

Human Embryology

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Self Evaluation on Histology Lab

18 May from 14-17:00

G1, G2 and G3

G1 will arrive at 14:00

G2 will arrive at 15:00

G5 will arrive at 16:00

19 May from 14-17:00

G3 will arrive at 14:00

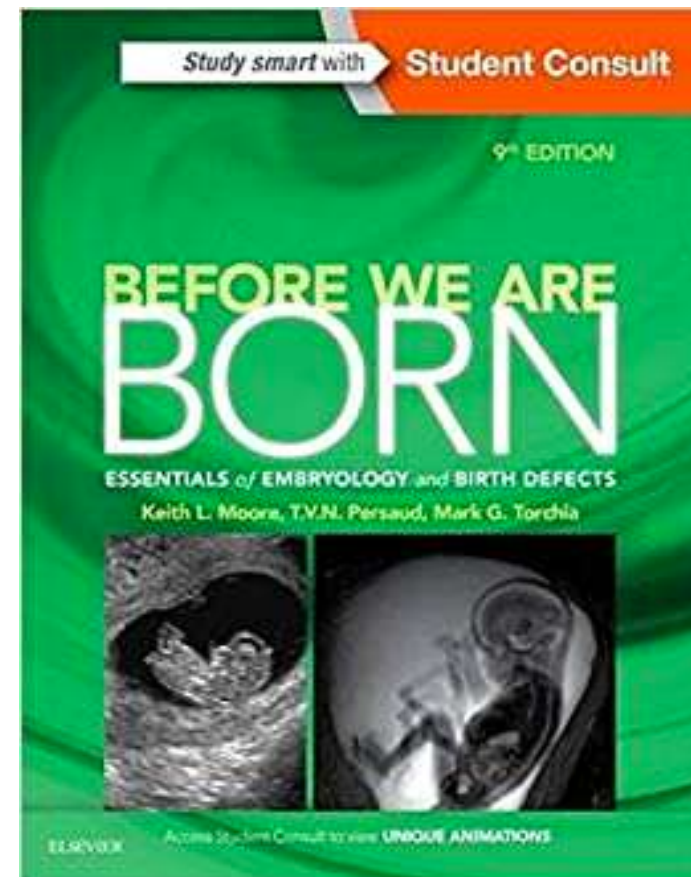
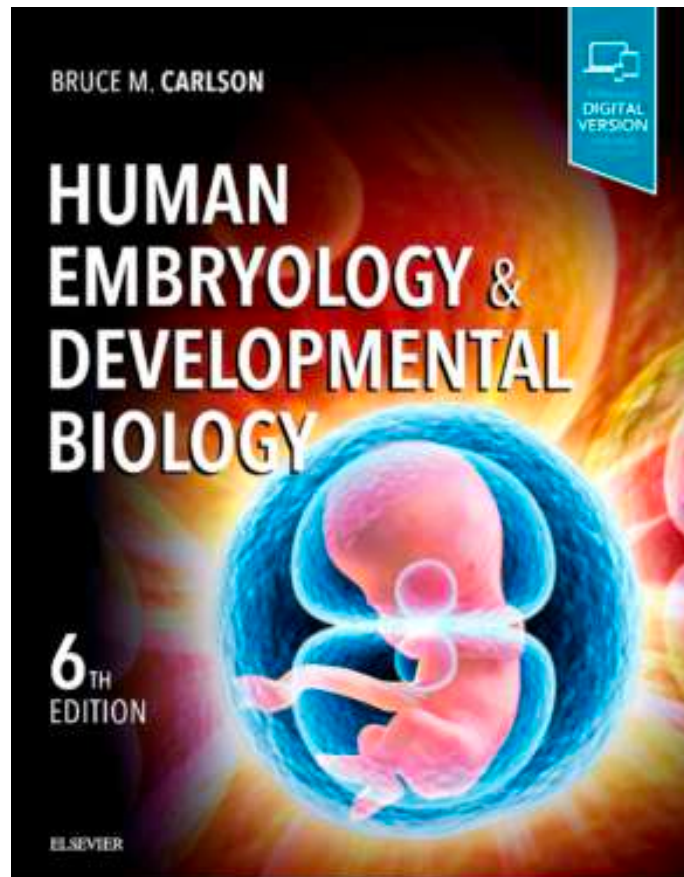
G4 will arrive at 15:00

G6 will arrive at 16:00

Program

- Human Reproduction
- Transport of Gametes and Fertilization
- Cleavage and Implantation
- Formation of Germ Layers and Early Derivatives
- Establishment of the Basic Embryonic Body Plan
- Placenta and Fetal Membranes
- Molecular Basis for Embryonic Development
- Oogenesis, embryonic development and offspring from female iPSC-derived PGCLCs.
- Self-Organization of the in vitro attached human embryo
- Ex utero mouse embryogenesis from pre-gastrulation to late organogenesis

Recommended Textbooks



Embryology

The branch of medicine that studies the Human Development

Development begins at fertilization, approximately 14 days after the last normal menstrual period. The continuous process begins when a sperm penetrates an oocyte (ovum) and forms a zygote (first week).

The **embryonic period** covers the first 8 weeks of development.

The **fetal period** begins in the 9th week. Most visible advances occur during the 3rd to 8th week.

