Chapter 2
Gametogenesis

2.1. Meiosis

Most **somatic cells** are diploid (2N), with each chromosome having a homolog. During meiosis the number of chromosomes is reduced by one half, to the **haploid** condition (1N), where there is only one of each different chromosome. Gametes are Haploid and Somatic (or body) cells are usually **diploid**. Prior to meiosis the sex cells **proliferate**, increasing their numbers by mitosis. These proliferating cells of the testes are called **spermatogonia** and in the ovary are called **oogonia**. In addition to undergoing meiosis, gamete development also includes **differentiation** of cytoplasmic structures. Thus, the egg accumulates stores of nutritive material and the sperm acquires a means of locomotion.

2.1.1. Phases of Meiosis

The **first meiotic prophase** is extended and is divisible into a number of **stages**: ***leptotene, zygotene, pachytene, diplotene*** and ***diakinesis***. Recall that the nuclear envelope breaks down during prophase. During **leptotene** the chromosomes first become visible as thin chromatin threads. During **zygotene** the homologous chromosomes pair up (or synapse). During **pachytene** the chromosomes of each pair of homologous chromosomes twist around each other and become indistinguishable from one another. During this stage each chromosome divides lengthwise into two **chromatids.** (The actual replication of DNA occurred during the S phase of interphase). Each homologous chromosome pair (each chromosome with 2 chromatids) makes up a bivalent (or tetrad). While the chromosomes are in pachytene they may undergo crossing over, where they exchange homologous parts. During **diplotene** the homologous chromosomes, as well as their chromatids, repel each other and are seen as four distinct threads of chromatin per bivalent. Finally, during **diakinesis** the chromatids become shorter and thicker, and are typically short stout rods.

The chromosomes are now ready for the first meiotic division, which is also called the **reduction division**, because the chromosomes are reduced from diploid to haploid. During **Metaphase I** (metaphase of 1st meiotic division). Chromosomes line up at equator of cell, as homologous pairs, called bivalents. During **Anaphase I** the homologous chromosomes separate and migrate to opposite poles. During **Telophase I** cytokinesis occurs giving two haploid cells. The cells may enter a brief **Interkinesis**, but there is **no further DNA replication**. Each cell now goes through the **Second Meiotic Division**:**Prophase II, Metaphase II, Anaphase II** - chromatids separate and become chromosomes and **Telophase II** - the end result is 4 haploid cells.

To summarize meiosis, it is two consecutive cell divisions (1st and 2nd meiotic divisions) preceded by a single replication of the chromosomes (which occurs during the S phase of Interphase, prior to Prophase I)

