same way. In plants and in coelenterates such as the Hydra and jellyfishes, missing parts are replaced by reorganization of preexisting ones. The wound is healed, and the neighbouring tissues reorganize themselves into whatever parts may have been cut off. This process of reorganization, called morphallaxis, is the most efficient way for simple organisms to regenerate. Higher animals, with more complex bodies, regenerate parts differently, usually by the production of a specialized bud, or blastema, at the site of amputation. The blastema, made up of cells that look very much alike despite their often diverse origins, made its first appearance evolutionarily in flatworms and is encountered in the regenerative processes of all higher animals. It provides the tissue that will form the regenerated part.

4.5.2 Types of Regeneration:

Regeneration is of three types

A. **Physiological: -** There is a constant loss of many kinds of calls due to wear and tear caused by day-to-day activities. The replacement of these cells is known as physiological regeneration. Example: Replacement of R.B.C's. The worn out R.B.C's are deposited in the spleen and new R.B.C's regularly produced from the bone marrow cells, since the life span of R.B.C's is only 120days. Replacement of Epidermal Cells of the Skin (The cells from the outer layers of epidermis are regularly peeled off by wear and tear. These are constantly being replaced by new cells added by the malpighian layer of the skin).

B. **Reproductive: -** This is the replacement of lost parts or repair of damaged body organs. In this type of regeneration, wound is repaired or closed by the expansion of the adjoining epidermis over the wound. Example: Regeneration of limbs in salamanders, Regeneration of lost tail in lizard, Healing of wound, Replacement of damaged cells.

C. **Autotomy: -** In some animals like starfish, some part of the body is broken off on being threatened by a predator. This phenomenon of self-mutilation of the body is called autotomy. Example: Crabs break off their leg on approaching of the enemy, Holothurians throw off their internal viscera, Star fish breaks off an arm.

The mechanism of regeneration can be studied from limb regeneration in salamander. This involves the following stages

i) **Wound healing:** The epidermal cells from the edges of the wound migrate exposed surface. This is known as wound healing

ii) **Blastema formation:** A few days later, undifferentiated cells accumulate inside the epidermis, resulting in a bulge. This is known as regeneration bud or blastema

iii) **Redifferentiation and morphogenesis:** The blastema develops rudiments of the lost organ, like the digits which grow into new digits.

iv) **Growth:** The regenerated limb increases and attains the size of a normal limb.

In planarians and in *Hydra*, there are undifferentiated cells called neoblasts which multiply and then migrate from the deeper parts of the body to the cut surface.

4.5.3 Regeneration Process

Following amputation, an appendage capable of regeneration develops a blastema from tissues in the stump just behind the level of amputation. These tissues undergo drastic changes. Their cells, once specialized as muscle, bone, or cartilage, lose the characteristics by which they are normally identified (dedifferentiation); they then begin to migrate toward, and accumulate beneath, the wound epidermis, forming a rounded bud (blastema) that bulges out from the stump. Cells nearest the tip of the bud continue to multiply, while those situated closest to the old tissues of the stump differentiate into muscle or cartilage, depending upon their location. Development continues until the final structures at the tip of the regenerated appendage are differentiated, and all the proliferating cells are used up in the process.

The blastema cells seem to differentiate into the same kind of cells they were before, or into closely related types. Cells may perhaps change their roles under certain conditions, but apparently rarely do so. If a limb blastema is transplanted to the back of the same animal, it may continue its development into a limb. Similarly,

a tail blastema transplanted elsewhere on the body will become a tail. Thus, the cells of a blastema seem to bear the indelible stamp of the appendage from which they were produced and into which they are destined to develop. If a tail blastema is transplanted to the stump of a limb, however, the structure that regenerates will be a composite of the two appendages.

4.5.4 Range of Regeneration

A) In Invertebrates:

a) ***Coelenterates:*** The vast majority of research on coelenterates has been focussed on Hydra and some of the colonial hydroids. If a *Hydra* is cut in half, the head cnd reconstitutes a new foot, while the basal portion regenerates a new hydranth with mouth and tentacles. This seemingly straightforward process is deceptively simple. From tiny fragments of the organism whole animals can be reconstituted. Even if a *Hydra* is minced and the pieces scrambled, the fragments grow together and reorganize themselves into a complete whole.

In colonial hydroids, such as Tubularia, there is a series of branching stems, each of which bears a hydranth on its end. If these hydranths are amputated they grow back within a few days. In fact, the organism normally sheds its hydranths from time to time and regenerates new ones naturally.

b) ***Flatworms:*** Planarian flatworms are well-known for their ability to regenerate heads and tails from cut ends. In the case of head regeneration, some blastema cells become brain tissues, others develop into the eyes, and still others differentiate as muscle or intestine. In a week or so, the new head functions almost as well as the original.

The blastema that normally gives rise to a single head is, under certain circumstances, even capable of becoming two heads if the stump of a decapitated flatworm is divided in two by a longitudinal cut. Each of the two halves then gives rise to a complete head. Thus, each blastema develops into an entire structure regardless of its size or position in relation to the rest of the animal.

c) ***Annelids:*** The segmented worms exhibit variable degrees of regeneration. The leeches, as already noted, are wholly lacking in the ability to replace lost segments, whereas the earthworms and various marine annelids (polychaetes) can often regenerate forward and backward. The expression of such

regenerative capacities depends very much on the level of amputation. Anteriorly directed regeneration usually occurs best from cuts made through the front end of the worm, with little or no growth taking place from progressively more posterior bisections. Posteriorly directed regeneration is generally more common and extensive. Some species of worms replace the same number of segments as were lost. Hypomeric fewer segments are produced than were removed, is more common, however.

Anterior regeneration depends upon the presence of the central nerve cord. If this is cut of deflected from the wound surface, little or no forward regeneration may take place. Posterior regeneration requires the presence of the intestine, removal of which precludes the formation of hind segments. Thus, it would seem that no head will regenerate without a central nervous system, nor a tail without an opening.

d) ***Arthropods:*** Many insects and crustaceans regenerate legs, claws, or antennas with apparent ease. When insect legs regenerate, the new growth is not visible externally because it develops within the next proximal segment in the stump. Not until the following molt is it released from its confinement to unfold as a fully developed leg only slightly smaller than the original. In the case of crabs, regenerating legs bulge outward from the amputation stump. They are curled up within a cuticular sheath, not to be extended until the sheath is molted. Lobsters and crayfish regenerate claws and legs in a straightforward manner as direct outgrowths from the stumps. As in other crustaceans, however, these regenerates lie immobile within an enveloping cuticle and do not become functional until their sheath is shed at the next molt.

In all arthropods regeneration is associated with molting, and therefore takes place only during larval or young stages. Most insects do not initiate leg regeneration unless there remains ample time prior to the next scheduled molt for the new leg to complete its development. If amputation is performed too late in the intermolt period, the onset of regeneration is delayed until after shedding; the regenerate then does not appear until the second molt. Metamorphosis into the adult stage marks the end of molting in insects, and adults accordingly do not regenerate amputated appendages.

Crustaceans often tend to molt and grow throughout life. They therefore never lose the ability to grow back missing appendages. When a leg is lost,

a new outgrowth appears even if the animal is not destined to molt for many months. Following a period of basal growth, during which a diminutive limb is produced, the regenerated part eventually ceases to elongate. Not until a few weeks before the next molt does it resume growth and complete its development, triggered by the hormones that induce molting.

4.5.5 In Vertebrates

a) ***Fisher:*** Many different parts of the fish's body will grow back. Plucked scales are promptly replaced by new ones, and amputated gill filaments can regenerate easily. The "whiskers," or taste buubels, of the cattisti grow back as perfect replicas of the originals. The most conspicuous regenerating structures in fishes, however, are the fins. When any of these are amputated, new fins grow out from the stumps and soon restore everything that was missing. Even the coloured stripes or spors that adorn some fins are reconstituted by new pigment cells that repopulate the regenerated part. Fin regeneration depends on an adequate nerve supply. If the nerves are cut leading into the tin, regeneration of neither the amputated fin nor excised pieces of the bony fin rays can take place

b) ***Amphibians:*** Salamanders are remarkable for their ability to regenerate limbs. Larval frogs, or tadpoles, also possess this ability, but usually lose it when they become frogs. It is not known why frog legs do not regenerate, and under appropriate stimuli they can be induced to do so.