Human pregnancy periods

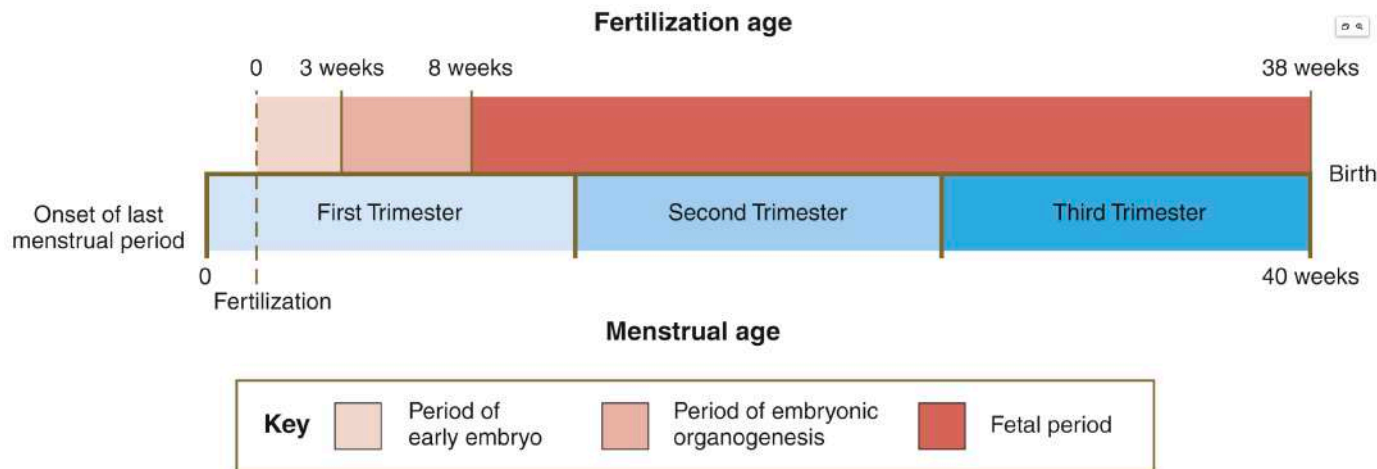
Physicians typically use trimesters: three-month periods (zero to three months, three to six months, and six to nine months) starting with the date of the onset of the last menstrual period (a memorable landmark) and ending at birth.

Human embryologists use intervals called periods:

1- the period of the egg (generally from fertilization to the end of the third week)

2- the period of the embryo (generally from the beginning of the fourth week to the end of the eighth week),

3- the period of the fetus (from the beginning of the third month to birth).