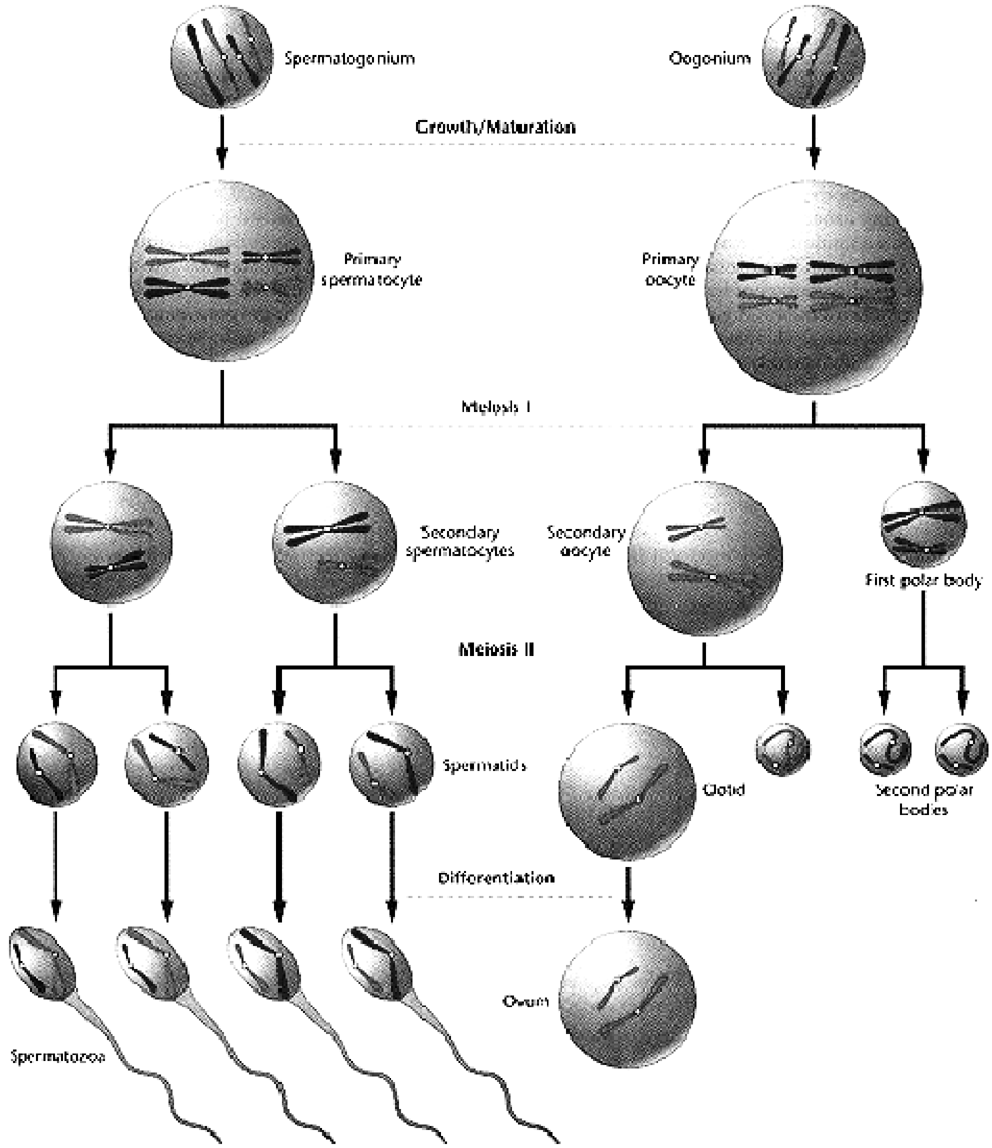

Fig.2.1. Overview of Gametogenesis

2.2. Spermatogenesis

Spermatogenesis is the development of **spermatozoa**. Because this is a continuous process, all the stages are present at one time. In **insects** and **vertebrates**, spermatogenesis occurs in **seminiferous tubules**, which converge to a common duct (vas deferens). In insects the seminiferous tubules are divided into little compartments (cysts) by partitions (septa). These compartments (cysts) pass down the tubules while spermatogenesis is occurring in the cells within them. Therefore, in insects spermatogenesis can be observed, from start to finish, by observing a longitudinal section of a seminiferous tubule. In the vertebrates, all stages of spermatogenesis can be observed at any level of the seminiferous tubules, because, along its full length, spermatogenesis is occurring from the outside of the tubule toward the middle.

