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Introduction : History and types of embryology

All cells arise from pre-existing cells. There is no other way for a cell to be produced unless scientists succeed in synthesizing cells from simple molecules in laboratory. Every living species whether virus, bacterium, plant or animal has the innate capacity to give rise to similar new individual to balance the loss as a consequence of adverse environmental conditions or ageing. Thus **reproduction** in organisms denotes formation of new individual which essentially resembles the parents. *The entire chain of developmental events and changes in an animal is referred to as* ***development***. Since during developmental events the developing stage of an animal is termed as an embryo, this branch of Science in biological sciences is termed as **Embryology**.

The developmental events can easily be followed in sexual reproduction and life-cycle of higher animals. Further, the entire life-cycle of an higher form can be divided into two phases: **pre-natal period** and **post-natal period**; that is, periods before and after birth, respectively.

The **pre-natal period** includes all events and changes which occur from *fertilization* of the egg till *hatching or birth* of the young offspring. All developmental changes which occur after hatching or birth in a developing young one are included in the **post-natal period**. Both the periods, that is, developmental changes from fertilized egg or zygote to the formation of fully formed individual are jointly referred to as **ontogenetic development or ontogeny**. Ontogenetic development is of two types—**embryogenesis** and **blastogenesis**. Embryonic development from zygote till the formation of a new individual is referred to as **embryogenesis**, while developmental changes which occur in the formation of a new individual as a result of asexual reproduction (budding, body segmentation, etc.) are referred to as **blastogenesis**. Thus the branch of life-science in which embryogenesis and blastogenesis among animals is studied is defined as embryology or **developmental biology**. Development generally does not stop with the formation of a new organism but continues during the postnatal period also. A newly-formed young individual at the time of hatching or birth besides being small in size is also not in many ways fully developed. For example, a new-born infant lacks teeth and its reproductive system is still not mature. Similarly, in organisms formed as a consequence of blastogenesis development is

considered to be incomplete during the initial phases. The development, involving many internal and external changes, therefore, continues in such a newly-formed organism and as a result it ultimately transforms into a sexually mature adult. Even after attaining full development and maturity, somatic changes continue progressively in an individual. Thus after achieving a critical limit of growth, **degenerative changes** begin to commence in an organism and as a result in the end the individual dies. These degenerative changes in an organism comprise the phenomenon of **ageing**. The study of the various progressive and organized changes, which occur in the entire life-cycle of organisms is referred to as **developmental biology**. In relative terms, the scope of developmental biology, therefore, is obviously wider than **embryology** because in embryology only events which occur during the pre-natal stage are included while in developmental biology changes which occur both during the pre-natal and post-natal stages of an organism are studied.

Besides ontogenetic development, in organismal studies, **phylogenetic development** is also considered. However, both are completely different from each other. The historic and evolutionary development of a species is referred to as **phylogenetic development** or **phylogeny. Ontogenetic development** or **ontogeny**, therefore, confines itself only to changes which take place during the life-time of an individual organism. In other words, it can also be said that ontogeny is the study of changes which occur in a given single generation within the chain of many generations of a species.

Phases of Embryonic Development

In the embryonic development of multicellular animals the following chief sequential phases of development are essentially present in one form or the other:

1. Gametogenesis: In the embryonic development of all animals a single cell, the **zygote**, is transformed into a fully-formed daughter organism. Also, the zygote in turn is formed as a result of fusion between two germ-cells. Thus the commencement of embryonic development is taken as the phase in which formation of the germ-cells takes place. The germ cells are also referred to as **gametes**. Male gametes are called **spermatozoa** *(sing.* spermatozoon) and female gametes are referred to as ova *(sing.* ovum). Both spermatozoa and ova are highly modified and specialized cells and are totally different than the rest of the somatic or body cells of an organism. Although the gametes or germ cells originate from ordinary somatic or body cells, their formation involves highly complex and radical alterations, and it is only due to these significantly fundamental changes. These cells are able to successfully perform their assigned function. Formation of gametes which takes place in the reproductive organs, or **gonads** of an adult animal is referred to as **gametogenesis**. The spermatozoa or sperms are formed in the testis and the process of their formation is called **spermatogenesis**. Likewise, eggs or ova originate in the ovary and the process of their formation is thus referred to as **oogenesis**. In both, spermatogenesis and oogenesis, reduction division or meiosis takes place which is considered as one of the most important events of gametogenesis. It is because of meiotic division that each sperm or ovum receives only one set of chromosomes, that is, gametes are

haploid (= n) cells while the parent cell is *diploid (= 2n)* containing a duplicate set of chromosomes.

2. Fertilization : In the next phase of embryonic development fusion of an ovum with a sperm occurs. The process of fusion of gametes is termed **fertilization** and as the result, a united cell called **zygote**, is formed. In the zygote itself the development of the embryo commences. The most significant event in the fertilization process is the fusion of nuclei of the sperm and ovum referred to **as karyogamy** or **amphimixis**. The re-establishment of the diploid number of chromosomes in the zygote is called **diplosis**. In the fertilization process along with karyogamy, fusion or mixing of the cytoplasm of the two gametes or plasmogamy also occurs. The process of **plasmogamy** is also called cytogamy.

3. Cleavage: Immediately after fertilization, the zygote begins to divide rapidly and progressively into daughter cells. This phase of division is referred to as **segmentation** or **cleavage**. All cleavage cell-divisions are **mitotic** in nature. Thus each daughter cell from the numerical, structural and functional point of view has an identical set of chromosomes like the zygote. As a result of repeated cleavages, the so-formed daughter cells called **blastomeres** transform into a solid ball-like structure termed the **morula**. The blastomeres act as building-blocks for various body organs and parts of the future multicellular organisms.

4. Blastulation: As a result of continuous cleavage, the blastomeres of the morula soon arrange themselves in a hollow ball containing a central fluid-filled cavity. The central cavity is called the **blastocoel** and the so formed developmental stage is referred to as the **blastula**. The single or multilayered epithelial layer of blastomeres is called as the **blastoderm**. The entire process of formation of the blastula from the morula stage is called **blastulation**: In the blastoderm the primordia of the various organs of the future embryo are formed.

5. Gastrulation: In the embryonic development of animals, this stage is considered as one of the most important phases. During this phase extensive migrations in the blastodermal cells take place. These migrations or movements are referred to as **formative movements** or **morphogenetic movements**. As a result of rearrangement of blastomeres the three *primary germ layers* are established, which are **ectoderm, mesoderm** and **endoderm**. Further, besides differentiation of the three primary germ layers from the undifferentiated blastomeres, by morphogenetic movements these germ layers also come to occupy their designated locations. The process of development at this phase is referred to as **gastrulation** and the resultant embryonic stage is described as the **gastrula**. The fully-formed gastrula contains a central cavity, the **archenteron**. The archenteron is lined by endoderm layer and opens to the outside by a **blastopore**. The archenteron will form the lumen of the *alimentary canal* and the blastopore will modify into either the *mouth* or the *anus* of the future animal. Animal groups where the biastopore forms the mouth are designated as **Protostomia** (all invertebrate groups excluding Echinodermata and Hemichordata). Animal groups where the blastopore modifies to form the anus are described as **Deuterostomia**. Although in different animals variations in morphogenetic movements

during the gastrulation process are present but ultimately in all animals the three primary germ-layers are formed and which migrate to their designated locations in the developing embryo.

6. Organogenesis: The process of formation of various organs and organ-systems from the three primary germ-layers of a. developing embryo is called **organogenesis**. After gastrulation, in the developing embryo, cells in numerous small groups separate from the, three primary germ layers to form the **primary organ rudiments**. Each primary organ rudiment during the course of progressive development forms a specific organ or part of an organ of the animal. Further, the primary organ rudiments also redivide into secondary organ rudiments and the process of formation of single organ or their parts continues to transform by the process of organogenesis, a developing embryo into a fully-formed animal.

7. Morphogenesis: Along with organogenesis, the external shape and physical form of the embryo also undergoes progressive alterations and it begins to resemble more and more the fully-formed animal. This process is described as **morphogenesis**. The meaning of morphogenesis is generally considered wide-ranging. Thus in morphogenesis, alteration in the external morphology and shape is not involved alone but the term includes all developmental changes which occur in the internal as well as external structures of a developing animal, since external morphology is directly related to the structure of the internal organs.

8. Differentiation: With the development and differentiation of various tissues and organs, their constituent cells also begin to gradually differentiate according to the structure and function of the concerned tissue or organ. In other words, muscle cells, nerve cells, epithelial cells, skeletal cells, etc. begin to differentiate. Ultimately every type of cell modifies to perform a specific function. The process of differentiation of similar type of cells into structurally and functionally different types is referred to as **differentiation**. All cells of an embryo are formed as a result of mitotic divisions from a single cell or zygote. Thus in the beginning all cells are basically similar from the functional, structural and genetic points of view; however, after gastrulation variations in these cells begin to appear. In the future developmental process, this differentiation progressively continues because without differentiation, organogenesis and morphogenesis will not be possible in a developing embryo. Therefore, differentiation is an extremely significant event of embryonic development. Differentiation is categorised into three main types:

(i) *Morphological differentiation (structural differentiation) or Cytological differentiation.*

(ii) *Physiological differentiation.*

(iii) *Chemical differentiation.*

The structural specialization of a cell is called as **structural differentiation** or **cytological differentiation**. By this process, similar cells gradually become different in structure. For example, the profound structural differences found between muscle cells and nerve cells are actually due to the process of **morphological differentiation**.

Although in all types of cells similar type of basic metabolic processes, take place but besides these, various differentiated cells also perform different specific functions. For example, nerve cells transmit impulses, gland cells secrete specific secretions, muscle cells undergo contractions, etc. The phase of differentiation in which the different cells acquire the ability to perform specific functions is called **physiological differentiation**. Morphological differentiation and physiological differentiation which occur in cells are actually due to chemical differentiation. **Chemical differentiation** refers to the production of such specific organic compounds which distinguish one type of cells with another. The synthesis of different types of organic substances by cells is because of the presence of specific enzymes. Chemically, enzymes are proteins, and therefore, it can also be stated that chemical differentiation is basically formation of specific types of proteins by cells.

9. Growth: Due to continuous cell-divisions, the number of cells in the developing organism continues to increase. The volume of the daughter cells also increases. Different types of cells manufacture different kinds of organic substances and as a consequence, the volume of the embryo also progressively increases. This phenomenon is described as growth. **Growth** denotes increase in size, and through growth the embryo ultimately acquires the shape of the parent.

Amongst the above described phases of embryonic development, gametogenesis, fertilization, cleavage, blastulation and gastrulation are progressively sequential but distinct stages, that is, these phases progressively follow each other. However, organogenesis, morphogenesis, differentiation and growth are not sequential events. These phases take place simultaneously for a considerable length of time. These events continue even in the larva and adult. In cells, chemical differentiation commences along with cleavage and it continues during blastulation and gastrulation. Morphogenesis begins during the process of gastrulation.

History of Embryology

Reproduction and embryonic development have been subjects of human interest from the very early times. However, knowledge of embryonic development could only be provided after establishment of the **cell-theory** during the 19th century. Therefore, history of embryology is generally studied in two steps : **ancient period and modern period.**

I. Ancient Period

1. Ancient Thoughts: Approximately 3000 - 1500 B.C. the Egyptians presented several concepts related to the formation and growth of the embryo. The Egyptians also gave certain methods to control birth and for abortion. These ancient Egyptian writings are considered to be the oldest documentation on Embryology. In ancient religious writings dating around 600 B.C. from India, there is also mention of three methods for the formation of animals, that is, from *eggs, organisms* and *germ*. In the text "Sushrut a Samhita" of India which dates back to 2nd or 3rd century A.D. there is a mention of the development of the human young in the womb of the mother.

2. Greek Thoughts: Various Greek philosophers had also presented thoughts on the development of organisms. These include thoughts of Empidocles, Hippocrates, Aristotle, Herophilos and Galen. In the 5th century **B.C. Empedocles** presented the concept that formation of a young is due to partial contributions from both the male and female parents. Hippocrates (460-377 B.C.) studied the development of the egg of a chick. Aristotle (384-322 B.C.) besides the chick, also studied the embryology of several animals and presented his work in a book entitled **De Generatione Animalium**. Therefore, it is Aristotle who is considered to be the founder father of Embryology and he coined the term "Embryon". According to Aristotle, for the development of embryo, it is the female organism which provides the necessary material and the semen of the male parent which initiates and directs embryonic development. **Herophilos** and **Galen** gave the concept that it is the ovary of the female which provides the material for embryogenesis.

From the time of Galen till about the 17th century, like in other sciences, in embryological knowledge also there did not occur any apparent progress due to the onset of "dark-ages" in Europe.

II. Modern Period

The microscope was invented in the 17th century, and thus, the knowledge of the presence of spermatozoa and ova was known. However, at that period they were not considered as cells. The progressive history of modern Embryology can somewhat be briefly described on the following lines:

1. Theory of Pre-formation: In 17th century the Dutch scientist Swammerdam gave the hypothesis that in the reproductive or germ cells is present the preformed miniature of the embryo or of the adult animal. In this context **Malpighi** (1673), **Haller** (1707-77), **Bonnet** (1720-1793), **Spallazani** (1729-1799), etc. gave the opinion that this miniature form was present in the ovum or egg which is stimulated for growth by the sperm. In contrast, **Leeuwenhoek** (1632-1723), **Harsocker** (1656-1725), etc. said that instead of the ovum, the miniature form is located in the head of the sperm. They called this hypothetical miniature form as homunculus.

2. Theory of Epigenesis : The above described improbable theory of preformation was opposed by **Wolff** (1759). On the basis of his studies on the developing Embryo of the chick he proved that the development of the embryo takes place as a result of successive progressive formation of new organs, and hence organs are never pre-formed. The successive formation of new organs was referred to as **epigenesis**. The 19th century scientists later confirmed the theory of epigenesis.

3. Theory of Germ-layer : In the early part of the 18th century, cleavage in the zygote of frog was observed for the first time by **Spallazani**. Later on several scientists with their work established that cleavage is a basic feature in the embryonic development of all animals. In 1817 **Pander** described the formation of the three **primary germ layers**, viz., *ectoderm, endoderm* and *mesoderm* in the chick embryo and also demonstrated that all tissues and organs of the body develop from these three germ-layers. **Kowalevski** (1846-1901) described the process of development

of the **primary germ layers** in *Amphioxus (= Branchiostoma)*. Kowalevski also clarified that during development of *Amphioxus*, the zygote first modifies into a single-layered hollow **blastula** which as a result of invagination is converted into a double-layered **gastrula**. Currently this sequence of events is well-established in the embryonic development of all animals.

4. Baer's Law: Karl Ernst Von Baer (1792-1876) on the basis of his extensive studies on the embryorlic development of several animals presented an extremely significant concept according to which: during embryonic development of an animal, the general features of larger taxonomic groups appear first and relatively later the specific characteristics of the animal itself make their appearance. This concept is now known as **Baer's Law**. Von Baer is universally accepted as the father of modern embryology.