Tadpoles and salamanders can replace amputated tails. Tadpole tails have a stiff rod called the notochord for support, whereas salamanders possess a backbone, composed of vertebrae. Both tails contain a spinal cord. When the salamander regenerates its tail, the spinal cord grows back and segmental nerve-cell clusters (ganglia) differentiate. Tadpoles also regenerate their spinal cords, but not the associated ganglia. If the spinal cord is removed or destroyed in the salamander, no tail regeneration occurs; if it is removed from the tadpole tail, however, regeneration can proceed without it.

c) ***Reptiles:*** Lizards also regenerate their tails, especially in those species that have evolved a mechanism for breaking off the original tail when it is grasped by an enemy. When the lizard tail regenerates, however, it does not replace the segmented vertebrae. Instead, there develops a long tapering cartilaginous tube within which the spinal cord is located and outside of which are segmented muscles. The spinal cord of the lizard tail is necessary for regeneration, but the regenerated tail does not reproduce the ganglia that are

normally associated with it. Occasionally, a side tail may be produced if the original tail is broken but not lost.

d) ***Birds:*** Regeneration of amputated appendages in birds is not known to occur; however, they do replace their feathers as a matter of course. While most species shed and regenerate feathers one at a time so as not to be grounded, lightless birds, such as penguins, may molt once. Male puttins cast off their colorful beaks aller the mating season, but grow new ones the following year. In like manner, the dorsal keel on the upper beaks of male pelicans is shed and replaced annually.

e) ***Mammals:*** Although mammals are incapable of regenerating limbs and tails, there are a few exceptional cases in which lost tissues are in fact regenerated. Not the least of these cases is the annual replacement of antlers in deer. These remarkable structures, which normally grow on the heads of male deer, consist of an inner core of bone enveloped by a layer of skin and nourished by a copious blood supply. During the growing season the antlers elongate by the proliferation of tissues at their growing tips. The rate of growth in some of the larger species may surpass one centimetre (0.39 inch) per day; the maximum rate of growth recorded for the elk is 2.75 centimetres (1.05 inches) per day. When the antlers have reached their full extent, the blood supply is constricted, and the skin, or velvet, peels off, thus revealing the hard, dead, bony antlers produced by the male deer in time for the autumn mating season. The regeneration of elk antlers spans about seven months. The following spring, the old antlers are shed and new ones grow to replace them.

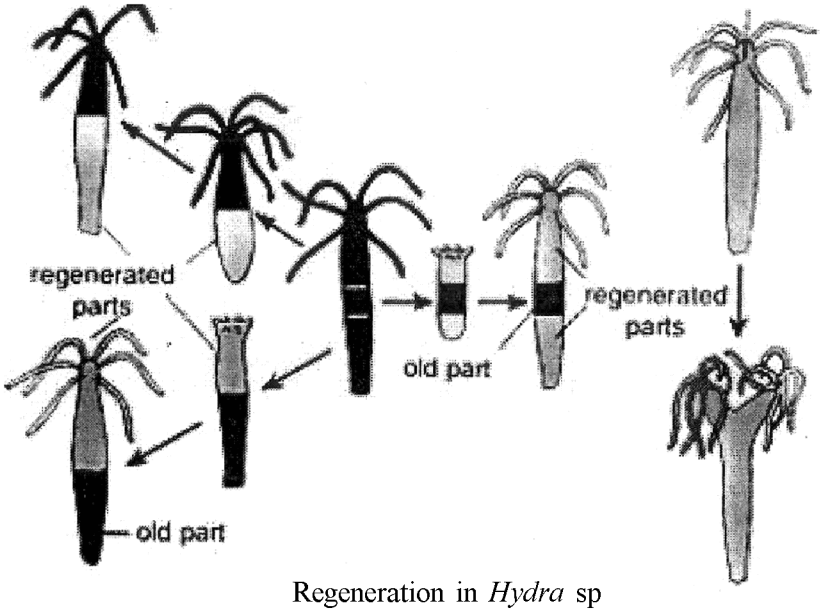
Still another example of mammalian regeneration occurs in the case of the rabbit's ear. When a hole is punched through the external ear of the rabbit, tissue grows in from around the edges until the original opening is reduced or obliterated altogether. This regeneration is achieved by the production of new skin and cartilage from the margins of the original hole. A similar phenomenon occurs in the case of the bat's wing membrane.

4.5.6 Regeneration of *Hydra* sp

Hydra has the considerable power of regeneration. Trembley (1744 or 1745) first of all demonstrated that an individual Hydra can be cut into several pieces, and each will regenerate the lost parts, developing a whole new individual. The parts usually retain their original polarity, with oral ends developing tentacles and aboral ends, basal discs.

Parts of two different individuals, often of different species, may be brought together and grafted together in various arrangements. The germ layers, however, will not mix. The epidermis will only fuse with epidermis and gastro dermis with gastro dermis.

Trembley (1744 or 1745) also demonstrated that if the head end of 11ydru i split into two stile the parts are slightly separated it results into a Y shaped //ydra or two headed individual kaving, two mouths and two sets of tentacles. Loch head may be nynin split in a similar manner in the way Trembley succeeded in producing a seven headed *Hydra*.



Regeneration in *Hydra* sp

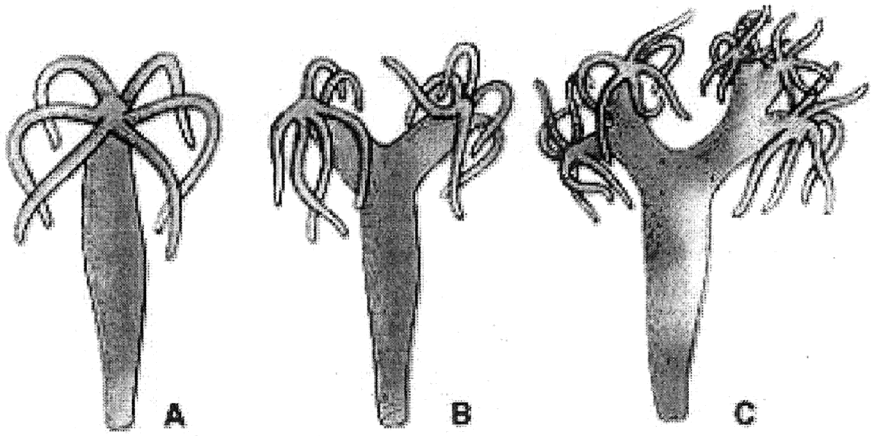


Fig. 4.11 : Showing the formation of multi-headed specimen by regeneration in *Hydra* sp (A) Single Headed *Hydra*; (B) Two-headed *Hydra* and (C) Four headed *Hydra*.

Occasionally, even in nature, a Hydra becomes turned inside out. In the accomplished mechanically or by overdoses of glutathione. Trembley (1744) thought that under these conditions the epidermis becomes gastrodermis and gastrodermis becomes epidermis.

More modern studies, however, demonstrate conclusively that this does not occur. Rather, the Hydra usually turns itself right side again, but if it does not, the layers switch location by migration of cells through the mesogloea.

4.6 Questions

1. What is amphibian metamorphosis?
2. What amphibians go through metamorphosis?
3. Which hormone is responsible for metamorphosis? What happens during amphibian metamorphosis?
4. What are the 3 stages of metamorphosis? Do all amphibians go through metamorphosis?
5. Briefly describe the hormonal regulation of amphibian metamorphosis.
6. What controls insect metamorphosis?
7. What insect does not go through metamorphosis?
8. What is the difference between molting and metamorphosis?
9. What insect goes through complete metamorphosis?
10. What is insect molting?
11. What is regeneration? Describe the process of regeneration in Hydra sp.
12. What are the characters of an advanced tadpole larva? Describe regressive and progressive changes takes place in amphibian metamorphosis.
13. Distinguish between complete and incomplete metamorphosis in insects. Describe different types of insect metamorphosis.
14. What is hypermetabolous development?
15. Define regeneration. Describe the role of hormones in regeneration.
16. What do you mean by autotomy and wound healing?

