

DEVELOPMENTAL BIOLOGY

GAMETOGENESIS

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**Key Words : Gonad, Ovary ,Testes, Sertoli cells, Leydig cells , Primordial Germ cells ,Gametes
Spermatogenesis, Spermiogenesis, Spermatocyte, Spermatid, Spermatozoa, Acrosome,
Capacitation , Oogenesis, Oocyte , Ovum, Graafian follicle ,Antrum, Vitellogenesis, Egg
polarity, Egg membranes, Ovulation , Gonadotrophins, Animal pole, Vegetal pole**

Reproduction is the biologic process which maintains the continuity of life by the generation of new individual of a species. Many organisms reproduce asexually by budding, fragmentation, fission or by spore formation. Most of the metazoans reproduce sexually by the fusion of highly specialised cells called **gametes** (gameto- marry) produced by two different individuals, the male and female. Metazoans reproducing sexually start their life from a single cell, a fertilized egg or **zygote** (zygosis-joining) formed by fusion of male and female gametes. Protozoans also reproduce sexually but by conjugation, autogamy or cytogamy.

Sexually reproducing metazoans pass through the stages of **gametogenesis, fertilisation, cleavage, blastulation, gastrulation and organogenesis**. In the sequence of these developmental events, generation of gametes or the gametogenesis is the first step. The male gamete, spermatozoa and the female gamete, the ova are produced from the gonads, the testes and the ovary respectively which are held in the peritoneal cavity by mesorchium and mesovarium. When the gametes are produced by different sexes of the individual, it is called dioecious condition. If the single individual takes the charge of producing both the gametes, it is called monoecious or hermaphrodite (example Herdmania, a protochordate) condition. In some fishes, sequential hermaphroditism is seen as the fish becomes female in one season and male in other. In man, hermaphroditism condition is rare and the individuals are usually sterile.

Primordial germ cells (PGC) arising from the yolk sac endoderm or epiblast (Hahnel and Eddy, 1986) gradually migrate to the genital ridges and localise in the gonads (Fig1.1). The gonadal germ cells called **gametogonia** proliferate there, undergo development process and transform to unique cells, the **gametes**. This process of formation of gametes is called **gametogenesis** which includes both the **spermatogenesis** (sperm production) and **oogenesis** (ovum or egg production).

The migration of precursors of germ cells or PGC in amphibians and mammals is by amoeboid movement whereas in birds and reptiles PGC are carried by blood vessels. They squeeze between the endothelial cells of small blood vessels by a process called **diapedesis**. A number of glycol conjugates have been localised on the surfaces of the PGC of different animals and galactosyl transferase has important role in PGC migration. In man, PGC are first formed in the endoderm epithelia of yolk sac in the vicinity of yolk stalk. They show high alkaline phosphatase activity and from there, they migrate to the adjacent mesenchyme and reach the genital ridges. PGC then incorporate in the sex cords and are the only cells in the gonad forming the gametes. The mesodermal cells in the sex cord form the gonadal somatic cells.

Male Gonad Structure: Reproduction is an orchestrated process requiring the coordinated preparation of many gonadal and extra gonadal tissues. Male gonad **testes** are located in the **scrotal sacs** in most of eutherian mammals. The testes are the tireless production factories of sperms as spermatogenesis is a continuous process (starting from puberty) except in seasonal breeders. A section of testes showing germ cells in various stages of development corroborates this fact (Fig1.2). Testes is a composite gland both exocrine and endocrine in nature. The exocrine product is a sex cell for which the testes are also called **cytogenic** gland. It consists of highly convoluted **seminiferous tubules**, (semen - seed, ferre-carry) 0.2mm in diameter and 30-70cm in length with an orderly arrangement of spermatogonia, in various development stages. The mature gametes are distal in position ready to take off for their storage sites in **epididymus** which lies along the posterior border of testes passing by rete

testes and vas efferentia. The delivery of mature sperms in the tubule is called **spermiation**. It is assisted by peritubular connective tissue having some characters of smooth muscles or myoid cells which by their contractions aid in the movement of spermatozoa. From epididymus, sperms are carried by vasdeferens to urethra for ejaculation. In one of the principal methods of male sterilisation, vasectomy, vasdeferens is cut (Fig.1.3); therefore sperm production continues in the testes but they cannot reach the exterior as the duct is cut so the sperms degenerate. In the epididymus, testicular spermatozoa acquire the ability of forward progression and capability to fertilize the ova. Various epididymal controlling factors are involved in the functional maturation of sperms such as pH, cAMP, mono and divalent cations, forward motility factors, acidic epididymal glycoproteins, immobiline, zona binding proteins and epididymal secretory proteins, etc. The spermatozoa selectively accumulate acetylcarnitine from blood while passing through epididymus which is their energy reservoir. **Sertoli** (after Fenriou Sertoli) cells or **sustentacular** cells are the somatic cells in between the germ cells which support and nourish the germ cells, produce fluid for sperm transport, secrete the hormone inhibin and regulate the sperm production. They are supporting cells, few in number and are spaced along the tubule at regular intervals. They are pillar like cells with irregular outline and have a basal pale nucleus. Spermatogenic germ cells are bound to Sertoli cells by N-cadherin molecules present on both the cells and by Galactosyl transferase molecule on spermatogenic cell only that binds to carbohydrate receptors on Sertoli cell. Mature or nearly mature spermatozoa are observed with their heads in close association with the cytoplasmic processes of Sertoli cells and their tails extending to the lumen (Fig.1.2). Free spermatozoa in lumen are rare because they pass to the epididymus as soon as they are detached from the seminiferous tubules. Tight junctions between Sertoli cells near the basal lamina form blood-testes barrier and prevent many large molecules from passing through interstitial tissue towards the region of tubular lumen. In between the seminiferous tubules are Leydig cells secreting testosterone which is an important male androgen. It is lipid soluble and diffuses out of the cells into the interstitial fluid and enters the bloodstream. It is converted to more potent form in the prostate gland and seminal vesicles.

Cyclostomes, fishes and urodele amphibians lack seminiferous tubules in the testes but have cyst like seminiferous ampulla. In cyclostomes, testes are unpaired gonads compared to paired testes in other vertebrates. A duct to carry sperms, vas deferens, is unique in mammals, whereas in lower vertebrates a common urinogenital duct carries sperms to the exterior.

Morphology of Spermatozoon: Male gametes or spermatozoa (sperma-seed, zoon- animal) is a misleading word embalming the historical fact that they were thought to be parasitic animals. Leuwonhock (1678) considered spermatozoon to be parasitic animals in semen (Spermatozoa means sperm animals) but its role in reproduction was given by Spallanzini in 1700. The term spermatozoon was coined for the male gamete by Von Baer in 1827. Sperm is a highly specialised and atypical cell which is designed to subserve the role of reaching the egg and donating paternal genetic dowry to ovum, resulting in the final arousal of quiescent egg (activation) for further development. Sperm has two main parts, **the head and the tail** (Fig .1.4,15). Head consists of nucleus which has the condensed mass of chromatin and a cap of **acrosome**. It has enzymes for species- specific attachment to the egg and penetration through egg envelopes because of which it was earlier called perforatorium. Acrosome and nucleus determine the shape of sperm. It may be ovoid and flattened from both sides (In man and bull), drawn into pointed tip (rodents and amphibia) or spirally twisted like a cork screw (In birds and some mammals) or may be round in bivalve molluscs. The shape has no apparent mechanical role to play in fertilisation since the apical

segment which contributes to the shape of the head is lost during fertilization. The shape of sperm head is species- specific.