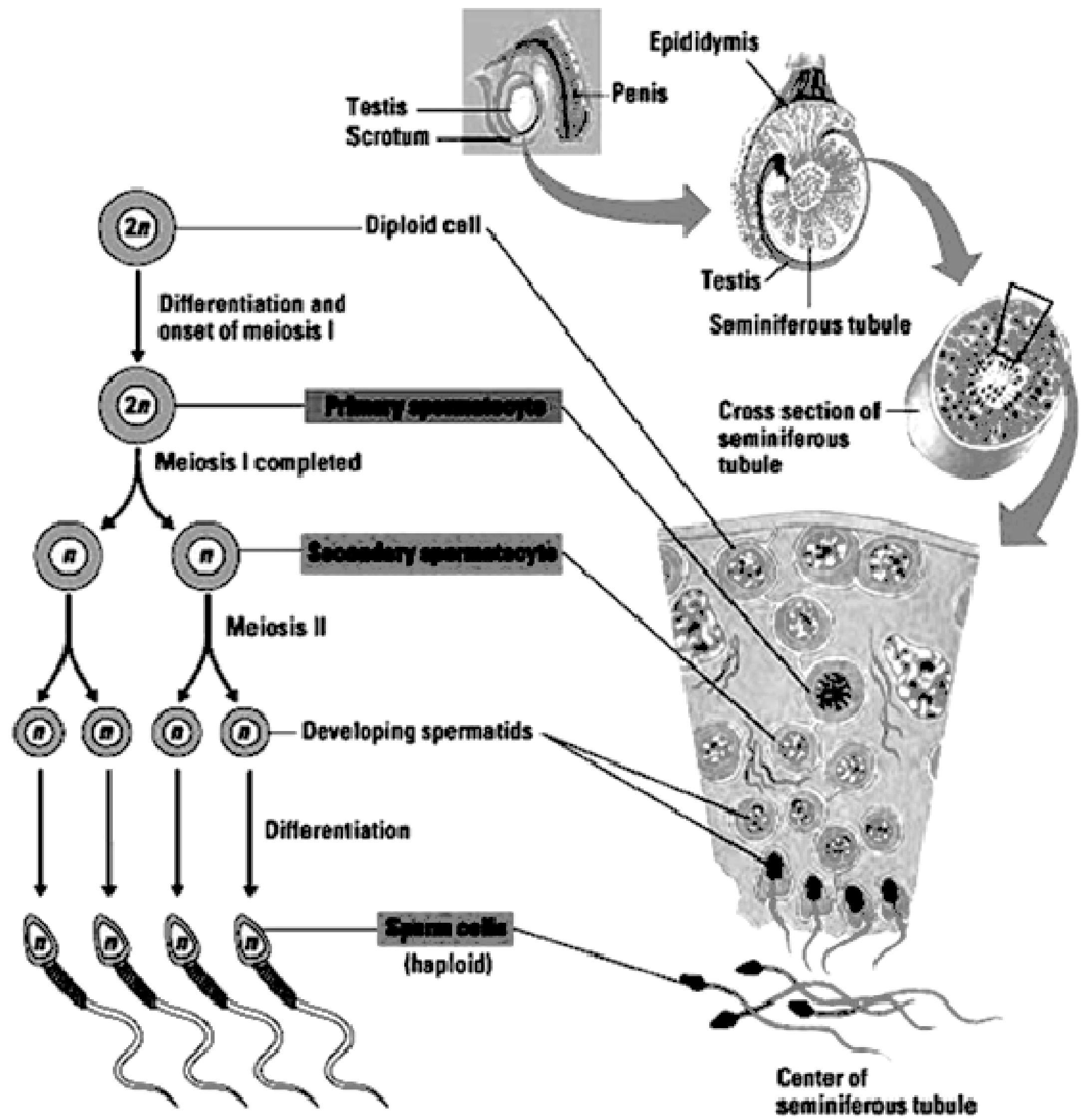

Fig.2.2. The Process of Spermatogenesis

(a) Phases of Spermatogenesis

Spermatogonia proliferate by cell division involving **mitosis**. In insects these are found only at the closed ends of the seminiferous tubules. In vertebrates these are found around the outside of the seminiferous tubules. Some of the spermatogonia grow and become primary **(1°) spermatocytes**. The 1° spermatocytes undergo the **1st meiotic division** and become secondary **(2°) spermatocytes**. 2° spermatocytes are equal in size and have the haploid number of chromosomes (*but each chromosome has 2 chromatids*). Both 2° spermatocytes undergo a 2nd meiotic division, producing a grand total of 4 **spermatids**. Each spermatid is equal in size and has the haploid number of chromosomes, but each chromosome is single, no longer having 2 chromatids. The **spermatids** are then differentiated into **spermatozoa**. **Spermiogenesis** is the differentiation of spermatozoa.

(b) Transformation Process (Spermiogenesis)

The nucleus of the spermatid takes on the normal interphase appearance with the chromatin material dispersed. The nucleus shrinks in size by reducing its water content. It also removes other excess components (*i.e.* nucleolus and other RNA). In somatic cells, DNA is wound around **nucleosomes** (made of histones). During **spermiogenesis** histones are replaced by protamines, forming a protamine-DNA complex, which is more dense and is genetically inactive (*i.e.* no transcription). This occurs early in spermiogenesis, therefore **transcription** stops by early spermiogenesis. However, because protein synthesis **does** occur during spermiogenesis, there must be a post-transcriptional delay mechanism.The acrosome forms from the golgi apparatus. A **proacrosomal granule** (or several) appear in vacuoles within the golgi. If there is more than one proacrosomal granule, they combine. The proacrosomal granule is composed chiefly of mucopolysaccharides. It continues to grow and becomes the **acrosomal granule**. The acrosomal granule occupies a vacuole in the golgi (acrosomal vesicle). This vacuole is surrounded by a double membrane from the golgi. The acrosome positions itself anterior to the nucleus. The remainder of the golgi is called the **golgi rest (or remnant)** and is eventually extruded with the excess cytoplasm. The **two centrioles** in the spermatid move to the posterior pole of the nucleus. The **proximal centriole** remains in close contact with the nucleus. The other centriole is the **distal centriole**; it gives rise to the axial filament of the flagellum and becomes continuous with it. The mitochondria become dispersed and regroup around the base of the axial filament. In many animals the mitochondria combine to form a mitochondrial spiral around the axial filament. Most of the cytoplasm of the spermatid is discarded during **spermiogenesis**. The excess cytoplasm including the golgi remnant flows to the area around the middle piece, where it eventually pinches off and disintegrates. As spermatogonia undergo mitosis cytokinesis may be incomplete; this forms syncytial clones with intercellular bridges. Because this is widespread, it is probably fundamentally important, but the importance not understood.

