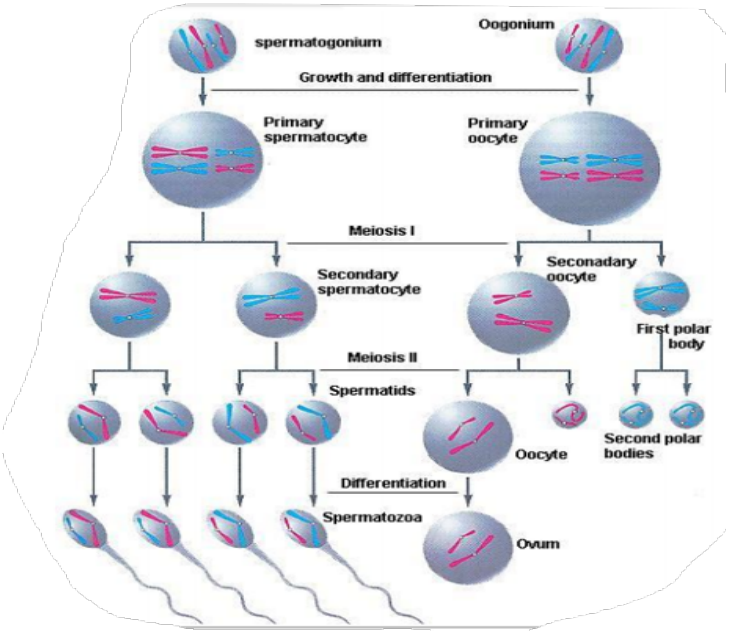
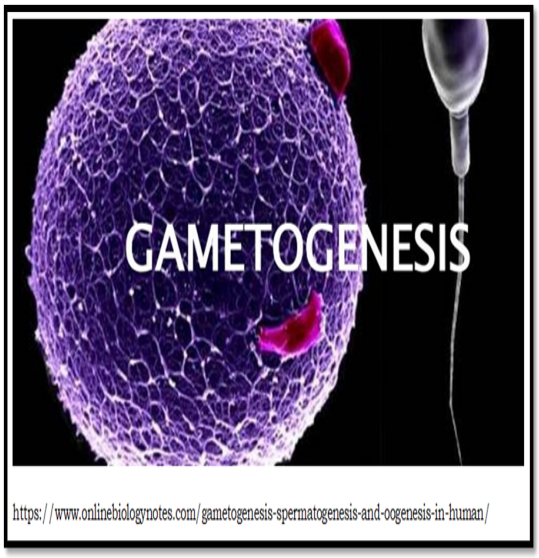
Module 3

GAMETOGENESIS

This module will look into the formation of gametes (**gametogenesis**) which are the key players in the development of sexually reproducing animals. Development begins first with the male and female parents producing the gametes sperm and eggs, respectively in their gonads. Once there are gametes, the next stage of development is **fertilization** which will be covered in the next module, as well as the transport of the gametes that are ready for fertilization and after fertilization





Learning Objectives: At the end of this lesson, the student should be able to:

1. Discuss the formation of gametes in both male and female representative vertebrates (frog, and amniotes).
2. Compare and contrast oogenesis from spermatogenesis.

Introduction

In gametogenesis, the germ plasm which has the hereditary endowment (in contrast to somatoplasm) is converted into highly specialized sex cells in the gonads that are capable of fusing at fertilization and produce a new being, a new life. According to Carlson (1996), gametogenesis is divided into four phases:

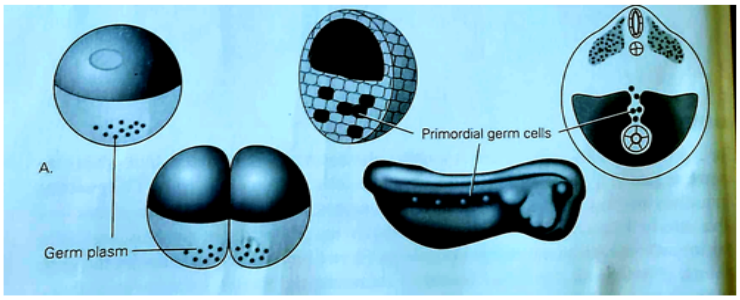
1. The origin of the primordial germ cells and their migration to the gonads.

2. The multiplication of the germ cells in the gonads through mitosis.
3. Reduction of the chromosome number by half during meiosis.
4. The final stages of maturation and differentiation of the gametes into spermatozoa and ova.

1. Primordial germ cells (PGCs) and their migration to the gonads

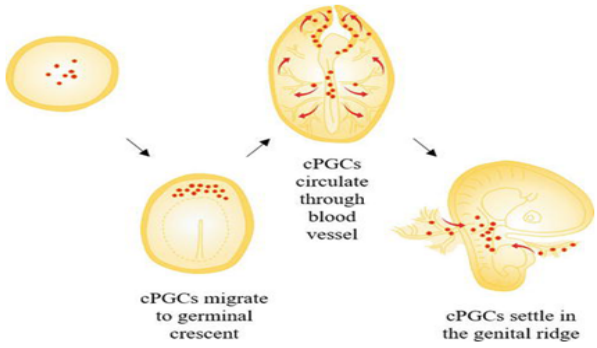
The PGCs that will give rise to the gametes are recognizable at an early stage of development of vertebrates.

In anuran amphibians, the germ plasm/PGCs can be recognized in the vegetal region of a fertilized egg, or during cleavage then into the entodermal floor of the primitive gut.

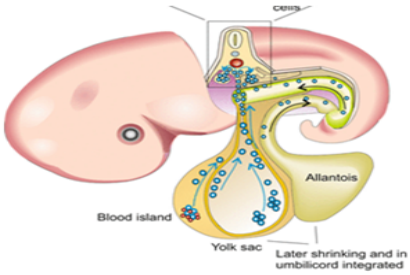


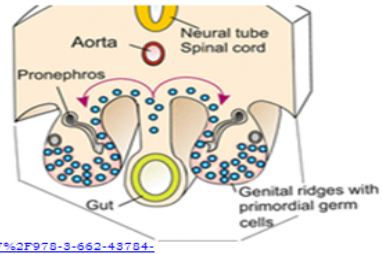
Among amniotes (reptiles, birds and mammals), the PGCs arise in the epiblast of the early embryo and take temporary residence in extra-embryonic tissues then later on return to the body of the embryo proper.

In birds, the PGCs are recognizable in the *germinal crescent*, which is located beyond the future head region of the embryo. And migrate through the vitelline veins until they settle in the genital ridge, the future gonad of the organism.



In mammals, the PGCs are found in the posterior wall of the yolk sac near the origin of the allantois. These cells reach the future gonads by migration around the wall of the posterior gut and then through the dorsal mesentery.





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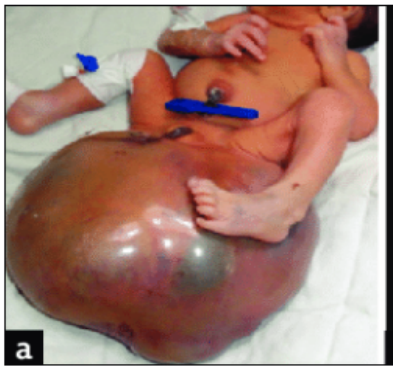
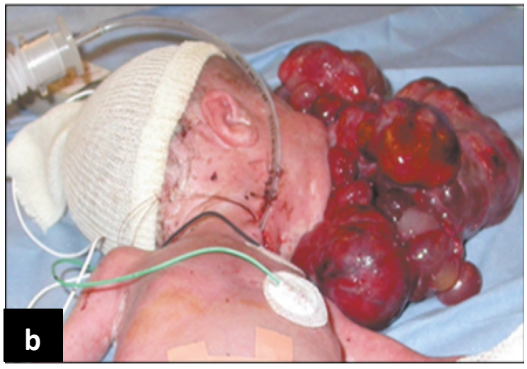
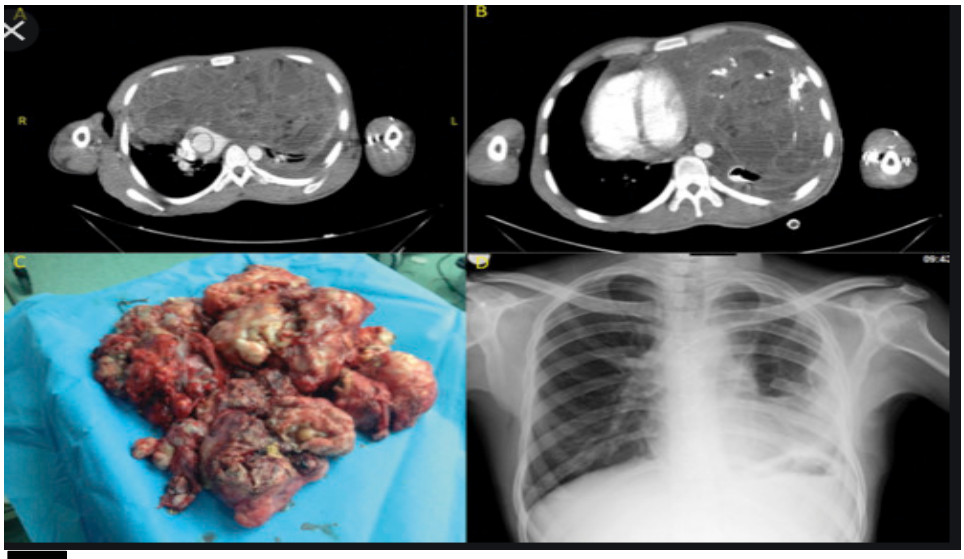
When PGCs migrating along the gut are misdirected to the gonads, meaning they go elsewhere, these cells may develop into **teratomas** (see image below). Teratomas are bizzare growths of a mixture of highly differentiated tissues such as skin, hair, cartilage, teeth, etc in one site. These teratomas can be found in the sarcoccygeal region (a), the oropharyngeal region (b) and the mediastinum (c).