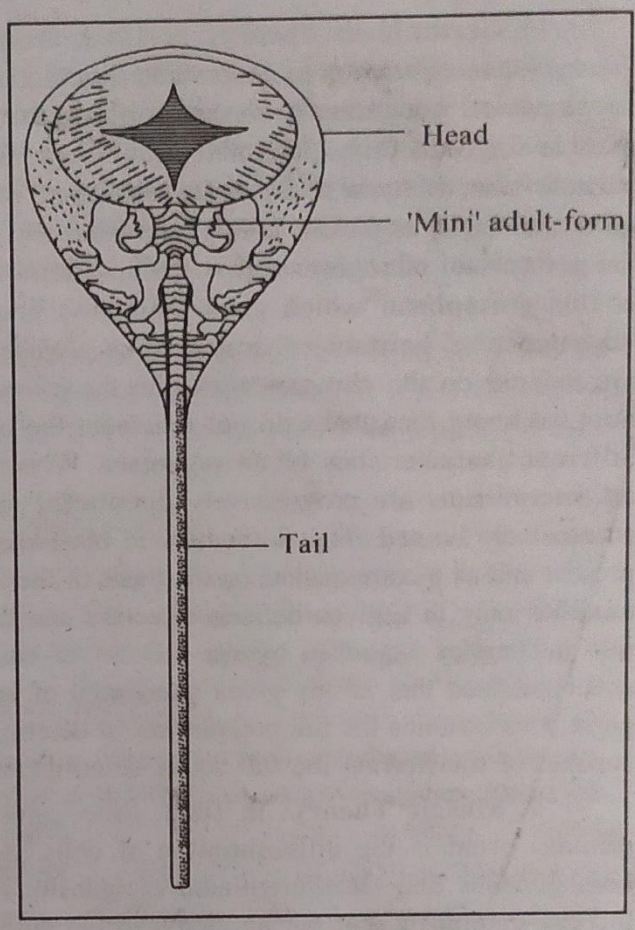

Fig. 1.1 Presence of hypothetical 'homanculus' in a sperm

5. Theory of Recapitulation or Biogenetic Law: Miller (1864) and **Haeckel** (1868) analysed and applied Baer's law to phylogenetic concepts. As a result, the recapitulation hypothesis of Haeckel was presented. According to this hypothesis during the embryonic development of an animal the primitive features develop first and the relatively recent characteristics then make their progressive appearance. In other words, *embryonic or ontogenetic development recapitulates the successive evolutionary or phylogenetic history of an animal species.* Thus in short, *ontogeny repeats phylogeny.*

After the presentation of the 'Cell-theory' by **Schleiden** and **Schwann** (1838, 1839), the cellular nature of the ovum and sperm was also established. For the first time entry of the sperm into the ovum during fertilization was observed by **Newport** (1854) in the frog. This observation and other similar studies gave the concept that in sexual reproduction fusion of germ cells takes place. Oscar **Hertwig** (1875), **Fol** (1877), **Strassburger** (1877) and other workers proved the fact that in the fertilization process the fusion of nuclei of ovum and sperm is the most essential and significant event.

6. Germplasm Theory: In 1904, **August Weismann** with his *germ-plasm theory*, made an attempt to understand the relationship between heredity and embryonic development. According to the germ-plasm theory, in the germ-cell of an organism a particle for each body part of the adult organism (animal or plant) is present. This particle was referred to as **determinant**. Accordingly, all the determinants in the germ cell of an organism jointly represent the entire body of the adult form, that is, the germplasm of a germ cell is the sum total of all determinants of an organism. It is this germplasm which goes from one generation to the next and causes the inheritance of heritable characteristics. According to **weismann**, the determinants are located on the chromosomes like the *genes* of modern day Genetics. However, now we know that genes do not represent the body parts of an animal but define the different characteristics of an organism. Weismann also stated that during cleavage as determinants are progressively distributed into different blastomeres they become successively limited. Thus ultimately in blastomeres only few definite determinants are present and as a consequence on the basis of the contained determinants each blastomere modifies only in a given definite direction and thus all the blastomeres jointly develop into a complex organism by the process of embryonic development. Weismann also conceptualized that in any given generation of an organism only the germplasm of the germ cells contains the full compliment of determinants, because only the germ cells are capable of transferring the full set of determinants into the next generation.

7. Mosaic Theory: In 1888, Roux gave the concept that in development the primary event is the differentiation of cells. As ovum before fertilization contains homogeneous and undifferentiated cytoplasm. Fertilization causes rearrangement of the egg-cytoplasm and during cleavage the cytoplasmic substances are distributed in such a way that in the blastula for the development of specific tissues and organs different areas become determined. In other words, with reference to different cellular areas the blastula is like a *mosaic*.

8. Regulative Theory: Roux observed that the fate of each cell of an early embryo is pre-determined and it is thus responsible for the development of an specific area or part or an organism. However, in 1891, **Driesch** hypothesized that each cell of an early embryo has the capability to develop into a complete embryo and the fate of these cells is dependent upon their location in the developing organism. Such type of development was referred to as regulative development.

9. Gradient Theory: The credit of this theory goes to **Boveri** (1901) and later to **Child** (1940). According to this theory, there exists a *metabolic axial gradient* between the 'active' animal pole and the 'inactive' vegetal pole of an ovum which regulates morphogenetic development or morphogenesis. **Horstadium** (1955) and **Runnstrom** (1967), however, proposed the presence of two gradients in the ovum which control morphogenesis. One active gradient is present between the animal and vegetal poles and the other, inactive gradient runs from the vegetal pole towards the animal pole of an ovum. On the fundamental basis of the gradient theory many of the problems related to cleavage, gastrulation and morphogenesis have now been satisfactorily explained.

10. Organizer Theory: In the beginning of the 20th century, **Hans Spemann**, on the basis of his extensive and painstaking experiments on amphibian embryos proved that one embryonic tissue stimulates or induces another adjacent tissue to develop into a specific structure. The tissue which induces; is referred to as the **inductor** or **organizer** and the morphogenetic effect exerted by it is called **induction**. According to Spemann, induction is the basic fundamental mechanism in embryonic development. For this path finding discovery Spemann was awarded the Nobel Prize in 1935.

In short this is the history of developmental biology. The rapid progress of this science is considered mainly due to the availability different types of highly sophisticated microtomes and microscopes and the development of highly precise techniques for the study of embryos.

Types of Embryology

Modern embryology or developmental biology can be described as a scientific crossroad where the various branches of science like physics, chemistry, zoology, botany, microbiology, genetics, physiology, mathematics, etc. converge and join. In other words, for a proper understanding of developmental biology all the sciences have been utilized and shall continue to be utilized in future also. With the increase in scientific knowledge, embryology has also accordingly progressed and as a result from an academic point of view it has also diversified into many branches. Some of the important branches of embryology are:

1. Descriptive Embryology: From the above described early history of embryology it can easily be appreciated that descriptive embryology which includes the study or description of the individual development of various animal forms began with the commencement of scientific knowledge itself. For example, the famous Greek philosopher Aristotle gave a description of the development of hen's egg in 340 B.C. However, the factual history of embryology was obtained only after the establishment of the cell-theory and several scientists from the 17th and 18th century provided the description of embryology of various animals, especially insects and vertebrates. An excellent summation of the available studies on the embryology of various animals was provided in 1828 by **Karl Ernst Von Baer** in his book **Veber Entwicklungs geschichte der tiere, Beobachtung und Reflexion**. On the basis of studies made by other authors and his own extensive investigations Baer also presented a theory which became famous as **Baer's law**. According to Baer's law-during the embryonic life of an animal the more general features which are universally present in all members of the animal group to which it belongs develop first while later on those characteristics make their appearance which are specific to the animal-type itself. For example, in the development of a bird the more common features of general vertebrates like brain, spinal cord, segmented muscle bundles, endoskeleton, etc. appear first and later on the specific characteristics of birds like feathers, wings, beak, etc. develop.

At the time when Baer's law was presented the concept of organic evolution had little acceptance. However, when after sometime organic evolution received its due recognition then Baer's law was also redescribed on the basis of the concept of organic evolution and it came to be known as the **biogenetic law of Muller-Haeckel**. It was **Muller** who in 1864 redefined Baer's law on the basis of his extensive observations on crustacean development and larvae and **Haeckel** in 1868 first established the modified concept. According to the biogenetic law **ontogeny repeats phylogeny**. Thus during embryonic development those general features appear first which had originated first during the course of organic evolution and later the species characteristics appear which have a more recent evolutionary origin.

2. Comparative Embryology: This branch of embryology is closely related to descriptive embryology because in comparative embryology besides the basic study of embryology of individual animals an attempt is also made to compare the embryology of various animals with a view to gain a better understanding of developmental biology as a biological phenomenon.

After the establishment of Baer's law and of the Recapitulation Hypothesis, the embryonic development of a large number of representative types from various groups of the Animal Kingdom was extensively studied in great detail. This type of comparative study of animal embryology was made by numerous authors of the 19th and 20th century. These fundamental studies are considered to be highly significant contributions in embryology.

3. Experimental Embryology: Although descriptive and comparative embryologies are complete in themselves, but as a consequence of these fundamental studies many points were also raised which could not be explained on the basis of these studies alone. The answer to these problems was possible only through experimental analysis. Thus, the third branch of embryology, namely, experimental embryology evolved naturally on its own.

Wilhelm Roux (1850-1924) was the first author who initiated experiments on the developing embryo. One of the experiments he performed on the egg of frog (1888) was to understand whether each blastomere of a cleaving zygote has the capacity to develop into an entire fully-formed animal or it is capable of forming only those body parts for which it is pre-determined. Since the earlier experiments of **Roux** were grossly crude, they largely met with expected failure. However, similar but more sophisticated experiments under controlled conditions by later scientists exhibited that if the two blastomeres of a fertilized egg are completely separated then both the blastomeres are capable of independently developing into normal embryos by the process of cleavage. This remarkable success was first described by **H. Driesch** (1891) in sea urchin eggs and later on by **Endres** (1895) and **Spemann** (1901, 1903) with the eggs of the newt (urodelan amphibian) and much later by **Schmidt** (1931) with the eggs of frog. These early experimentations were the beginning of experimental embryology. Understandably experimental embryology has now become an inseparable and essential branch of developmental biology since without experimental proof no concept can be established.

4. Chemical Embryology: To gain a better understanding of developmental biology, along with experimental embryology the importance of biochemical and physiological aspects of embryogenesis is increasingly appreciated, and therefore, chemical embryology has also become an important branch of modern embryology. This speical branch is also referred to as **physiological embryology**. The major pioneer contributors to chemical embryology are **Needham** (1931), **Brachet** (1940), **Lehmann** (1945), etc.

5. Analytical Embryology: Modern developmental biology is also referred to as **analytical embryology** in which developmental biologists review or critically analyse the various developmental phenomena either singly or jointly including aspects of descriptive, comparative, experimental embryology, etc. with a view to understand the complex knowledge of the fundamental principles of embryology. Some of the more recent wellknown analytical embryologists include **Berill, Balinsky, Witschi, Nalbandov,** etc.

Scope of Developmental Biology

1. The study of developmental biology allows us to understand better the fundamental aspects of the various branches of biological sciences. Developmental biology has especially made it easier to appreciate the available knowledge in genetics, cell-biology, physiology, evolution etc.

2. Developmental biology has assisted in establishing phylogenetic relationship between the various animal groups.

3. Developmental biology has also helped us in knowing the origin of many congenital and pathogenic diseases. In fact, medical science is considered to rest mainly on the knowledge gained through studies or developmental biology.

4. Human welfare including family-welfare, fertility, improvement of breeds of domestic and farm animals, etc. is directly related to studies on developmental biology.

5. A decisive role of developmental biology is acknowledged in the control of pests and vectors of diseases.

To sum, developmental biology, is not only of academic interest but also has great applied value.

QUESTIONS

1. Define:

1. Blastogenesis
2. Embryogenesis
3. Gametogenesis
4. Morphogenesis
5. Organogenesis
6. Epigenesis
7. Oogenesis
8. Spermatogenesis
9. Amphimixis
10. Karyogamy
11. Plasmogamy
12. Cytogamy
13. Biogenetic Law
14. Baer's Law.

2

Gametogenesis : Structure and differentiation of gametes

Gametogenesis

We are already aware that by **reduction-division** or meiosis the number of chromosomes in the daughter cells reduces to half, the cells become haploid *(n)* from diploid *(2n)*. Meiosis takes place in the **germinal cells** which are present in the **gonads** and all the haploid cells which are so-formed are called as **gametes**. This special process of cell-division is, therefore, called **gametogenesis**. The male gametes which are formed in the testes of male animals are known as **spermatozoa** and the gametogenic process is called **spermatogenesis**. Similarly, the female gametes formed in the ovaries of female animals are called ova and the process of gamete formation is thus called **oogenesis**. The gametes have a special and essential— importance in sexual reproduction and production of new generation because in the process of **fertilization**, the male and female gametes unite to form a diploid zygote and it is this zygote which undergoes embryonic development to form a new animal which possesses all the heritable characteristics of both the male and female parents.

The male gamete or **sperm** and the female gamete or **ovum** radically differ from each other both in form and structure but the formative stages of both the types are more or less similar. Therefore, both spermatogenesis and oogenesis are studied by dividing them into the following three stages :

1. Multiplication phase,
2. Growth phase,
3. Maturation phase.

In the following description of spermatogenesis and oogenesis both the processes have been described after dividing them into the above referred three phases (Fig. 2.1).

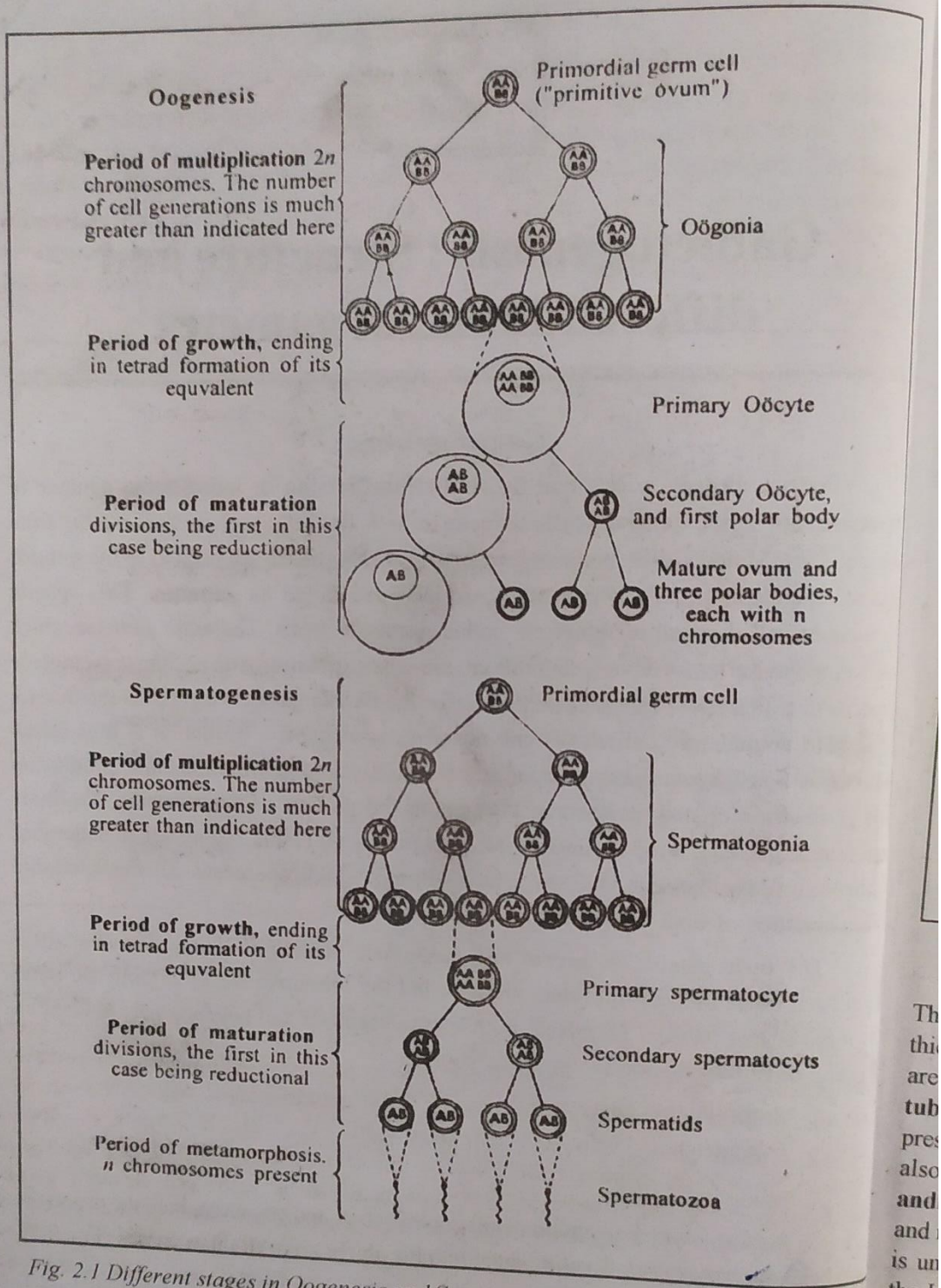

Fig. 2.1 Different stages in Oogenesis and Spermatogenesis of a typical gametogonium

Spermatogenesis: Formation of Sperm

Before discussing the process of spermatogenesis, a description of the structu of the testis and the spermatozoon would prove to be helpful.