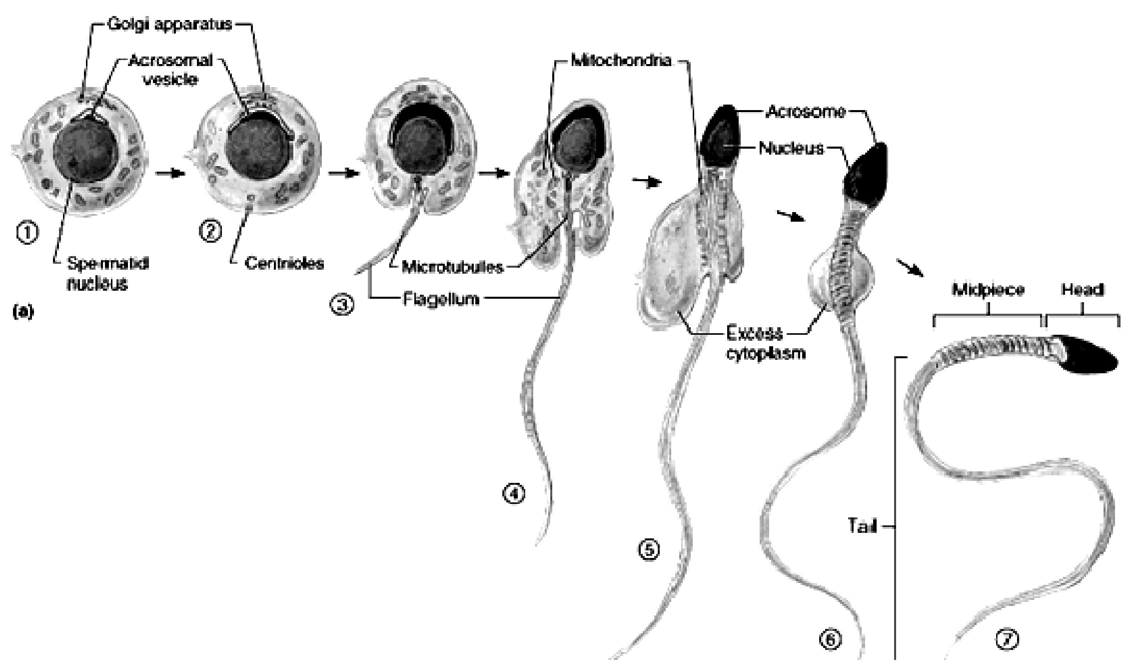

Fig.2.3. Spermiogenesis

(c) Structure of a Mature Spermatozoon

It consists of 2 major parts: **head** and **tail (flagellum)**. The tail has 4 major regions: **neck,**

middle piece, principle piece and end piece. At the anterior tip of the **head** is the **acrosome**, which is used for penetration of the egg membrane. Most of the head is occupied by the **nucleus**, which contains a haploid set of genetic information. The **proximal centriole** is also found in the head. According to the time-honoured tradition proposed by **Theodor Boveri,** it initiates cell division in the fertilized egg. However, in 1975, **Fawcett** found that the **proximal centriole** is lost in some mammalian species. **Longo** and **Anderson,** 1968, found that both sperm centrioles enter the egg during sea urchin fertilization. The **neck** is the slender connection between the head and middle piece. The **middle piece** contains the **distal centriole**, the base of the **axoneme**, and the mitochondrial spiral around it. The **axoneme** is made of microtubules. The mitochondrion provides the energy needed for moving the flagellum. At the posterior end of the middle piece is the ring *centriole* or **annulus**, which is not a centriole; its function is not known. The **principle piece** of the tail has cytoplasm around the axoneme.
The **End piece** is the naked, exposed part of axial filament (axoneme).