(a) https://www.researchgate.net/figure/a-Clinical-picture-of-giant-sacrococcygeal-teratoma-b-and-c-Solid-white-arrow-and_fig1_338861929
(b) https://www.ajronline.org/doi/pdf/10.2214/ajr.183.2.1830493
(c) https://www.sciencedirect.com/science/article/abs/pii/S000349751630371X

2.The multiplication of the germ cells in the gonads through mitosis.

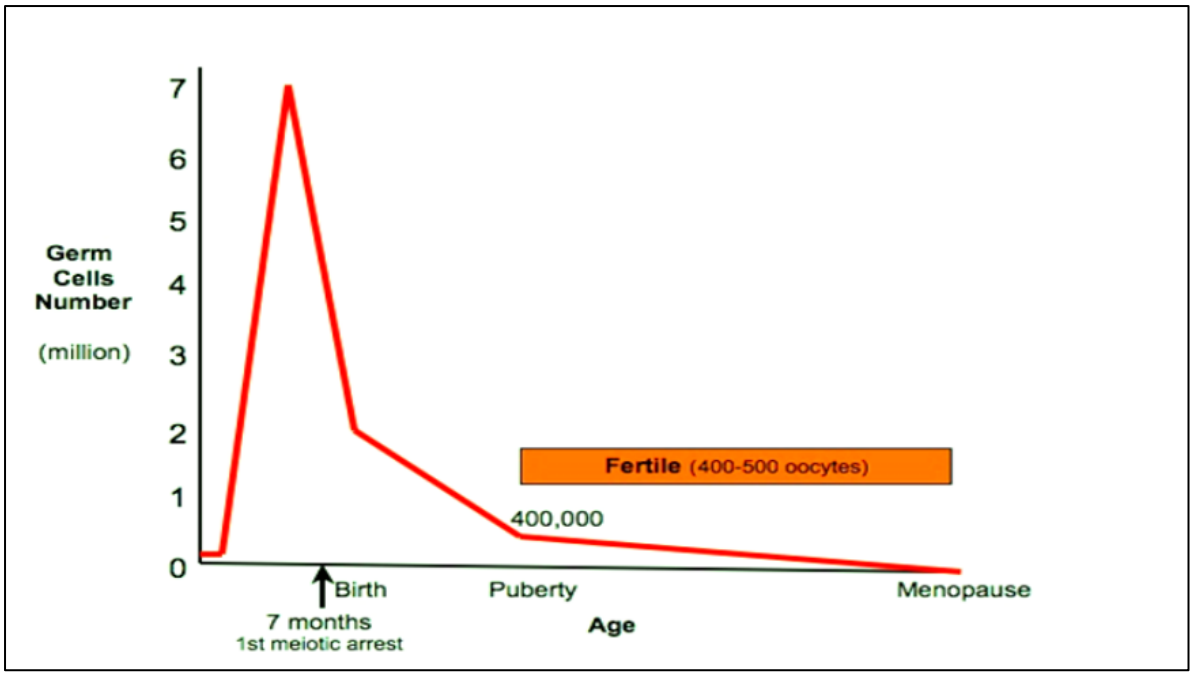
Once the PGCs are established and settled in the embryonic gonad, they proliferate in numbers by mitosis. The actively dividing germ cells in the female are called **oogonia** and in the male, they are called **spermatogonia.**

As what you know about **mitosis**, each germ cell that divides produces two **diploid** daughter cells. Each resulting daughter cell is genetically equal with each other, and with their parent cell. These mitotic divisions by the germ cells increases their population from hundreds to millions. The fate of the oogonia and spermatogonia of

humans are different from each other (Carlson, 1994). The cell cycle and stages of mitosis must be clear to all by this time because we will not go through with them here anymore.

OOGONIA	SPERMATOGONIA
1. Intense mitosis in embryonic ovary from the 2nd through the 5th month of pregnancy.	1. Mitosis begin in the embryonic testes and continue to divide throughout the human male's life.
2. At this time, the increase is from a few thousand to nearly 7 million, which then stops here.	2. The seminiferous tubules of the testes are lined with a germinative population of spermatogonia
3. Then from 7M, these number drastically drop to about 1M-2M by birth time.	3. At puberty, there is a wave of mitosis in subpopulations of spermatogonia.
4. Then further drops to about 400,000 by 10 years old and by age 50, the number has declined to almost zero. Decline is due to atresia.	4. The progeny of these divisions enter meiosis as synchronous groups at the onset of puberty.
5. And in the reproductive life of a woman, there are about 400-500 oocytes that can be ovulated and be fertilized.	

Below is a graphical representation of the "story" of human oogonia given in the table above. This graph was lifted from Carlson (1996).

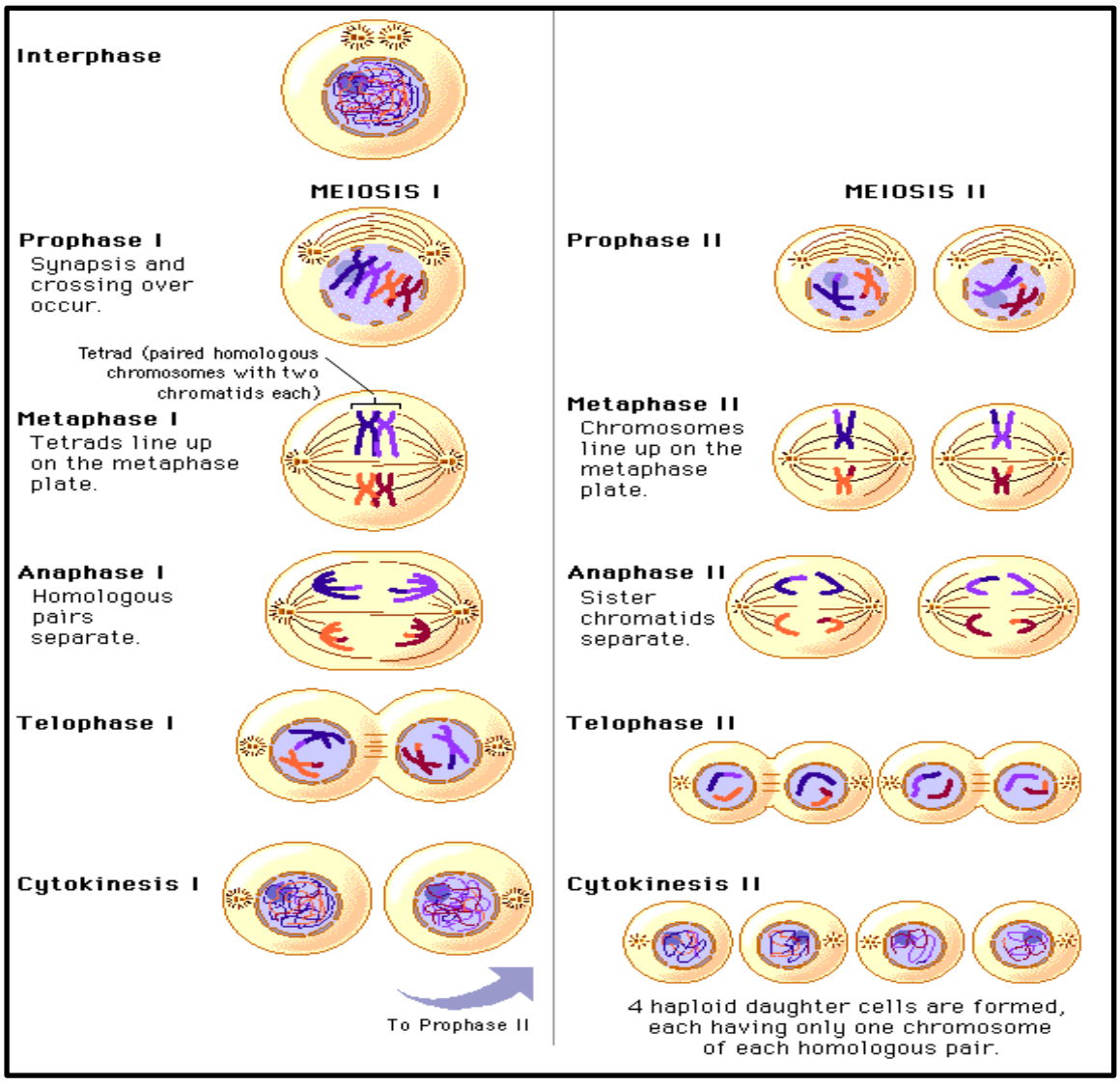


3. Reduction of the chromosome number by half during meiosis.

In all animals that reproduce sexually, **meiosis** is a must for all the sex cells. The biological significance of meiosis in man is the same for all other species. The significances are as follows and the figure at the bottom is a summary of the events of meiosis.

a. Reduction of the chromosome numbers from diploid (2n) to **haploid (n)** to maintain the species number of chromosomes from generation to generation.
b. Independent reassortment of maternal and paternal chromosomes for better mixing of genetic characteristics (genetic variability).
c. Further redistribution of lesser amounts of paternal and maternal genetic information through the process of **crossing-over** during the first meiotic division.

Shown below is a summary of events in the two phases of meiosis. Other information about each phase will be in the succeeding pages.