MCQ

1. The stages between successive molts of insects are called

   a) Larvae b) Instars c) Nymphs d) Imago

2. Hormones playing crucial role in insect metamorphosis is/are–

   a) Prothoracicotrophic hormone b) Juvenile hormone c) Ecdysone d) All above

3. Molting in insects is controlled by what hormone?

   a) Ecdysone b) Juvenile hormone c) Cytokinin d) Growth hormone

4. Metamorphic changes are most prominent in group amphibians

   a) The urodels b) The caecelians c) The anurans

5. Process of regeneration by repatterning of existing tissues is called

   a) Compensatory b) Morphallaxis c) Epimorphosis d) Autotomy

6. Morphallaxis regeneration is best seen in

   a) Planaria b) Urodel amphibians c) Hydra d) Lizards

7. Type of regeneration in Planaria is

   a) Compensatory b) Morphallaxis c) Epimorphosis d) Autotomy

8. Following amputation of urodel limb the tissues develop is

   a) Wound epidermis b) Apical endodermal cap c) Regeneration blastema d) All of above

Unit - 5 □ Implications of Developmental Biology

Structure

5.1 Objective

The primary objective of this unit are:

- application of the study of developmental biology.
- to learn the effects of different chemical compound on the developmental process or teratogenesis.
- to learn how the mutation of the genetic system alters the developmental process.
- to learn the mechanism of In vitro fertilization and its benefit.
- to learn the details of the stenn cell biology and their impact on the developmental processes.

5.2 Introduction

With the advancement in genetics, molucular biology and genomicsthe developmental biologists are gradually unveiling the mystry of the developmental process in modern days. Similarly this unit also elaborates the abnormalities of the physiological development. Teratogenesis is also elaborately disssussed, means the effect of the substances that may cause birth defects on an mbreyo. In this unit stem cell biology is also elaborately disussed including the roles and application of the 'In Vitro Fertilization' with their risk factors as well as their benefits.

5.3 Teratogenesis

Teratology is the study of abnormalities of physiological development. It is often thought of as the study of human congenital abnormalities, but it is broader than that, taking into account other non-birth developmental stages, including puberty; and other organisms, including plants. The related term developmental toxicity includes all manifestations of abnormal development that are caused by environmental insult. These may include growth retardation, delayed mental development or other congenital disorders without any structural malformations.

Teratogens are substances that may cause birth defects via a toxic effect on an embryo or fetus. Teratogenesis is quite complex process which involves different factors - called teratogens - that affect the normal process of prenatal development. Not each of the teratogen exposures actually leads to formation of some defect; there are many other factors that will influence the final result of the exposure.

5.3.1 Environmental Mutagens and Teratogens

A. Physical mutagens

i. **Radiation:** Ionizing radiation represents a radiation with shorter wavelength and larger energy in comparison with other type of radiation (X-ray, gamma-ray, cosmic rays).

Above radiation exhibits high energy and high penetration through the tissues. Ionizing radiation by passing through the tissue colides with atoms with subsequent release of the electrons; along the trace of the radiation free radicals and ions, capable of reacting with biological macromolecules, including DNA, arise (H", OH). Ionizing radiation may directly attack DNA itself. This kind of radiation induces oxidation of DNA bases and disrupts pentose-phosphate bond in DNA helix. Mutagenic effect depends on the amount of arising ions.

Ionizing radiation induces gene mutations, chromosomal aberrations and, ultimately, chromosomal translocations. There is no threshold for ionizing radiation, even small quantities may induce mutations. The quantity of radiation, which may duplicate the amount of mutations in humans, is important in genetics for prediction of the risk.

ii. **Ultraviolet radiation:** Damage of DNA molecule induced by UV radiation UV radiation exhibits lower energy than ionizing radiation, but even UV is capable to cause electron excitation. UV radiation is absorbed by several organic molecules, namely by pyrimidines and purines. UV acts as potent mutagen in unicellular organisms, in more complex organisms it alterates cells on the surface. In humans UV induces or contributes to the induction of skin neoplasia (carcinoma, melanoma). The risk of exposure to UV radiation increases with decreasing ozone content in the atmosphere. UV radiation induces mutations mainly due to generation of hydrated purines and pyrimidine dimers. Thymine alterations are mutagenic due to: a) distortion of DNA double-helical structure hinders the procedure of DNA polymerase along the template with subsequent block of DNA replication; b) in the course of the

repair of altered thymines a base mispairing often occurs. The repeated interruption of DNA replication due to thymidine dimers and their incomplete repair cause gaps in newly synthesized DNA chain with subsequent chromosomal breaks. Thymidine dimers may give rise base substitutions and/ or del etions.

B. Chemical mutagens

Chemical mutagens are chemicals exerting mutagenic effects. They comprise: a) food stains based on acridine; b) combustion products in cigarette smoke (more than 400 carcinogens and mutagens); c) chemicals in car exhausts; d) monomers in plastics industry (polychlorinated biphenyls, styrene, butadiene, vinyl chloride etc.).

Mode of action of chemical mutagens:

1. Compounds those are mutagenic only during replication (base analogues and acridine stains).
2. Compounds those are mutagenic by attacking DNA unless this is replicated.
3. Compounds causing alkylation, deamination and hydroxylation of bases.

a) Base analogues: These compounds are structurally related to nucleotides and are therefore mis- incorporated into DNA during replication. Their differences in comparison with physiological nucleotides cause base mispairing and mutations. These compounds are employed in investigation of mutagenic processes and as anticancer drugs (2-aminouracil, 5-bromouracil, 5-fluorouracil). 5-bromouracil is analogous to thymin. Br atom replaces methyl group on C5 of pyrimidine and increases a chance of tautomeric shin if in enot form, 5 BU pairs with guanine If 5-BU in enol form is incorporated into a new strand, during the following replication 5 0) in keto form pairs with adenine and GCAT transition arises. Acridine stains (such as proflavin and acridine blue) induce a shift in the reading frame Molecules of bases are incorporated in between base pairs and the double-helix conformation of DNA is altered during the replication During the replication insertion or deletion of one or more bases occurs with all associated phenotype consequences.

b) Alkylation compounds: Many chemicals may be donors of alkyl groups. Yperit (or its nitroso derivative) was the first reported mutagen. Nitroso guanidine, on the other hand, belongs among the most potent mutagens. Alkylating agents cause mispairing by attaching the functional group (methyl-, ethyl- etc.) to the nucleophilic centers of purines and pyrimidines.

Alkylating agents may induce all kinds of mutations and result ultimately in chromosomal aberrations and translocations

c) **Hydroxylating agents:** May convert cytosin into hydroxy aminocytosine, which pairs with adenine forming CG:AT transition.

C. Biological mutagens

i. **Viruses:** In the course of lysogenic cycle viruses may become incorporated into the DNA of the host. Incorporation of virus into the sequence of the gene affects substantially its function, the gene loses its function with subsequent consequences, such as chromosomal breaks, tumors.

ii. **Transposons:** Represent elements capable to transpose from one site of the genome to the other. In human genome there are two classes of transposable elements: LINE (long interspred nuclear element) and SINE (short interspred nuclear element). Their shifts within a genome may have mutagenic effects.

iii. **Testing of mutagens:** Most mutations negatively affect human health and, additionally, mutagenic compounds are often teratogenic and carcinogenic. Testing of the new compounds for their tentative mutagenic effect is a standard procedure within obligatory a tests prior to the compounds is released on market.

iv. **Teratogens:** Teratogens are external factors capable to cause (or substantially of) congenital malformations. Alike mutagens, teratogens may arbitrarily classified into three major groups: biological teratogens, chemical teratogens and physical teratogens.

5.3.2 Classes of Teratogens

A) **Biological teratogens:** Several pathogenic viruses are members of this class. Proven teratogens are following viruses: Rubi virus (rubella), Cytomegalovirus, Herpes virus, Parvo virus B-19, influenza virus, HIV and others, but also bacteria Treponema pallidum (syphilis) and protozoon Toxoplasma gondii (toxoplasmosis). Teratogenic risk may also be elevated by serious diseases of the mother, such as diabetes mellitus, phenylketonuria, myasthenia gravis and others.

B) **Chemical teratogens:** These classes of teratogens comprise several industrially and agriculturally employed chemicals (organic solvents, polychlorinated biphenyls, heavy metals etc.). Particularly important group of chemical teratogens is constituted by drugs and medicaments, where prominent teratogens are cytostatics, several antibiotics (namely tetracyclins), antiepileptics

(fynytoin, valproate), lithium, warfarin, thalidomide, ACE-inhibitors, steroids, retinoids etc. Teratogenic effects have been spotted in the case of ethyl alcohol (its abuse in gravidity causes foetal alcohol syndrome) and drugs such as pervitin.