Structure of Testis

The primary male reproductive organs of animals are a pair of testes in which spermatozoa are formed by the process of **spermatogenesis**. The testes are located either in the abdominal cavity itself or as in most mammals are present outside the abdominal cavity in sac-like structures called the **scrotal sacs** or **scrotum**, which are located just below the anal aperture. It is generally agreed that at least in mammals since for spermatogenesis and formation of spermatozoa relatively lower temperatures than the abdomen are required, they are present in the relatively cooler extra-abdominal scrotum.

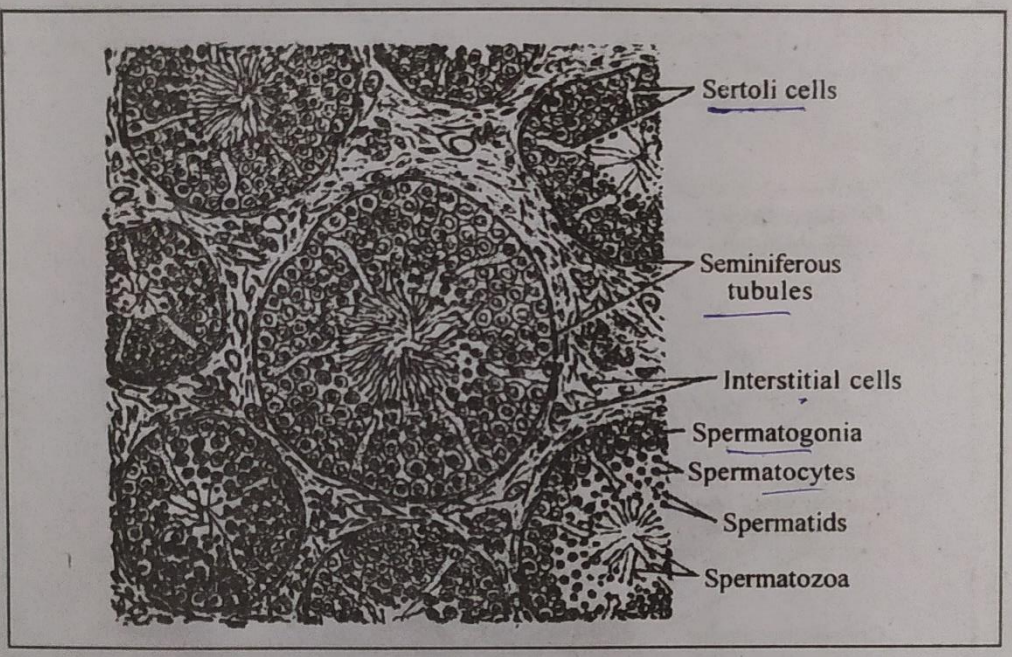

Fig. 2.2 Mammal : Cross section of testis (partial view)

The testis is covered by two layers- outer peritoneum and inner tunica albuginea. The peritoneum is a thin single cell-layer of the lining coelomic epithelium. The relatively thick and hard tunica albuginea is made of connective tissue. Below the testicular lining are present embedded in interstitial connective tissue coiled **seminiferous** or **germinal tubules**. In the interestitial tissue, besides blood capillaries and nerve fibres are also present special type of cells, the **interstitial cells** (Fig. 2.2). The interstitial cells are also referred to as **Leydig** cells. The Leydig cells secrete the **male hormones** or **androgens** the **testosterone**, etc. Spermatogenesis in the testis and the development and maintenance of the accessory reproductive organs and secondary sexual characters is under the regulatory control of the androgens. In turn, the functional regulation of the Leydig cells, that is, formation of androgens is under the control of the Interstitial Cell Stimulating Hormone (ICSH) Luteinizing Hormone (LH) synthesized by the anterior lobe of the pituitary gland.

The seminiferous tubule is lined by a thin covering, the **basement membrane** or the **tunica propria**. Below the tunica propria rests a single celled layer, the **germinal**

epithelium. The germinal layer is mainly constituted by nearly round cells, the **spermatogonia**. During spermatogenesis, each spermatogonium as a result of division and maturation successively gives rise to **spermatocytes, spermatids** and finally **spermatozoa**. Some cells inter-spaced between the spermatogonia are of a relatively large size. These are the **sertoli cells** (Fig. 2.3). During the spermatogenic process, all the successive developmental stages of the germ cells including the spermatozoa are embedded in the Sertoli cells for the likely purpose of nutrition. Spermatogenesis in the seminiferous tubules is under the regulatory control of the Follicle Stimulating Hormone (FSH) secreted by the anterior lobe of the pituitary gland.

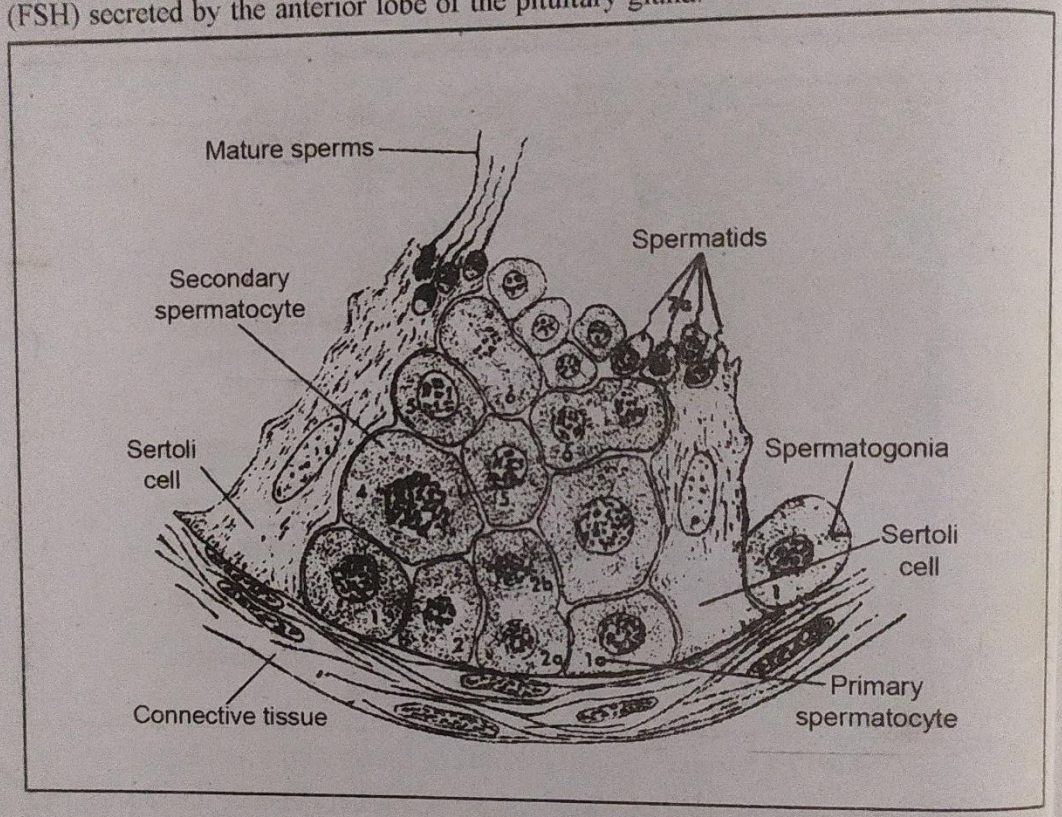

Fig. 2.3 Portion of seminiferous tubule.

Structure of a Spermatozoon

The structure of a spermatozoon is ideally suited to its function. The function of a spermatozoon is to reach up to the ovum, fuse with it, initiate early embryonic development in the egg and transfer paternal genes to the developing ovum. To accomplish its function the spermatozoon must be highly motile. In reality, the structure of a typical spermatozoon is comparable to an efficient, active and highly motile body. Sperms were first discovered by Leeuwenhoek but the term sperm was coined by Von-Bear. A typical spermatozoon is divisible into 3 regions: head, middle piece and tail or **flagellum**.

Head : The head representing the nucleus, is ovoid, although its shape and size varies from species to species (Fig. 2.5), and compressed from side to side and measures about one twelfth of the entire spermatozoa. The anteriormost part of the

head is occupied by the structure called **acrosome**. The function of acrosome is to penetrate the egg membranes and thus allow the sperm to establish contact with the egg cytoplasm during fertilization because of the presence of sperm lysins. The acrosome is formed by Golgi body and it is bound by a unit membrane; acrosome of various sperms are in Fig. 2.6. The larger part of the remainder of the head is occupied by the nucleus which has halpoid set of chromosomes.

Since the nucleus contains genes, it is responsible for transfer of heritable paternal characters to the developing egg. In the posterior region of the head, the **centrioles** are present. The first centriole is arranged at right angles to the longitudinal axis of the sperm. It is called the proximal **centriole** and it is essential for the induction of cell division in the fertilized egg. Immediately behind, the second or distal centriole is located. From the **distal centriole** arises the axial filament of the tail and thus it forms the **basal granule** of the axial filament (Fig. 2.4).

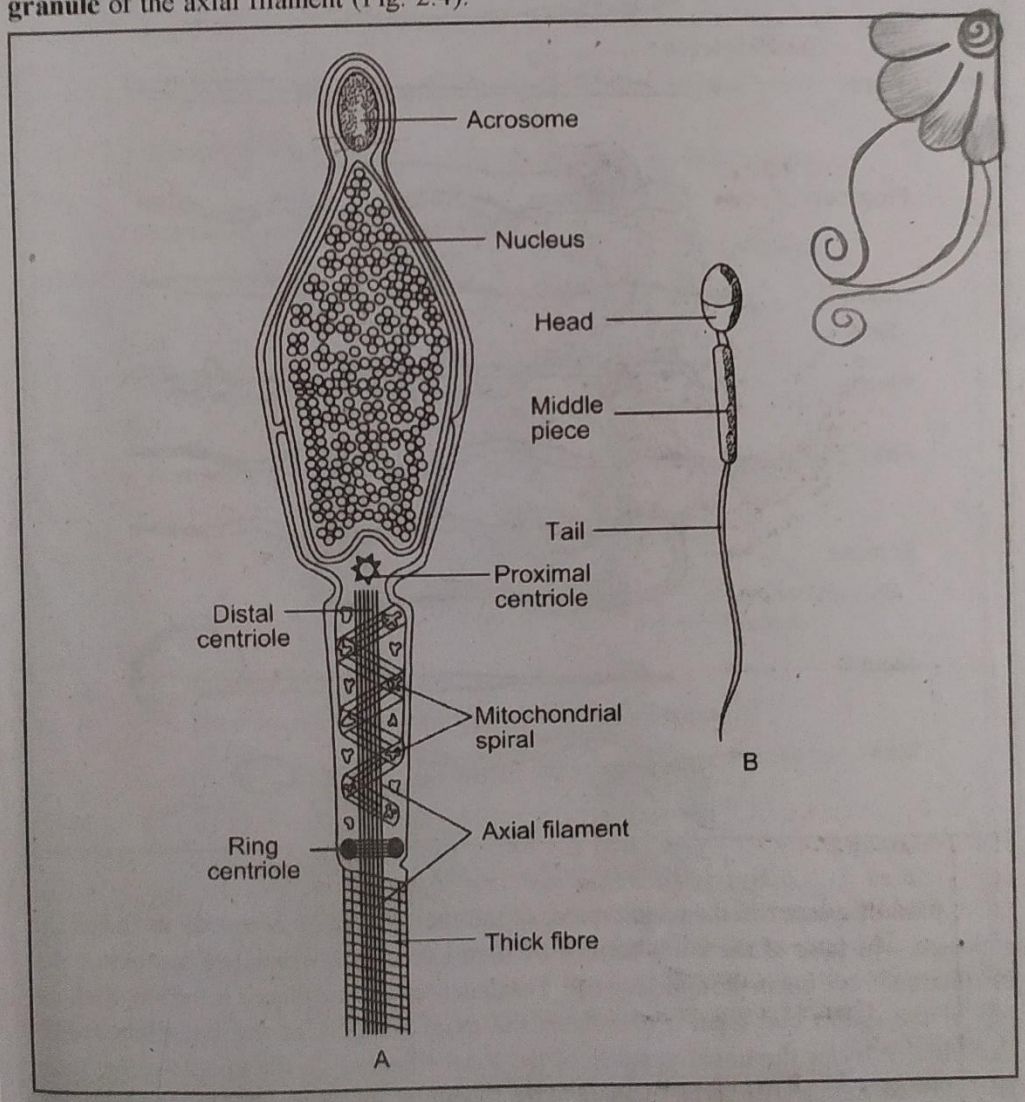

Fig. 2.4 Mummalian sperm : A. Under clectran microscope
B. Sperm under light microscope : Flat surface view

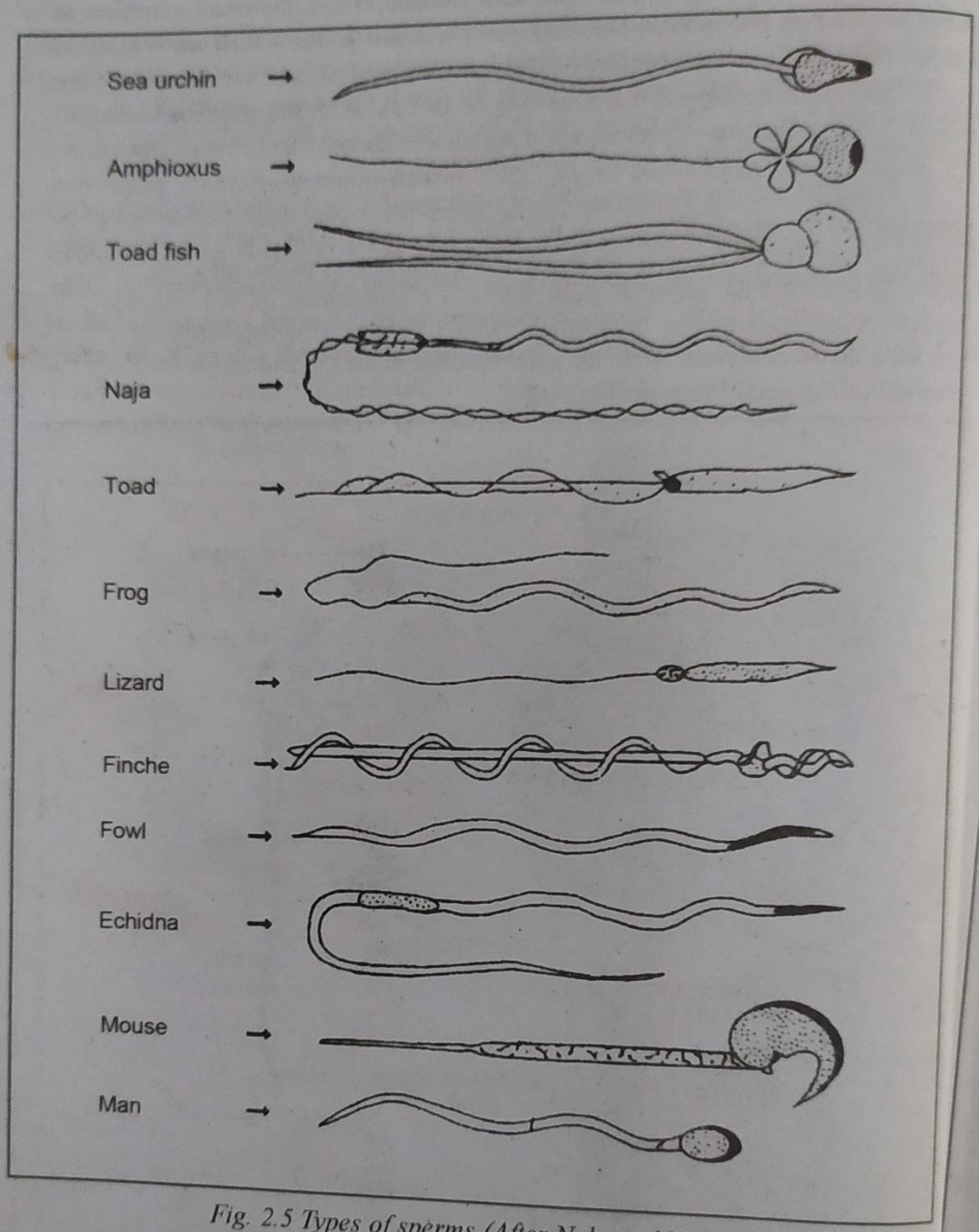

Fig. 2.5 Types of sperms (After Nelson, 1953)