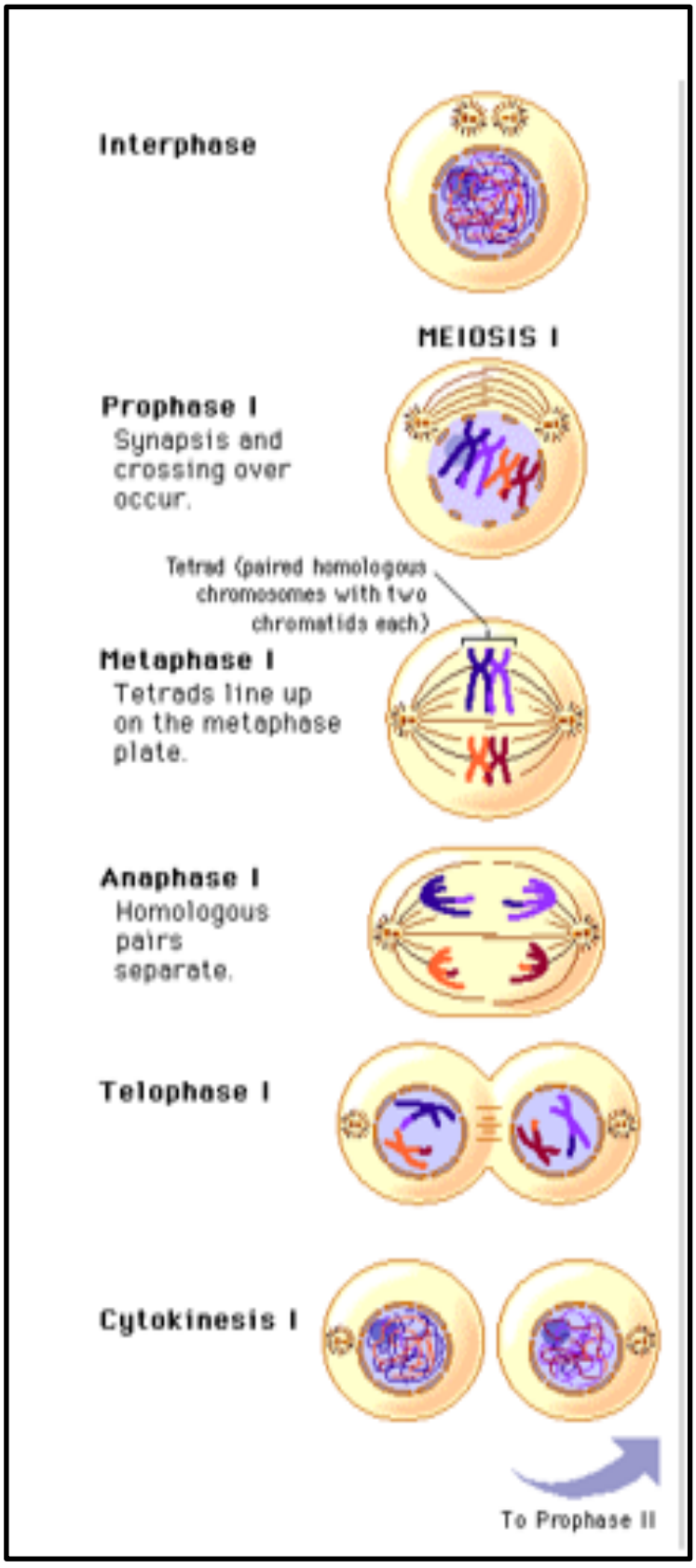


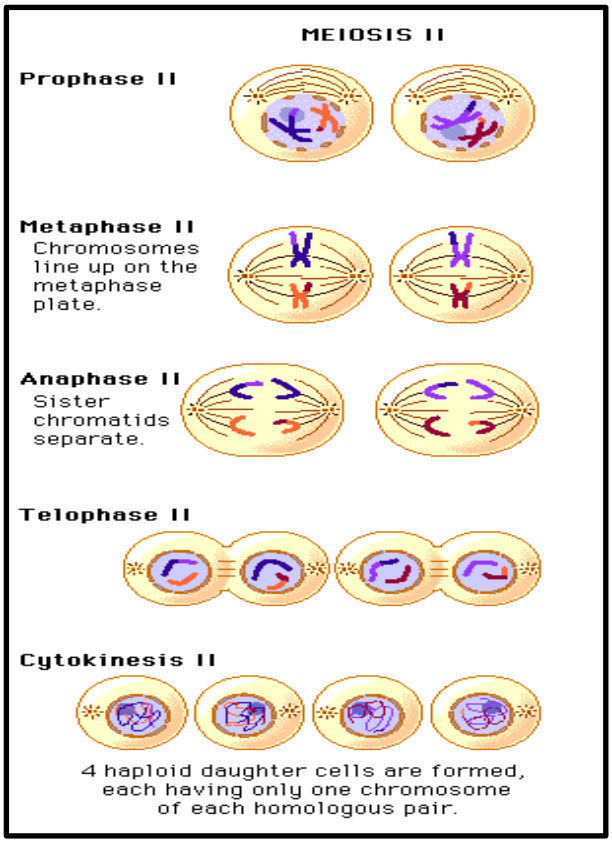
http://www.phschool.com/science/biology_place/labbench/lab3/concepts2.html

Meiosis has two phases.

The first phase is: (a) **Meiosis I** which has a prolonged prophase. It is in **prophase I** that there is pairing (**synapsis**) of homologous chromosomes and frequent crossing-over that results in the ***exchange of segments*** between members of the paired chromosomes. The homologous chromosomes or pairs (called **tetrads)** line up in the equatorial plate during **metaphase I** so that at **anaphase I**, one chromosome of the homologous pair moves toward one pole of the spindle and the other chromosomes move toward the opposite pole. Take note that when there is complete cytokinesis at the end of **telophase I**, each daughter cell contains the haploid (1n) number of chromosomes, but each chromosome still consists of two chromatids (2c) connected by a centromere.

Take note also, that before meiotic division I starts, DNA replication has already happened. So, the cell is 2n,4c (diploid state (2n), but because of replication, its DNA content (2c) has already doubled the normal amount, 4c).

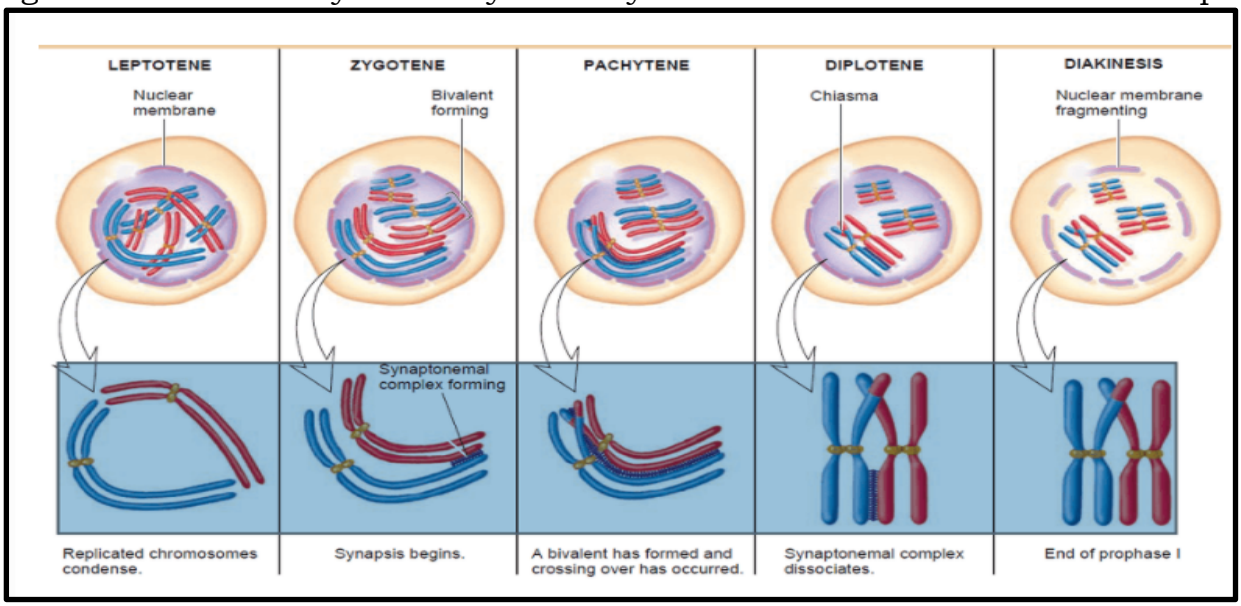




The second phase of meiosis is called (b) **Meiosis II** which is basically similar to an ordinary mitotic division. The only difference is that the resulting daughter cells are haploid (1n, 2c). No DNA replication occurs prior to **prophase II.**

At **metaphase II**, the chromosomes line up along the equatorial plate. The sister chromatids separate at the centromere, and with the spindle fibers attached to the kinetochores of each sister chromatids, they separate and migrate to opposite poles at **anaphase II.** The resulting daughter cells at the end of **telophase II** are now fully haploid (1n, 1c). **Remember**, the genetic makeup of the chromosomes has already been modified due to crossing-over at prophase I.

It was said earlier that prophase I is a long phase. This is because it has five subphases, which are: **leptotene, zygotene, pachytene, diplotene** and **diakinesis.** The details of their features will be tackled in the laboratory, but the figure below shows you briefly the key events and features of each subphase.