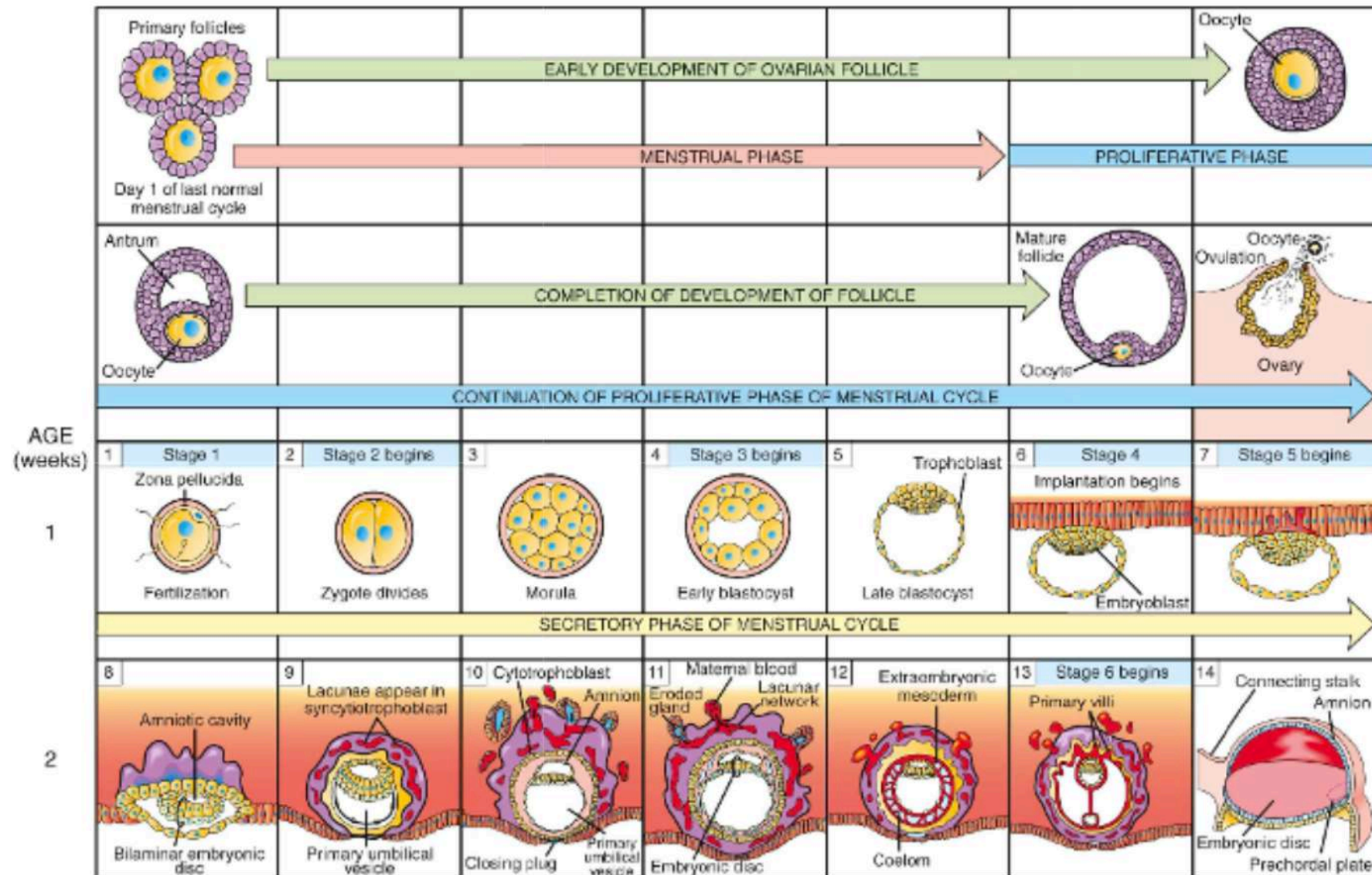


Phases of human embryogenesis

Human embryologists also identify

- Gametogenesis: the formation of the gametes, the egg, and sperm
- Fertilization: the joining of the gametes to form the zygote
- Cleavage: a series of rapid cell divisions that result first in the formation of the morula, a small cluster of cells resembling a mulberry, and then in the formation of the blastocyst, a hollow ball of cells containing a central cavity
- Gastrulation: the rearrangement of cells in the embryonic region of the implanted blastocyst into three primary germ layers—ectoderm, mesoderm, and endoderm—to form the embryonic disc
- Formation of the tube-within-a-tube body plan: conversion, through body folding, of the embryonic disc into a C-shaped embryonic body consisting of an outer ectodermal tube (the future skin) and an inner endodermal tube (the gut tube), with the mesoderm interposed between the two tubes
- Organogenesis: the formation of organ rudiments and organ systems

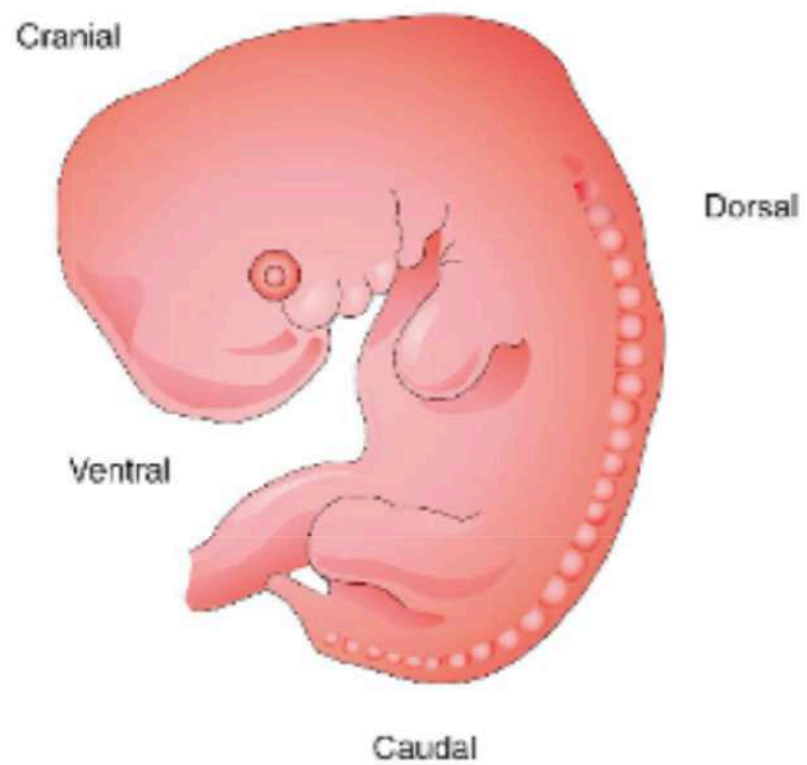
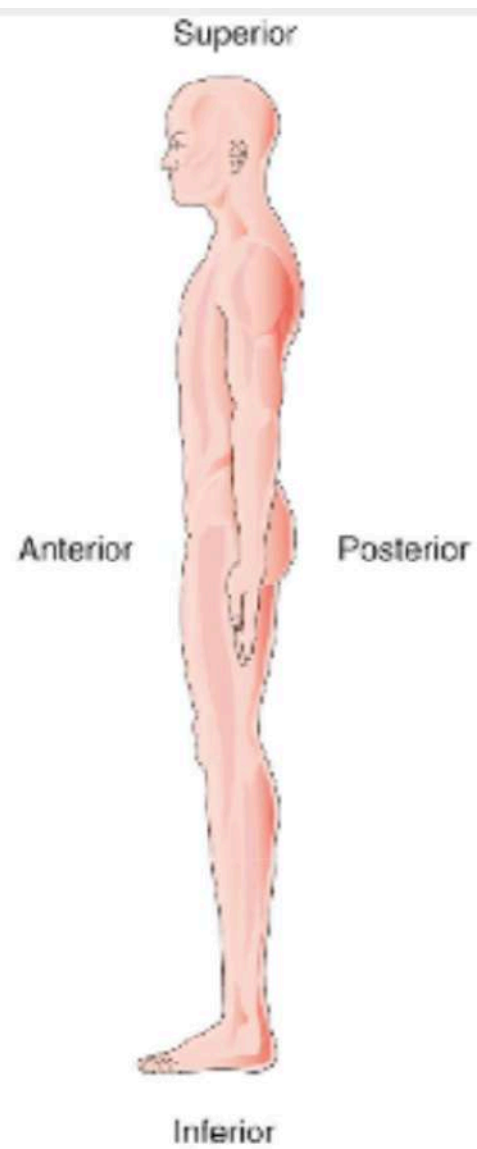
Time table of Prenatal Development



Developmental Biology Frontiers

Researchers continue to learn how, when and where selected genes are activated and expressed in the embryo during normal and abnormal development.