C) **Physical teratogens:** This class of teratogens involves various kinds of radiation (X-rays, gamma-radiation), high temperature and mechanical teratogens.

5.3.3 Teratogenesis

Studies designed to test the teratogenic potential of environmental agents use animal model systems (e.g., rat, mouse, rabbit, dog, and monkey). Early teratologists exposed pregnant animals to environmental agents and observed the fetuses for gross visceral and skeletal abnormalities. While this is still part of the teratological evaluation procedures today, the field of Teratology is moving to a more molecular level, seeking the mechanism(s) of action by which these agents act. Genetically modified mice are commonly used for this purpose. In addition, pregnancy registries are large, prospective studies that monitor exposures women receive during their pregnancies and record the outcome of their births. These studies provide information about possible risks of medications or other exposures in human pregnancies.

Understanding how a teratogen causes its effect is not only important in preventing congenital abnormalities but also has the potential for developing new therapeutic drugs safe for use with pregnant women.

Humans: In humans, congenital disorders resulted in about 510,000 deaths globally in 2010. About 3% of newborns have a "major physical anomaly", meaning a physical anomaly that has cosmetic or functional significance.

Vaccinating while pregnant: In humans, vaccination has become readily available, and is important to the prevention of some diseases like polio, rubella, and smallpox, among others. There has been no association between congenital malformations and vaccination, as shown in Finland in which expecting mothers received the oral polio vaccine and saw no difference in infant outcomes than mothers who had not received the vaccine. However, it is still not recommended to vaccinate for polio while pregnant due to possible complications for the mother. Another important implication of this includes the ability to get the influenza vaccine while pregnant. During the 1918 and 1957 influenza pandemics, mortality in pregnant women was 45%. However, even with prevention through vaccination, influenza vaccination in pregnant women remains low at 12%.

5.3.4 Effects on Embryonic Development

The toxicants which cause teratogenesis are known as teratogenic agents. A gestating-embryo exhibits great dynamicity of the living cells. The embryonic cells multiply and differentiate at a tremendous rate making the embryo more susceptible to the drugs.

5.3.5 Stage Sensitivity for Teratogenicity

i. Pre-Differentiation Stage

During this stage the embryo is not susceptible to teratogenic agents. These agents either cause death to the embryo by killing all or most of the cells, or have no apparent effect on the embryo. Even when some widely harmful effects have been produced, the surviving cells can compensate and form a normal embryo. This resistant stage varies from 5-9 days depending on the species.

ii. Embryonic Stage

In fact this is the period when the cells undergo intensive differentiation, mobilization and organization. It is during this period that most of the organogenesis takes place. As a result, the embryo becomes most susceptible to the effects of various teratogens.

This period generally ends sometimes from the 10th-14th day in rodents and in the 14th week of the gestation period in humans. All organs are, however, not susceptible in the same period of the pregnancy. Rat embryo is most susceptible between days 8 and 12 for most organs, but the palate and urinogenital organs are more susceptible at a later stage for teratogens.

J. G. Wilson (1965) observed teratogenic treatment on the 10th day of gestation which resulted in the following incidences of malformations in rat: Brain defects - 35%, Eye defects – 33%, Heart defects – 24%, Skeletal defects - 18%, Urinogential defects - 6%.

iii. Fetal Stage

This stage is characterized by growth and functional maturation. Teratogens are thus unlikely to cause morphological defects during this stage, but they may induce functional abnormalities. Whereas, morphologic defects are, in general, readily detected at birth or shortly thereafter functional abnormalities, viz., CNS impairment, may not be diagnosed for some time even after birth.

5.3.6 Various mechanisms are involved in teratogenic effects

i. Interference with Nucleic Acids

Various teratogenic agents interfere with nucleic acid replication, transcription, or RNA translation. These include alkylating agents, antimetabolites, intercalating agents and amino acid antagonists.

ii. Inhibition of Enzymes

Inhibitors of enzymes, e.g. 5-flourouracil, may induce malformation through interference with differentiation or growth by inhibiting thymidylate synthatase. Other examples include 6aminonicotinamide, which inhibits glucose-6-phosphate dehydrogenase, and folate antagonists which inhibit dihydrofolate reductase.

iii. Deficiency of Energy Supply and Osmolarity

Certain teratogens can affect the energy supply for the metabolism by restricting the availability of substrates either directly (e.g., dietary deficiencies) or through the presence of analogs for antagonists of vitamins, essential amino acids, and others.

In addition, hypoxia and agents i.e., CO and CO2, can be teratogenic by depriving the metabolic process of the required O2 and probably also by the production of osmolar imbalances. These can induce edema, which, in turn, cause mechanical distortion and tissue ischemia. Physical agents that can cause malformations include radiation, hypothermia, hyperthermia and mechanical trauma.

It shall not be out of place to mention that the mode of action of many teratogens is yet uncertain. Furthermore, a potential teratogen may or may not exert teratogenic effects depending on such factors as bio-activating mechanism, stability and detoxifying capability of the embryonic tissues. Appropriate experimental testing for the teratogenicity of toxicants is, therefore, essential.

5.4 *In vitro* Fertilization

In vitro fertilization (IVF). is process of fertilization where an egg is combined with sperm outside the body, in vitro. The process involves monitoring and stimulating a woman's ovulatory process, removing ova (egg or eggs) from the woman's ovaries and letting sperm fertilize them in a liquid in a laboratory. The fertilized egg (zygote)

undergoes embryo culture for 2–6 days, and is then transferred to the same or another woman's uterus, with the intention of establishing a successful pregnancy.

IVF is a type of assisted reproductive technology used for infertility treatment and gestational surrogacy, in which a fertilized egg is implanted into a surrogate's uterus, and the resulting child is genetically unrelated to the surrogate. Some countries banned or otherwise regulate the availability of IVF treatment. Restrictions on availability of IVF include costs and age to carry a healthy pregnancy to term. IVF is mostly attempted if less invasive or expensive options have failed or are unlikely to work.

The first successful birth of a child after IVF treatment, Louise Brown, occurred in 1978. Louise Brown was born as a result of natural cycle IVF where no stimulation was made. The procedure took place at Dr. Kershaw's Cottage Hospital (now Dr. Kershaw's Hospice) in Royton, Oldham. Robert G. Edwards was awarded the Nobel Prize in Physiology or Medicine in 2010, the physiologist who co-developed the treatment together with Patrick Steptoe; Steptoe was not eligible for consideration as the Nobel Prize is not awarded posthumously. With egg donation and IVF, women who are past their reproductive years, have infertile male partners, have idiopathic female-fertility issues, or have reached menopause can still become pregnant. Adriana Iliescu held the record as the oldest woman to give birth using IVF and donated egg, when she gave birth in 2004 at the age of 66, a record passed in 2006. After the IVF treatment some couples are able to get pregnant without any fertility treatments. In 2012 it was estimated that five million children had been born worldwide using IVF and other assisted reproduction techniques.

5.4.1 Medical Uses of IVF

IVF may be used to overcome female infertility where it is due to problems with the fallopian tubes, making in vivo fertilization difficult. It can also assist in male infertility, in those cases where there is a defect in sperm quality; in such situations **intra-cytoplasmic sperm injection (ICSI)** may be used, where a sperm cell is injected directly into the egg cell. This is used when sperm has difficulty penetrating the egg, and in these cases the partner's or a donor's sperm may be used. ICSI is also used when sperm numbers are very low. When indicated, the use of ICSI has been found to increase the success rates of IVF.

According to UK's NICE guidelines, IVF treatment is appropriate in cases of unexplained infertility for women that have not conceived after 2 years of regular unprotected sexual intercourse.

5.4.2 Risks Factors of IVF

Specific steps of an in vitro fertilization (IVF) cycle carry risks, including: -

i. **Multiple births:** IVF increases the risk of multiple births if more than one embryo is implanted in the uterus. A pregnancy with multiple fetuses carries a higher risk of early labor and low birth weight than pregnancy with a single fetus does.

ii. **Premature delivery and low birth weight:** Research suggests that use of IVF slightly increases the risk that a baby will be born early or with a low birth weight

iii. **Ovarian hyperstimulation syndrome:** Use of injectable fertility drugs, such as human chorionic gonadotropin (HCG), 10 induce ovulation can cause ovarian hyper-stimulation syndrome, in which your ovaries become swollen and painful.