Middle-piece : In the middle-piece of the sperm the base of the tail or flagellum is located. The base of the tail is formed by **distal centriole** which lies just below the proximal centriole but in the middle-piece. The distal centriole is placed in the longitudinal axis of the sperm and from it originates the axial filament of the flagellum (tail). Therefore, it forms the **basal granule** of the axial filament. In the middle-piece, both the distal centriole and the axial filament of the flagellum are surrounded by mitochondria which are fused together. In mammals, the mitochondria fuse together to form a

helical coil around the axial filament. In other animal groups, the fused mitochondria may be present in the middle-piece as one or **more lobes** which are known as **mitochondrial bodies** or **nebenkern**. The mitochondria christae are completely lost in the nebenkern. The nebenkern is closely associated with the axonemal complex, suggesting their role in energy transfer and mobility of sperm. The mitochondria contain oxidative enzymes and enzymes for oxidative phosphorylation which are responsible for providing the necessary energy to the highly motile active flagellum or tail of the spermatozoon. The cytoplasm surrounds the middle piece as thin layer and is known as **manchette**. Manchette also surrounds the posterior part of the head of spermatozoon. In the spermatozoa of some animals, a dark ring is present at the posterior end of the middle-piece. It is sometimes referred to as the **ring centriole** and it forms the boundary between the middle-piece and the tail. Electron microscopic studies have shown that in structure the ring does not resemble the centriole. The function of this body is also not known.

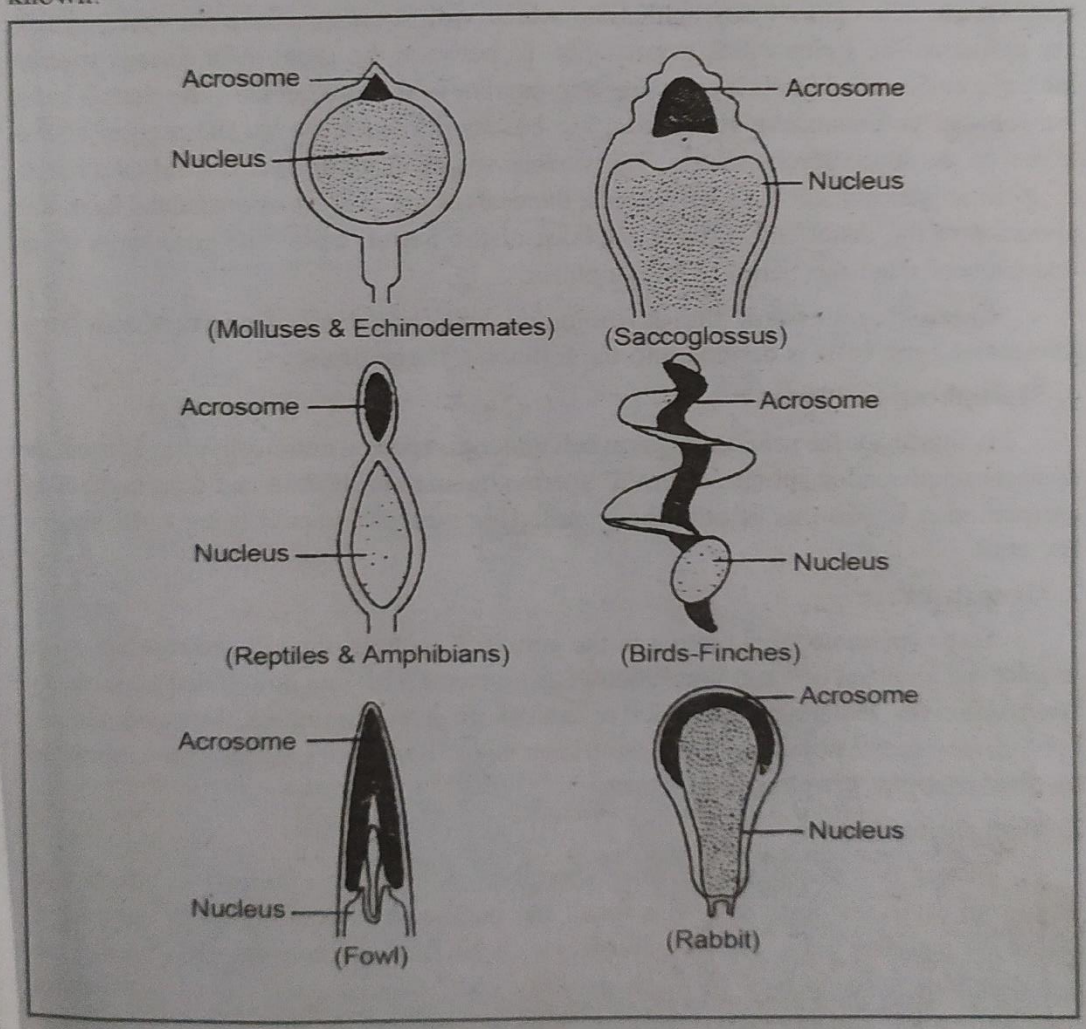

Fig. 2.6. Acrosome of various sperms (Berrill-1971)

Tail : Tail or flagellum is the longest part of the spermatozoon. With the help of the tail the spermatozoon actively swims about. The most important part of the tail is the **axial filament** surrounded by axial sheath. The end piece *i.e.* the terminal part contains only axial filament—the axial shealth is absent (human beings). The microscopic structure of the axial filament is similar to the cilium and flagellum of other forms. The entire spermatozoon is covered by an outer cytoplasmic layer or **plasmalemma**.

Spermatogenesis

The formation of spermatozoa or **spermatogenesis** takes place in the **germinal** or **seminiferous tubules** of the **male gonad** or testis. Generally, spermatogenesis is a continuous process, and therefore, the various formative step by step stages of spermatogenesis can be examined at a given time in the seminiferous tubules of an adult testis.

The **basement membrane** of the seminiferous tubules is lined by the **germinal epithelium**. The cells of this epithelium which will ultimately form the spermatozoa are called as the **primordial germ cells**. In between the germ cells certain special nutritive cells called **Sertoli cells** are also present in this epithelium. The Sertoli cells are tall and columnar extending from the basement membrane till the margin of the lumen of the seminiferous tubule. The various stages of germ cells formed as a result of spermatogenesis and the spermatozoa themselves are present as embedded from the proximal to the distal end in the cytoplasm of the Sertoli cells. The germ cells draw nourishment from the Sertoli cell cytoplasm.

Spermatogenesis or the development and formation of spermatozoa from primordial germ cells is divided into the following **three phases** :

1. Multiplication Phase:

In this phase the primordial germ cell undergo repeated mitotic division to produce numerous cells called **spermatogonia**. Spermatogonia are **diploid** and their number of chromosomes is like that of other body cells (for example: human being = 46; frog = 26, etc.)

2. Growth Phase:

Some spermatogonia remain in the germinal epithelium while the rest move-up. acquire material and become almost double in diameter. They are now called as **primary spermatocytes**. In the spermatogonial cells although growth is limited, their modification into primary spermatocytes is very significant since these germinal cells now represent the first phase or **prophase** of meiosis.

3. Maturation Phase:

During this phase, the primary spermatocytes undergo meiosis in which two sequential divisions occur and as a result the number of chromosomes is reduced to half in the daughter cells. The first division is called **first maturation division** and the two daughter cells formed by each primary spermatocytes are called **secondary spermatocytes**. The secondary spermatocytes immediately divide again. This division is known as the **second maturation division** and the daughter cells which are s

produced are referred to as **spermatids**. Spermatids are **haploid**. Therefore, at the end of meiosis every diploid primary spermatocyte **forms four haploid spermatids** (Fig. 2.7).

The conversion of germinal cells from diploid to haploid by meiosis is very significant and necessary since this is the only available method by which the number of chromosomes is stopped from continuously doubling and increasing. Further, during meiosis exchange of genetic material takes place as a result of which the heritable characters of both the parents are transferred in an equitable manner to the offspring.

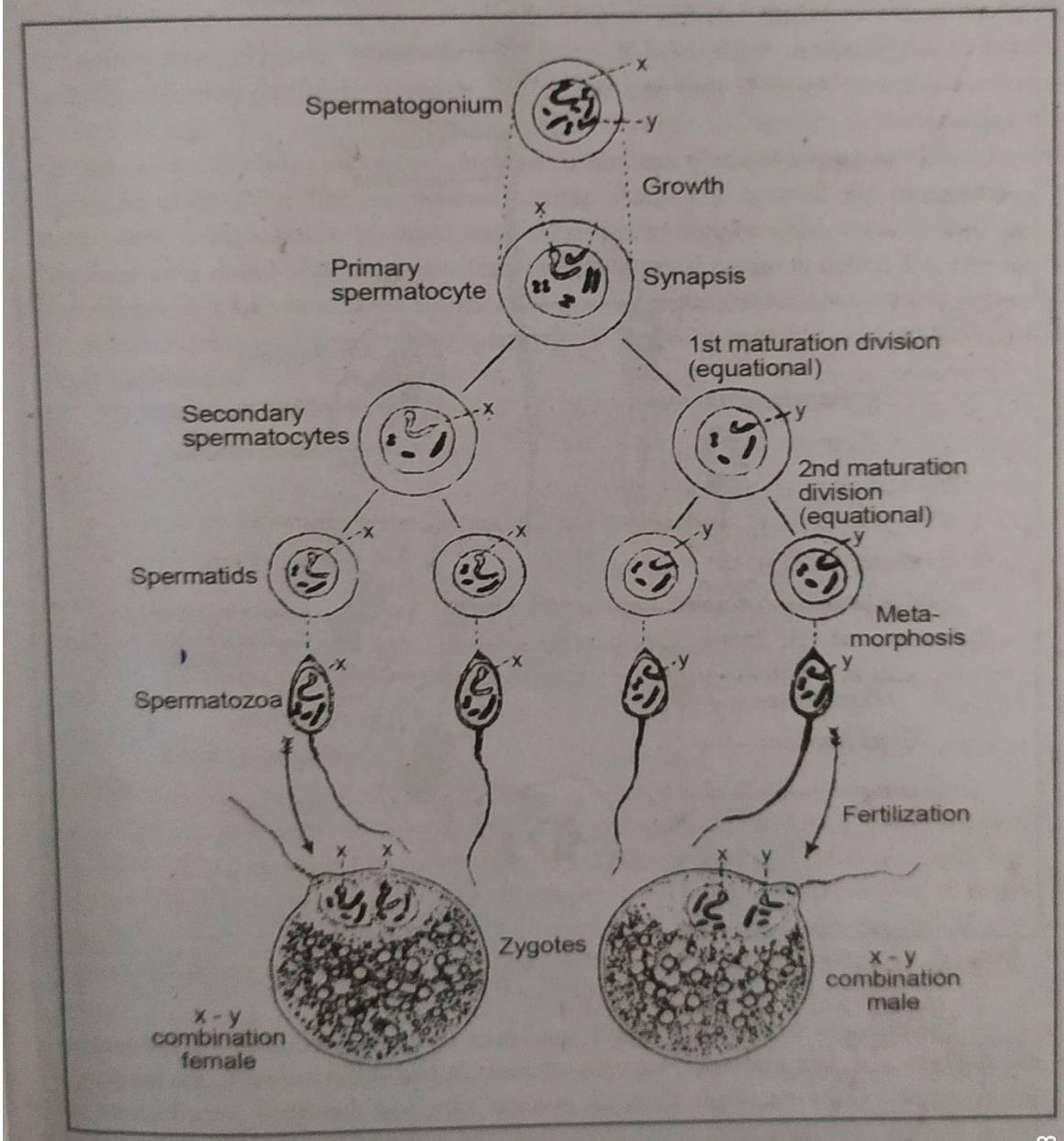

Fig. 2.7 Diagram showing the separation of the members of the sex chromosome pair in maturation and their recombinations in fertilization.

Spermiogenesis: Differentiation of Spermatozoa

Although in spermatids the number of chromosomes is reduced to half, they do not have the capacity to act as viable male gametes. Therefore, the spermatids undergo differentiation to form viable male gametes or spermatozoa. The process of differentiation of spermatids into spermatozoa is called **spermiogenesis** or **spermateliosis** (Fig. 2.8).

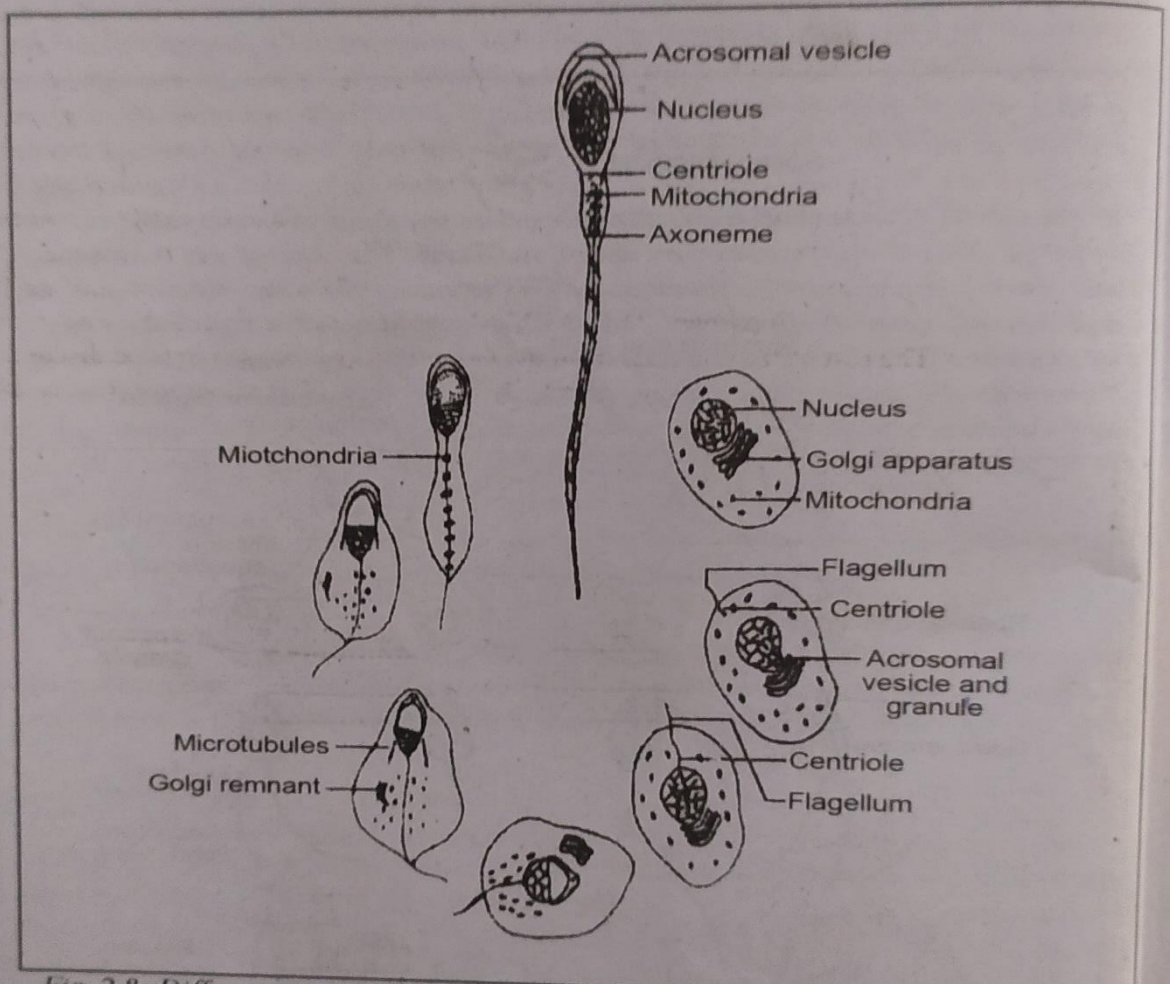

Fig. 2.8. Different stages in the spermiogenesis of a mammalian sperm (Redrawn from Gillbert 1997)

The changes which a spermatid undergoes in the formation of a spermatozoon are fundamental, and therefore, very significant. In this differentiation, the nucleus of the spermatid loses its water from its nuclear sap, and therefore, shrinks with the result that the chromosomes come close together and arrange themselves to occupy lesser space. This is essential to reduce the weight of the motile spermatozoon. Not only this, all the unwanted material from the nucleus is also discarded, for example

RNA etc. The nucleus ultimately contains only DNA, that is, the genetic material. The shape of the nucleus also changes. It is streamlined from round to long and narrow in shape for easy swimming. The shape of the head of the spermatozoon varies in different animals and depends upon the shape of the nucleus. For example, it is oval and laterally flat in man and bull, scythe-like in rats and frog, spiral in birds, etc.