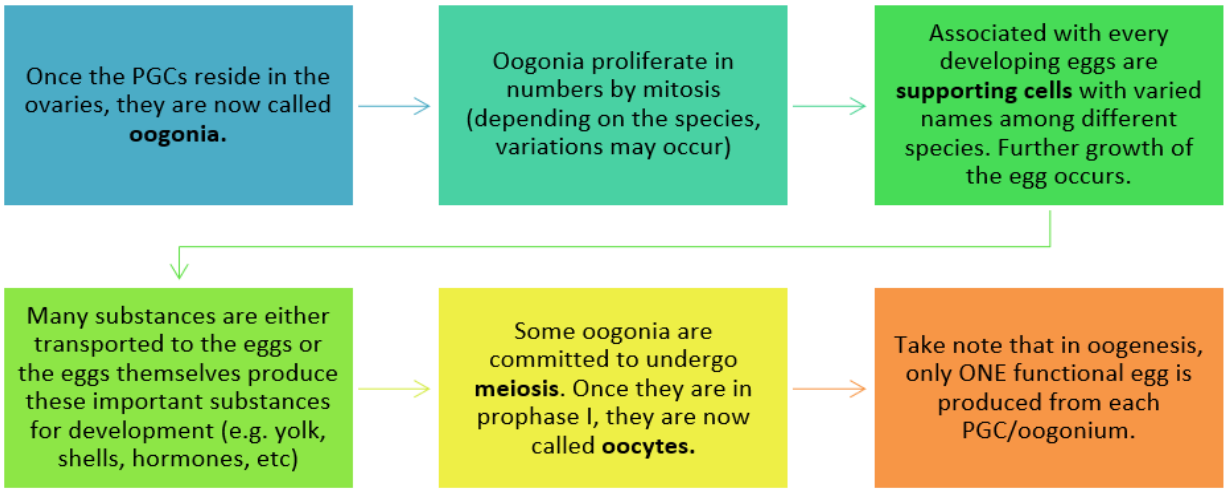


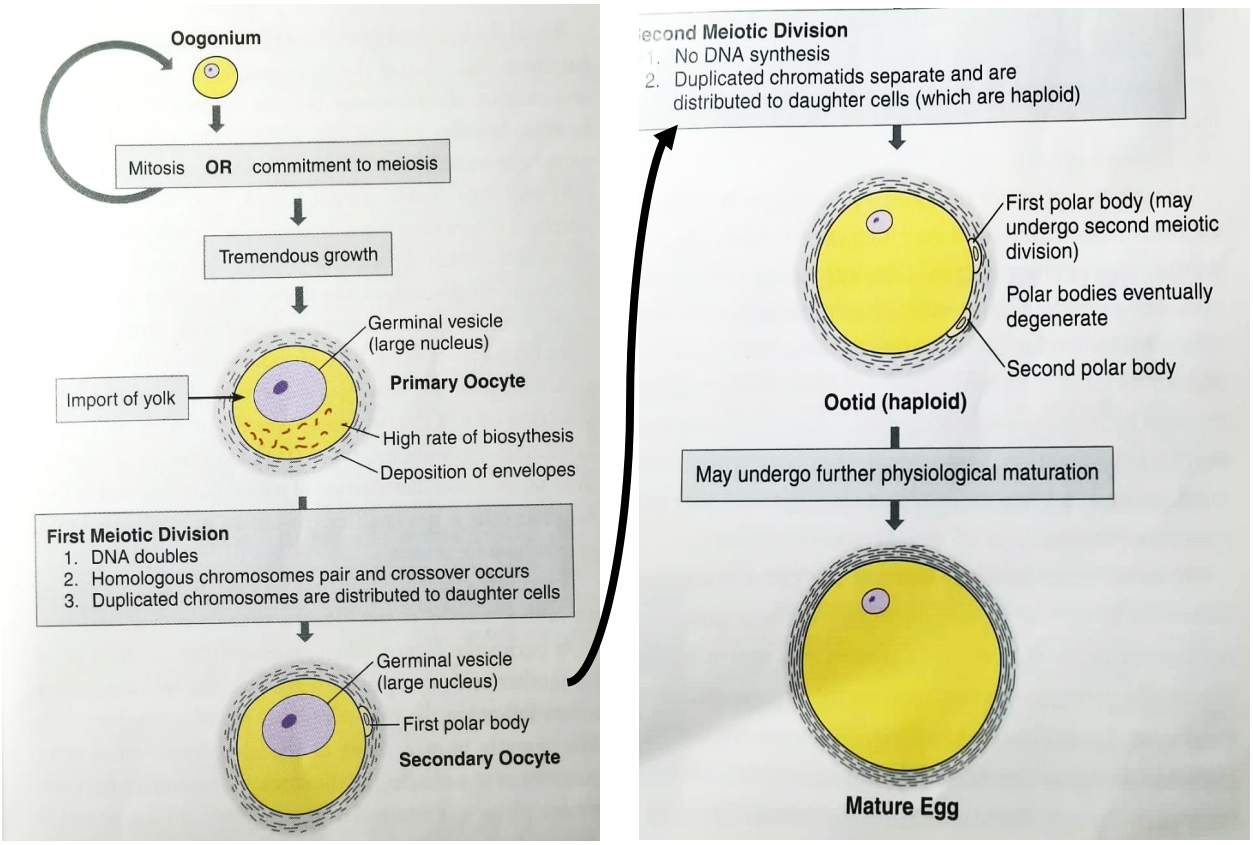
https://microbenotes.com/prophase

*Discussion 3.1. What is **synapsis?** Differentiate it from **synaptonemal complex.** Differentiate synapsis from **chiasma** (pl. chiasmata). What can happen if there is no chiasma formation during meiosis? How is the term **bivalent** different from the term **tetrad?** What is What is **nondisjunction** and **aneuploidy?** Answer in not more than five sentences.*

4. The final stages of maturation and differentiation of the gametes into ova and spermatozoa.

a. **OOGENESIS** occurs in the ovaries of several animal creatures and there will be varying features and events taking place in each of them. However, there is a basic plan which all of them go through.

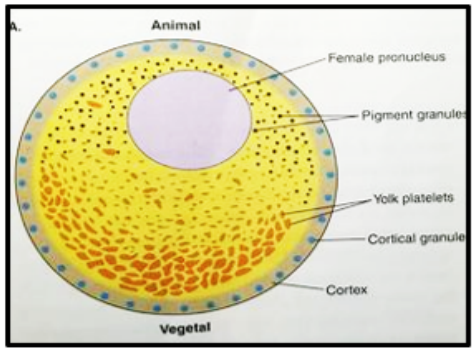


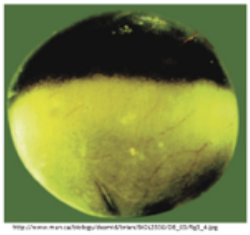


The functional ovum produced by oogenesis is highly organized. The large amounts of materials stored inside the egg that are essential in the development of a new being are so well-arranged. Again, variations may exist in the organization of an egg in different species of animals.

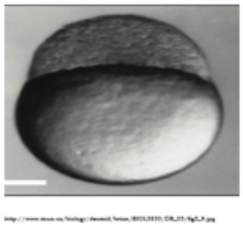
In the laboratory, you will be looking at photomicrographs of oogenesis in frogs and humans. These will be the histological views of their ovaries as well as the different stages of oogenesis in these representative vertebrates.

In general, the generic organization of an ovum is shown in the figure below:

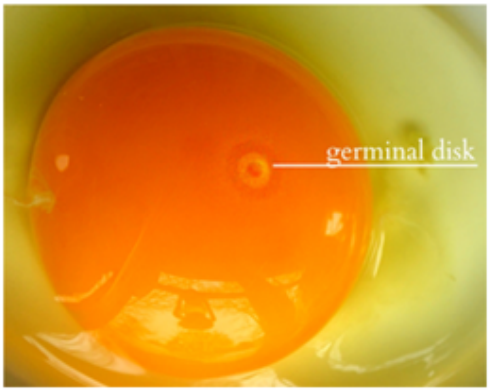




(a) Frog



(b) zebrafish



https://www.communitychickens.com/reading-germinal-disk-breeding/

(c) bird

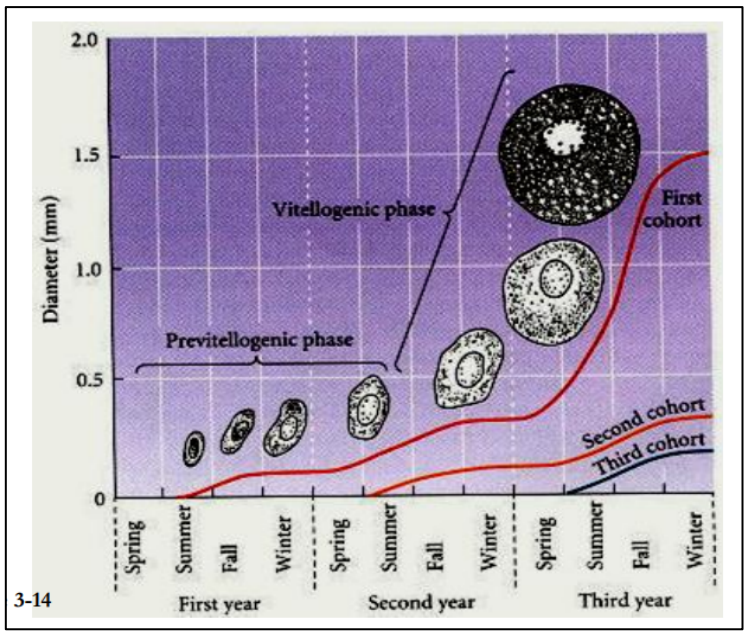
1. The yolk material (great source of nutrients) is assembled in organelles called **yolk platelets** that are unevenly distributed. Recall the types of eggs based on the amount and distribution of yolk: *alecithal or microlecithal amount of yolk = isolecithal distribution; mesolecithal and macrolecithal amount of yolk = telolecithal distribution.*
2. In an egg with uneven distribution of yolk, the yok-rich side or region of the egg is often called **vegetal pole**, while the less yolky region is called **animal pole**. In the frog and zebrafish eggs shown on the left, the top part is the animal pole and the bottom part is the vegetal. In the bird egg at the bottom left, the **germinal disk** is the region of the animal pole and the rest of the egg where yolk is abundant is the vegetal pole. REMEMBER, the albumen is not part of the egg cell, it is an extra-cellular covering.
3. As you will see in the laboratory, amphibian eggs have **pigment granules** of various hues/shades that may be localized.
4. The egg nucleus is somewhat large and is often called the **germinal vesicle.**
5. Abundant vesicles called **cortical granules** are located near the surface of the egg. We will see the roles of these granules during fertilization.
6. Under the electron microscope, the cytoplasmic periphery of the egg is ultra-structurally different from the inner cytoplasm. This area is called the **cortex.**

Discussion link 3.2 Why is yolk an imported material? What are the two molecules that represent the precursor of the yolk in frogs, vitellogenin? Answer in not more than five sentences.

Let us now look at how oogenesis take place in selected vertebrates which as mentioned earlier, will be studied thoroughly in the laboratory.