- The critical role of genes, **signaling molecules**, receptors, and other molecular factors in regulating early embryonic development is rapidly being delineated. In 1995, Edward B. Lewis, Christiane Nüsslein-Volhard, and Eric F. Wieschaus were awarded the **Nobel Prize** in Physiology or Medicine for their discovery of genes that control embryonic development. Such discoveries are contributing to a better understanding of the causes of spontaneous abortion and birth defects.
- In 1997, Ian Wilmut and colleagues were the first to produce a mammal (a sheep dubbed **Dolly**) by cloning using the technique of somatic cell nuclear transfer. Since then, other animals have been cloned successfully from cultured differentiated adult cells. Interest in human cloning has generated considerable debate because of social, ethical, and legal implications. Moreover, there is concern that cloning may result in an increase in the number of neonates (newborns) with birth defects and serious diseases.
- In 2021, **Ex utero mouse embryogenesis from pre-gastrulation to late organogenesis**



Getting Ready for Pregnancy

The Reproductive Organs

Produce and transport germ cells (**gametes**) from the gonads (**testes or ovaries**) to the site of fertilization that usually occurs in the uterine tubes (ampulla).

Vagina serves as excretory passage for menstrual fluid and forms the inferior part of the birth canal.

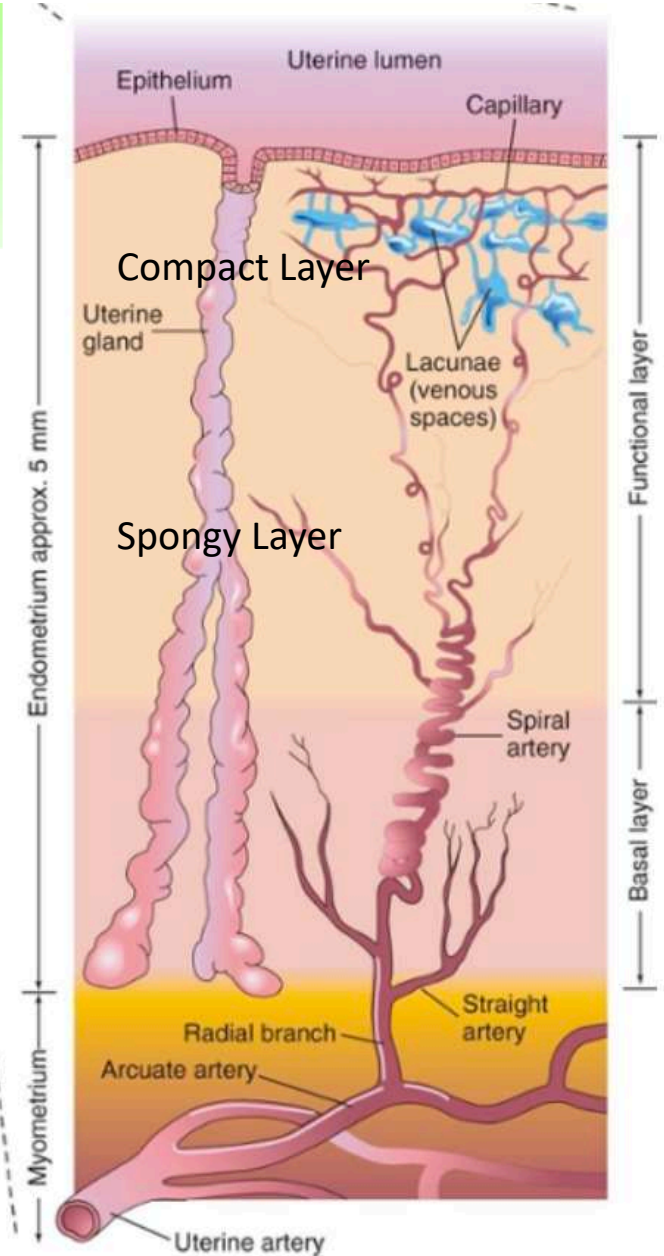
Uterus: pear-shaped organ composed of two parts:

- The body - the expanded superior two thirds
- The cervix, the cylindrical inferior third

Uterus

The wall of the body of the uterus consists of three layers

- 1- Perimetrium: a thin external peritoneal layer
- 2- Myometrium: a thick smooth muscle layer
- 3- Endometrium: the internal layer composed of
 - A compact layer, consisting of densely packed connective tissue around the terminal duct of the uterine gland
 - A spongy layer, composed of highly vascularized connective tissue containing dilated, uterine glands
 - Basal layer containing the blind end of the uterine glands