The **acrosome** of sperm is formed by differentiation of the Golgi bodies. Golgi body of the spermatid is made up of numerous membrane-bound vesicles. During differentiation one (or more) of the vesicles commences to increase in diameter. Simultaneously, in the cavity of this acrosomal vesicle, a minute dense body, the **proacrosomal granule**, makes its apparance. If more than one vesicle and granules are formed then they fuse together and at the end only a single granule-containing vesicle is left. The granule-containing vesicle attaches to the apex of the nucleus. The proacrosomal granule continues to increase in size and now it is known as the **acrosomal granule** (Fig. 2.9). The acrosomal granule forms the core of the acrosome. The acrosomal vesicle loses its fluid and its membrane covers the anterior half of the nucleus as a double-layered cap. This double-layered cover is called the **cap** of the spermatozoa. The rest of the material from the Golgi body of the spermatid is discarded. Acrosome contains important enzymes which at the time of fertilization dissolve the egg membranes.

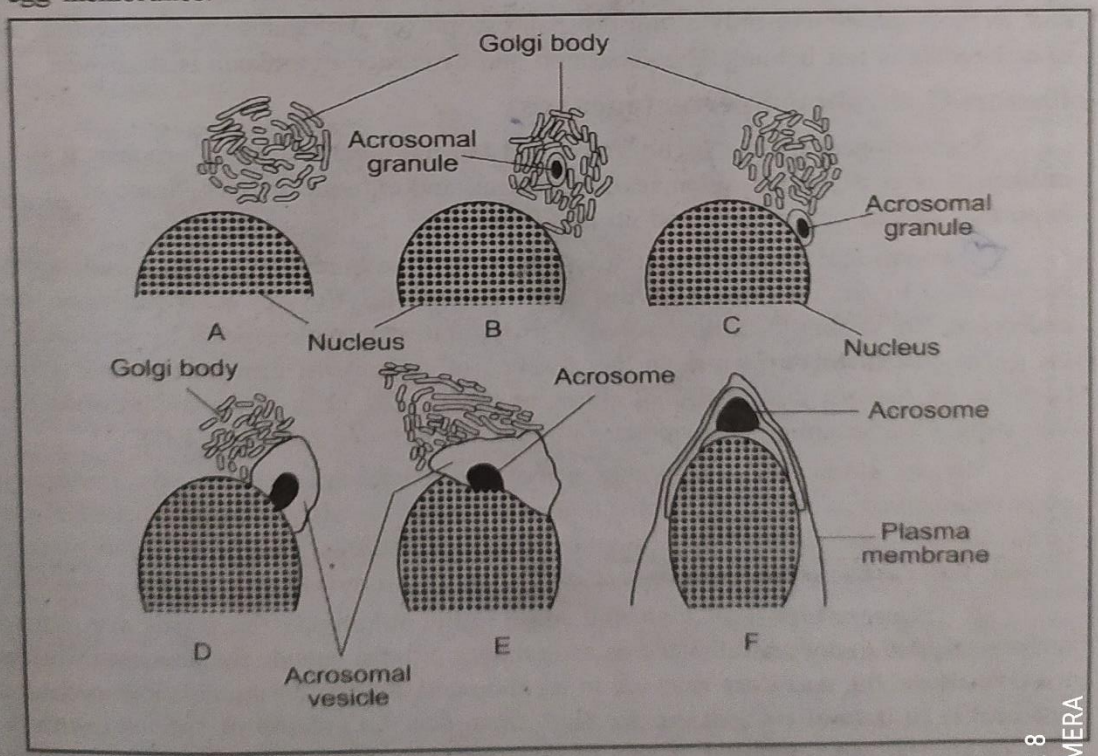

Fig. 2.9. Sequential development of acrosome and cap of sperm during spermitogenesis (A to F).

The **centrosome** of the spermatid is made up of two tubular centrioles which are arranged at right angles to each other. At the mid-posterior surface of the nucleus a groove is formed in which one centriole is arranged at right angles to the longitudinal axis of the sperm. It is called as the **proximal centriole**; the other centriole, that is, the **distal centriole** is arranged just below the proximal centriole but in longitudinal axis of the sperm. The distal centriole, which is located in the middle-piece of the sperm, forms the **axial filament** of the flagellum (tail) of the sperm, that is, it is the basal granule itself of the axial filament. The axial filament is the major part of the flagellum or tail of the sperm. In its simple form, the tail is made up of only the axial filament lined by a thin layer of cytoplasm and outer plasmalemma, but in its complex form the tail of sperm also contains additional fibres (for example, mammals).

A major part of the cytoplasm of the spermatid is useless to the spermatozoon, and therefore, it is discarded. At times, when the acrosome is differentiating above the nucleus, the cytoplasm of the spermatid flows out in the opposite direction as a result of which over the acrosome and nucleus only a very thin layer of plasmalemma is left behind. At the same time when the tail is differentiated from the middle-piece the remainder of cytoplasm of the spermatid is incorporated into the middle-piece. After this, the mitochondria arrange around the basal region of the axial filament, the rest of the cytoplasm along with the **"golgi rest"** is separated from the spermatozoa. In the end, in the middle-piece only a thin layer of cytoplasm, the manchette surrounding the mitochondria is left behind. The separated and discarded cytoplasm is destroyed.

Factors Controlling Spermatogenesis

Spermatogenesis is a highly important but extremely complex process. It is influenced or is dependent upon several internal and external factors. Some of important factors can be summed up as following:

1. Hormonal Dependence: It is known that the male hormones or **androgens** are secreted by the interstitial Leydig cells of the testis. Besides other functions, the androgens (for example, testosterone) also regulate spermatogenesis by influencing the **germ-cell differentiation**. In the absence of androgens, spermatogenesis is not possible. In its turn the functional status of the Leydig cells is dependent upon the Interstitial Cell Stimulating Hormone = Luteinizing Hormone [ICSH = LH].

Various studies have also shown that for normal spermatogenesis, hormones other than androgens and ICSH, some other substances are also required. These include prolactin and follicle stimulating hormone (FSH) from the anterior pituitary, epinephrine, insulin, fatty acids, protein, glucose, etc.

2. Temperature : In man and many other mammals the testes are extra-abdominal, that is, they are located in scrotal sacs present outside the abdomen. If due to some cause, the testes are retained in an abdomen, that is, during development they are unable to normally reach the scrotum, then also the process of spermatogenesis and the consequent formation of sperms does not take place. Researches have shown that on the average the temperature of the scrotum is about 4°C less than the abdomen and that this lower temperature is suitable for normal spermatogenesis. This fact is

also been experimentally demonstrated. However, in contrast in some mammals like the elephant, whale, seal, etc., which lack scrotal sacs, the testis remain abdominal throughout life but exhibit normal spermatogenesis. Similarly, in the permanently abdominal testes of birds and lower forms normal spermatogenesis takes place. Thus, the effect of temperature on spermatogenesis of animals without scrotal sacs is still not clear. The sperms in Sperm Bank are stored at -179°C.

3. Nutrition : It is a well-known fact that malnutrition adversely affects the reproductive system of both the sexes. Lack of vitamins, minerals (Zn) and certain other nutritive substances causes cessation of spermatogenesis in the testis. This adverse effect on spermatogenesis is considered to be possibly due to the malfunctioning of the Leydig cells and the consequent stoppage of androgen synthesis. For example, lack of vitamin B adversely affects the functional status of the testis with cessation of spermatogenesis.

Similarly, absence of the required amount of protein and essential amino-acids in food also has a deleterious effect on gonadal function. For example, feeding young rats with protein-free diet causes retardation of sexual maturity of the gonads. Similarly, absence of casein in the diet of rats also adversely affects the spermatogenetic process. There are some disorders of defective spermatogenesis. A condition when the sperms are nonmotile is known as **necrospermia. Azospermia** is a condition when the sperms are totally absent in the semen. But if the sperms are less in number than the normal number the condition is called as **oligospermia**.

Types of Spermatozoa :

The above described structure is of a typical spermatozoon. However, different animals exhibit different structural types of spermatozoa. Broadly, they can be divided into two major types:

Flagellated Spermatozoa and *Non-flagellated Spermatozoa.*

1. Flagellate Spermatozoa: In this type, the spermatozoon possesses one or two *tails* or *flagella.* Typically a flagellated spermatozoon bears only a single tail or flagellum and such types are called *monoflagellated spermatozoa* (for example, vertebrate spermatozoa). Exceptionally in some forms like the **toad fish**, the spermatozoa may have two tails or flagella and these are then called *biflagellated spermatozoa.* Under abnormal conditions, sometimes biflagellated spermatozoa are produced by animals which normally form monoflagellated spermatozoa (Fig. 2.5).

In some animals the heads of two or more spermatozoa clump together. Such spermatozoa are referred to as *conjugate spermatozoa.* For example, the conjugate spermatozoa of grasshopper form a 'sperm-boat'. However, all varieties of conjugate spermatozoa separate from one another before fertilization in the female reproductive tract.

2. Non-flagellate spermatozoa : In this type of spermatozoa, the tail or flagellum is absent and they appear like ordinary body cells. Such type are commonly seen in nematodes (for example, amoeboid spermatozoa of *Ascaris)* and some crustaceans (Fig. 2.10).

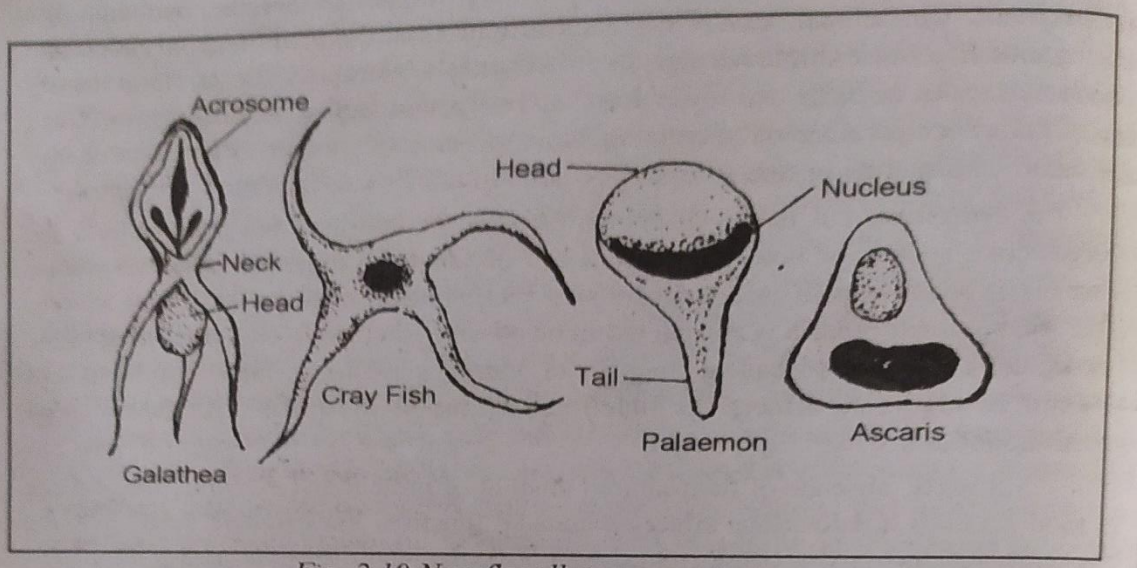

Fig. 2.10 Non-flagellate spermatozoa

Oogenesis: Formation of Egg

The process of oogenesis occurs in the ovaries of the female animal. Like the testis, the ovary is also lined by the **germinal epithelium**. The cells of the germinal epithelium are referred to as **primordial germ cells** which by division and maturation under the process of oogenesis develop into ova or eggs. Before gaining an understanding of oogenesis, for orientation, the study of typical ovary and ovum would be useful.

Structure of Ovary

Typically a pair of ovaries is present in the abdominal cavity of vertebrates, excepting in a majority of avian species where only a single ovary is present. The mammalian ovary is generally a small whitish oval structure. Each **ovary** is lined by a double-layered ovarian **capsule made up of the outer peritoneum** which is thin single cell-thick epithelial layer of flat cells and inner **tunica albuginea** which also is a thin layer made up of connective tissue. Below the capsule is present the **germinal epithelium** composed of cuboidal cells. The rest of the ovary is filled with connective tissue. The germinal cells and the connective tissue of the ovary is jointly referred to as the stroma (Fig. 2.11). The connective tissue of the ovary besides containing blood capillaries, nerve-fibres, lymph capillaries also contains numerous ovarian follicles at various stages of development and maturity. The ovarian follicle is formed by the **germinal epithelial cells**. In the beginning, each **ovarian follicle** arises as a small group of small germinal cells or **oogonia** which subsequently separates from the germinal epithelium and begins to sink into the ovarian stroma. One of the cells of this bunch of cells begins to increase in size and is now designated as the **developing oocyte**. The remainder of the small cells surround the oocyte to form a covering around it. These cells are now called follicular cells and the entire bunch of cells is referred to as the primary follicle. The follicular cells provide nourishment to the developing oocyte.

The follicular cells of the primary follicle undergo rapid celldivisions and soon a multilayered cell-envelope encloses the developing oocyte. This multilayered covering is called the **membrana granulosa**. In the oocyte of follicle besides growth, the process of **oogenesis** also commences and as a consequence it begins to develop into an ovum. The process of oogenesis has been described in detail under a separate heading later in the chapter. As a result of cell-divisions and consequent increase in the number of follicular cells, the follicle itself also continues to increase in diameter. Gradually a fluid-filled space begins to form in between the oocyte and the membrana granulosa. The space is called the **follicular space** and the contained fluid is referred to as the **follicular fluid**. In the maturing follicle, several follicular cells form a lining around the developing oocyte and at a point these cells form a connecting bridge-like structure between the oocyte and the rest of the covering follicular cells. Thus at this stage the developing oocyte appears as hanging in the follicular fluid by a solid stalk made up of follicle-cells. This solid cellular stalk is referred to as the **discus proligerus** and the layer of follicular cells enclosing the oocyte is called the **corona radiata**. Just below the corona radiata the developing egg is lined by a thin transparent membrane, the **zona pellucida**. At this stage the follicle reaches its peak of growth and is now referred to as a mature follicle. The mature follicle is called the Graafian follicle.