Signs and symptoms typically last a week and include mild abdominal pain, bloating, nausea, vomiting and diarrhea. If you become pregnant, however, your symptoms might last several weeks. Rarely, it's possible to develop a more-severe form of ovarian hyper-stimulation syndrome that can also cause rapid weight gain and shortness of breath.

iv. **Miscarriage:** The rate of miscarriage for women who conceive using IVF with fresh embryos is similar to that of women who conceive naturally about 15 to 25 percent but the rate increases with maternal age.

v. **Egg-retrieval procedure complications:** Use of an aspirating needle to collect eggs could possibly cause bleeding, infection or damage to the bowel, bladder or a blood vessel. Risks are also associated with general anesthesia, if used.

vi. **Ectopic pregnancy:** About 2 to 5 percent of women who use IVF will have an ectopic pregnancy when the fertilized egg implants outside the uterus, usually in a fallopian tube. The fertilized egg can't survive outside the uterus, and there's no way to continue the pregnancy.

vii. **Birth defects:** The age of the mother is the primary risk factor in the development of birth defects, no matter how the child is conceived. More research is needed to determine whether babies conceived using IVF might be at increased risk of certain birth defects. Some experts believe that the use of IVF does not increase the risk of having a baby with birth defects.

viii. **Ovarian cancer:** Although some early studies suggested there may be a link between certain medications used to stimulate egg growth and the development of a specific type of ovarian tumor, more recent studies do not support these findings.

ix. **Stress:** Use of IT can be financially, physically and emotionally draining. Support from counselors, family and friends can help you and your partner through the ups and downs of infertility treatment

5.4.3 Preparation

When choosing an in vitro fertilization (IVF) clinic, keep in mind that a clinic's success rate depends on many factors, such as patients' ages and medical issues, as well as the clinic's treatment population and treatment approaches.

Before beginning a cycle of IVF using our own eggs and sperm, we and our partner will likely need various screenings, including: -

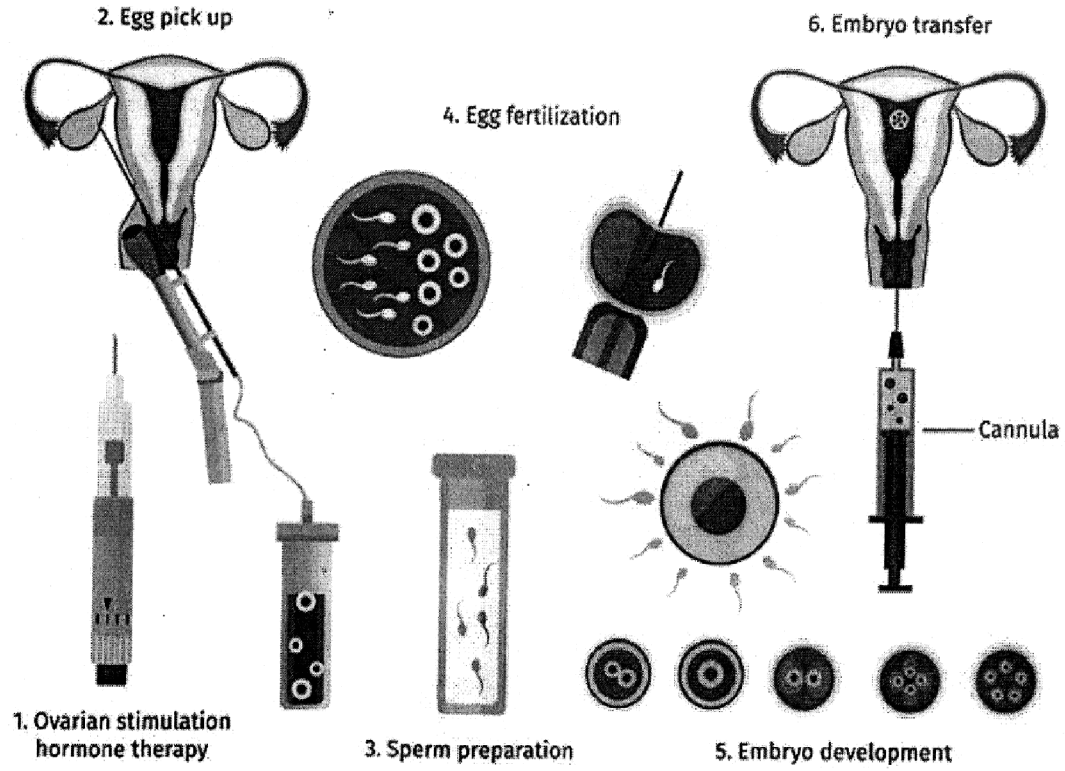


Fig. 5.1 : Process of In Vitro Fertilization (IVF)

i) **Ovarian reserve testing:** To determine the quantity and quality of eggs, doctor might test the concentration of follicle-stimulating hormone (FSH), estradiol (estrogen) and antimullerian hormone in blood during the first few days of menstrual cycle. Test results, often used together with an ultrasound of ovaries, can help predict how ovaries will respond to fertility medication.

ii) **Semen analysis:** If not done as part of initial fertility evaluation, doctor will conduct a semen analysis shortly before the start of an IVF treatment cycle.

iii) **Infectious disease screening:** Both of the partners will both be screened for infectious diseases, including HIV.

iv) **Uterine cavity exam:** Doctor will examine uterine cavity before start IVF. This might involve a sonohysterography — in which fluid is injected through the cervix into uterus, and an ultrasound to create images of uterine cavity. Or it might include a hysteroscopy in which a thin, flexible, lighted telescope (hysteroscope) is inserted through vagina and cervix into uterus.

5.4.4 Steps of IVF

In vitro fertilization (IVF) involves several steps ovulation induction, egg retrieval, sperm retrieval, fertilization and embryo transfer. One cycle of IVF can take about two weeks, and more than one cycle may be required.

A. **Ovulation induction:** If we're using our own eggs during IVF, at the start of a cycle we'll begin treatment with synthetic hormones to stimulate our ovaries to produce multiple eggs rather than the single egg that normally develops each month. Multiple eggs are needed because some eggs won't fertilize or develop normally after fertilization. We may need several different medications, such as:

Medications for ovarian stimulation: To stimulate ovaries, we might receive an injectable medication containing a follicle-stimulating hormone (FSH), a luteinizing hormone (LH) or a combination of both. These medications stimulate more than one egg to develop at a time.

Medications for oocyte maturation: When the follicles are ready for egg retrieval- generally after eight to 14 days — we will take human chorionic gonadotropin (HCG) or other medications to help the eggs mature.

Medications to prevent premature ovulation: These medications prevent our body from releasing the developing eggs too soon.

Medications to prepare the lining of uterus: On the day of egg retrieval or at the time of embryo transfer, doctor might recommend that begin taking progesterone supplements to make the lining of your uterus more receptive to implantation.

Sometimes IVF cycles need to be canceled before egg retrieval for one of these reasons:

- Inadequate number of follicles developing.
- Premature ovulation.
- Too many follicles developing, creating a risk of ovarian hyper-stimulation syndrome.
- Other medical issues.

B. Egg retrieval: Egg retrieval can be done in doctor's office or a clinic 34 to 36 hours after the final injection and before ovulation.

i Transvaginal ultrasound aspiration is usual retrieval method. An ultrasound probe is inserted into vagina to identify follicles. Then a thin needle is inserted into an ultrasound guide to go through the vagina and into the follicles to retrieve the eggs.

ii. If ovaries aren't accessible through transvaginal ultrasound, an abdominal surgery or laparoscopy, a procedure in which a tiny incision is made near navel and a slender viewing instrument (laparoscope) is inserted may be used to guide the needle.

iii. The eggs are removed from the follicles through a needle connected to a suction device. Multiple eggs can be removed in about 20 minutes.

iv. Mature eggs are placed in a nutritive liquid (culture medium) and incubated. Eggs that appear healthy and mature will be mixed with sperm to attempt to create embryos. However, not all eggs may be successfully fertilized.

C. Sperm retrieval: If we're using our partner's sperm, he'll provide a semen sample at our doctor's office or a clinic through masturbation the morning of egg retrieval. Other methods, such as testicular aspiration, the use of a needle or surgical procedure to extract sperm directly from the testicle— are sometimes required. Donor sperm also can be used. Sperm are separated from the semen fluid in the lab.