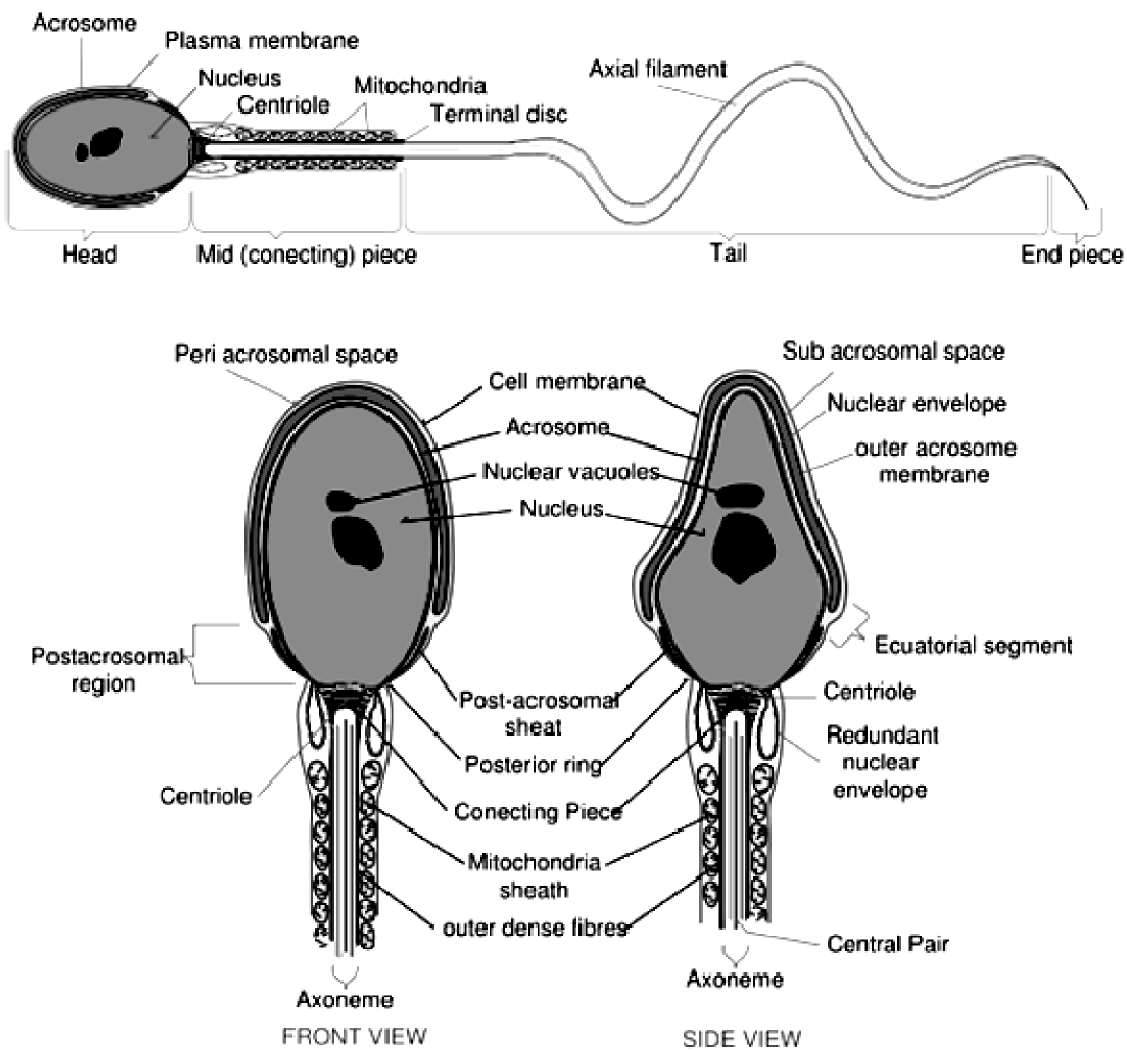

Fig.2.4. Structure of Spermatozoon

(c) Hormonal Control of Spermatogenesis

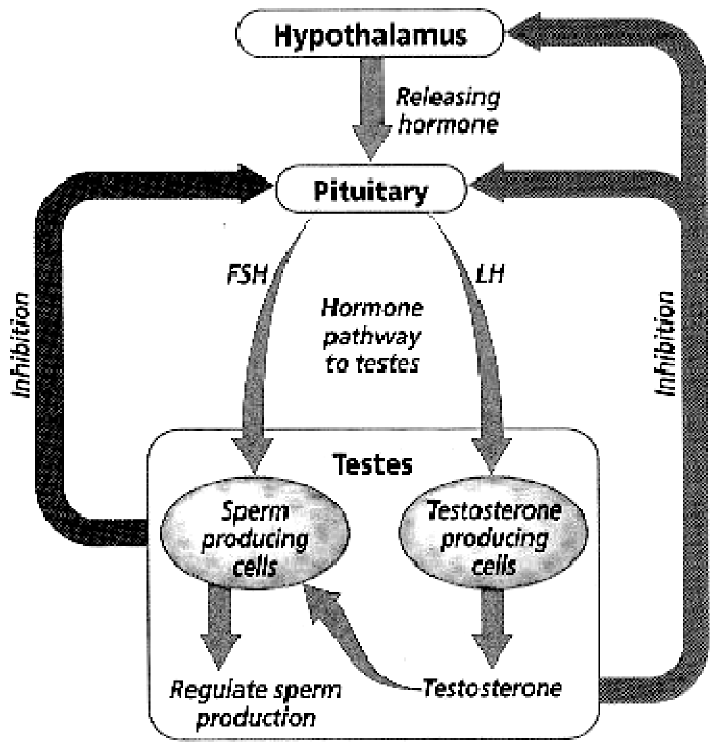

Fig.2.5. Hormonal control of Spermatogenesis

2.3. Oogenesis (Development of Mature Ova)

(a) Stages of Oogenesis

Oogonia proliferate by cell division, involving **mitosis**. They then enter a growth period and are transformed into **1° oocytes**. Apparently, in mammals, the formation of 1° oocytes is restricted to embryonic life and that all the ova produced by a female during her reproductive life are derived from 1° oocytes already present in the ovaries at birth. The 1° oocyte undergoes the 1st meiotic division, forming one **2° oocyte** and the **1st polar body**. First polar body and 2° oocyte both have the same number of chromosomes (haploid number) but the polar body has little cytoplasm and eventually dies. Only seldom does the 1st polar body undergo a second meiotic division. The 2° oocyte undergoes the second meiotic division producing the **mature ovum** and the **2nd polar body**. Both of which have the same number of chromosomes, but again the 2nd polar body has little cytoplasm and dies. Therefore, the *primary oocyte produces only one mature ovum*.