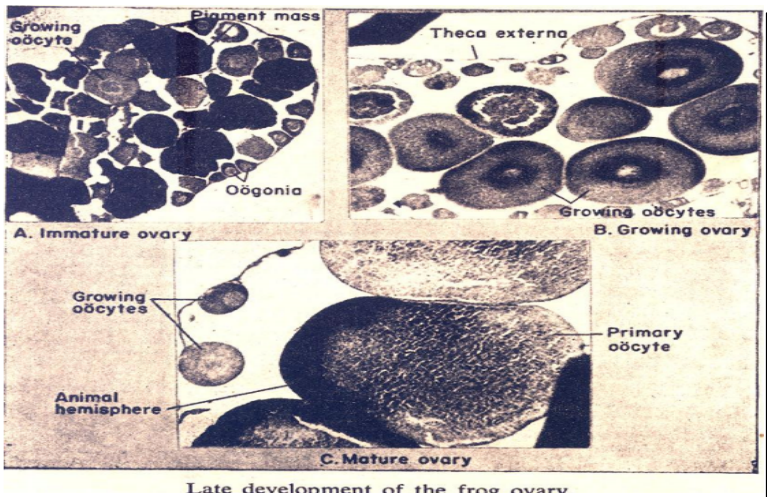
a.1 Amphibians

Since the fertilization of the amphibian eggs are external, a large number of eggs and their release at one time during the year is their reproductive pattern. This pattern is quite different from most amniotes such as birds and mammals, as we will see later. A graph lifted from Carlson (1996) shows the growth of oocytes in the frog during the first three years of its life. The graph also shows that inside the ovaries of a frog, three batches of eggs are formed. It also clearly shows that the maturation of amphibian egg cells has three phases: **previtellogenic** (before the deposition of yolk) **phase, vitellogenic** (major period of yolk deposition) **phase** and **maturation phase** (oocyte is released from its meiotic block through the action of progesterone)**.** The table below summarizes the key events in each phase of amphibian oogenesis.

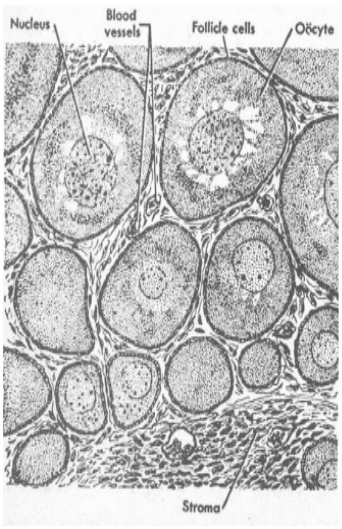


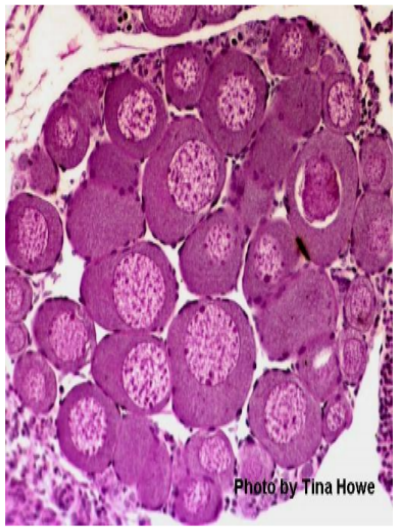
Previtellogenic	Vitellogenic	Maturation
Start to diplotene phase of meiosis	More changes are seen in the cytoplasm that are associated with the formation of yolk	The **progesterone** released by the follicular cells around the oocytes releases the egg from its first meiotic block.
Increase in number of mitochondria, RNA synthesis	Proteinaceous material is stored in the membrane bound **yolk platelets**; lipid is stored in inclusions called **lipochondria**; and carbohydrate is accumulated as **glycogen granules.**	As maturation proceeds, the oocyte can now be called an **ovum** or **secondary oocyte.**
Intense nuclear activity that nucleus diameter increase in size (called as germinal vesicle)	The yolk precursor **vitellogenin** is produced in the **liver** then pass through the **follicular epithelium** to reach the egg.	The maturing egg goes to another meiotic block (**at metaphase II**) due to the action of a **cytostatic factor.**
After the pachytene stage, chromosomes form large number of loops called **lampbrush chromosomes (LBC)**	Other cellular organelles increase in number at this phase. Numerous pigment granules are situated in the **animal pole**.	The release from this second meiotic block occurs only when a sperm has penetrated the egg during fertilization.
The LBC stage exposes 5% of the oocyte as template for RNA synthesis	Less heavily pigmented but more yolk laden is the **vegetal pole.**	Prolonged exposure to progesterone causes the germinal vesicle to breakdown. This breakdown allows mixing of the nucleoplasm and cytoplasm for the egg to undergo cleavage.
Large numbers of **nucleoli** are seen inside the nucleus.	The transition between these two poles is the **marginal zone.**	

Below are images of sectioned frog ovaries showing different structures and stages of egg development that you will see in the laboratory. On the right, is a flow diagram of frog oogenesis in general. Follicle cells are somatic cells that surround each 1° oocyte.

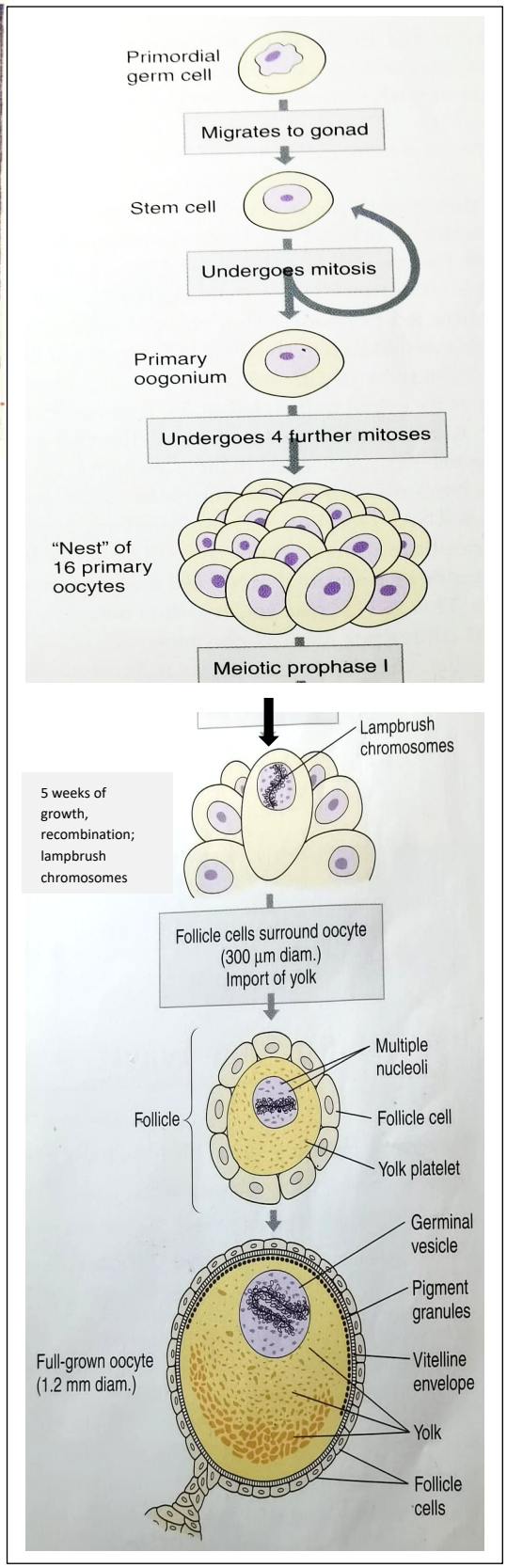


Late development of the frog ovary





Below are a male frog (smaller and on top) and a female frog performing **amplexus** to release the eggs and sperms for (external) fertilization.



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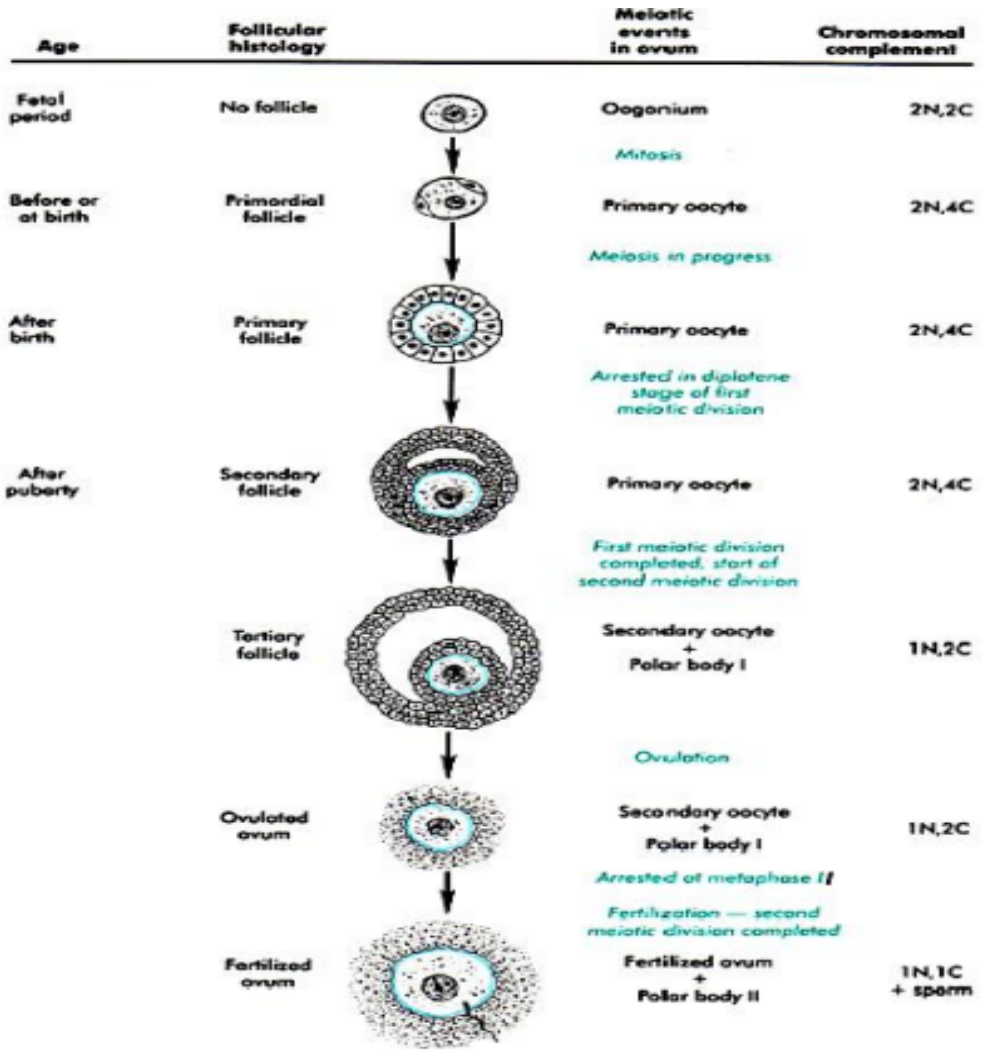
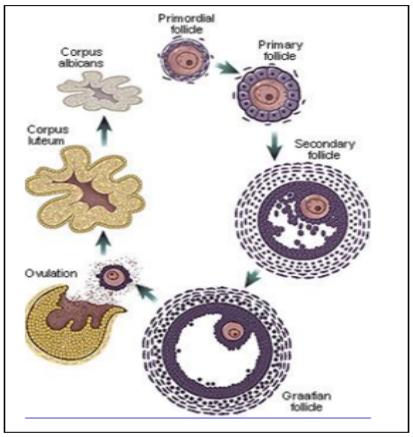
a.2 Mammals