Tail consists of **neck, middle piece, principal piece and end piece**. Neck provides articulation to the tail and head. Middle piece consists of proximal and distal centriole. Distal centriole is behind the proximal centriole coinciding with the longitudinal axis of spermatozoon. The proximal centriole is of importance during first cleavage of fertilised egg. The axoneme or axial filament of tail is anchored to the distal centriole and is wrapped by mitochondrial spiral in mammals providing energy for locomotion. It is called **nebenkern** if however, in other animals, spiral arrangement is lacking and they are joined into one or more massive clumps called **mitochondrial bodies**. Thin cytoplasmic sheath covering around the mitochondria is called **manchette**. **Axoneme** consists of structural protein **tubulin** and **dynein** protofilaments having ATPase activity to convert chemical energy to mechanical energy. Electron microscopical studies show that it consists of two central microtubules surrounded by an array of nine doublet microtubules, sub unit A and B out of which sub unit A is a complete circle and B sub unit is C shaped (Fig 1.5) . A sub unit bears **dynein arms** and radial spokes. The essential role of dynein in sperm motility is demonstrated by immotile cilia syndrome called kartagener traid which is a genetic syndrome resulting in lack of dynein. Ring centriole or Jenson's ring or annulus appears sometimes at the posterior end of middle piece. The function of ring centriole is unclear probably it prevents mitochondria from slipping into principal piece (Wheater et al, 1979). Principal piece begins posterior to ring centriole and is characterised by absence of mitochondrial spiral .Endpiece is the short terminal portion of tail. In few groups of animals such as nematode and decapod crustaceans, the spermatozoa do not have flagella and are incapable of swimming or lashing movements. Decapod spermatozoa lack centriole and are devoid of mitochondria which shows that they do not require large amount of energy for movement. The mechanism of penetration of non- flagellate spermatozoa in to the egg is different from that of flagellate spermatozoa. In most of the vertebrates the sperms are monoflagellate but in few species they are biflagellate as in toad fish. In spermatozoa of some fishes and amphibians , undulating membrane is present along the tail.

Process of Spermatogenesis and Spermiogenesis or Spermateleosis: Formation of a mature sperm is called spermatogenesis. It is broadly classified into three phases (Fig1.6).

1. **Proliferation Phase:** The undifferentiated primordial germ cells undergo repeated mitotic divisions and produce large number of diploid cells called **spermatogonia**(sperm mother cell).
2. **Growth Phase:** Spermatogonia increase in volume during growth of each cell. The cells at the end of the growth period are called **primary spermatocyte** which are diploid.
3. **Maturation Phase:** Primary spermatocytes enter the maturation phase during which the cells divide meiotically to form **secondary spermatocytes**. Primary spermatocytes pass through a short interphase and then enter second meiotic period. Finally four **spermatids** which are formed are half the size of secondary spermatocytes and with haploid number of chromosome.

The undifferentiated primordial germ cells, divide to form type A, Spermatogonia which are smaller than PGC and have ovoid nucleus. These stem cells continue dividing to produce A₁, A₂, A₃, A₄, spermatogonia. The continuous mitosis is important to maintain the germ cell number as, 280×10^6 sperms leave the human body during single ejaculation.

Type A₄ has three options: It can form A₄ type for self renewal; it can undergo cell death; or apoptosis or it can form Type B spermatogonia. Type B spermatogonia divide once followed by a very brief growth period to form primary spermatocytes which enter meiosis to form secondary spermatocytes and spermatids. Primary spermatocytes are the largest germ cells within the seminiferous tubules. Secondary spermatocytes are approximately half the volume of primary spermatocytes and lie near the lumen of the tubule. They are rarely seen in the sections of the seminiferous tubules as they are short-lived and divide quickly to produce spermatids. The newly formed spermatid contains a central spherical nucleus; Golgi apparatus, numerous mitochondria and a pair of centrioles. Transition from spermatogonia to spermatocyte is by Glial cell line derived neurotrophic factor GDNF secreted by Sertoli cells. The level of GDNF determines whether dividing spermatogonia will remain gonial or enter the spermatocyte pathway. Low levels of GDNF favour spermatocyte formation regulated by FSH. The initiation of spermatogenesis during puberty is regulated by BMP8B gene product which is synthesised by spermatogonia. When BMP8B reaches a certain concentration, the spermatogonia enter into meiotic division and differentiate into spermatids, haploid gametes.

Meiosis consists of two successive nuclear divisions called **reduction division** (meiosis I) and **equational division** (meiosis II). During reduction division, homologous chromosomes undergo synapsis and crossing over that result in two haploid daughter cells. These cells are genetically different from each other and from parent cells. Therefore, reduction and crossing over furnish the basis for variations in the hereditary pattern that is expressed when the sperms and ova pool their simple set of chromosome in a zygote. During the equational division the two haploid daughter cells divide to form four haploid cells. Maturation promotion factor (MPF) induces cell division, both mitotic and meiotic (Fig.1.7). In human males, if DAZ gene located on long arm of Y chromosome and expressed exclusively in male germ cells is deleted then sperms are not formed that results in sterility.

Mitotic and meiotic divisions are important in gametogenesis, but abnormal chromosomes can be produced in the germ line of either parent through errors in meiosis or mitosis. The gametes thus produced may contain missing or extra chromosome. The absence of a specific chromosome in a gamete and fusion with the normal gamete, may result in monosomy. Conversely, presence of two of the same kind of chromosomes in one of the gametes may cause Trisomy or Down's syndrome (Fig.1.8). New techniques like Southern blotting using DNA probes with known sequences have led to fine analysis of DNA structure making it possible to know which parent is the source of defective chromosome. Abnormalities due to change in number of chromosomes can be detected during fetal life by amniocentesis and chorionic villi sampling.

It is important to note that during spermatogonial divisions, cytokinesis is not complete but the cells form a syncytium whereby each cell communicates with each other by cytoplasmic bridges about 1 μm in diameter (Fig.1.9). These bridges facilitate movement of various molecules and assure the synchronised maturation of the spermatocytes resulting in production of several mature sperms at exactly the same time. Cytoplasmic connections are lost as the cells move to the lumen of seminiferous tubules. A major protein AKAP82 on the fibrous sheath of mouse sperm is required for organisation of sperm tail and is the site for various enzymes. It is transcribed after meiotic division by only X bearing sperm. The cytoplasmic bridges allow this protein to cross from one cell to the other for equal distribution. Further high temperature reduces the spermatogenic activity. Therefore, in the

warm blooded mammals, the testes are outside the abdominal cavity enclosed in the scrotal sacs ; as development of sperms is inhibited by high temperature of pelvic cavity. An abnormal condition when the testes fail to descend to scrotal sacs is called **cryptorchidism**. Untreated cryptorchidism results in sterility in males. Some groups of mammals e.g. Monotremata (spiny ant eater), Proboscidea (elephants), Cetacea (whales) have intraabdominal testes, therefore scrotal sacs do not develop. In other mammals like squirrels and bats, the testes descend temporarily in the scrotal sacs during breeding season. Bird's testes are not enclosed in scrotal sacs but are covered by air sacs to reduce the temperature. Mammalian testes are surrounded by network of veins called **pampiniform plexus** which pre-cools the arterial blood by counter current heat exchange mechanism. Scrotal sacs are connected to abdominal cavity by inguinal canal.