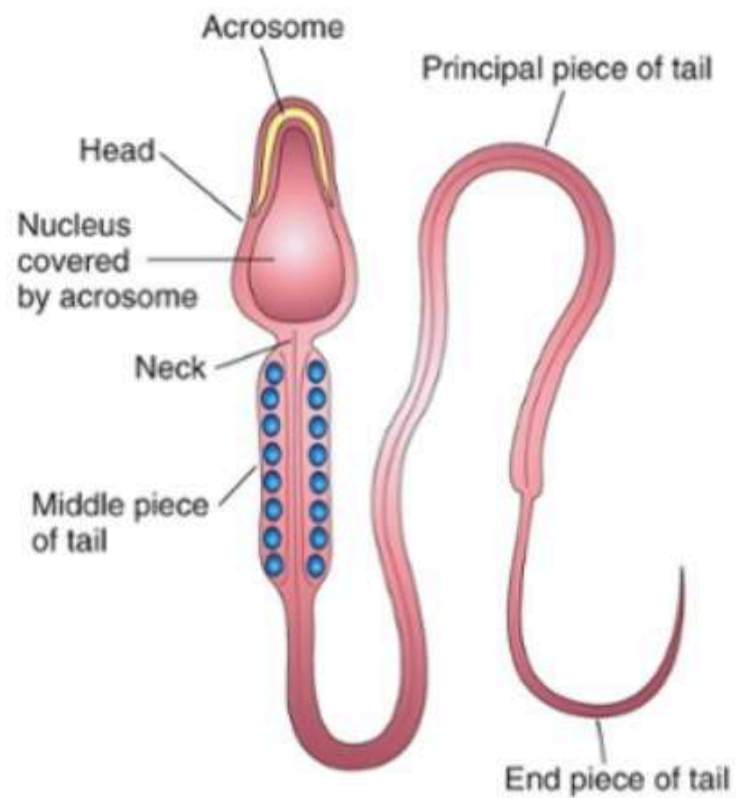
Gametogenesis

Is the formation of germ cells.

Spermatogenesis in male

- The sperms and oocytes are highly specialized gametes - germ cells
- 23 chromosomes instead of 46: obtained with meiosis