D. Fertilization: Fertilization can be attempted using two common methods:

a) **Insemination.** During insemination, healthy sperm and mature eggs are mixed and incubated overnight

b) **Intracytoplasmic sperm injection (ICSI):** In ICSI, a single healthy sperm is injected directly into each mature egg. ICSI is often used when semen quality or number is a problem or if fertilization attempts during prior IVF cycles failed.

c) **Assisted hatching.** About five to six days after fertilization, an embryo "hatches" from its surrounding membrane (zona pellucida), allowing it to implant into the lining of the uterus.

d) **Preimplantation genetic testing:** Embryos are allowed to develop in the incubator until they reach a stage where a small sample can be removed and tested for specific genetic diseases or the correct number of chromosomes, typically after five to six days of development. Embryos that don't contain affected genes or chromosomes can be transferred to uterus.

e) **Embryo transfer:** Embryo transfer is done at doctor's office or a clinic and usually takes place two to six days after egg retrieval. If successful, an embryo will implant in the lining of uterus about six to 10 days after egg retrieval.

After the embryo transfer, we can resume our normal daily activities. However, our ovaries may still be enlarged. Consider avoiding vigorous activity, which could cause discomfort.

5.5 Stem Cell (ESC)

Stem Cell (ESC)

Stem cells have the remarkable potential to develop into many different cell types in the body during early life and growth. In addition, in many tissues they serve as a sort of internal repair system, dividing essentially without limit to replenish other cells as long as the person or animal is still alive. When a stem cell divides, each new cell has the potential either to remain a stem cell or become another type of cell with a more specialized function, such as a muscle cell, a red blood cell, or a brain cell.

Stem cells are distinguished from other cell types by two important characteristics. First, they are unspecialized cells capable of renewing themselves through cell

division, sometimes after long periods of inactivity. Second, under certain physiologic or experimental conditions, they can be induced to become tissue- or organ-specific cells with special functions. In some organs, such as the gut and bone marrow, stem cells regularly divide to repair and replace worn out or damaged tissues. In other organs, however, such as the pancreas and the heart, stem cells only divide under special conditions.

Until recently, scientists primarily worked with two kinds of stem cells from animals and humans: embryonic stem cells and non-embryonic "somatic" or "adult" stem cells. Scientists discovered ways to derive embryonic stem cells from early mouse embryos more than 30 years ago, in 1981. The detailed study of the biology of mouse stem cells led to the discovery, in 1998, of a method to derive stem cells from human embryos and grow the cells in the laboratory. These cells are called human embryonic stem cells. The embryos used in these studies were created for reproductive purposes through in vitro fertilization procedures. When they were no longer needed for that purpose, they were donated for research with the informed consent of the donor. In 2006, researchers made another breakthrough by identifying conditions that would allow some specialized adult cells to be "reprogrammed" genetically to assume a stem cell-like state. This new type of stem cell, called induced pluripotent stem cells (iPSCs), will be discussed in a later section of this document.

5.5.1 Definition

Stem cells are a class of undifferentiated cells that are able to differentiate into specialized cell types. Commonly, stem cells come from two main sources: Embryos formed during the blastocyst phase of embryological development (embryonic stem cells) and Adult tissue (adult stem cells). Both types are generally characterized by their potency, or potential to differentiate into different cell types (such as skin, muscle, bone, etc.).

5.5.2 Properties Stem Cells

Stem cells differ from other kinds of cells in the body. All stem cells—regardless of their source—have three general properties: they are capable of dividing and renewing themselves for long periods; they are unspecialized; and they can give rise to specialized cell types.

Stem cells are capable of dividing and renewing themselves for long periods. Unlike muscle cells, blood cells, or nerve cells—which do not normally replicate themselves stem cells may replicate many times, or proliferate. A starting population

of stem cells that proliferates for many months in the laboratory can yield millions of cells. If the resulting cells continue to be unspecialized, like the parent stem cells, the cells are said to be capable of long-term selfrenewal.

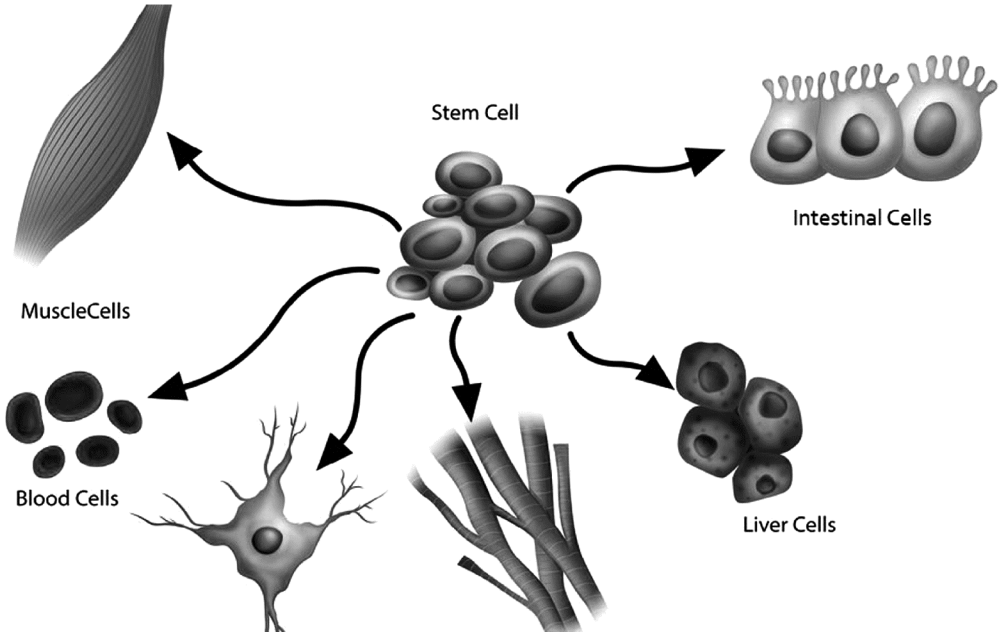


Fig. 5.2 : Stem cells and their derivatives

The specific factors and conditions that allow stem cells to remain unspecialized are of great interest to scientists. It has taken scientists many years of trial and error to learn to derive and maintain stem cells in the laboratory without them spontaneously differentiating into specific cell types. For example, it took two decades to learn how to grow human embryonic stem cells in the laboratory following the development of conditions for growing mouse stem cells. Likewise, scientists must first understand the signals that enable a non-embryonic (adult) stem cell population to proliferate and remain unspecialized before they will be able to grow large numbers of unspecialized adult stem cells in the laboratory.

5.5.3 What stages of early embryonic development are important for generating embryonic stem cells?

Embryonic stem cells, as their name suggests, are derived from embryos. Most embryonic stem cells are derived from embryos that develop from eggs that have been fertilized in *vitro*-in an in *vitro* fertilization clinic and then donated for research purposes with informed consent of the donors. They are not derived from eggs fertilized in a woman's body.

5.5.4 How are embryonic stem cells grown in the laboratory?

Growing cells in the laboratory is known as cell culture. **Human embryonic stem cells** (hESCs) are generated by transferring cells from a **pre-implantation** stage embryo into a plastic laboratory culture dish that contains a nutrient broth known as culture medium. The cells divide and spread over the surface of the dish. In the original protocol, the inner surface of the culture dish was coated with mouse embryonic skin cells specially treated so they will not divide. This coating layer of cells is called a **feeder layer**. The mouse cells in the bottom of the culture dish provide the cells a sticky surface to which they can attach. Also, the feeder cells release nutrients into the culture medium. Researchers have now devised ways to grow embryonic stem cells without mouse feeder cells. This is a significant scientific advance because of the risk that viruses or other macromolecules in the mouse cells may be transmitted to the human cells.

The process of generating an embryonic stem cell line is somewhat inefficient, so lines are not produced each time cells from the preimplantation-stage embryo are placed into a culture dish. However, if the plated cells survive, divide and multiply enough to crowd the dish, they are removed gently and plated into several fresh culture dishes. The process of re-plating or subculturing the cells is repeated many times and for many months. Each cycle of subculturing the cells is referred to as a passage. Once the cell line is established, the original cells yield millions of embryonic stem cells. Embryonic stem cells that have proliferated in cell culture for six or more months without differentiating, are pluripotent, and appear genetically normal are referred to as an embryonic stem cell line. At any stage in the process, batches of cells can be frozen and shipped to other laboratories for further culture and experimentation.