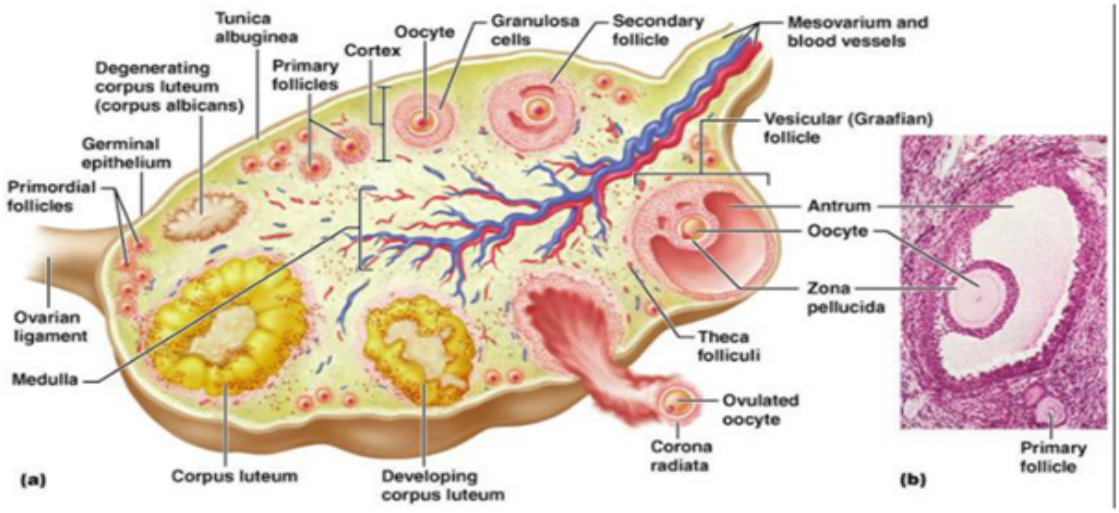
Mammals are different from other vertebrates because they cannot replenish the stored oocytes produced in the ovary before birth. Lifting from Carlson (1994), the summary of the major events in human oogenesis and follicular development (**folliculogenesis)** are shown below. Again, a follicle is the oocyte and surrounding follicular cells.





1. Primordial follicle – single layer of ovarian cells (called **follicular** or **granulosa** cells) surrounding a primary oocyte.
2. Primary follicle – at birth primary oocyte surrounded by one to two layers of follicular cells.
3. Secondary follicle – more than two layers of granulosa cells around the oocyte and start of the formation and recognition of a space called **antrum.**
3. Tertiary or Graafian follicle – a mature follicle seen prior to ovulation, with the 2° oocyte surrounded by several layer of granulosa cells and protrudes into the antrum as the **cumulus oophorus.** The antrum is filled with fluid called **liquor folliculi.**
4. Corpus luteum – Once the ovum is ovulated, the surrounding granulosa cells and the theca interna are left behind in the ovary and form this yellowish body. *What is the endocrine role of this body?*
5. Corpus albicans – a degenerated corpus luteum, appearing as a whitish scar tissue in the ovary.

Below is a schematic diagram of a human ovary showing sequence of events in origin, growth, and rupture of the ovarian (Graafian follicle) and in the formation and regression of the corpus luteum. The details of the structures and appearances associated with the formation of the mammalian ovum will be thoroughly studied in the laboratory.



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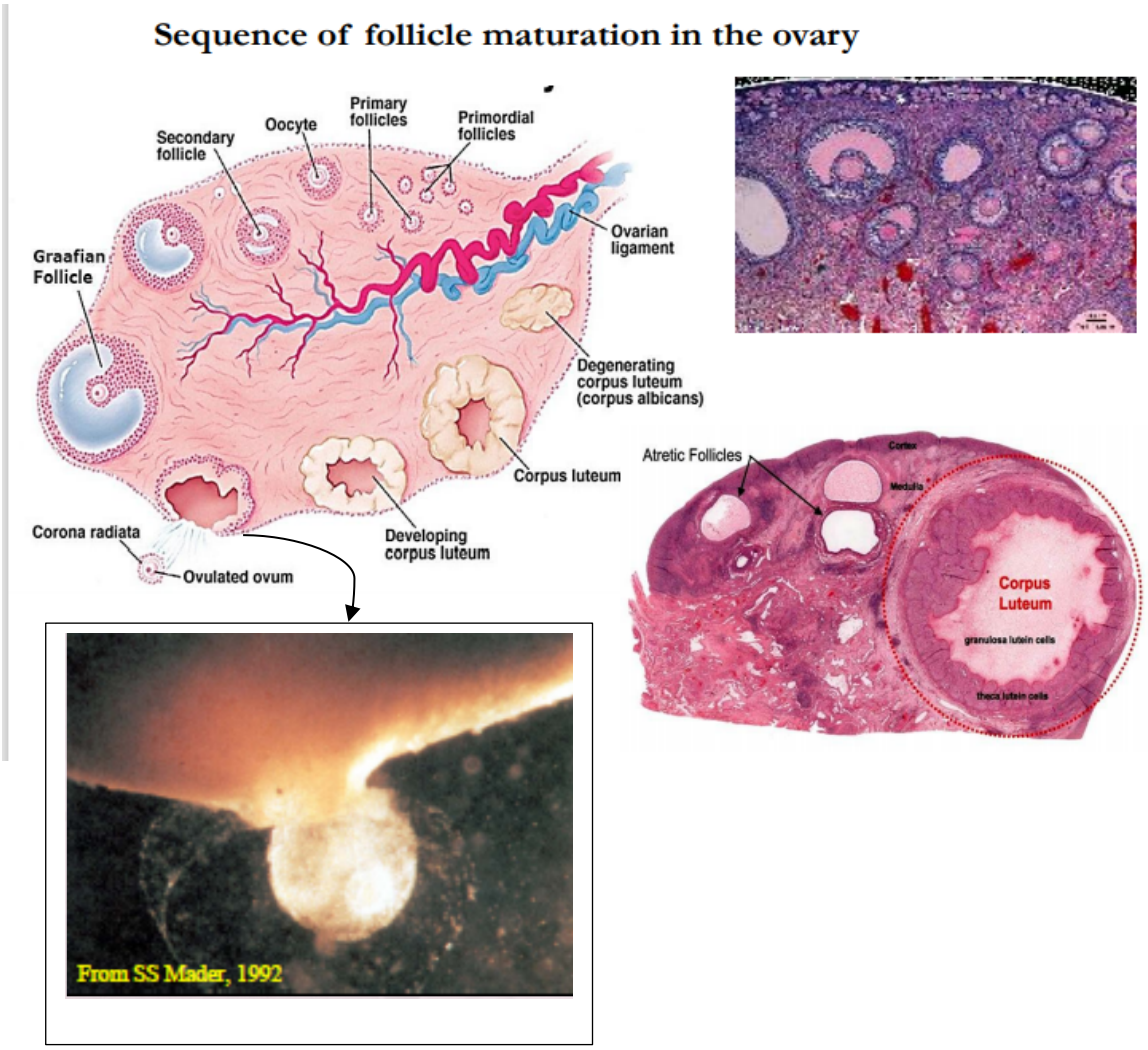