Maturation of spermatid is important as it reduces the diploid number of chromosomes to haploid number. The spermatids are not functional male gametes though morphologically they are mature. They have to undergo the process of differentiation to become spermatozoa. Mature male gamete, **spermatid**, undergoes intracellular redesigning and superficial disguising of certain cell organelles that reduce it to a small motile cell devoid of stored food and protective envelopes. Mature sperm can be compared to a Roman soldier without impediment because most of its superfluous structures which might add weight and reduce its speed, are sacrificed to form a cell carrying nucleus mainly. All the preparatory changes from the spermatid to differentiated motile spermatozoa are called **spermiogenesis**. Functional maturation (changes in cell surface proteins) of sperms in mammals is acquired on its way to epididymus, but the sperms emerging from testes are fertile in most animal species. Final permit to meet the egg cell is given on its journey to female genital tract and this is called **capacitation**. The molecular changes during capacitation are loss of cholesterol from sperm cell membrane, loss of some surface proteins, unmasking of the sperm recognition sites for zona proteins of egg , change in membrane permeability as Ca^{++} channels open up and activation of cAMP, facilitating fusion of membrane during acrosome reactions. First, the sperm cell membrane is altered by the removal of cholesterol by albumin proteins in the female reproductive tract. The loss of cholesterol leads to rise in pH which enables the sperm to undergo acrosome reaction. Second, the surface proteins or carbohydrates are lost during capacitation. It is possible that these compounds block the recognition sites for the proteins that bind to the zona pellucida. It has been suggested that the unmasking of these sites might be one of the effects of the cholesterol depletion. Third, the membrane potential of the sperm cell becomes more negative as potassium ions leave the sperm. This change in membrane potential may allow calcium channels to be opened and permit calcium to enter the sperm. Calcium and bicarbonate ions may be critical in activating the cAMP production and in facilitating the membrane fusion events of acrosome reaction . Fourth is the phosphorylation of surface, the female genital tract is not only a simple route for transport of sperm but brings surface modifications in sperm for fertilisation. Spermatozoa used in vitro fertilisation are artificially capacitated. Various important changes occur in the cell organelles of the spermatid for their packaging and final transport to the female genital tract . These changes mainly involve nucleus, Golgi bodies and mitochondria. Structural changes occur in the nucleus such as formation of acrosome and tail, by sacrificing some cell organelles, thereby making the male gamete light and adapted for fast locomotion. In addition, the nucleus loses fluid content, RNA, nucleolus and most of proteins. The nucleus elongates and this morphological change in shape of the nucleus is attributed to the production of microtubules that wrap the nucleus in a double helical arrangement. Other major change is the replacement of histone proteins by protamines (Brachet, 1974). They are relatively small proteins which shut down transcription in nucleus and facilitate formation of its crystalline structure. Protamines bind to DNA, neutralise its negative charges and

help in coiling the complex DNA into tight circles which form a dough net type structure. Each doughnet represents one DNA loop attached to nuclear matrix. This condensation is important for packing the nucleus for its transport to female gamete without damage.

The **acrosome** of spermatozoon is derived from the Golgi apparatus of a spermatid (Fig1.10, 1.11). The Golgi apparatus consists of series of cisternae arranged concentrically around an aggregation of small vacuoles. During acrosome formation, one or more vacuoles, start enlarging and within the vacuole appears a small dense body called proacrosomal granule or **acroblast**. The contents of vacuole and granule give a positive staining reaction for mucopolysaccharide. The vacuole fuses with other small vacuoles containing proacrosomal granule and attaches to tip of elongated nucleus. The liquid content of vacuole is lost and its wall spreads over the **acrosomal granule** in front of nucleus. Therefore, nucleus is covered with double layer, one of the plasma membrane and other of the acrosomal vesicle forming an **acrosome cap**. Acrosome has number of hydrolytic enzymes called sperm lysins (Brachet, 1974) which are phosphatase, cathepsin, hyaluronidase and acrosin. The remaining part of Golgi apparatus is gradually discarded as **Golgi rest**. Acrosome is called a specialized lysosome (De Robertis and De Robertis, 1980) because it originates from the Golgi apparatus. Acrosomal protein in seaurchin is **bindin** which helps the sperm in the species-specific recognition of the egg (Glabe and Lennarz, 1979). With the formation of acrosome (acro-extremity, soma-body) at the anterior end, cytoplasm moves in the opposite direction, leaving only a very thin layer of cytoplasm. Mitochondria also concentrate in the midpiece of sperm as mitochondrial spiral or mitochondrial bodies. The two centrioles arrange behind the nucleus. The distal centriole gives rise to axial filament or axoneme of spermatozoon. Centrioles are lost in the insect sperm during spermiogenesis.

The small size of sperm is a result of sacrifice of metabolic machinery for shedding weight for fast motility but is compensated by production in large numbers for assured encounter with the egg. In man, the sperms produced during sexual life may be 340 billions approximately while the eggs that come to maturity during the lifetime of human female is hardly exceeding 400. This makes the ratio of possible sperm to egg in man as $8.5 \times 10^8 : 1$. Due to less cytoplasm, functional life of sperm is short which may vary from 6 hours (mouse) to few days. Sperms of fresh water and marine invertebrates undergoing external fertilisation are **primitive** with small acrosome which tends to produce acrosome process during sperm egg interaction. The acrosome process has species specific bindin proteins on it. Internal fertilization using **intromittent organs** in many chordates has ensured good survival of sperms and fertilized egg without production of large number of eggs.

The duration of the spermatogenic cycle varies in different animals in mouse, it takes 34.5 days whereas in man, each spermatogenic cycle takes 64 days, mitosis (16 days), 1st meiotic 8 days, 2nd meiotic 16 days, spermiogenesis 24 days. Gonadotrophins from **pituitary** and testosterone from **Leydig cells** of testes regulate and coordinate the spermatogenic activity. Genetic control of spermatogenesis is elegantly demonstrated by defective spermatogenesis caused by autosomal recessive mutation resulting in non motile sperms. The germ cells during their differentiation also require the helpers which are the neighbouring somatic cells (Sertoli cells). Three important types of signals are involved in regulating the spermatogenesis. The first important signal is **the endocrine secretions** (Fig. 1.12). At the onset of puberty, the anterior pituitary increases secretion of gonadotrophic hormone, that is **Leutinisng hormone (LH)** and **Follicle Stimulating Hormone (FSH)** which is controlled by gonadotrophin release is the principal androgen regulating

spermatogenesis. FSH acts indirectly to stimulate spermatogenesis. FSH and testosterone act synergistically on sustentacular cells to stimulate secretion of **Androgen Binding Protein (ABP)** into the lumen of seminiferous tubules. ABP binds to testosterone and keeps the testosterone levels high. These hormones regulate transcription of genes. Before birth testosterone stimulates development of male reproductive system and descent of testes. At puberty, it brings about the development of secondary sexual characters. Inhibin, a protein hormone secreted by Sertoli cells, inhibits FSH secretion and spermatogenesis. The germ cells lack FSH receptors, but supporting Sertoli cells have the FSH receptors which secrete ABP protein. Sertoli cells also produce Seminiferous Growth Factor (SGF), which stimulates blood vessel production in testes during fetal and postnatal development. The intimate relation between germ cells and Sertoli cells involves the **paracrine signal** for the spermatogenic activity. The third type of signal is the **autocrine**, as Sertoli cells respond to the production of SGF by producing sulfated glycoprotein SGP-2, also called clusterin. It protects the germ cells from apoptotic cell death. Signalling molecules like **Bone Morphogenetic proteins (BMP)**, are important for the resumption of germ cell proliferation at puberty and maintenance of germ cells in the adult. In many lower mammals spermatogenesis occurs in a definite cyclic waves along the length of seminiferous tubule but in man, the waves are less distinct and because of the cyclic spermatogenesis every stage can not be seen at the same time at a given point along the seminiferous tubule.