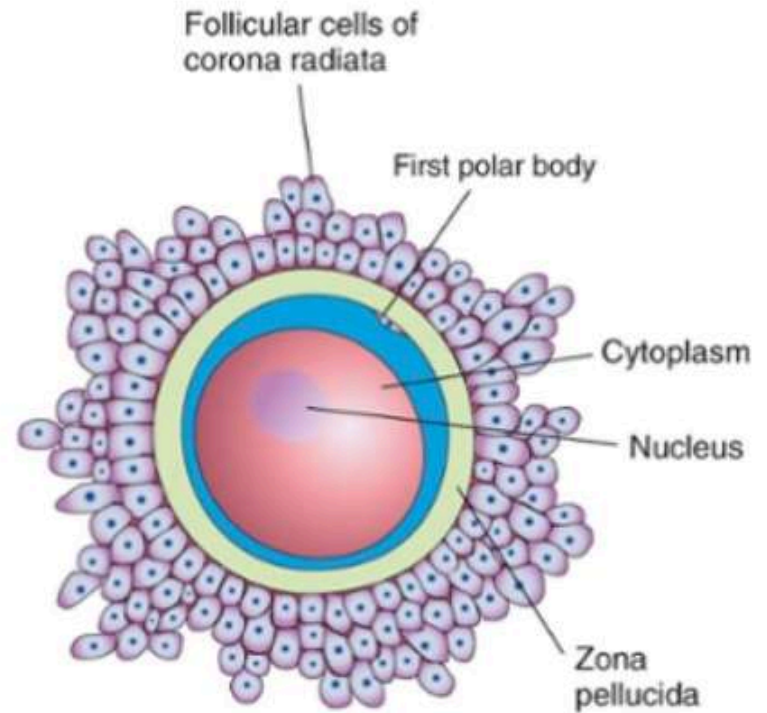
Gametogenesis



A

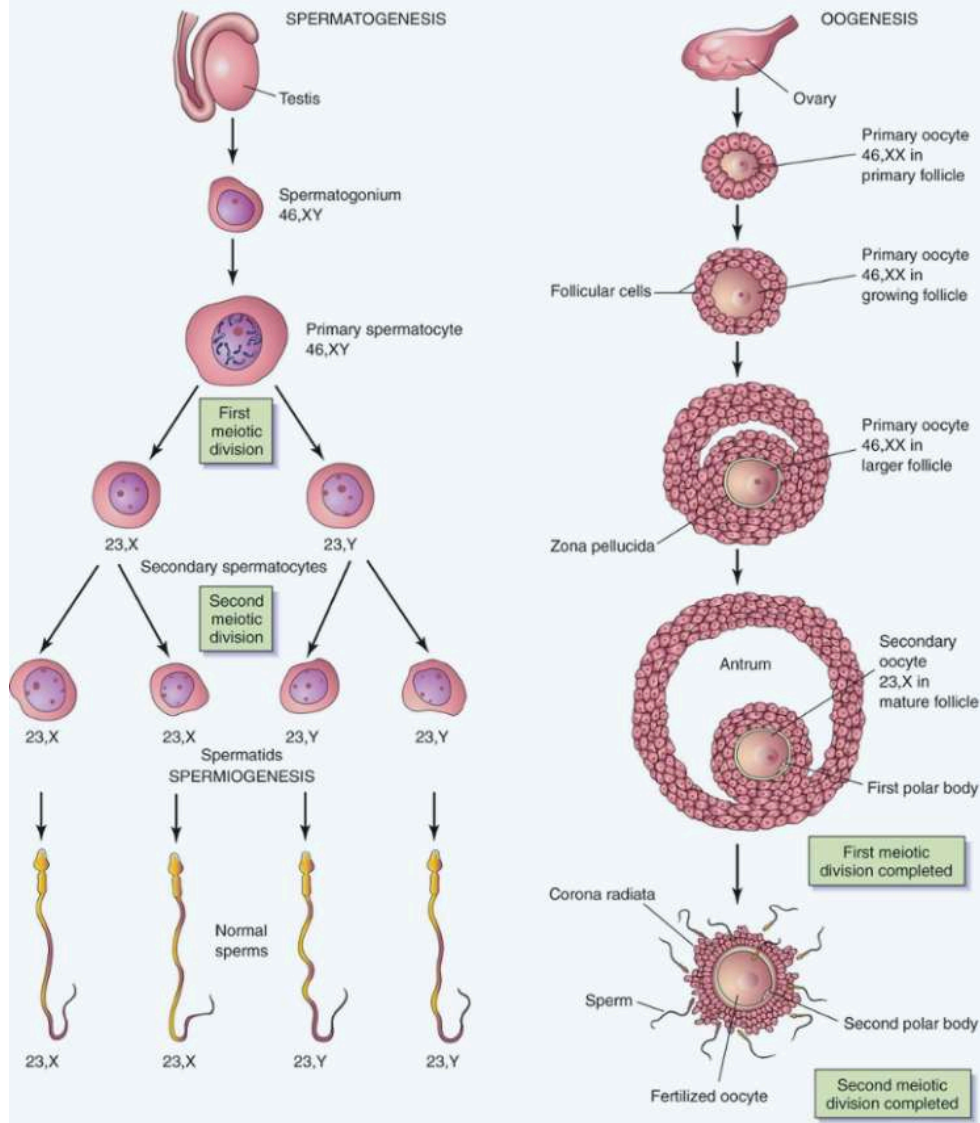


B



C

NORMAL GAMETOGENESIS



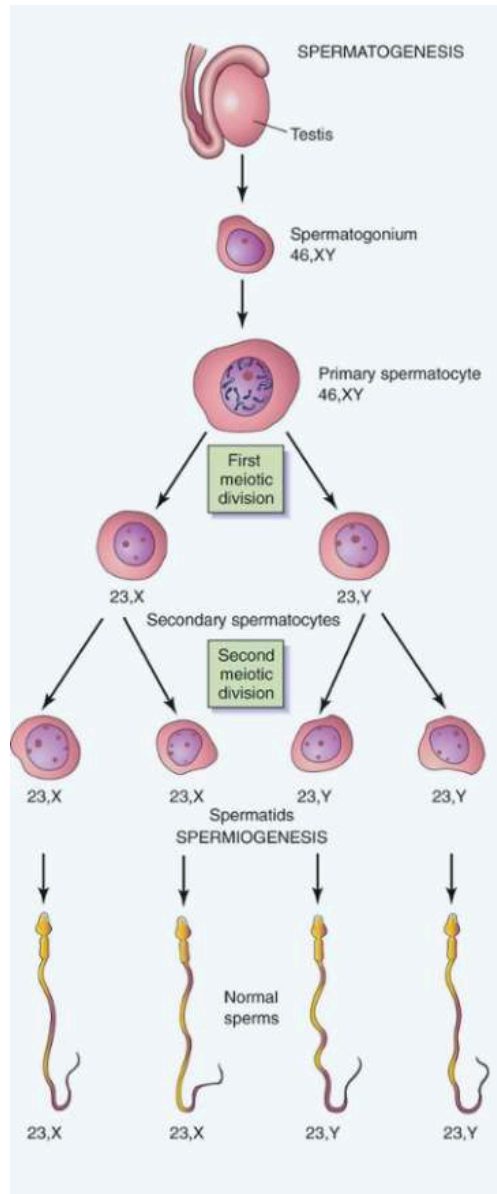
The sperms and oocytes contains half the number of required chromosomes (i.e., 23 instead of 46).

The number of chromosomes is reduced during a special type of cell division—**meiosis**.

This type of cell division occurs only during **gametogenesis**

In male, this process is termed **spermatogenesis**; in female, is **oogenesis**

Spermatogenesis



- Primordial germ cells (PGC), that derive from the **epiblast** (blastoderm) remain dormant from the 6th week of embryonic development till puberty.
- Primordial sperms (spermatogonia) remain dormant in the seminiferous tubules of the testes.
- After puberty they increase the number with several mitotic cell divisions, the sperms grow and undergo gradual changes that transform them into primary spermatocytes.
- Each primary spermatocyte subsequently undergo a reduction division - the first meiotic division (to form two haploid secondary spermatocytes), half the size of primary spermatocytes.
- Second meiotic division to form spermatids (half size)