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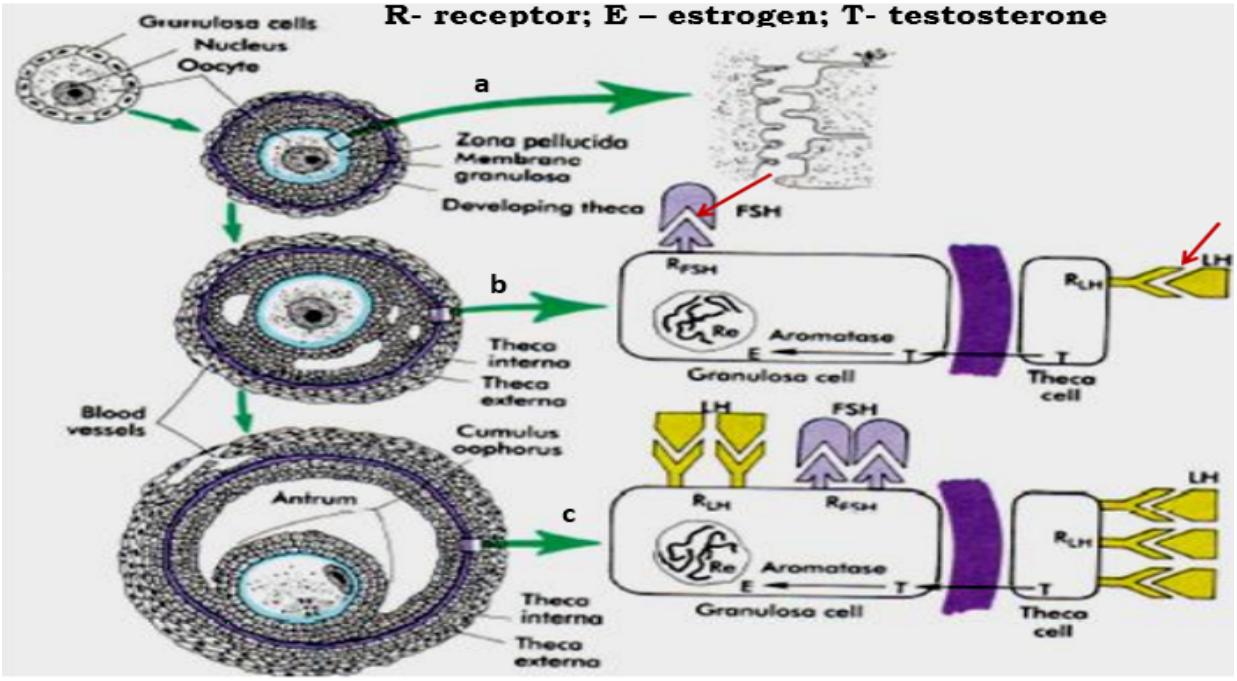
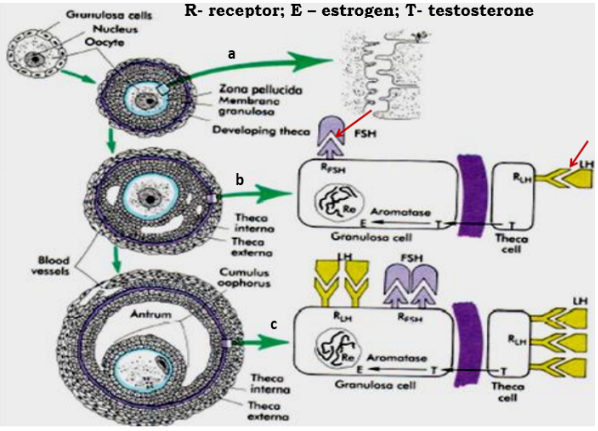
The development and maturation of mammalian egg cells as mentioned in Module 2, requires hormonal interactions. Make sure you still remember and have understood by this time the ovarian and menstrual cycle of primate mammals, especially the humans. But let us look again at the roles of hormones in the growth and maturation of the mammalian eggs. Below is a figure lifted from Carlson (1994) showing the growth and maturation of a follicle and the actual interactions of hormones in the process.



1. In the embryonic ovary, the oogonia are naked. But once meiosis begins, the primary oocytes are surrounded now by follicular cells, and are now called primary follicles.
2. Looking at part (a) above, there are prominent *microvilli* and *gap junctions* between the oocyte and the follicular cells. There is also now the presence of a **zona pellucida** between them, which has a number of functions as we will know in Module 4.
3. The gap junctions allow the exchange of amino acids and glucose metabolites needed for the growth of the oocyte.
4. **Meiotic inhibitory factor** is responsible for maintaining the first arrest of meiosis at the diplotene stage. The disruption of these gap junctions will stop the arrest.
5. Many of the primary follicles enlarge at prepubertal years due to increase in size of oocyte and the number of follicular cells.
6. The several layers follicular or granulosa cells around the oocyte rest on the **membrana granulosa.** This membrane forms a barrier to capillaries, thus making the oocyte and granulosa cells depend on it for diffusion of oxygen and nutrients.
7. Note the developing **theca** in (a). This additional set of cellular coverings are derived from the ovarian connective tissue (**stroma)** and increases its thickness as development progresses. Growth of the theca is permitted by the **angiogenesis factor** released by follicular cells themselves. More blood vessels that are formed, more nutritional support is given.
8. This thecal layer differentiates into two layers: the **theca interna** (highly vascular and glandular); and the **theca externa (**a capsule of connective tissue).
9. In part (b) which happens as puberty approaches, hormones are now influencing the further growth and maturation of the follicle. The **FSH** released by the _____



attaches to the receptors (R) on the follicular cells. This then stimulates the granulosa cells to produce small amounts of **estrogens** stimulating further the growth of the follicle. An obvious indication of further development is the formation of the **antrum**, a cavity filled with **liquor folliculi** or **antral fluid**. This fluid is initially formed by the follicular cells but later comes as transudate from the capillaries on the other side of the membrana granulosa. Once an antrum is formed and very visible, the follicle is now called a **secondary follicle.**

10. The secondary follicles produce significant amounts of estrogen. Also, at this time, the theca interna cells with receptors for **LH**, produce **androgens (testosterone)** and find their way to the granulosa cells.
11. The granulosa cells as influenced by FSH, produces **aromatase**, an enzyme that converts the theca-derived androgens into estrogens (mainly 17β-estradiol).
12. Estradiol leave the follicle thru blood vessels and exert important effects on other parts of the body. *Can you enumerate these effects?*
13. Estradiol also stimulates the formation of LH receptors on the granulosa cells (part c). In this way, the follicular cells can respond to surges of LH immediately before ovulation.
14. With the influences of several hormones, the antrum literally becomes "huge" and presses the follicle against the inner surface of the ovary. Again, this stage of the follicle is the Graafian follicle.
15. Remember, 10 to 12 hours before ovulation, meiosis is released from the arrest at diplotene stage.
16. The hormonal actions of FSH and LH prepare a follicle to be ovulated and possibly be fertilized.
17. Why only one follicle matures?

- as many as 50 follicles develop early in the cycle but only about 3 reach the size as big as 8mm
- only one follicle develops more receptors for FSH and LH
- this enlarging follicle releases inhibin that suppresses further release of FSH thus making the other follicles atretic.

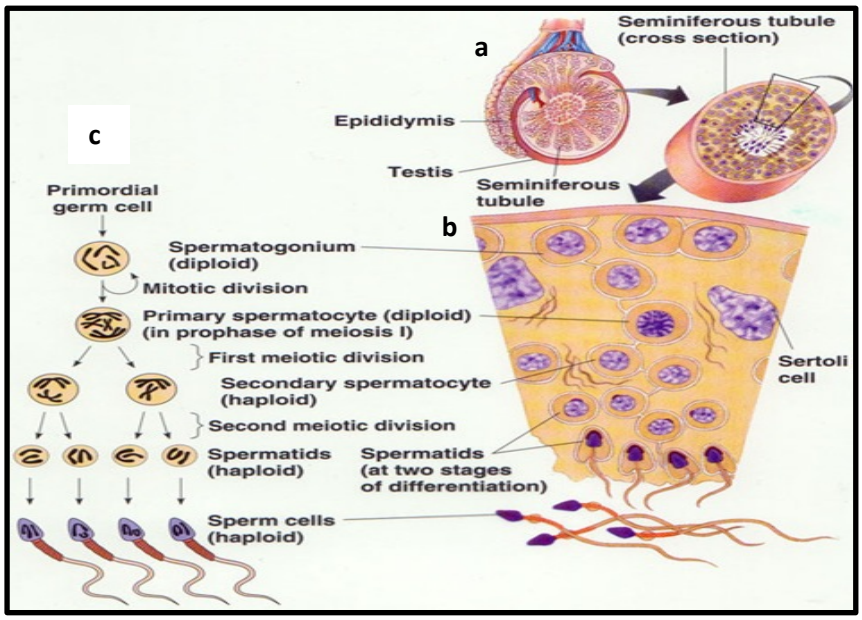
WAIT!!! There is more..... 😊 ☹ 😊 ☹

b. **SPERMATOGENESIS** is the formation of male gametes in the testes. Recalling the discussion in Module 2 about the male gonad, let us look again at its structure and how spermatogenesis takes place in it. Again, details of the amphibian and mammalian spermatogenesis will be seen in the laboratory.

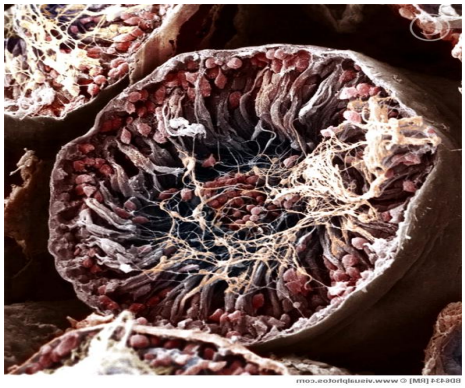
a – The testis is made of several seminiferous tubules where spermatogenesis takes place.

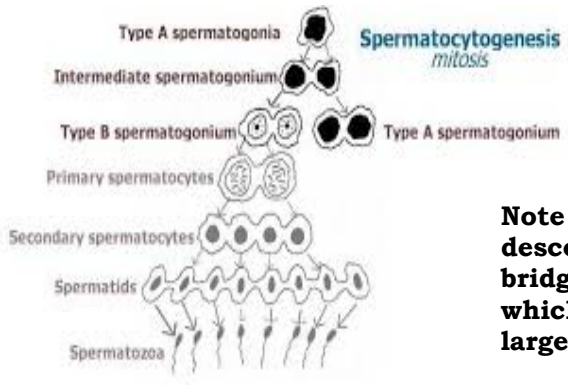
b – The different stages of sperm development taking place in each tubule.

c – The summary of the mitotic and meiotic divisions of the PGC and how they become functional sperm cells.



For now, let us focus with the mammalian (human) spermatogenesis. As mentioned earlier, this process is a lifelong process in males. In contrast to females, oogenesis stops at menopause.