Thorough understanding of the spermatogenesis is important for its application in therapy of male infertility and for the management of assisted reproduction. The fertilisation potential of the human sperm depends on volume of the ejaculate, total number of sperms in the ejaculate, number of motile sperms and percentage of the abnormal sperms.

Female gonad : Ovary is the female gonad and ever changing multicompartiment organ located in the pelvic cavity on each side of uterus held by fold of peritoneum called **mesovarium (Fig. 1.13)**. It is lined by germinal epithelium, which is a misnomer as it does not give rise to oocytes. The peripheral cortex has follicles (follicle-bag) in various stages of development viz. **primordial follicle, primary follicle, secondary follicle, graafian follicle, corpus luteum and corpus albicans (Fig.1.14)**. Corresponding to the various spermatogenic stages, egg cell also passes through oögonia, primary oocyte, secondary oocyte and ovum. The developmental changes from oögonia to ovum is called oögenesis. The three major steps for oögenesis are

- (1) Proliferation Phase- **oögonium**
- (2) Growth Phase- **primary oocyte**. It is very long in females
- (3) Maturation Phase- **secondary oocyte and ovum**

Mature Egg is an highly ordered large fertilisable cell which determines individual embryonic components. All the eggs are surrounded by cell membrane or plasmalemma. It is spherical with large eccentric nucleus called **germinal vesicle** containing nucleolus (Fig. 1.15). The cytoplasm is rich with other organelles as mitochondria, Golgi apparatus, annulate lamellae, centrosome, etc and also accumulates reserve food material in the form of yolk. The egg cytoplasm is not homogenous but has ooplasmic determinants or **morphogenetic determinants** that foreshadow and condition the development events. These determinants define the cleavage pattern and fate of the cells after cleavage. Important component of oocytes produced during oögenesis, is **germplasm** which is responsible for distribution of the germ cell determinants in zygote and for maintaining cell line in the next generation. The germ cell determinants are localised towards the vegetal pole in the form of clusters. The movement of these masses of germplasm is dependent on kinesin like protein (Savage and

Danilchick, 1993;Robb et al 1996). In addition, eggs may also contain pigment granules which are either uniformly distributed (in Arbacia punctulata, a sea urchin)or differentially distributed in amphibians. It is an easily recognisable marker of cytoplasmic asymmetry of the egg. Therefore, frog's egg has been the most favourable material for the research. . The egg is also protected by various types of egg membranes. The size of ripe ovum varies in different animals. The human ovum is approx 0.14mm in diameter cat ovum is 0.18 mm, in mouse the size is 0.06 mm whereas in frog it is 1.5mm. Sperm enters the egg at different stages of development. , it is in the primary oocyte stage in Ascaris, Neries, dogs and fox. In the mollusc , Dentalium, insects, starfish , sperm enters in the the first metaphase stage but in fishes, amphibians and most of the mammals it is in the second metaphase stage. In sea urchin, even the second metaphase stage is complete, so that nucleus is haploid at the time of fertilisation.

The mature egg thus serves the following purpose :

1. It supplies haploid set of chromosomes to the embryo.
2. It supplies maximum amount of cytoplasm to zygote so that it can develop into embryo without difficulty.
3. It supplies sufficient amount of food reserve in the form of yolk for the developing embryo where the development is outside the body of mother .
4. It develops egg membranes to protect the growing embryo.

Mammalian oogenesis

Oogenesis in mammals is different from that of the other vertebrates as most of the mammals are viviparous .The number of oocytes in the ovary is fixed at birth. As the egg cell grows and matures it is surrounded by the follicular and thecal layers during oogenesis.The mature egg has very less amount of yolk and it does determine egg polarity. The different egg membranes cover the egg. ZP proteins are also produced during this period .

The ovary consists of following follicles depending upon the stage of ovarian cycle:

1. Primordial follicle- oocyte surrounded by a single layer of low cuboidal epithelium
2. Primary follicle- large size oocyte surrounded by single layered cuboidal epithelium .
3. Maturing follicle- number of follicular layers increases around the oocyte.
4. Mature graafian follicle- follicular layers differentiate as zona pellucida, corona radiata, theca externa and theca interna .
5. Corpus luteum- ovulatory product.
6. Corpus albicans- after demise of corpus luteum.
7. Atretic follicle – abortive oocyte.

In the section of ovary, several follicles in various stages of development are seen but only one ovulates.The follicle that produces maximum estrogen in response to FSH will mature and ovulate. Various oogenic stages are safely enclosed in the ovarian follicles where the follicular investments change the packing pattern of immature to mature follicle stage. During the early fetal development, primordial germ cells migrate from the endoderm of yolk sac to the ovaries. The germ cells differentiate within the ovaries into oogonia. Oogonia are diploid (2N) cells and divide mitotically to produce more germ cells. Even before birth many

of the germ cells undergo degeneration which is called **atresia**. A few develop into larger cells called primary oocyte that enter the prophase of reduction division (meiosis) during fetal development but do not complete until puberty. At birth 200000 ---2000000 oogonia and primary oocytes remain in each ovary maintained till puberty in diplotene of meiosis called **dictyate** stage. Only 400 oocytes will mature and ovulate during the women's reproductive life and the rest undergo degeneration or atresia. Irreversible attrition progressively diminishes the ovarian germ cell endowment which begins from six months of gestation till the reproductive life cycle of female. At puberty, the primary oocytes complete meiosis I which produces **secondary oocyte** and **polocyte or polar body** with less cytoplasm because of unequal cytokinesis. The secondary oocyte begins equatorial division (meiosis II) but stops in metaphase. The secondary oocyte is ovulated and reassumes meiotic equational division after fertilisation. It splits into **ovum** and a **second polocyte** which dies off. If fertilisation does not occur, the secondary oocyte degenerates. So, the primary oocyte divides to form a single haploid ovum and three haploid polocytes compared to four sperms by spermatogonia. It helps to conserve most of the cytoplasm and reserve food for the developing embryo.