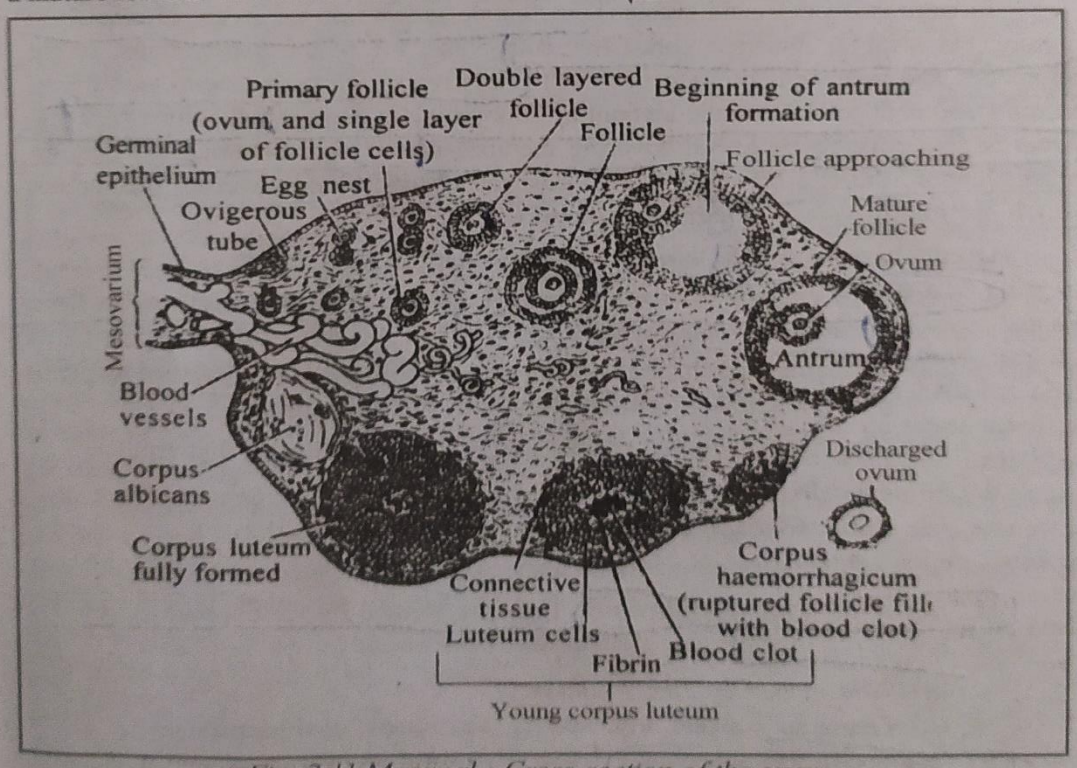

Fig. 2.11 Mammal : Cross-section of the ovary

Formation and maturation of the Graafian follicle and the process of oogenesis is regulated by the Follicle Stimulating Hormone (FSH) from the anterior pituitary gland.

The follicular cells of the maturing follicles secrete the female hormones or estrogens (for example, estradiol). The estrogens cause the development and maintenance of the female accessory reproductive structures and secondary sexual characters.

The mature Graafian follicles reach the surface of the ovary and then burst and release the contained egg or **ovum** into the abdominal cavity. The process of release of ovum from the Graafian follicle is called **ovulation**. The empty Graafian follicle does not degenerate but immediately modifies into new but temporary endocrine structure, the corpus luteum. After the release of the ovum, the broken area of the mature follicle closes and in the cavity of the follicle the blood from the surrounding blood capillaries collects to form a clot. It is around this clot nearly all the cells of the membrana granulosa convert into the hormone-secreting lutein cells. Lutein cells which are relatively larger in diameter and round in shape secrete another female hormone, progesterone. Progesterone is responsible for maintenance of the functional relationship between the developing embryo and the mother, that is, it is responsible for maintenance of pregnancy. The process of ovulation and formation of corpus luteium is regulated mainly by Luteinizing Hormone (LH) secreted by the anterior lobe of the pituitary gland.

Structure of an Ovum

In contrast to the male gamete, the female gamete or the ovum of animals is nothing but a larger nutritive yolk-filled cell. Thus, the ovum also contains all the organelles and inclusions of an animal cell. The size of the ovum varies in different animals and is dependent on the amount of yolk which is present. Like other cells, the ovum is also covered by a thin covering membrane, the plasmalemma. Besides, the plasmalemma, the egg or ovum is also covered by additional covering membranes which are jointly known as **egg-membranes**.

On the basis of origin, the egg membranes are of three types:

I. **Primary egg membranes:** They are formed by oocyte itself within the ovary. They are found in the form of :
   (a) Vitelline membrane, *e.g.*, insects, echinoderms, molluscs, proto chordates, fishes, amphibians, birds and mammals.
   (b) Chorion or Chorona radiata, *e.g.*, fishes, amphibians and reptiles.
   (c) Zona pellucida, *e.g.*, mammals.
   (d) Zona radiata, *e.g.*, mammals.
   (e) Jelly coat, *e.g.*, echinoderms.

II. **Secondary egg membranes:** They are formed by the ovarian tissues within the ovary. They are found in the form of :
   (a) Jelly coat, *e.g.*, fishes.
   (b) Chitinous capsule, *e.g.* insects, asccidians, cyclostomes.

III. **Tertiary egg membranes:** They are formed by the various regions of oviduct/uterus and are found in the form of :
   (a) Jelly coat, *e.g.*, amphibians.

(b) Chalazae, *e.g.*, birds.

(c) Albumin layer, *e.g.*, Nematodes, birds.

(d) Shell membranes-Inner and outer, *e.g.*, birds.

(e) Shell, *e.g.*, sharks, birds.

Ova are generally round in shape but in some animals they may be elongated and ovoid. In relation to body cells, the size of eggs is generally quite large. However, the size or diameter of eggs is also very variable. For example, the diameter of vertebrate ova varies from 0.06 mm (mouse) to 85 mm (ostrich). The colour of ova is dependent upon the type of pigment granules present in the ova.

Yolk : As stated above, the size of ovum is dependent on the amount of yolk which is present in the cytoplasm. Yolk is an extremely important component of the ovum. Yolk is the stored nutrient of the egg cell and provides nourishment to the cleaving egg and embryo. Chemically, the major constituents of yolk are *proteins* and *fats*, however, small or trace amounts of carbohydrates, mineral salts and vitamins are also present. Yolk granules can be small or large and their shape can vary from round to discoidal. Large yolk granules which are ovoid in shape are generally referred to as *yolk platelets*. During early embryonic development it is the yolk which provides the necessary nourishment to the embryo. The size of ovum, type of cleavage, blastula, gastrulation, and the course of embryonic development itself is greatly influenced by the yolk. The role of yolk in the development process has been described at the appropriate place in the following description.

Polarity of ovum : The various substances and cellular constituents in a developed oocyte exhibit a definite pattern of arrangement and thus the egg shows a **polarity**, that is, an unequal distribution of inclusions at the two opposing poles of the egg as well as in relation to the main axis of the egg; the main axis being the line which connects the two poles. The nucleus of the egg is present near one pole of the egg. This pole is referred to as the **animal pole**. The opposite pole is termed the **vegetal pole**, because the yolk present in this region provides nourishment to the developing embryo (Fig. 2.12). When the oocyte undergoes meiosis, the cell-nucleus moves towards the animal pole and the polar bodies are **always** discharged at the animal pole. The seemingly unequal distribution of inclusions in the oocyte is referred to as *bipole differentiation* or *polarity* of the egg and this imaginary main axis which cuts through the animal and vegetal poles of the egg is called the *polar axis* or the *animalvegetal axis*. This axis is also the axis of symmetry of the oocyte on which the organization of the oocyte is dependent.

The nucleus is present near the animal pole and hence it is considered the active part of the egg. Besides, being the seat of divisional activity, generally the anterior region of the embryo differentiates from this region. The developing oocyte is generally attached to the ovarian follicle by its animal pole. At this pole the egg-cytoplasm is maximally concentrated and the amount of yolk is the least. The metabolic rate at this region is also maximal. In contrast, the vegetal pole is relatively less active. The vegetal pole generally differentiates into the posterior part of the embryo.

In the vegetal pole the yolk is maximally concentrated but the amount of cytoplasm is the least and the rate of metabolism is also the least in this region. It is evident that a *concentration gradient* and a *metabolic gradient* is present between the two poles of the oocyte.

Pigment : The presence of pigment in the mature eggs of many animals is a specific characteristic. For example, amphibian eggs contain dark brown or black pigment material and accordingly the colour of the egg ranges from dark chocolate brown to black. Most of the pigment granules are concentrated near the periphery of the egg cytoplasm but they may also be present in the interior of the egg. One of the characteristic features of the amphibian egg is that in it the pigment is not evenly distributed but is relatively concentrated in the animal hemisphere whereas in the vegetal half it is scarce. Thus the animal half of the egg appears dark brown or black and the vegetal half appears whitish in appearance.

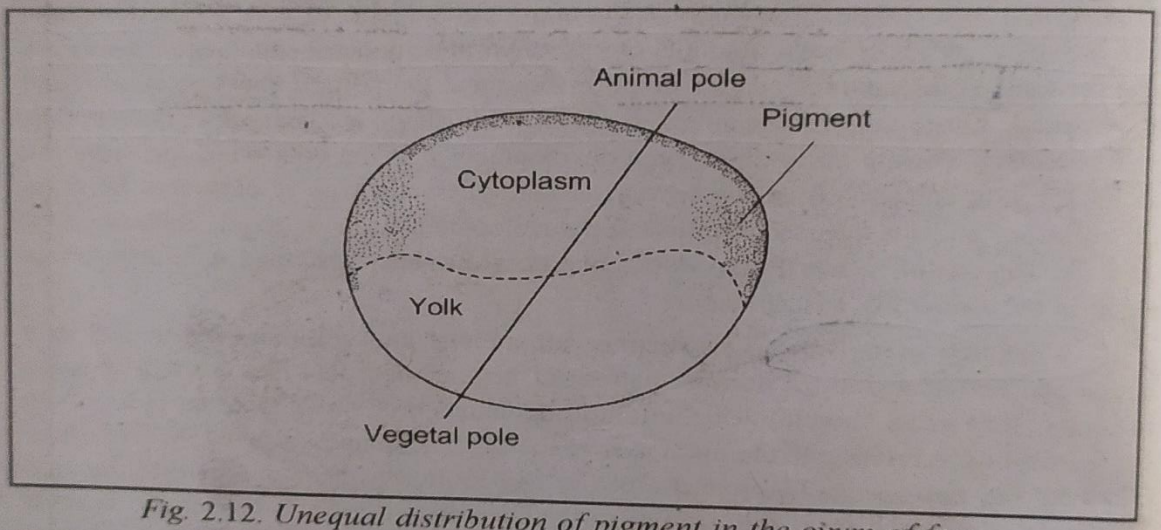

Fig. 2.12. Unequal distribution of pigment in the ovum of frog.

It is generally agreed that probably the pigment substance of the egg has little or no role to play in the developmental process as such because in some animal eggs pigments is present while in others it is totally lacking but in all instances embryonic development occurs normally.

Oogenesis

Oogenesis or differentiation of egg takes place in the female gonad, the **ovary**. Like the testis, the ovary is also lined by the germinal epithelium, the cells of which are known as **primordial germinal cells**. The primordial germ cells through **oogenesis** form the **ova**. Like spermatogenesis, oogenesis is also divisible into the **multiplication, growth** and **maturation phases**.

1. Multiplication Phase:

During this phase the **primordial germinal cells** undergo many mitotic divisions to produce a large number of daughter cells referred to as the **oogonia**. The oogonia arrange themselves into many small groups called **follicles** near the germinal epithelium.

Oogonia are **diploid** as the number of chromosomes in these cells is similar to those of the other body cells. The oogonia in turn also undergo mitosis to form cells called the **primary oocytes** (Fig. 2.13).

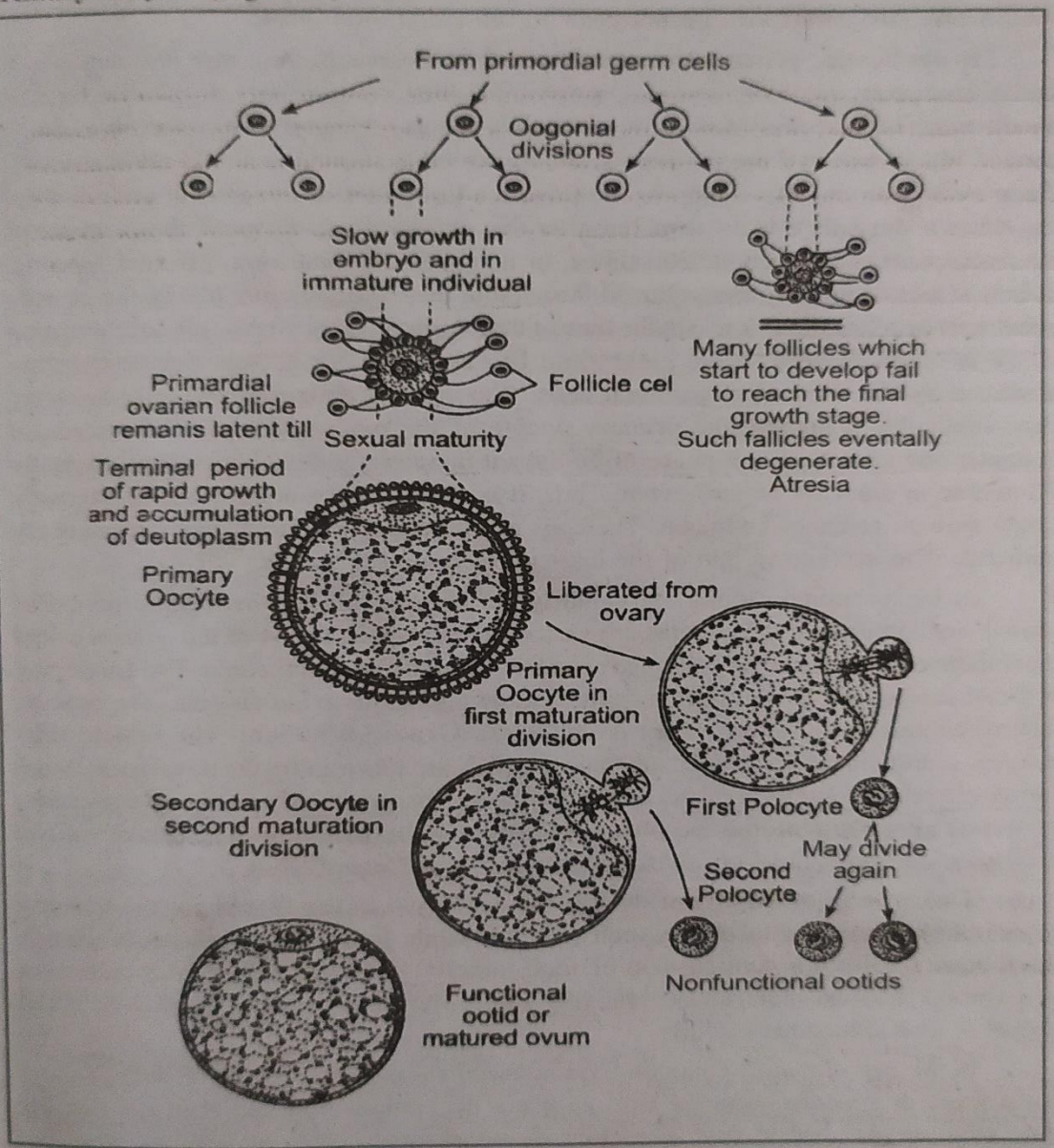

Fig. 2.13. Diagram to show the main steps in the growth and maturation of the ovum.