5.5.5 Adult Stem Cells

An adult stem cell is thought to be an undifferentiated cell, found among differentiated cells in a tissue or organ. The adult stem cell can renew itself and can differentiate to yield some or all of the major specialized cell types of the tissue or organ. The primary roles of adult stem cells in a living organism are to maintain and repair the tissue in which they are found. Scientists also use the term somatic stem cell instead of adult stem cell, where somatic refers to cells of the body (not the germ cells, sperm or eggs). Unlike embryonic stem cells, which are defined by their origin (cells from the pre-implantation stage embryo), the origin of adult stem cells in some mature tissues is still under investigation.

5.5.6 Tests for identification of stem cells

Scientists often use one or more of the following methods to identify adult stem cells: (1) label the cells in a living tissue with molecular markers and then determine the specialized cell types they generate; (2) remove the cells from a living animal, label them in cell culture, and transplant them back into another animal to determine whether the cells replace (or "repopulate") their tissue of origin.

Importantly, scientists must demonstrate that a single adult stem cell can generate a line of genetically identical cells that then gives rise to all the appropriate differentiated cell types of the tissue. To confirm experimentally that a putative adult stem cell is indeed a stem cell, scientists tend to show either that the cell can give rise to these genetically identical cells in culture, and/or that a purified population of these candidate stem cells can repopulate or reform the tissue after transplant into an animal.

5.5.7 Stem cell differentiation

As indicated above, scientists have reported that adult stem cells occur in many tissues and that they enter normal differentiation pathways to form the specialized cell types of the tissue in which they reside. Normal differentiation pathways of adult stem cells. In a living animal, adult stem cells are available to divide for a long period, when needed, and can give rise to mature cell types that have characteristic shapes and specialized structures and functions of a particular tissue. The following are examples of differentiation pathways of adult stem cells that have been demonstrated in vitro or in vivo.

Hematopoietic stem cells give rise to all the types of blood cells: red blood cells, B lymphocytes, I lymphocytes, natural killer cells, neutrophils, basophils, eosinophils, monocytes, and macrophages.

Mesenchymal stem cells have been reported to be present in many tissues. Those from bone marrow (bone marrow stromal stem cells, skeletal stem cells) give rise to a variety of cell types: bone cells (osteoblasts and osteocytes), cartilage cells (chondrocytes), fat cells (adipocytes stromal cells that support blood formation. However, it is not yet clear how similar or dissimilar mesenchymal cells derived from non-bone marrow Sources are to those from bone marrow stroma.

Neural stem cells in the brain give rise to its three major cell types: nerve cells (neurons) and two categories of non-neuronal cells—astrocytes and oligodendrocytes.

Epithelial stem cells in the lining of the digestive tract occur in deep crypts and give rise to several cell types: absorptive cells, goblet cells, Paneth cells, and enteroendocrine cells.

Skin stem cells occur in the basal layer of the epidermis and at the base of hair follicles. The epidermal stem cells give rise to keratinocytes, which migrate to the surface of the skin and form a protective layer. The follicular stem cells can give rise to both the hair follicle and to the epidermis.

5.5.8 Similarities and differences between embryonic and adult stem cells

Human embryonic and adult stem cells each have advantages and disadvantages regarding potential use for cell-based regenerative therapies. One major difference between adult and embryonic stem cells is their different abilities in the number and type of differentiated cell types they can become. Embryonic stem cells can become all cell types of the body because they are pluripotent. Adult stem cells are thought to be limited to differentiating into different cell types of their tissue of origin.

Embryonic stem cells can be grown relatively easily in culture. Adult stem cells are rare in mature tissues, so isolating these cells from an adult tissue is challenging, and methods to expand their numbers in cell culture have not yet been worked out. This is an important distinction, as large numbers of cells are needed for stem cell replacement therapies.

Scientists believe that tissues derived from embryonic and adult stem cells may differ in the likelihood of being rejected after transplantation. We don't yet know for certain whether tissues derived from embryonic stem cells would cause transplant rejection, since relatively few clinical trials have tested the safety of transplanted cells derived from hESCS.

Adult stem cells, and tissues derived from them, are currently believed less likely to initiate rejection after transplantation. This is because a patient's own cells could be expanded in culture, coaxed into assuming a specific cell type (differentiation), and then reintroduced into the patient. The use of adult stem cells and tissues derived from the patient's own adult stem cells would mean that the cells are less likely to be rejected by the immune system. This represents a significant advantage, as immune rejection can be circumvented only by continuous administration of immunosuppressive drugs, and the drugs themselves may cause deleterious side effects.

5.5.9 Uses of human stem cells and the obstacles

There are many ways in which human stem cells can be used in research and the clinic. Studies of human embryonic stem cells will yield information about the complex events that occur during human development. A primary goal of this work is to identify how undifferentiated stem cells become the differentiated cells that form the tissues and organs. Scientists know that turning genes on and off is central to this process. Some of the most serious medical conditions, such as cancer and birth defects, are due to abnormal cell division and differentiation. A more complete understanding of the genetic and molecular controls of these processes may yield information about how such diseases arise and suggest new strategies for therapy. Predictably controlling cell proliferation and differentiation requires additional basic research on the molecular and genetic signals that regulate cell division and specialization. While recent developments with iPS cells suggest some of the specific factors that may be involved, techniques must be devised to introduce these factors safely into the cells and control the processes that are induced by these factors.

Human stem cells are currently being used to test new drugs. New medications are tested for safety on differentiated cells generated from human pluripotent cell lines. Other kinds of cell lines have a long history of being used in this way. Cancer cell lines, for example, are used to screen potential anti-tumor drugs. The availability of pluripotent stem cells would allow drug testing in a wider range of cell types. However, to screen drugs effectively, the conditions must be identical when comparing different drugs. Therefore, scientists must be able to precisely control the differentiation of stem cells into the specific cell type on which drugs will be tested. For some cell types and tissues, current knowledge of the signals controlling differentiation falls short of being able to mimic these conditions precisely to generate pure populations of differentiated cells for each drug being tested.

Perhaps the most important potential application of human stem cells is the generation of cells and tissues that could be used for cell-based therapies. Today, donated organs and tissues are often used to replace ailing or destroyed tissue, but the need for transplantable tissues and organs far outweighs the available supply. Stem cells, directed to differentiate into specific cell types, offer the possibility of a renewable source of replacement cells and tissues to treat diseases including macular degeneration, spinal cord injury, stroke, burns, heart disease, diabetes, osteoarthritis, and rheumatoid arthritis.