Mammalian ovary is compact compared to saccular ovary of amphibian having large spaces in the medulla

Large size maturing follicles tends to invade medulla of ovary. Medulla has connective tissue, blood vessels called helicine arteries and nerves. Folliculogenesis in ovary is controlled by gonadotrophins. Follicular rupture releasing ova is called **ovulation** (equivalent to breaking of a boil) and is under the influence of LH surge which changes the left follicular structure to corpus luteum. Mature graafian follicle (about 10mm in diameter) is surrounded by **zona pellucida** and **corona radiata**. It encloses a space **antrum** which is filled with liquor folliculi. It is a viscous fluid rich in Hyaluronic acid. The antrum lined by granulosa layer is surrounded by the theca layers. The theca interna is the innermost layer and is richly vascularised whereas theca externa is the outermost layer. Theca interna produces an important hormone estrogen. The ovum, surrounded by group of follicular cells, is localised to one side of follicle and forms a definite projection into the antral cavity. This eccentric mound of follicular cells is known as **cumulus oophorus**. Small irregular spaces filled with the fluid appear between the cells of cumulus oophorus at follicular maturity thus weaken the connection. Mature follicle increases in size, occupies the cortex region and bulges on the free surface of ovary where theca layer and connective tissue of ovary is attenuated. This point is called **stigma**. Increased secretion of liquor causes further expansion of the follicle which is termed as preovulatory swelling. Finally, the follicle ruptures at stigma and follicular fluid oozes into the peritoneal cavity. The ovum surrounded by corona radiata is torn away from cumulus and is discharged with liquor which is called **ovulation**. The physical detachment and expulsion of oocytes is due to the LH induced increase in collagenase, plasminogen activator, and prostaglandins within the follicle. LH surge activates the mRNA for the plasminogen activator. Prostaglandins increase follicular pressure by localised contraction of the smooth muscles (Koos and Clark, 1982). The free ovum retains the capacity to be fertilised for only 24 hrs. After ovulation, the remaining follicle changes to **corpus luteum** which has **theca lutein** and **granulosa lutein** cells. Corpus luteum enlarges to form important glandular structure if the female conceives. It produces the hormone progesterone which prepares the uterus for the implantation of growing embryo to the uterine wall. If there is no fertilisation then it regresses to a vascular hyalinised structure called **corpus albicans**. Corpus luteum of man is yellow in colour due to lipochrome pigment in lutein cells; the corpus luteum of cow is orange whereas that of dogs and cat is colourless. In addition to corpus luteum, there are number of abortive oocytes which fail to reach the

graafian follicle stage, they undergo degeneration and are called **atretic follicles**. After menopause, the follicles disappear and the ovarian cortex eventually consists of connective tissue only.

The female embryo (man) has fixed number of oogonia at birth as mitotic activity of oogonia is terminated at seventh month of gestation which means the proliferative phase is intrauterine. In the reproductive period of female from menarche to menopause only 400 ripe egg are ovulated. After the egg has deposited its yolk, it is ready for ovulation and in higher animals it is transported into oviduct. The time of ovulation is directly related to the breeding cycle of the animals. There are two quite different controls for ovulation. One is the **environmental** control as a result, the animals ovulate during a brief period once a year according to season. The other is an **internal control** that establishes a rhythm independent of season so that animals ovulate once in a given period. Most mammals have a periodic ovulation pattern in which female ovulates only at the specific times of the year and this ovulatory period is called **estrous**. Human females have cyclical ovulation and is called **menstrual** cycle as there is periodic shedding of uterine tissue at monthly intervals. Ovulation occurs at regular periodic intervals which is highly variable in the different orders and genera of mammals. Thus, in rats ovulation occurs every five days, in guinea pig every sixteen days, in man every twenty eight days, in dogs twice a year and in rabbit it occurs after copulation. Since reproduction is the most important factor in the preservation of species, these cyclical recurrences of ovulation are usually accompanied by the sexual urge in females. This is the common phenomenon observed in domestic animals. **Anovulatory** cycle which means ovulation fails to occur sometimes before menopause is observed in man. As the human egg after ovulation is carried to the oviduct where it is viable for two days for fertilisation, abstinence from coitus during this period is one of the contraceptive rhythm method used for family planning.

The cyclical recurrences of ovulation are integrated with environmental factors for rearing of the young, for example wolves have only one breeding season in the year which is late fall or early winter. The young are born in late winter and are reared during most favourable time of the year that is spring and summer. Moreover, the hormones of pituitary, hypothalamus and ovary are important in regulating oogenesis (Fig.1.18,1.19). Estrogens secreted by follicle cells promote the development of female reproductive tract and secondary sexual characters. Progesterone secreted by corpus luteum acts synergistically with estrogens to prepare endometrium of uterus for implantation of fertilised ovum and mammary glands for milk production. High levels of estrogen stimulate release of more GnRH and LH from hypothalamus and pituitary. GnRH facilitates release of FSH and more of LH. LH surge brings about ovulation. LH surge also removes the meiotic block probably by decreasing the cAMP levels which has some role in the phosphorylation of oocyte proteins. Negative feedback on anterior pituitary reduces secretion of FSH and LH and signals hypothalamus to inhibit GnRH which prevents further follicular development. By adjusting the hormone levels, it is possible to interfere with gametogenesis and implantation of fertilised ovum in the uterus. This may be accomplished by using oral contraceptive pills where surgical method like tubectomy and others are not preferred. Synthetic estrogens and progesterones are employed to take the advantage of feed back inhibition that curtails gonadotrophin release by the pituitary. There is little doubt that granulosa and theca cells also elaborate large number of proteins like inhibin, activin, follistatin (single chain polypeptide of 315 amino acids) having some role in ovarian physiology. The animals that have many enemies and are attacked by number of predacious forms have several litters of young. The size of the litter depends on the number of ova released after maturation and the number of eggs fertilised.

In mammals after growth and maturation of the oocyte that is at ovulation, oocyte completes its first meiotic division and proceeds to metaphase of second division but the second maturation division in the egg occurs at the time of fertilisation only. It is suggested that the calcium ion flux during fertilisation inactivate cytotstatic factor facilitating completion of meiosis. In order to conserve its rich cytoplasm, there is asymmetric fractioning of egg cell forming a **definite oocyte** and three **diminutive polocyte** or polar bodies which degenerate as they are unable to bear the injustice of unequal cytoplasmic distribution .

Gene regulation in oogenesis is also highly unusual whereas the spermatocyte is the only diploid cell without active X chromosome but premeiotic oocyte is the diploid cell where more than one X chromosome is active . At this stage of oogenesis, how inactive X chromosome is reactivated is not clear but failure to do so is deleterious to oogenesis. In humans with Turner's syndrome, XO , there exist only one X chromosome and the individuals are sterile.

Nonmammalian oogenesis Oogenesis is different in other animals from mammals in various respects. As the development is not intrauterine the proliferative phase is not for a fixed period. The animals which lay large yolky eggs have long growth periods for the synthesis of yolk. As the egg takes charge of synthetic activity so the growth phase is very long (amphibian egg three years, *Drosophila* three days , mouse sixteen days) unlike spermatogenesis. The period of growth of oocyte may vary in different animals. In frog *Rana pipiens*, the growth of oocytes is slow during first two seasons but becomes more rapid in third year . In this growth period of three years, young oocyte may increase in size from 0.05 mm. to about 1.5 mm (Fig. 1.17). The egg is surrounded by different egg membranes during this process. The large size lampbrush chromosomes are observed in the yolky eggs of these animals.

The frog ovary is quite large containing approximately 2000 eggs, and is attached by membrane to the kidney. After attaining maturity, the eggs break out of their restraining follicles and fall into body cavity or coelom. The rhythmic ciliary motion sweeps ova to the opening of oviduct that lies close to lung. The egg passes through the body cavity to enter the ostium of oviduct. As it passes through oviduct, the egg is covered by egg membranes. The eggs finally rest in ovisac and the entire mass of egg is released at the time the male liberates sperms as fertilisation occurs externally in water (Fig.1.20). The sexual embrace of male and female frog is called **amplexus or pseudocopulation** . During the breeding season, the male frog's vocal sac enlarges for mating calls and they develop thumb pads also. Sexual dimorphism becomes clear during breeding season. Gonadal dysgenesis or conversion of testes to ovary is reported in male frogs by the weedicide Atrazine which induces enzyme aromatase converting testosterone to estrogen (Hayes et al., 2003). In mammals, the distance of ovary and oviduct is less and the egg is directed to the oviduct by the fimbriae surrounding oviducal funnels and egg traverses by movement of cilia in oviduct. Ovulation is controlled by amount of light relative to amount of darkness each day in many birds and mammals. This periodicity in light which controls sexual cycle is called Sexual periodicity. Light also affects the ovulation and the reproductive rhythm in birds (Fig.1.21). In both birds and certain mammals, reception of photic stimuli produces sexual activation.