In eggs the period of **growth** & **differentiation** occurs before the 1st meiotic division. During this time the oocyte increases tremendously in volume (i.e. 27,000 fold increase in some **amphibians** (*viz.* Rana, the grass frog). During this growth phase the nucleus swells in size, mainly due to an increase in **karyoplasm** (nuclear sap). (The nucleus is sometimes called the **germinal vesicle**). The chromosomes during this time enter diplonema of prophase I, with the homologous chromosomes synapsed, and remain in this stage throughout the growth period (which may be years).

In some large-egged species, the chromosomes give out thin loops which are thought to be loops of uncoiled chromosomes. These loops give the chromosome the appearance of lampbrushes, therefore they are called **lampbrush chromosomes**. These loops are the **synthesis site** for **mRNA**, using RNA polymerase. Newly synthesized RNA complexes with proteins forming **RNP (ribonucleoprotein)**.

Some mRNA would then go to the ribosomes in the cytoplasm and produce the storage proteins the egg is accumulating. Some of this RNA is pre-mRNA. Highly repetitive DNA is sometimes transcribed along with structural genes. Lampbrush chromosomes are nearly universal but not essential, because of its absences in some species. In young oocytes large amounts of RNA are present, in the form of granules. Large numbers of ribosomes are synthesized during oogenesis. The genes which code for the molecules which make up the ribosomes may actually duplicate themselves. This increase in the number of genes, without mitosis is called **amplification** of the genes concerned.

For instance, the genes which code for 5S, 18S and 28S rRNA molecules undergo amplification. tRNA is also synthesized in large amounts. Ribosomal proteins are synthesized in large amounts. The nucleoli synthesize rRNA and assemble ribosomes. During oogenesis nucleoli may increase in number. Large amounts of DNA can be found in egg cytoplasm, as shown in amphibians, sea urchins and insects.

Mitochondria have circular molecules of DNA within them. Mitochondria are relatively scarce in young **oocytes**, but the numbers of mitochondria may increase considerably as the oocytes age. Large numbers of mitochondria are produced and saved for post-fertilization development. (*e.g.* *Xenopus* eggs have 10^5 times more mitochondria than somatic cells). The mitochondria form juxta-nuclear aggregates, known as yolk nuclei, **mitochondrial clouds** or **Balbiani bodies**.

B. Follicle Development in the Ovary Sheet

In chordates, and especially in mammals, the primary oocytes are surrounded by follicle cells. In mammals, the follicle cells proliferate until they are several cell layers thick around the oocyte. The follicle cells and oocyte produce finger-like microvilli which interdigitate one with the other. This space occupied by microvilli between the follicle cells and oocyte is called the ***zona radiata***. At this time, fluid-filled spaces appear between the follicle cells. These spaces unite to form a single fluid filled space, the **Antrum** (cavity of the follicle). As the follicle continues to grow the oocyte becomes more displaced to one side of the Antrum. The oocyte becomes covered with a mound of follicle cells, collectively called the ***cumulus oÖphorus***. The follicle cells, as a whole, are arranged as a stratified epithelium called the ***stratum granulosum***. Around the stratum granulosum is a layer of ovarian connective tissue, called the ***theca folliculi***. The follicle is now mature and comes into contact with the ovary wall. The follicle then ruptures, along with the ovary wall, extruding the oocyte from the ovary into the **Fallopian tube (oviduct)**, which leads to the **uterus**. In mammals the egg being discharged from the ovary carries with it a layer of follicle cells, referred to as the ***corona radiata***. These are later lost as the egg descends the oviduct. The empty ruptured follicle becomes the ***corpus luteum*** which degenerates further to form the ***corpus albicans*** which eventually degenerates completely.