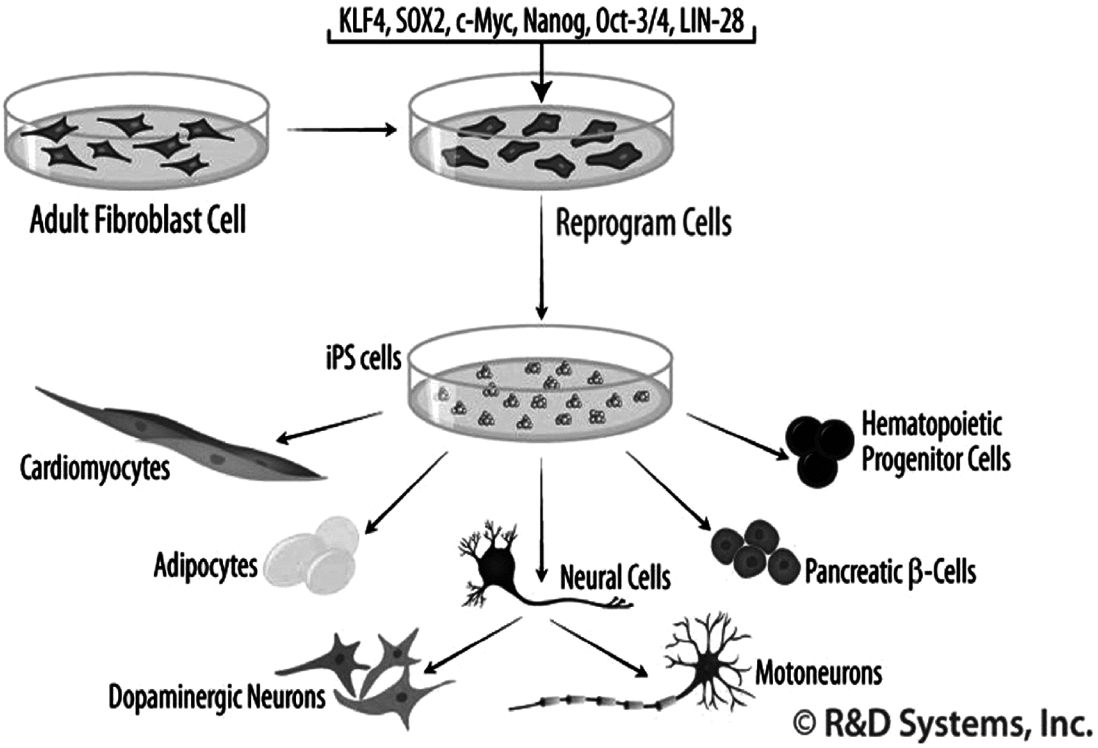


Fig. 5.3 : Use of iPS cell to generate adult tissue cells

5.6 Amniocentosis

Amniocentesis (also referred to as **amniotic fluid test**) is a medical procedure used in prenatal diagnosis of chromosomal abnormalities and fetal infections, and also for sex determination, in which a small amount of amniotic fluid, which contains fetal tissues, is sampled from the amniotic sae surrounding a developing fetus, and then the fetal DNA is examined for genetic abnormalities. The most common reason to have an "amnio" is to determine whether a baby has certain genetic disorders as Down syndrome. or chromosomal abnormality, such a Amniocentesis (or another procedure, called **chorionic villus sampling** (CVS)) can diagnose these problems in the womb. Amniocentesis is performed when a woman is between 14 and 16 weeks gestation. Women who choose to have this test are primarily those at increased risk for genetic and chromosomal problems, in part because the test is invasive and carries a small risk of miscarriage. This process can be used for prenatal sex discernment and hence this procedure has legal restrictions in some countries.

An amniocentesis test is a procedure where a doctor takes a small sample of amniotic fluid from our uterus. It helps find certain birth defects.

5.6.1 What is amniocentesis?

Amniocentesis tests our amniotic fluid— the fluid that surrounds and protects the fetus inside your uterus. Cells from the fetus that float in the fluid can be examined for chromosomal defects, like Down syndrome. The fluid can also be tested for neural tube defects, such as spina bifida.

The test results are usually ready within a few weeks. They are more than 99 percent accurate in spotting chromosome defects, such as Down syndrome. They also detect nearly all open neural tube defects.

Amniocentesis is usually done between 15th and 20th weeks of pregnancy. Amniocentesis is usually painless and safe. But there is a small risk of miscarriage after amniocentesis. Fewer than one out of 100 women who have it will have a miscarriage. You get to decide which, if any, genetic tests you want to have done.

5.6.2 How is amniocentesis done?

Our doctor puts a long, thin needle through our belly and into our uterus and takes out a small sample of fluid. They look at an ultrasound to help guide the needle. Amniocentesis usually doesn't hurt. Most people say they have a little discomfort or no pain at all.

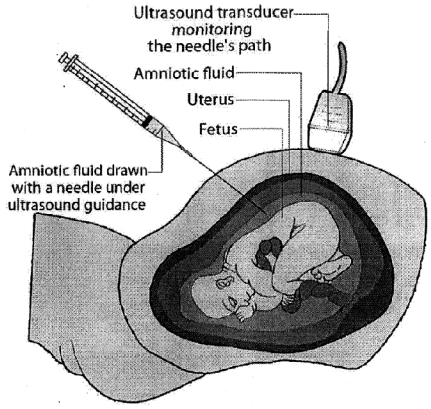
5.6.3 Procedure

Before the start of the procedure, a local anesthetic can be given to the mother in order to relieve the pain felt during the insertion of the needle used to withdraw the fluid. After the local anesthetic is in effect, a needle is usually inserted through the mother's abdominal wall, then through the wall of the uterus, and finally into the amniotic sac. With the aid of ultrasoundguidance, a physician punctures the sac in an area away from the fetus and extracts approximately 20ml of amniotic fluid. If used for prenatal genetic diagnosis, fetal cells are separated from the extracted sample. The cells are grown in a culture medium, then fixed and stained. Under a microscope the chromosomes are examined for abnormalities. The most common abnormalities detected are Down syndrome (trisomy 21), Edwards syndrome (trisomy 18), and Turner syndrome (monosomy X). In regard to the fetus, the puncture seals and the amniotic sac replenishes the liquid over the next 24-48 hours.

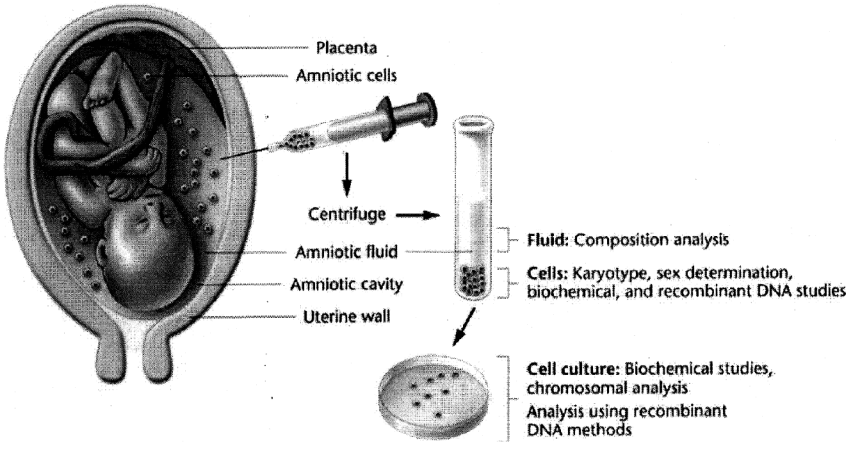
5.6.4 Risks Factors

Amniocentesis is performed between the 15th and 20th week of pregnancy; performing this test earlier may result in fetal injury. The term "early amniocentesis" is sometimes used to describe use of the process between weeks 11 and 13.

Complications of amniocentesis include preterm labor and delivery, respiratory distress, postural deformities, chorioamnionitis, fetal trauma of the mother (rhesus disease). Studies from the 1970s originally estimated the risk of amniocentesis-related miscarriage at around 1 in 200 (0.5%). Three more recent studies from 2000-2006 estimated the procedure-related pregnancy loss at 0.6-0.86%. A more recent study (2006) has indicated this may actually be much lower, perhaps as low as 1 in 1,600



(A)



(B)

Fig. 5.4 : A-B : Process of amniocentosis

(0.06%). Unlike the previous studies, the number in this study only reflects the loss that resulted from amniocentesis complications and excluded the cases when parents decided for an abortion following the test results. In contrast to amniocentesis, the risk of miscarriage from chorionic villus sampling (CVS) is believed to be approximately 1 in 100, although CVS may be done up to four weeks earlier, and may be preferable if the possibility of zenetic defects is thought to be higher.

5.7 Questions

1. What do you mean by teratology and teratogens?
2. Describe the different types of physical mutagens.
3. Write about the mode of action of chemical mutagens.
4. What are biological mutagens?
5. Write the effect of teratogen on human body.
6. Describe various mechanisms that are involved in teratogenic effects.
7. Describe the preparatory phase of in vitro fertilization.
8. Define stem cell. Write the different properties of stem cell.
9. Write the similarities and differences between embryonic and adult stem cells.
10. What is amniocentesis? Describe the process of it.

MCQ

1. External factors capable to cause (or substantially increase a risk of) congenital malformation is called

   a) Teratogens b) Biological mutagen c) Viruses d) None of these.

2. Studies designed to test the teratogenic potential of environmental agents is called

   a) Teratogenesis b) Embryogenesis c) Physical factors d) Chemical compound

3. A process of fertilization where an egg is combined with sperm out side the body is called

a) In vitro fertilization b) In vivo fertilization c) Internal fertilization d) None of these.

4. The cells which have the remarkable potential to develop into many different cell types in the body during early life and growth is known as

   a) Embryonic cell b) Stem cell c) Epithelial cell d) Nerve cell

5. A medical procedure used in potential diagnosis of chromosomal infection is called

   a) Amniocentesis b) In vitro fertilization c) Metabolism d) Fertilization

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