Vitellogenesis ;

Maturation of ovum is a complicated process as besides producing haploid nuclei it also stores reserve food material in the form of yolk, collects packets of mRNA called **informosomes** , stores preassembled nuclear pore complexes as **annulate lamellae**, stockpile some membranes, structural proteins, morphogenetic determinants and mitochondria to be used immediately after fertilisation. The mRNA produced is for immediate use in the oocyte

and for use during early development after fertilisation as the embryonic genes do not begin active translation until midblastula (Davidson, 1986). Stockpiling of mitochondria is by autonomous replication of their own circular DNA. In many oocytes, mitochondria collect near the nucleus during early previtellogenic stages but later on they disperse in the cytoplasm. The nucleolus is the principal cell organelle for ribosome biosynthesis and assembly. Species with small and yolk poor eggs, for example sponges and echinoderms usually contain one large metabolically active nucleolus while those with yolk rich eggs as of amphibians have thousands of nucleoli. Gene amplification which means one set of genes is replicated selectively is seen in rDNA of nucleolar organisms of amphibian oocytes. rDNA is used to make ribosomes during oogenesis. Peripheral egg cytoplasm is 2-3 μ thick and is gelled called **egg cortex** or **cortical layer** of egg. It has high concentration of globular actin molecules which form microfilaments required for cell division during cleavage. It is also bordered by an array of mucopolysaccharide granules called cortical granules which are spherical bodies varying in diameter from 0.8 μ to 2 μ . They are the products of endoplasmic reticulum and golgi complex which burst at the time of fertilisation and the contents surround the egg thus help to form fertilisation membrane which blocks entry of more than one sperm into egg and also hold the blastomeres together at the time of cleavage by forming hyaline layer. They are absent in molluscs, insects, urodeles. The cortical granules contain peroxidase enzyme to harden the fertilisation membrane (Faerder and Shapiro, 1977). In mammals, cortical granules do not form fertilisation membrane. Growing diplotene oocytes also actively translate genes for Zona Pellucida proteins in mammals.

The important task of synthesis of reserve food called yolk from **vitellogenin** (precursors of yolk) is called **vitellogenesis** which occurs when oocyte reaches diplotene of meiotic prophase. Stored yolk fulfills nutritional requirement of embryo until it develops its own mechanism for acquiring nutrients. The egg cell increases in size when large amount of food stuffs are stored. Vitellogenesis can be by autosynthesis when raw material is procured from egg cell or by heterosynthesis when raw material is collected from outside the ovary (liver in man or fat body in insects). Amphibians use both autosynthetic and heterosynthetic mechanisms of yolk production. In amphibia, vitellogenesis is stimulated by the Gonadotrophins which help in oocyte growth and differentiation resulting in increase in estrogens. Estrogens increase uptake of vitellogenin and help in yolk platelet formation. The increase in the progesterone helps in the maturation of oocytes.

Yolk is a term for assembled components may be proteins, phospholipids and fats in different combination. It may be stored in the form of flattened bodies called yolk platelets (amphibia) or yolk granules. Exogenously produced yolk precursor, vitellogenin is transported by blood to the ovary. Vitellogenin was earlier associated with the activity of special body in oocytes of spiders, amphibians and birds called **yolk nucleus of Balbiani** which are mitochondrial aggregates. Yolk platelets are found in proximity to mitochondria as it converts partially phosphorylated soluble form of phosvitin to fully phosphorylated insoluble form of phosvitin in the presence of protein kinase. Lipid is stored in lipochondria which are lipids surrounded by proteins. Eggs take the help of follicle cells and **nurse cells** for the extra ovarian transport of yolk. In the large eggs of certain animals, the chromosomes during this phase despiralise to look like lampbrushes for synthesis of mRNA and protein. Vitellogenesis is mediated by estrogen which instructs liver to secrete vitellogenin. In Drosophila, juvenile hormone secreted by corpus allatum promotes oocyte differentiation, regulates uptake of yolk proteins at oocyte surface and stimulates the ovary to produce ecdysone which is metabolised to its active form 20-hydroxyecdysone. It stimulates ovary and fat body to produce vitellogenin, so, Drosophila has dual origin for yolk production that is fat body and ovary. The uptake of yolk into the insect oocyte is a

receptor mediated endocytosis, the receptors for which are on the oocyte membrane at the base of microvilli. (Raikhel and Dhadialla, 1992).

The accumulation of dense yolk also gives the egg the polarity which is manifested in two ways. The region of high activity from where polar body is pinched off is called **animal pole** and sluggish cytoplasmic area storing yolk (deutoplasm) is called **vegetal pole**. The **animal pole** gives rise to anterior parts and **vegetal pole** to the posterior region of embryo. The imaginary line passing through animal and vegetal pole is called **polar axis**. As polarity endows the ovum with primary axis and is also the topographical mark of earliest symmetry of ovum which according to Child's metabolic gradient theory is explained as more metabolic vigour at the animal pole than vegetal pole. The initial egg polarities acquired during oogenesis act as a blue print for the future development of the embryo. No generalisation can be made about the origin of egg polarity. It also depends on the egg's position in the ovary, its relation to the nutritive cells and its attachment to the epithelium of ovary.

Growth and maturation of ovum go hand in hand with the development of follicles in ovary. The accumulation and distribution of yolk determines the patterns of cleavage and further development processes. The growing oocyte transcribes various genes for making products necessary by oocyte that is why during this period certain chromosomes stretch out large DNA loops so called lampbrush chromosomes. **Lampbrush chromosomes** were first observed by Flemming and were described in detail in the shark oocyte. In the oocyte of species of certain fishes, amphibians, reptiles and birds, there is uncoiling of chromosomes during diplotene stage as a result nucleus also enlarges giving appearance of interphase nucleus. Lampbrush chromosomes are homologous bivalents in which paternal and maternal. The long axis of each homologous chromosome is made of two chromatids and has series of chromomeres which are tight coils of principal axial thread but the lampbrushes are despiralised regions extending laterally. Each lateral loop has axis formed by single DNA molecule which is unfolded for intense RNA synthesis. Each loop is surrounded by matrix of ribonucleoproteins that is comprised of precursors of mRNA's. The loops fold back once the diplotene stage is over.

Egg types based on amount and distribution of yolk: Synthesis of cytoplasmic reserve material during growth period is under the imprint of genetic code carried by m-RNA resulting in different egg architecture of varied animal groups. **Based on amount of yolk**, three different types of eggs are recognized (1.22).

1. **Microlecithal** or **oligolecithal** are small sized eggs which contain very less amount of yolk. They are found in certain marine invertebrates such as hydra, sea urchin and in various chordates such as amphioxus, marsupial and eutherian mammals.
2. **Mesolecithal** eggs are the eggs which contain moderate amount of yolk and are found in annelid, mollusca, petromyzontia, dipnoi and amphibia.
3. **Megalecithal** or macrolecithal or polylecithal eggs are the egg types that contain enormous amount of yolk and are found in insects, myxinoidea, elasmobranchs, reptiles, birds and monotremata.