2. Growth Phase:

The primary oocytes enter into the growth phase. Here two points require special attention. Firstly, during embryonic development the various material contained in the

ovum have a special role, therefore, in relation to spermatogenesis during oogenesis the growth phase is extremely important. Secondly, in contrast to spermatogenesis, during oogenesis differentiation of ovum occurs simultaneously along with growth. In other words, differentiation occurs prior to the maturation phase.

In the female gamete, the growth phase is extremely long and the increase in size of the egg is also enormous in comparison to the male gamete; for example, in frog the diameter of the primordial germ cell is only about 50 micron but a mature **ovum** is about 1500 micron in diameter and its growth period is about 3 years. In other animals, the growth phase is not as long. In the fowl, the last phase of the growth period of the egg takes about only 6 to 14 days but after this period of rapid growth the diameter of the ovum increases by about 200 times. In mammals, a somewhat different situation exists. In mammals, ova are produced throughout their reproductive life by the already existing oocytes (in the ovaries) at the time of their birth. In other words, the multiplication phase is confined only to the embryonic life and after birth, new oocytes are not produced by the primordial germinal cells. The eggs of mammals are comparatively very small. For example, the primary oocyte of the mouse measures 20 micron in diameter and after a growth phase of 16 days it increases to become an approximately 70 micron in diameter mature ovum. Thus, it is clear that a mature ovum is relatively larger than an ordinary body cell. The eggs of birds, reptiles and sharks are relatively immense. The average weight of the egg-cell of a fowl is 55 gm.

In many animal groups, particularly vertebrates, during their entire period of growth and maturation the oocytes are surrounded by special cells of the ovaries called the **follicle cells**. The follicle cells also arise from the germinal epithelium. The developing egg-cell surrounded by the follicle cells is known as an **ovarian follicle**. The ovarian follicle containing a mature ovum is called the **Graafian follicle**. The follicle cells actively secrete many important substances which are taken in by the developing ovum for its growth. The growth of the ovum occurs by absorption of various **amino-acids, proteins, phosphoproteins** and **phospholipids** and also in turn by synthesis of various necessary end-products, from these substances by the ovum itself.

The growth of the ovum occurs in two steps: In the first phase the quantity of cytoplasm increases in the egg-cell (oocyte) while in the second phase of growth there occurs collection and storage of food material. Since in the oocyte, collection and storage of food material is relatively rapid, the second phase of growth of the oocyte is also completed rapidly.

In the egg, the most common food material stored are granules of **yolk**. Yolk is not a fixed or specific chemical substance and its composition and chemical content vary from animal egg to egg. The chief chemical constituents of yolk are **protein, phospholipids** and **fatty substances**.

3. Maturation Phase:

On completion of the growth-phase the primary oocyte enters into the final maturation phase. In this phase the diploid **primary oocyte** undergoes **meiosis** or **reduction division** involving two sequential cell divisions resulting in the formation of

four **haploid** daughter cells. However, there is a fundamental difference in the meiotic process in the male and female germinal cells. In the egg-cell of the female, in reduction division the cytoplasm is unequally divided and out of the 4 daughter cells only one cell receives almost all of the yolk-containing cytoplasm while the remaining three minute daughter cells receive only a token amount of cytoplasm (Fig. 2.14).

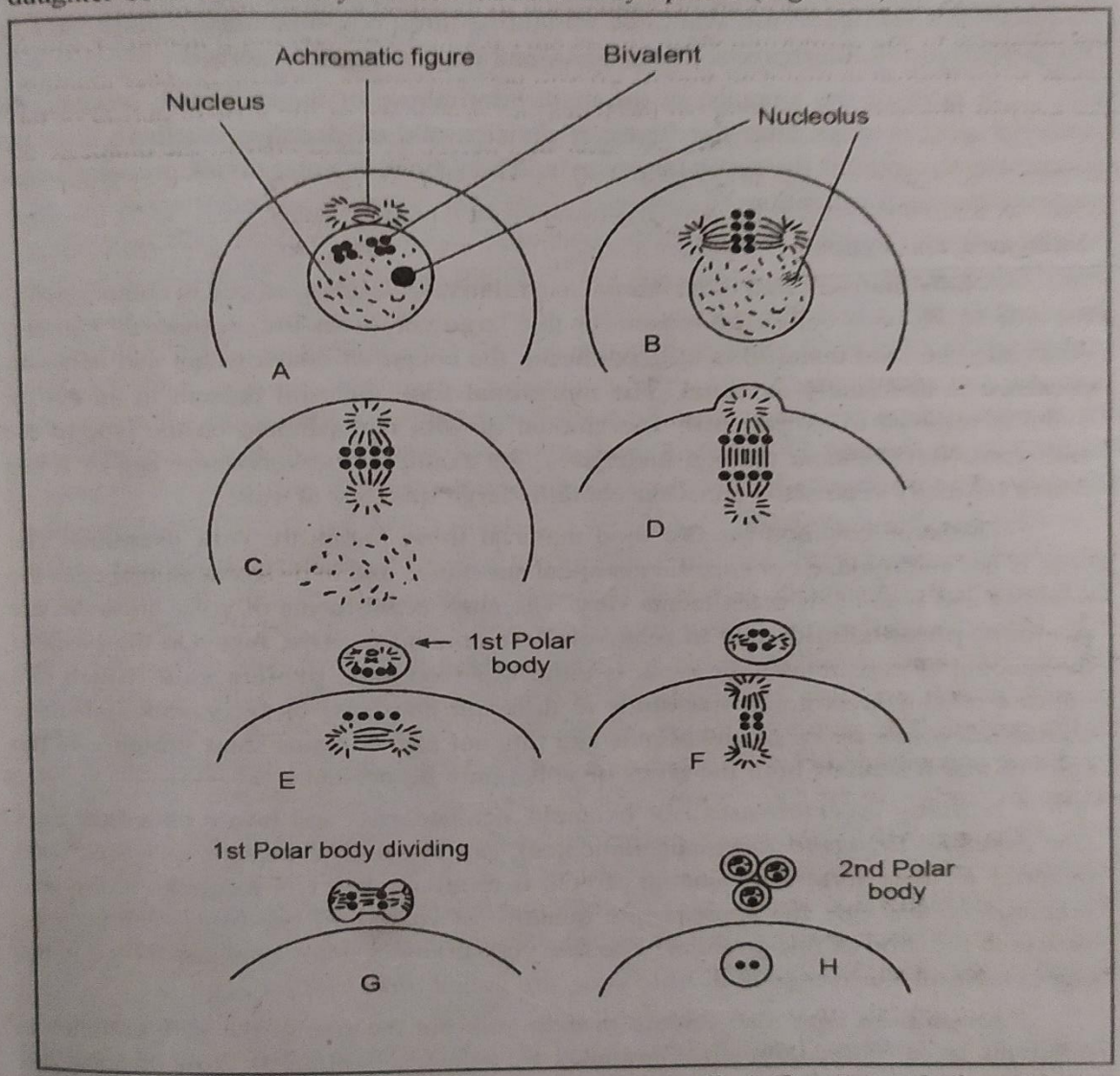

Fig.2.14. Maturation (reduction) division in an egg-cell: A. Oocyte just before meiosis; B, C, D. First reduction division; E. Removal of 1st polar body and preparation for second meiotic division; F, G. Second meiotic division; H. End of division.

The first cell division of meiosis divides the primary oocyte into two unequal daughter cells, one of which is an extremely large yolk-filled cell while the other is very minute. The large daughter cell is called the **secondary oocyte** and the minute daughter cell is known as the **first polar body**. The daughter cells now undergo the second

maturation division. The second meiotic division is also unequal and again a very large yolk-filled daughter cell and a minute daughter cell are produced by the secondary oocyte. The large daughter cell is called the **ootid** which is really the mature **ovum** or **egg**. The minute daughter cell is called the **second polar body**. Therefore, by oogenesis although 4 haploid daughter cells or tetrads are formed, but out of these only one differentiates into an **ovum** while the remaining three non-functional polar bodies do not participate in the reproductive process and are ultimately resorbed.

In most of the animals, in the maturation phase of the oogenetic process, the meiotic process stops after the first cell division and till fertilization there does not occur any division in the secondary oocyte. The second meiotic division occurs in the egg-cell during fertilization.

Yolk and Its Types:

A fully mature ovum of an animal is a relatively large-sized cell in comparison to the rest of the body cells. The reason for this large volume is the presence of nutritive material. The food material is utilized during the course of development and hence its presence is absolutely essential. The nutritional food material present in an egg or ovum is referred to as the **yolk**. The amount of yolk is dependent on the type of the embryonic development the egg undergoes, for example, the very large egg of a hen which basically represents an ovum contains large quantity of yolk.

The most common reserve food material in an ovum are **yolk granules**. The yolk is not an organized or specific chemical substance and in different animal eggs the quantity and type of its constituents vary. The chief constituents of yolk, however, are **proteins, phospholipids** and in relatively less amount **neutral fats**. On the basis of the amount of constituents, the yolk is either referred to as **protein yolk** (which is a mixture of chiefly proteins and lipids in different amounts) or **fatty yolk** (which is constituted primarily by phospholipids and fats but also contains some protein). In the eggs of many animals both the types of yolks may be present.

In **many invertebrates** (for example, echinoderms) and **lower chordate** eggs (for example, *Branchiostoma* and tunicates), *protein-yolk* is present as stored food material. In these forms, the amount of yolk is relatively less (for example, in the sea-urchin *Arabacia* egg, the approximate quantity of yolk is 27 per cent). The protein-yolk is in the form of fine granules. The fine yolk-granules are evenly distributed in the egg cytoplasm. Such eggs with little yolk, are called *oligolecithal eggs*.

Amphibian eggs also contain protein-yolk but the size of the yolk granules is relatively large. These large-sized granules are called yolk platelets. Yolk platelets are oval-shaped and are flat on one surface. The yolk-platelets are densely packed in the egg-cytoplasm and hence the relative quantity of yolk is very high. The distribution of yolk-platelets in the amphibian egg is also very different since the platelets are concentrated mostly in the vegetal half of the egg. Such type of eggs in which yolk is concentrated in one particular region are referred to as **telolecithal eggs**. The yolk-platelets of the amphibian eggs contain two types of protein containing substances: **phosvitin** and **lipovitellin**. Phosvitin is a protein with high phosphorus content (8 per cent) with a

molecular weight of 35,000. Lipovitellin is a protein with a very high molecular weight (4,00,000) containing a large amount of bound lipids (17.5 per cent). The yolk platelets are constituted by structural units of 2 molecules of phosvitin and one molecule of lipovitellin. These structural units are arranged in the platelet in the configuration of a granular reticulum. In the amphibian egg besides the yolk, the cytoplasm also contains lipids and glycogen as stored food material. In a mature amphibian ovum the approximate content of protein yolk is 45 per cent and the lipid and glycogen content is 25 and 8.1 percent, respectively.

In the eggs of **cyclostomes**, **elasmobranchs**, **gonoid** and lung-fishes, the distribution and type of yolk is like that of the amphibian egg. In contrast, among higher bony fishes or teleosts, the yolk is separate from the cytoplasm and becomes isolated inside the egg-cell around which the cytoplasm is present arranged as a thin covering excepting at the apical region of the egg where it is relatively thicker in the form of a cytoplasmic cap in which the egg-nucleus is present. The yolk of bony fishes is categorized as fatty yolk because the yolk contains fat-droplets of a relatively large diameter.

In the eggs of **reptiles** and **birds** the type (fatty yolk) and arrangement of yolk is very much like that of bony fishes. In these groups also the yolk is present as a separate dense central mass around which the cytoplasm is present as a covering layer and here also the egg-nucleus is present in the cytoplasmic cap. A major protion of the yolk is liquid but about 23 per cent is in the form of solid **yolk spheres**. Overall yolk is constituted by 48.7 per cent water, 16.6 per cent protein, 32.6 per cent phospholipids and fat and 1.0 per cent carbohydrate. The yolk-proteins of birds are very much similar to the amphibian yolk-proteins and their constituent structural units are also phosvitin and lipovitellin but in which one part of lipovitellin is joined to phosvitin. This part of the yolk-protein is insoluble in water, but the remainder of lipovitellin is soluble in water. The fatty portion of the avian yolk is in the form of neutral fats (50 per cent of dry yolk) and the remainder is made up by phosphatides and cholesterol.

In some invertebrates also, eggs with relatively large quantity of yolk are present. For example, in the cephalopods- and gastropods of the phylum Mollusca telolecithal eggs are present. Among Arthropoda, particularly in Insecta, the yolk exhibits a special kind of arrangement. In the insect egg the yolk is present as a dense central mass surrounded by a thin layer of cytoplasm. Besides, a small island of the egg-cytoplasm is also present in the centre of the egg enclosed by the surrounding yolk.

In this central cytoplasmic island the egg-nucleus is present. Such eggs are referred to as centrolecithal eggs.

Vitellogenesis:

From a very long time, it was universally believed that yolk-formation in the egg occurs through a specific body referred to as the **yolk-nucleus** or the **nucleus of Balbiani**. This body was initially discovered in the ova of spiders. Later, it was also located in the eggs of amphibians, birds and some mammals. During early stages, this body is present near the egg nucleus but in later stages it breaks into numerous pieces

which arrange themselves at the periphery of the egg. Since the yolk platelets appear first at the periphery of the egg it was concluded that vitellogenesis or yolk formation occurs in the yolk-nucleus.

However, currently this hypothesis is not considered applicable to all animals because electron microscopic studies have demonstrated that the structure of the yolk nucleus is not similar in all animals. For example, the yolk nucleus of the spider egg is made up of many mitochondria containing laminated membranes, or it is constituted of inner membranous sac-like structure which is surrounded by several membranes. Further, any definite evidence has also not been provided which would decisively establish that the yolk nucleus actually forms the yolk.

The yolk-nucleus, has been considered to be homologous to the **mitochondrial cloud** present in the eggs of amphibians. In the developing ova of amphibians and birds numerous mitochondria bunch together to form the mitochondrial cloud. In the egg of the anuran *Xenopus*, although the mitochondrial cloud is arranged around the egg periphery, this kind of mitochondrial distribution occurs later, that is, after the formation of the yolk-platelets indicating that the mitochondrial cloud is not involved in vitellogenesis.

In the cytoplasm of the mammalian egg, a mitochondrial cloud-like body is present near the nucleus, however, it is nothing but a collection of Golgi-like membranes. Therefore, this body is currently referred to as the Golgi material. The Golgi material does not participate in vitellogenesis because in the eggs of eutherian mammals there is complete absence of yolk-platelets.

Besides the yolk nucleus, various other cell-organelles of the egg-cell have been linked to vitellogenesis in different animals. In this context, the endoplasmic reticulum, Golgi bodies and mitochondria have been described to be involved in vitellogenesis, however, definite evidence to support the contention are still lacking.

According to the famous development biologist **Balinsky**, this problem is most likely to be solved when the actual site of the formation of protein-yolk is located. Other researches support the view that probably vitellogenesis does not occur in the egg but yolk is formed in some other part of the body from where it is transported in a soluble state by blood vascular system through the follicular cells to the ova where it is converted into its final storage form. Thus, for instance, it has been considered that yolk is probably synthesized in the liver of vertebrates.

In other animals such type of transport of preformed yolk into the egg has not been observed and in this situation synthesis of yolk takes place by the general process of protein-synthesis from simple precursor compounds, that is, yolk-synthesis is accomplished by the **ribosomes** of the egg itself. Such type of synthesis mostly likely occurs among members of the phylum Coelenterata which lack a vascular system. In this synthesis, the yolk granules make their first appearance in the Golgi body of the egg cytoplasm, however, it is believed that the actual synthesis of yolk granules occurs by the ribosomes located on the endoplasmic reticulum associated with the Golgi body. Here, the Golgi body probably act only as a 'packing organoid'. Further, the synthesis

of carbohydrate present in the yolk is done in the Golgi body. In Crustaceans, the yolk granules, appear first either in the laminar or vesicular endoplasmic reticulum, indicating that their synthesis mostly likely occurs in this region. Therefore, in conclusion it can be stated that at least in these animals, formation of yolk occurs by the normal method of protein-synthesis at the ribosome-containing endoplasmic reticulum and related regions of the Golgi-body.