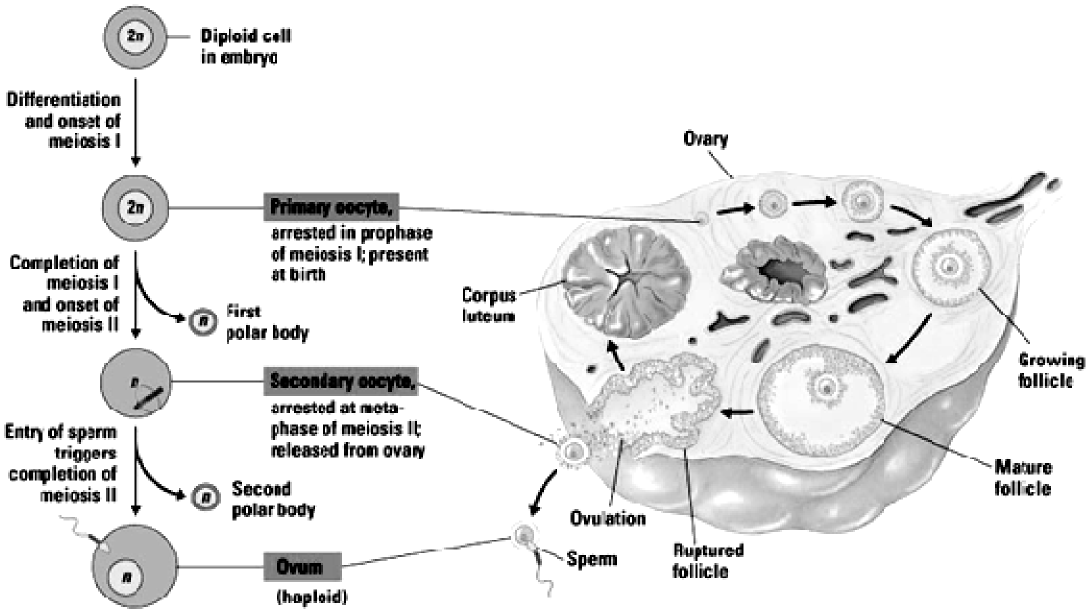

Fig.3.6. Ovary sheet and oogenesis

In some invertebrates (some insects, annelids and mollusks) there are **nurse cells** in addition to the follicle cells. The nurse cells are formed when the oogonium undergoes 4 mitotic divisions. This results in the formation of a group of 16 cells, which are surrounded by follicle cells. Fifteen (15) of these cells become nurse cells and one becomes the 1° oocyte and undergoes meiosis. Therefore nurse cells are not the same as follicle cells.

(c) Egg Differentiation

During the growth phase of oogenesis the oocyte accumulates great amounts of nutrient material which the embryo will need during development. In the vertebrates the egg proteins and phospholipids are thought to be made in the liver. They are then transported to the ovary by the blood. This also appears to be the case with fat bodies in insects. Preliminary data suggest that in cnidarians and crustaceans the egg may be capable of manufacturing some of its own storage proteins and lipids. Accompanying the accumulation of food reserves are some other cytoplasmic changes. In general, the organization of the cytoplasm increases in complexity. **Vitellogenesis** (yolk formation) is the most dominant structural change that occurs during oocyte maturation. ("**Yolk**" is a morphological or structural term and doesn't speak about the chemical structure).

The chemical composition of the yolk of different species varies. Yolk is composed of proteins, phospholipids and a smaller amount of neutral lipids and carbohydrates. Two of the more important yolk proteins are vitellin and phosvitin. **Vitellin** is large (MW of 350,000 to 500,000 daltons) and has a large amount of lipid bound to it (17.5%-amphibians; 30% in Crustaceans). **Phosvitin** is a smaller molecule (MW of 35,000 daltons) and has a large amount of phosphorus (8%-amphibians). The amount of yolk and its structural relationship with the functional cytoplasm differs with different species. The amount of yolk that is present in an egg also influences the mechanics of embryonic development.

Based on the amount of yolk and its of distribution, eggs are: **oligolecithal (isolecithal),**

moderately telolecithal, highly telolecithal or centrolecithal. **Oligolecithal** eggs contain little yolk which is uniformly dispersed throughout the cytoplasm; these are found in echinoderms, most mammals and in lower chordates (*viz. Amphioxus*, tunicates). Telolecithal eggs have more yolk and it is not equally dispersed, but rather, is more concentrated at one end of the egg.

1. In **moderately telolecithal** eggs, more yolk is found in the lower part of the egg (called the **vegetal pole**) and more cytoplasm is found in the upper part of the egg (called the **animal pole**); these are found in amphibians, cyclostomes, elasmobranchs & some bony fish).
2. In **highly telolecithal** eggs the functional cytoplasm is merely a small disc sitting atop the yolk, which nearly fills the remainder of the egg; these are found in birds, reptiles and most bony fish. *The "yellow" of a hen egg is actually the egg cell.*
3. In **centrolecithal** eggs a thin layer of cytoplasm envelops the centrally located yolk. In addition, at the center of the egg there is a small island of cytoplasm which contains the nucleus. These are found in arthropods, especially insects.

E. Advanced oocytes and ova display **polarity**. That is, various substances and cellular components are unequally distributed, in gradient fashion, from one side of the cell to the other or from one pole to the other. The nucleus is situated nearest to the **animal pole**. 2. The other pole is termed the **vegetal pole**. In telolecithal eggs more yolk is found at this pole. When the egg undergoes meiosis the nucleus migrates to the animal pole and thus the polar bodies are discharged at the animal pole. Therefore, it is possible to distinguish between the animal and vegetal poles, even in oligolecithal eggs. Many species have pigment granules in the eggs. These are, in many species, unevenly distributed from one pole to the other. This polar distribution is distinct in amphibians. The cortical cytoplasm of the animal pole is highly pigmented, whereas the vegetal pole is sparsely pigmented.