Based on the **distribution of yolk** three different egg types are recognized:

1. **Homolecithal** or isolecithal egg are the microlecithal eggs where the yolk is so little that it is found scattered uniformly through the egg cytoplasm.

2. The second type is **Heterolecithal** egg where the yolk due to its density is concentrated more in one hemisphere than in other . Because of the uneven distribution of yolk there is distinct polarity in the egg as the egg with the region having large amount of yolk is called **vegetal pole** and other side is **animal pole**. In **macrolecithal** egg, most of the space is occupied by yolk and active cytoplasm remains confined to a small cap in the animal pole. **Mesolecithal** eggs or moderately **telolecithal and macrolecithal** eggs are **highly telolecithal**
3. The third type of egg is the **centrolecithal** that is where the yolk is concentrated in the centre of the egg and active cytoplasm forms a thin peripheral layer around cytoplasm. The yolk which is the reserve food material serves the twin purpose of supply of energy and synthesis of products required for the elaboration of embryonic body. Besides influencing the egg size, cleavage patterns, morphogenetic movements , it also determines whether the development in eggs is direct or indirect that is with the intervention of larval stage or without it.

The eggs are also classified as **mosaic or determinate** eggs of molluscs, platyhelminthes, ascidians or **regulative** or **plastic** or **indeterminate** eggs as of vertebrates on the basis of prelocalisation of embryonic structures. In the mosaic egg, it loses its flexible organisation at an early stage of development and are also called determinate eggs. The mosaic eggs are formed as they depend on the ooplasmic determinants (intrinsic or autonomous factors) but the regulative eggs depend on extrinsic factors which are cell to cell interactions or so called conditional regulation.

As the egg undergoes growth and maturation and it accumulates lot of reserve food, so to protect its accumulated wealth it is surrounded by egg membranes or egg covers. They are named according to their origin. **Primary egg membrane** is first one to surround the egg. The **secondary egg membrane** is the second one in the race and **tertiary** is the third one released by genital tract when the egg passes through it (Fig.1.22).

- a) **Primary egg membranes** are laid between the egg plasma membrane and the follicle cells. They are formed by egg or the follicle cells or sometimes by both. They are of the following types:
 - **Vitelline membrane** is the noncellular transparent layer of mucoproteins which has tensile and elastic properties and is important for the species specific recognition of sperms . It remains intact through several stages of cleavage where it appears to hold the dividing cells together. In fishes, it is called **chorion** and in reptiles and mammals it is called **zone pellucida**. In amphibians, it is lifted up at the time of sperm penetration to the egg and it is called fertilisation membrane. Zone pellucida in mammals has ZP1, ZP2 and ZP3 proteins and sperms first bind to ZP3 glycoproteins during fertilisation. Zona pellucida is the docking site for the species specific binding of spermatozoa to the oocyte . It induces acrosome reaction in ZP bound spermatozoa, avoids polyspermy and protects the growing blastocyst before implantation. ZP proteins are named as ZP1, ZP2 ,ZP3 based on the mobility on SDS-PAGE. They are named as ZPA, ZPB, ZPC based on the size of the mRNA transcripts (Lefevre et al, 2004). It is the active area of the research for the production of ZP vaccines immunocontraceptive) for curtailment of fertility (Govind and Gupta). **Zona radiata** is the primary cover of sharks, bony fishes , some amphibians and reptiles which also represent the degraded microvilli of the growing oocyte.

- **Jelly envelope** is primary egg membrane of marine invertebrates which helps to keep the egg mass together and was earlier reported to release fertilizin to attract the sperms of the same species in water. The jelly cover has chemotactic molecules one of which is **Resact** identified in sea urchins for attracting species specific sperms(Ward *et al* , 1985) . All the primary egg membranes adhere closely to the surface of oocyte but at a later stage a space filled with fluid may appear between the egg plasma membrane and primary egg membrane and is called perivitelline space.
- b) **Secondary egg membrane** is secreted outside the primary egg membrane by a layer of follicle cells that surround the oocyte, Secondary egg membranes are usually tough and impermeable . It occurs in the form of chitinous shell surrounding the egg in insects, ascidians and cyclostomes and is called **chorion**. In many insects, the surface of chorion shows a complicated sculpture which is typical of each species. To facilitate sperm entrance secondary membranes have an opening called **micropile**. These membranes are not found in amphibians, reptiles, birds and even in mammalian egg. However, in mammals when ovulation occurs egg carries with it a layer of follicle cells called corona radiata (compared to sun's corona) which does not form a true membrane since its cells are peeled off as the egg descends the oviduct.
- c) **Tertiary egg membranes** are secreted by the cells of oviduct as the egg travels down the duct towards cloaca. They are very diversified in their form and these membranes are called **albumin , jelly coat, shell membrane and shell**.

In oviparous cartilaginous fishes the egg is surrounded by **albumin** and hard **horny capsule** which also develops twisted threads for attachment to the sea weeds. The horny egg capsule is secreted by shell glands of oviduct. It gives purse like shape to the egg and helps to attach to the plants. It is called **mermaid's purse**.

The amphibian egg is surrounded by the jelly cover as the egg travels down the oviduct. These **jelly_covers** hold the egg together giving buoyancy , make them unappetising to the predators, and anchor the eggs to the plants. In the reptiles and birds the different eggs membranes are **albumen or egg white, two shell membranes and a porous shell**. The shell in birds is laid down by the shell or nidamental gland in the oviduct . In the birds (chick) the fertilised ovum is surrounded by the egg membranes as it passes down the oviduct towards cloaca. First , it is covered by the egg white or albumen which is secreted by glandular wall of oviduct . The ovum rotates as it passes through the ducts so that the albumen is wrapped in layers with opaque twisted cords on each side called **chalazae**. It keeps the ovum in the centre of the albuminous cover then, the ovum is surrounded by semipermeable shell membrane and finally covered by shell in the posterior region of the oviduct. The shell gives ovoid contours to the egg. The shell membranes enclose a space called air space which increases as the egg becomes old that is why the fresh egg sinks in water but old egg will always float (Fig.1.22).

As the ovum or egg passes through the hen's reproductive tract, it is rotated for 20 hours at the rate of 10 – 12 revolutions per hour which shifts the yolk in such of way that its lighter component is beneath the blastoderm. This end of blastoderm becomes the posterior portion of the embryo that is the part where primitive streak is formed (Eyal – Gladi and Fabian ,1980).

For the passage of large yolky eggs, there is absence of pelvic symphysis in birds and production of these large eggs is done only by the ovary of left right side as the ovary of right side is reduced. Because of the presence of the porous shell, the eggs of reptiles and birds are also called **cleidoic** egg or box like **or amniote eggs** which allow only the exchange of gases with the outside environment and the eggs of lower chordates that is fishes and amphibians **are non cleidoic** eggs or **anamniote** egg as they do not develop foetal membranes. The ability to nest above the ground and to avoid large predators is due to the advantage of cleidoic eggs which has helped the birds to exploit the new aerial environment. The cleidoic egg is called amniote egg because of the formation of extra embryonic membranes . It is a complex egg and its evolution has involved many changes. These changes with respective advantages to the egg are listed as under :

1. Egg can be laid on land so reduces the threat of aquatic animals. Fertilisation inside the female reproductive tract makes fertilisation on land more certain .
2. Egg becomes large so there is less danger of desiccation though there is more problem on land.
3. Egg covers or egg membranes provide more protection.
4. More water absorbing proteins in the yolk help for more water retention.
5. Amnion and chorion membranes develop (Extra embryonic) for water retention and exchange of the gases.
6. Allantois, an extra embryonicmembrane to collect the waste and later on to help in exchangeof gases.
7. Calcium rich shell for added protection against predation and desiccation.