Mature sperms differentiation process known as spermiogenesis

Spermatogenesis

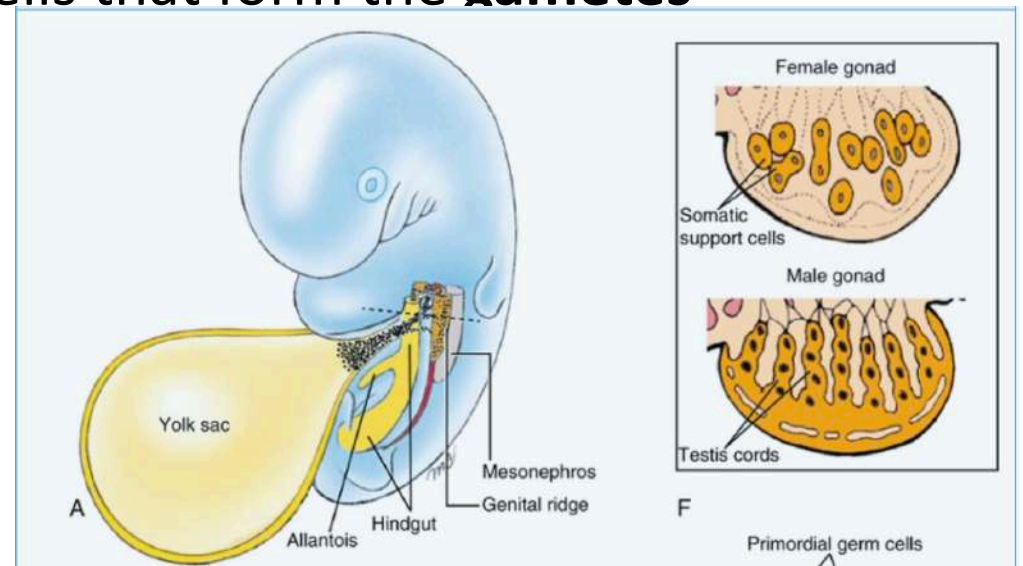
When spermiogenesis is complete, sperms enter the lumen of the **seminiferous tubules** in the testis. The sperms then move to the **epididymis**, where they are stored and become functionally mature. Spermatogenesis requires approximately 2 months for completion. Maturation of sperms—**spermatogenesis**—normally continues throughout the reproductive life of a male.

Primordial Germ Cells Reside in Yolk Sac

Cells that give rise to **gametes** in both males and females can be identified during the fourth-sixth week of gestation within an extraembryonic membrane called the **yolk sac**.

These cells are called **primordial germ cells (PGCs)**, and their lineage constitutes the **germ line**, a series of cells that form the **gametes**

one of the first things that happen in the developing embryo is that the germ line is set aside for the next generation!



- Primordial germ cells (PGCs), can be identified during the fourth-sixth week of gestation within...

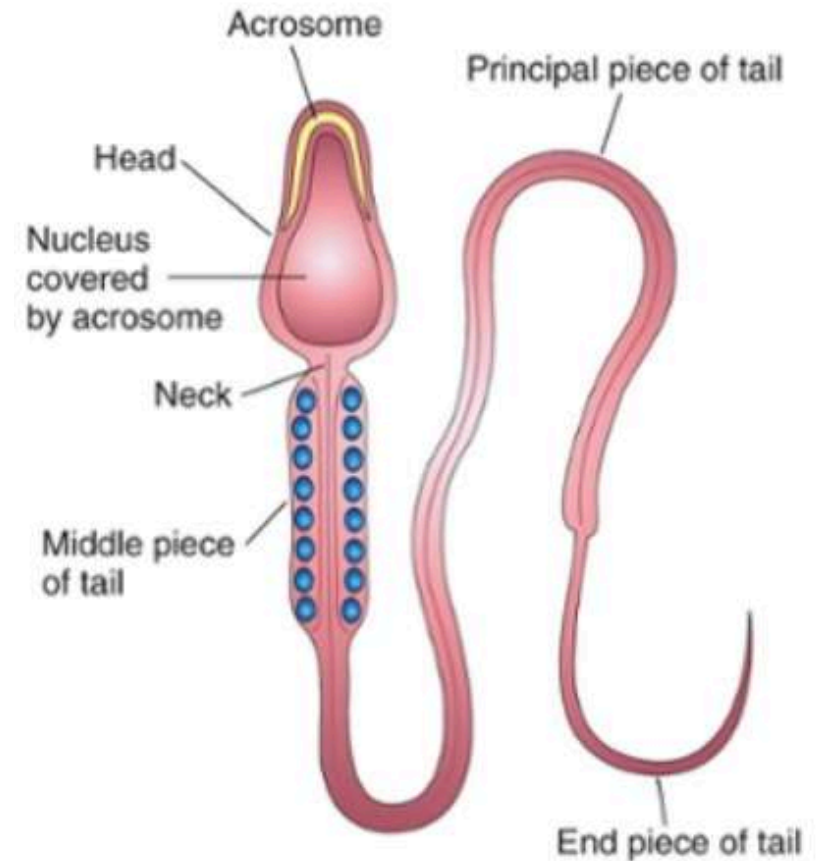
Spermatogenesis

Mature sperm cell:

- Acrosome contains enzymes that probably facilitate the sperm's penetration of the zona pellucida

Completed spermiogenesis (sperm cells differentiation) they move to:

- Seminiferous tubules
- Epididymus (storage and functional maturation)
- spermatogenesis



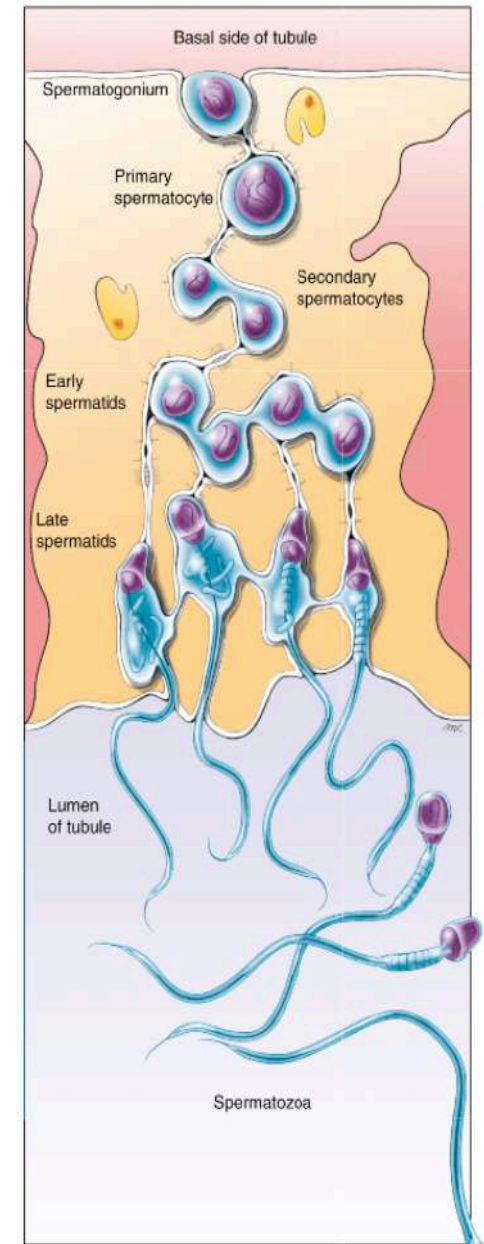
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Spermatogenesis