Besides the above described organelles, in the eggs of other animals the site of vitellogenesis has been shown to be the mitochondria. Convincing studies with the eggs of fishes and amphibians have definitely shown that the formation of yolk platelets occurs inside modified mitochondria. In this process, the inner membrane of the mitochondria separates and arranges itself into circular layers, and the space of the mitochondria is then seen to be occupied by the yolk-platelets. Such type of yolk-formation has been observed in gastropod molluses also.

It has already been described earlier that in some animals the synthesis of yolk does not occur in the ovum but in some other parts of the body (as in the liver of vertebrates) from where it is transported in a soluble state through the blood vascular system into the egg. In such a situation, the yolk is then stored in the ovum in the form of either yol kplatelets or yolk-granules. In this context, it becomes necessary to discuss the properties of the yolk-protein. When the yolk-protein **phosvitin** is *phosphorylated* then it is insoluble in water but when one part of phosphate is removed from it by enzymatic action of phosphatase, then phosvitin becomes soluble in water. Thus if in the egg the partially phosphorylated soluble phosvitin is made completely phosphorylated then it will become insoluble in water. In the egg of the frog an enzyme, *protein kinase*, has been discovered which actually converts the partially phosphorylated soluble phosvitin into fully phosphorylated insoluble phosvitin. In this reaction the phosphate is obtained by ATP. This reaction can be described by the following equation :

$$\text{Partially phosphorylated protein} + \text{ATP} \xrightleftharpoons[\text{Mg}^{++}]{\text{Protein kinase}} \text{Fully phosphorylated protein} + \text{ADP}$$

In the amphibian yolk the protein-kinase enzyme reaction is specific to only phosvitin. However, since in the yolk-granule, the other component, that is, **lipovitellin** is also attached to phosvitin, along with the conversion of phosvitin into the insoluble *state*, the lipovitellin also becomes stabilized. The point to appreciate here is that protein-kinase is a mitochondrial enzyme, and therefore, it is for this reason, formation of yolk-platelets is accomplished in the mitochondria because it is here that the yolk-protein is converted from a soluble to an insoluble state.

From the above description it is evident that for a proper understanding of vitellogenesis more exhaustive work is required. Further, our knowledge on vitellogenesis and fatty yolk of birds and reptiles is almost negligible.

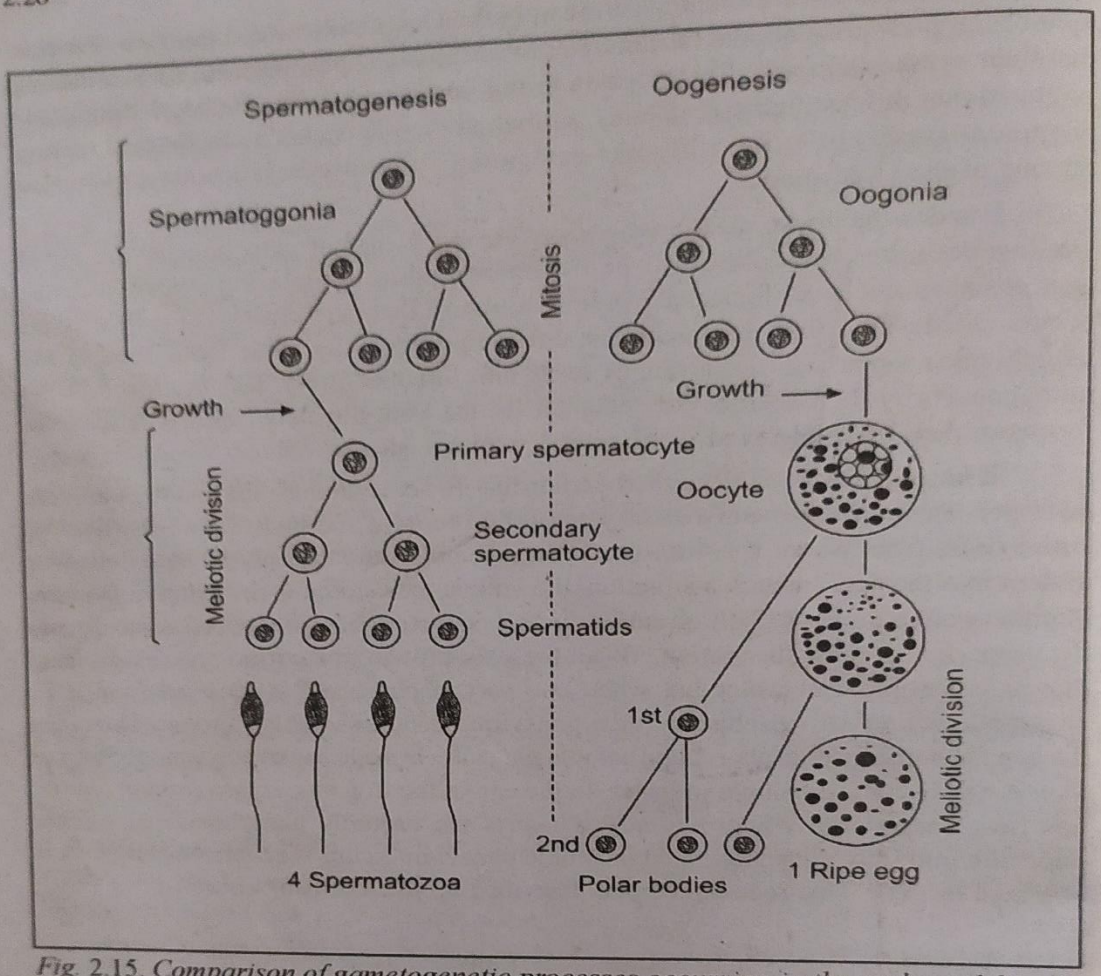

Fig. 2.15. Comparison of gametogenetic processes occurring in the male and female germ cells. At the end of the process in the male the" gametes (sperms) are functional but ill the female only one gamete (ovum) becomes functional.

Egg organization: Types of eggs and role of yolk

From the above study it is evident that there exist wide variations in the eggs of animals. Accordingly the basis of classification of types of eggs has also been different. The basis of categorization of types of eggs could be the (i) amount of yolk, (ii) distribution of yolk, (iii) presence and absence of shell and (iv) type of differentiation or development exhibited by the eggs.

On the basis of amount of yolk, eggs can be categorized as under:

1. Alecithal eggs: In many animals the amount of yolk in the egg is so small that it is negligible. Such eggs are described as *alecithal eggs*. For example, eggs of metatherian and eutherian mammals (Fig. 2.16). Since these are *viviparous*, embryonic development occurs within the mother from whom nourishment is obtained directly and hence yolk as such is not necessary for nourishment.

2. Oligolecithal (Microlecithal) egg: In such type of eggs, the fine granules of yolk are evenly distributed in the cytoplasm. In such eggs, the relative quantity of yolk is also very little. Such type of eggs are also called as micro lecithal eggs. Examples: Lower chordata *(Branchiostoma,* tunicates) and many invertebrate groups (Echinodermata).

3. Mesolecithal eggs: In such eggs the quantity of yolk is relatively more, and therefore, the size of the eggs is also relatively big. The size of yolk granules present in the cytoplasm is also large. These large yolk granules are known as platelets. In the egg-cytoplasm the yolk platelets are not evenly distributed, but are concentrated in the lower region (vegetal half) of the ovum. In such type of ova the quantity of yolk is of a medium category. Example: amphibians (frog) (Fig. 2.16), lung fishes, *Petromyzon.*

4. Macrolecithal eggs: In these type of eggs the quantity of yolk is very large, and therefore, the size of the ovum is also relatively enormous. In these eggs, the cytoplasm and yolk are segregated. Yolk is present in the centre of the ovum in the form of a compact mass and the cytoplasm is present around this yolk mass as a thin layer, and the upper region the cytoplasm is in the form of a thick cap-like structure called cytoplasmic cap. The cytoplasmic cap contains the nucleus of the ovum. In this type of egg, the yolk is in a liquid form containing about 23% solid yolk spheres in a suspended state. The entire yolk contains approximately 48.7% water, 16.6% proteins, 32.6% phospholipids and fat, and 1% carbohydrates. Such enormous yolk-filled eggs are also known as **megalecithal** or **polylecithal eggs** and are found in birds (fowl, Fig. 2.16), reptiles and bony fishes.

Eggs of some invertebrates are also of the **macrolecithal** variety containing a large amount of yolk (for example, egg of some insects). In insect eggs the yolk is confined to the middle of the ovum and it is surrounded by a thin layer of cytoplasm and in the centre of the yolk a small island of cytoplasm containing the nucleus is also present (Fig. 2.16).

Eggs are also categorized on the basis of **distribution of yolk.** In the description of *polarity of egg* is has been stated that there is a tendency of the yolk to concentrate near one pole (vegetal pole) of the egg and as the yolk is relatively heavier than other inclusions, the vegetal pole forms the lower pole of the egg. Therefore, on the basis of distribution of yolk, eggs are also typed into the following categories:

1. Homolecithal (Isolecithal) eggs: In microlecithal eggs the yolk granules are extremely fine, and therefore, they are evenly distributed in the egg cytoplasm and are not confined to one particular pole of the egg. Examples: Microlecithal eggs of protochordates *(Branchiostoma* and tunicates) and echinoderms, etc.

2. Moderately telolecithal eggs: In mesolecithal eggs of amphibians (Fig. 2.16), etc., the relatively larger granules of yolk exhibit a relatively higher concentration towards the vegetal hemisphere than the animal hemisphere of the egg. Such eggs are categorized as moderately telolecithal egg.

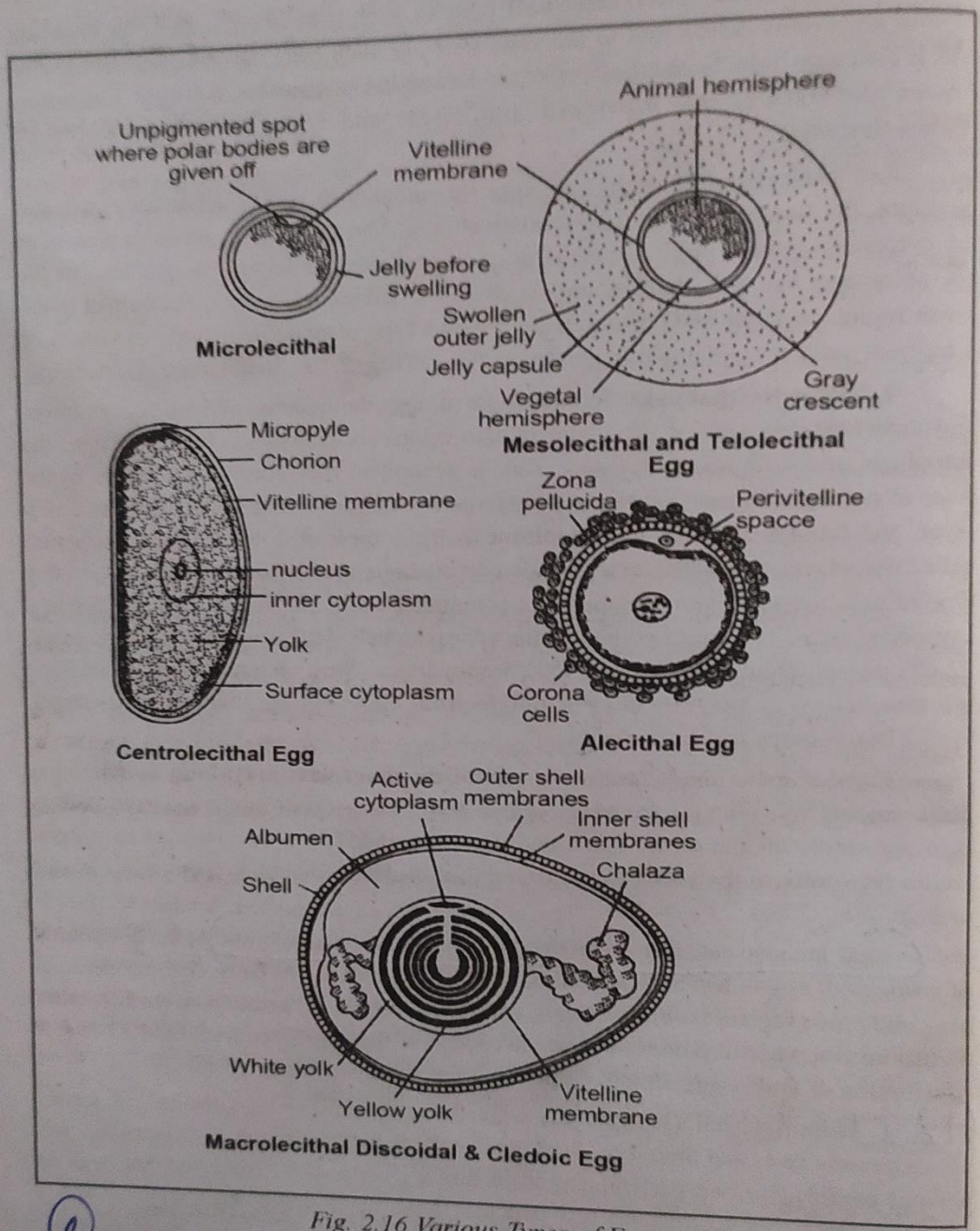

Fig. 2.16 Various Types of Eggs

3. Telolecithal eggs: In macro- or polylecithal eggs of reptiles and birds (Fig. 2.16), etc., the enormous amount of yolk is completely separated from the cytoplasm. In such eggs the yolk is present centrally as a compact mass which is enclosed by a

thin layer of cytoplasm but the upper region this layer of cytoplasm is in the form of a *cytoplasmic cap* containing the nucleus of the ovum. Such type of eggs with segregated yolk are referred to as telolecithal eggs.

4. Centrolecithal eggs: The eggs of insects are also of the macro or polylecithal variety. In insect eggs also the yolk is confined centrally and surrounded by a thin layer of cytoplasm. However, instead of a cytoplasmic cap, in the centre of the yolk, a small island of cytoplasm containing the nucleus is present (Fig. 2.16). Such eggs are typed as **centrolecithal eggs**.

According to some authors eggs are of two types:

I. Homolecithal (Isolecithal), and

II. Heterolecithal (Anisolecithal).

Heterolecithal eggs are of two types:

(a) Centrolecithal, and

(b) Telolecithal.

Telolecithal are again of two types:

(i) Mesolecithal, *e.g.*, amphibians, and

(ii) Meiolecithal *e.g.* reptiles and birds.

On the basis of presence or absence of a **shell, eggs** are also classified:

1. Cleidoic eggs: Eggs of terrestrial animals like reptiles and birds which are laid on ground, for protection are covered by a hard or leathery shell containing cafcium carbonate. Such eggs are referred to as cleidoic eggs (Fig. 2.16).

2. Non-cleidoic eggs: Eggs which are deposited in water (fishes, amphibians, etc.) or which undergo internal development (eutherian mammals) do not possess an outermost protective shell. Such eggs are called non-cleidoic eggs (Fig. 2.16).

On the basis of **embryonic development**, eggs are categorized into the following two types:

1. Determinate (mosaic) eggs: In eggs of some mammals the fate of each part of the egg is predetermined. Thus, if from such eggs a portion is removed, then in the developing embryo that particular organ, organs or parts will not differentiate. Such eggs are referred to as mosaic or determinate eggs. Example: Insect eggs.

2. Indeterminate (regulative) eggs: In majority of eggs, the fate of the parts of the egg is not predetermined. Thus, if from such eggs even if a portion or part is removed, then also the embryo will differentiate normally. Such eggs are called indeterminate or regulative eggs. Example : eggs of mammals.

QUESTIONS

I. Long Questions:

1. Explain with suitable examples the various types of egg membranes.
2. Briefly explain Vitellogenesis.
3. Compare between spermatogenesis and oogenesis with the help of suitable diagrams.