The egg membranes protect the growing embryo from various ecological hazards and also from the mechanical injuries. They check polyspermy and also maintain the normal cleavage pattern. These membranes are formed depending upon whether the development of egg is in water (may be fresh water, or marine,) or on land or in the maternal body, that is uterus. Mammals which are the highest vertebrate group selected the site of development of fertilized egg in the maternal body, so the mammals are called viviparous thus the eggs do not have shell membrane (amniote egg).

Mammals have bypassed the problem of food and water by undergoing uterine development (**Viviparity**). Land vertebrates like birds and reptiles required water conserving covers so variety of egg membranes surround the reptilian or bird's egg as development of fertilised egg is outside the mother's body (oviparity). The intermediate stage is called **ovoviviparity** when the egg is retained in the mother's body but without any close connection between the two, egg is saved of the environmental stresses (Example, Mustelus vulgaris). This also reflects the egg's adaptation for development on water and land (external fertilisation) or uterine development (internal fertilisation) . Thus, the gametes are among the most conservative of all the cells but are also the keys to unfold the chordate phylogeny.

Functions of accessory cells during growth of oocyte : In many groups of animals, notably in the chordates, the oocytes are surrounded during their entire growth and maturation by special cells of the ovary, the follicle cells.(Fig.1.23). In mammals, the follicle cells are derived from the germinal epithelium of ovaries and the young oocyte is surrounded by one layer of follicle cells. Later these cells increase in number and become stratified. The oocytes are surrounded by follicle cells not only in mammals but in other vertebrates as well though due to large size of egg, the follicle cells are not so conspicuous . The follicle cells help in the growth of oocyte by secreting substances that are taken up by the oocyte. The structural relationship between the follicle cells and oocytes develop in the form of desmosomes where the plasma membrane of the adjoining cells are thickened and the space is filled by denser substance. In this space later on elongated processes called microvilli develop which interdigitate with those of follicle cells . The microvilli increase the surface area of the oocyte. At the base of microvilli, small in-pocketings appear which are indicative of oocytes taking in fluid and dissolved substances by means of pinocytosis .

In the nest of follicle cells, the egg is helped by these accessory cells in the transport of yolk precursor vitellogenin from their source. As the follicle cells (somatic cells) surrounding the mammalian egg develop microvilli and are physiologically coupled by gap junctions for it, so the layer gives a striated appearance and is called **corona radiata** . The invertebrate eggs of some insects, molluscs and annelids are surrounded by **nurse cells** which are connected by small channels and unlike follicle cells they are derived from germ cell line . In certain insects, as in Drosophila where oogonial connections are retained it is called **meroistic oogenesis (Fig.1.24)**. No microvilli or cytoplasmic processes develop between the oocyte and the nurse cells but gaps appear between the two cell types. The cluster of oocyte and nurse cells is called **egg chamber**. The nurse cells are the abortive oocytes and are finally eaten by the growing egg cell. They are important as they provide nutrition to the growing oocyte and various building materials pass in through it so they are considered as the integral part of the metabolic apparatus of the oocyte. The meroistic eggs do not have lampbrush chromosome stage but the nurse cells quickly transport RNA also so that oogenesis in insects is a rapid process. The transcriptional efficiency of nurse cells is increased by polyteny. Oogenesis takes 100 days in cricket and 8 days in Drosophila. The oogenesis is the coordinated expression of genes in number of cells surrounding oocyte. Insect follicle cells play active role in vitellogenesis, as they can sequester yolk precursors from hemolymph for transport to oocyte and in some species they synthesise yolk precursors themselves. They also secrete vitelline envelope and chorion around insect egg.

Thus we see principal role of accessory cells in oogenesis :

1. They supply reserve food material during oocyte growth that is during vitellogenesis.
2. They act as a selective barrier between oocyte and surrounding tissues and mediate transfer of yolk precursors.
3. They form egg membranes.

The formation of gametes is the most intricate developmental processes known. The constituents of mature egg have both immediate and long term implications for reproduction . Unlike other differentiated cells fulfillment of the unique role in fertilisation by gametes is not the last function but the first step for designing a complete new individual (Fig.1.25).

The whole chapter will be a futile effort if in the end, the students are not able to capture the following points of differences between sperms and ova and also their process of transformation from the “raw” to “ripe” and processed forms that is “spermatogenesis” and “oogenesis”

It should be very clear that:

- sperm is a male gamete.
- Manufactured in testes and is of small size
- Production is in large numbers
- Active and motile.
- Only covered by plasma membrane
- Acrosome and tail present
- Two centrioles present
- Cytoplasm is less
- Nucleus compact with no nucleolus.

BUT

- ova is a female gamete
- Manufactured in the ovary. Very large in size, compared to sperm.
- Produced in less number.
- Inactive and non motile
- Jacket of egg membranes around the plasma membrane of egg
- Yolk present
- No acrosome and tail. Centrioles absent
- Cytoplasm abundant Nucleus large with several nucleoli

The simplified picture of the complex processes of spermatogenesis and oogenesis is presented below which will help the students to store the important but relevant points of the topics and to generate interest in it for further research. The **comparative account** of the two processes is listed below as :

SPERMATOGENESIS

- Occurs in the testis
- Insignificant growth phase
- No vitellagenic stage

- Both meiotic divisions occur in close succession
- The meiotic divisions are equal
- No polar bodies are produced
- Extensive redesigning of spermatid `Result of development event is **four functional sperms.**`

OÖGENESIS

- Occurs in the ovary.
- Growth phase is remarkably long and maturation and differentiation go side by side.
- Vitellogenic stage present
- In most vertebrates, second maturation division is completed only after fertilisation
- The meiotic divisions are unequal
- Polar bodies are produced

Morphological redesigning is not much as in sperms

- Result of the final event is a single functional egg out of four oocytes

In the end of the long story of **gametogenesis**, it should be very clear that :

- Male and female gametes called sperms and ova are **specialised germ cells** and sperm is a motile cell as it is the sperm reaching the egg cell and not the vice versa.
- Sperms are produced by testes, stored in epididymus, for potential motility and fertilisability , transported by vas deferens and modified in female genital tract.
- Ova is produced by ovary, released in the coelom, reaches the fallopian tube in internal fertilisation and is shed outside in external fertilisation.
- Spermatogenesis is essentially a continuous process as large number of sperms are lost during ejaculation In 3ml ejaculate of humans there are about 2.5×10^8 sperms present.
- Shelf life of sperm is less due to less cytoplasm.
- For the final event of fertilisation, germ cells spermatogonia or oogonia undergo structural and functional changes involving all the cell organelles, producing sperms and ova (gametogenesis , spermatogenesis , oogenesis,)
- Mature ovum is metabolically inert but is proud of its rich cytoplasmic wealth which is protectively locked in the egg covers.
- Various extrinsic (environment) and intrinsic factors (endocrine, Paracrine , autocrine) regulate the gametogenesis.
- The processes of embryonic development have challenged man's imaginations since period of classical antiquity but with the scientific advancements and new tools for investigations the important early processes of sexual reproduction (gametogenesis) can be explained at molecular level. It's thorough knowledge has helped us to apply it for therapeutic use and population control.

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