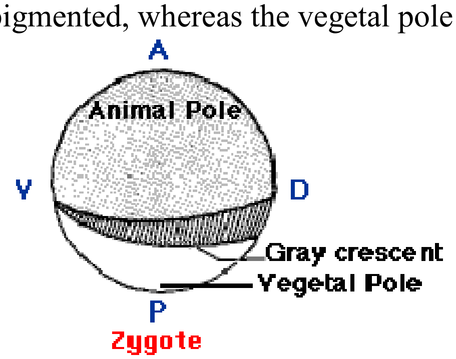

Fig.3.7. Amphibian egg

While the pigment granules may not be critical to the development of the embryo, they are important as landmarks in studying the development of the embryo. In young oocytes of frogs, RNA is concentrated at the animal pole. In mammals one side of egg *(which corresponds to the dorsal side of the embryo)* contains a higher concentration of mitochondria, RNA and proteins. The other side has more water and contains numerous vacuoles.

Just under the cell membrane of the egg is a thin layer of superficial cytoplasm called the **cortex**. The cortex is highly viscous (gelatinous), whereas the rest of the cytoplasm is less viscous. The cortex contains **microvilli**, which form **pinocytotic** vesicles (*cell drinking*). Cortical granules are important structures within the cortex. They are formed by the golgi apparatus. They are lined with simple membrane and contain mucopolysaccharides. They are found in many, but not all, animal eggs regardless of phylogeny, *i.e.* they are found in man, mice, hamster & rabbit, but not in rat and guinea pig. In the unfertilized mouse egg, there are 4000 cortical granules and in the unfertilized sea urchin egg, 15,000 cortical granules. Cortical granules play an important role at the time of fertilization.

During the growth process the oocyte remains in diplonema of prophase I. After growth, the oocyte now undergoes the remainder of meiosis. At the end of prophase the nuclear envelop breaks down, which results in the mixing of the karyoplasm (nuclear sap) with the cytoplasm. In some animals this mixing is an essential prerequisite to activation of the egg after fertilization.

(d) The Egg Membranes

In addition to the cell membrane (plasmalemma), most animal eggs have additional special membranes. These membranes are: 1° (primary) membranes *(are produced by the egg)*. 2° (secondary) membranes *(are produced by the Follicle cells)*.3° (tertiary) membranes (*are deposited around the egg by the oviducts or other accessory organs*).

Examples include: The **Zona Pellucida** (**fertilization envelope**) in mammals, which is a 1° membrane layer around the plasmalemma, made of **glycoprotein**. Amphibians secrete a jelly layer around the egg as it passes down the oviduct. Some sharks and rays have shell glands which secrete leathery shells around their eggs. The Avian egg has 5 membranes: The 1° membrane is the vitelline membrane; it covers the egg cell ("yellow" or "yolk" of the egg). *The remainder of the membranes are tertiary.* The second membrane is the Albumen (*or "white of the egg"*) and is secreted by the oviduct and uterus. The next two membranes are the inner and outer shell membranes. They adhere to one another over most of the egg except at the blunt end of the egg where they separate and form an air space between them. The fifth membrane is the shell, composed chiefly of $CaCO_3$.

2.4. Hormonal Control of Oogenesis

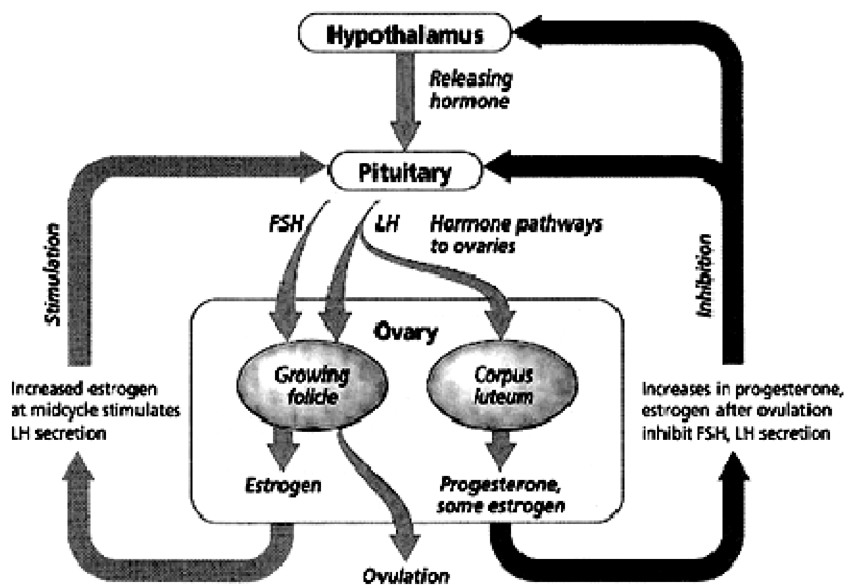

Fig.3.9. Hormonal Control of Oogenesis