At the onset of puberty, spermatogenesis begins at the seminiferous tubules of the testis. Again, primordial germ cells from the embryonic yolk sac migrates to the embryonic gonad, the genital ridges on both sides of the body. Here the PGCs proliferate by mitosis and at birth has developed into **spermatogonia** (sing., spermatogonium). These spermatogonia are located at the base of the **seminiferous epithelium.**

There are two populations of spermatogonia:

Type A: stem cell population that will continually divide throughout the human's life. Further, there are two subpopulations of Type A spermatogonia

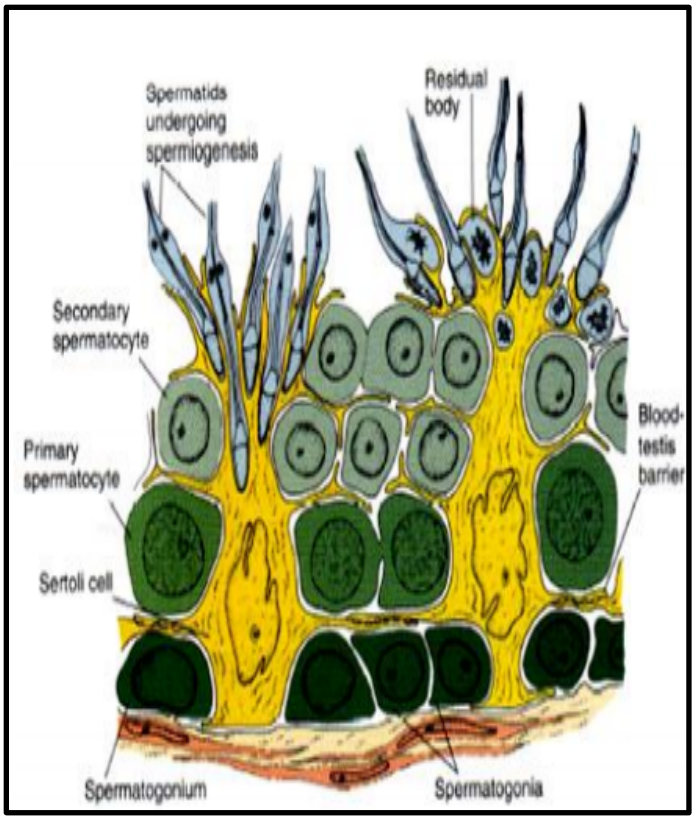
- Dark -noncycling cells; long term reserve population
- Pale –giving rise to Type B

Type B: undergoes differentiation to become functional spermatozoa

Note that the spermatogonia and their cellular descendants are connected by intercellular cytoplasmic bridges (due to incomplete cytokinesis after mitosis), which may maintain the synchronous development of large cluster of sperm cells.

https://www.google.com/url?sa=i&url=https%3A%2F%2Finstruction.cvhs.okstate.edu%2FHistology%2Fmr%2FHiMRP4.htm&psig=AOvVaw3dtT8TCxzVb5s4_wUEzmxR&ust=1604782455792000&source=images&cd=vfe&ved=0CAIQjRxqFwoTCIC1kNfm7uwCFQAAAAAdAAAAABAx

Look for the **Sertoli** cells on the image on the image on the right. These cells which may also be referred to as **sustentacular cells** are "nurse cells" that helps in the process of spermatogenesis.

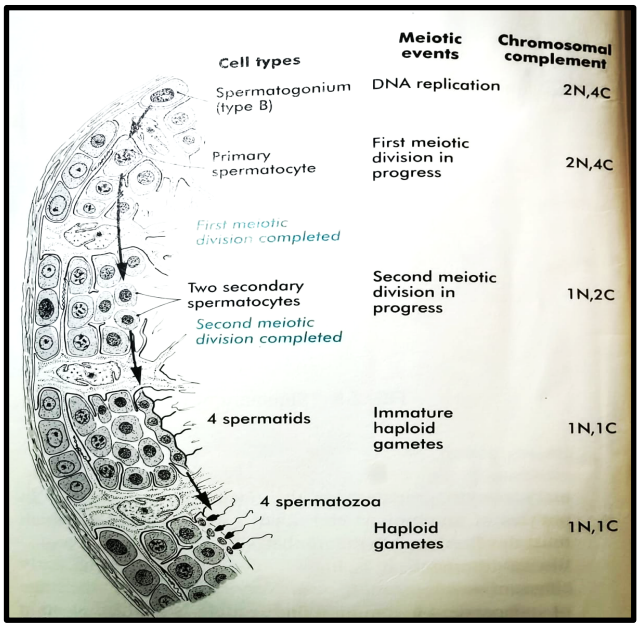


Other functions of the Sertoli cells are as follows:

- Target cells for FSH
- Synthesize the androgen binding protein (ABP), which maintains the high concentration of testosterone in the seminiferous tubules
- Maintain the blood-testis barrier
- Create an environment important in the differentiation of the sperm cells
- Facilitate the release of mature spermatozoa
- Degrades the residual cytoplasm shed during spermiogenesis

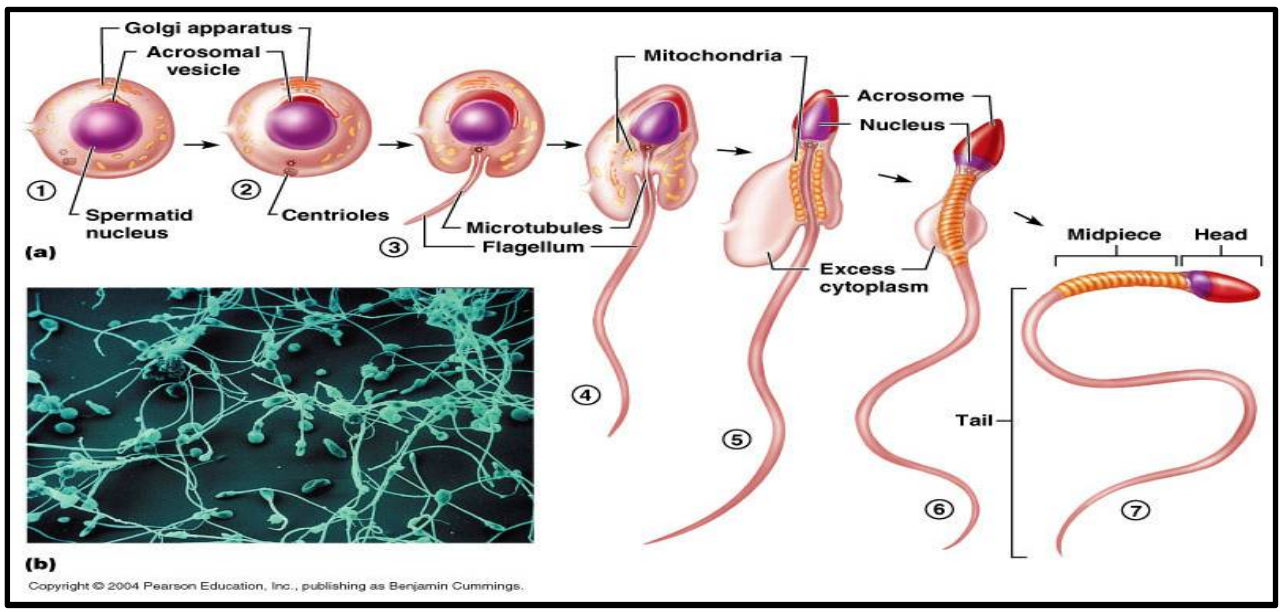
Since the processes of the Sertoli cells are very tightly joined and form an *immunological barrier* called the **blood-testis barrier** between the forming cells, and the rest of the body, including the spermatogonia. Therefore, once they begin meiosis, these developing sperm cells are immunologically different from the rest of the body. If the blood-testis barrier is damaged, it can cause autoimmune sterility.

Once the Type B spermatogonia enter the meiotic cycle as **primary spermatocytes,** they spend several weeks for the first meiotic division. The daughter cells of the first meiotic division of one primary spermatocyte give rise to two **secondary spermatocytes.** Then these secondary spermatocytes undergo the second meiotic division which can be completed in 8 hours. The resulting daughter cells are 4 **spermatids** with half the number of chromosomes (1N) and half the amount of the DNA content (1C). These spermatids are still immature and by **spermiogenesis** they become **spermatozoa.**



SPERMIOGENESIS. The spermatids resulting from the 2nd meiotic division of the secondary spermatocytes do not divide further. Instead, they undergo a series of profound changes that transforms them from relatively typical looking cells to highly specialized spermatozoa (sing. spermatozoon). This transformation process is called **spermiogenesis** or **spermatid metamorphosis.** These are the highlights of spermiogenesis:

1. Formation of the acrosome, which extends over half the nuclear surface.
2. Condensation of the nucleus.
3. Formation of the head, neck/middle piece, and tail.
4. Shedding of most of the cytoplasm.



In the figure above showing spermiogenesis, the resulting spermatids from the 2nd meiotic division of the secondary spermatocytes are initially ordinary looking cells (1). Notice the formation of the **acrosomal vesicle** and the **Golgi apparatus** at the apical end of the nucleus. The Golgi apparatus forms proacrosomal granules and fuse with the acrosomal vesicle (2-3) forming the **acrosome,** which contains enzymes that will play an important role during fertilization (4). The **centrioles** become conspicuous (2) and will become the anchorage for the developing **flagellum**. The microtubules of the distal centriole become continuous with the microtubules in the flagellum. The **nucleus** loses fluid resulting to a decrease in size and the chromatins become granular and compacted. The **mitochondria** begin to form a spiral investment around the proximal part of the flagellum (3-4). As spermiogenesis continues, the excess **cytoplasm** becomes aggregated into a remnant or **residual body** (5-6) which will be sloughed off and **phagocytized** by Sertoli cells. At the end of spermiogenesis, a sperm cell (7) consists of a **head** containing the nucleus and the acrosome: the **midpiece** containing the centrioles, the proximal part of the flagellum, and the mitochondrial helix; and the **tail**, which consists of the highly specialized flagellum.

The spermatozoa formed in the seminiferous tubules are immature morphologically speaking, because they are still nonmotile and incapable of fertilizing an egg. Physiological or biochemical maturation occurs when these cells travel to the

epididymis via the currents of the seminiferous tubules fluid. As they travel, glycoprotein substances are added to their surfaces. As we will learn in the next module on fertilization, these glycoproteins will be removed in the female reproductive tract in a phenomenon known as capacitation.

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