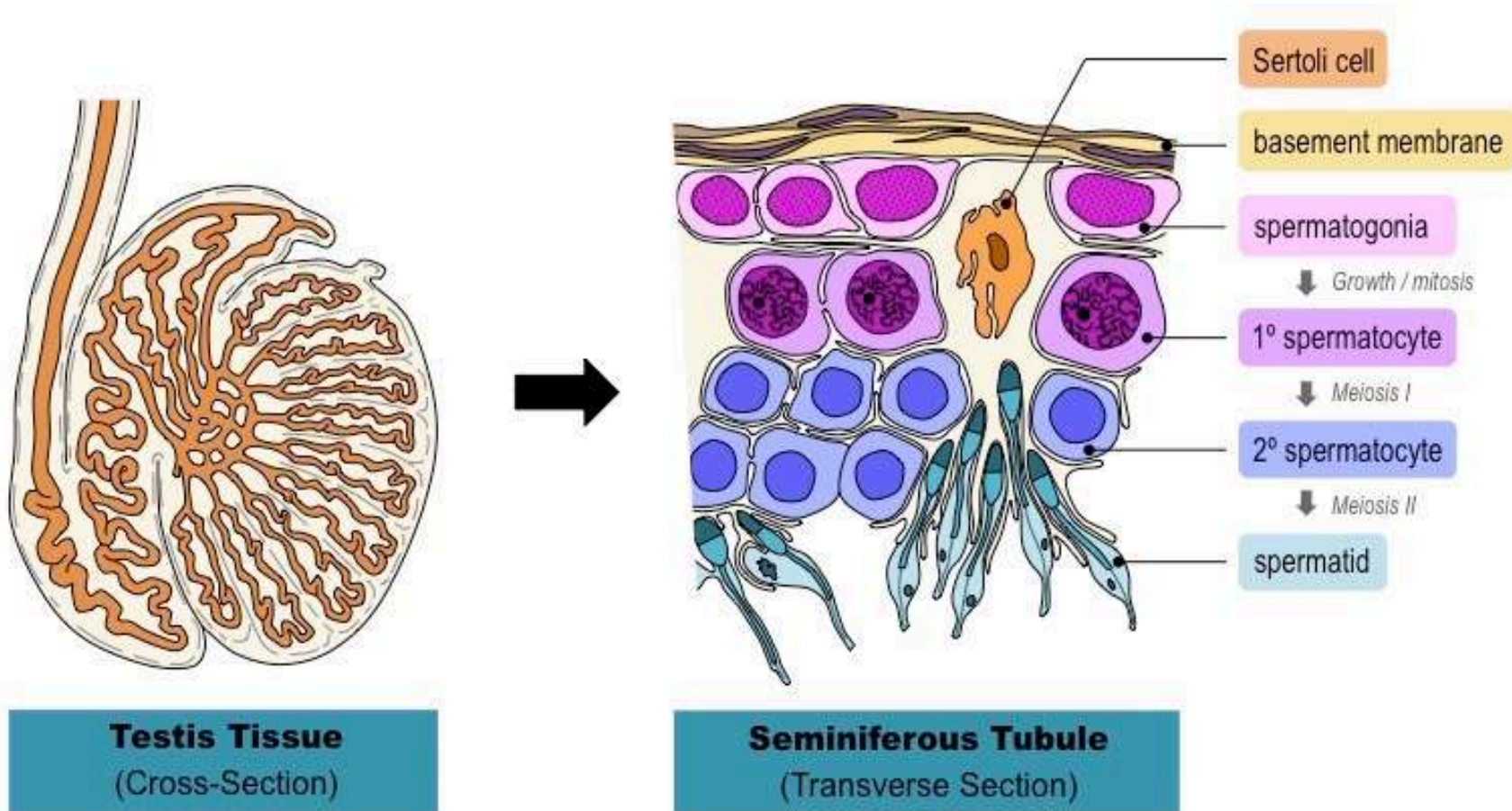
At puberty, **testosterone secretion** stimulates development of 11 sex characteristics, triggers growth of the testes, maturation of seminiferous tubules, and commencement of spermatogenesis, which results in the production of 150 to 275 million spermatozoa per day in humans.

- Sertoli cells differentiate into a system of seminiferous tubules. The dormant Primordial Germ Cells (PGCs) resume development, divide several times by mitosis, and then differentiate into spermatogonia.
- These spermatogonia are located immediately under the basement membrane surrounding the seminiferous tubules. Adjacent Sertoli cells are interconnected between the pockets by **tight junctions**, which help establish a **blood-testis barrier (the testis in immune privilege)**

Thus, developing spermatogonia reside within an immune privileged site during their development in the testes.

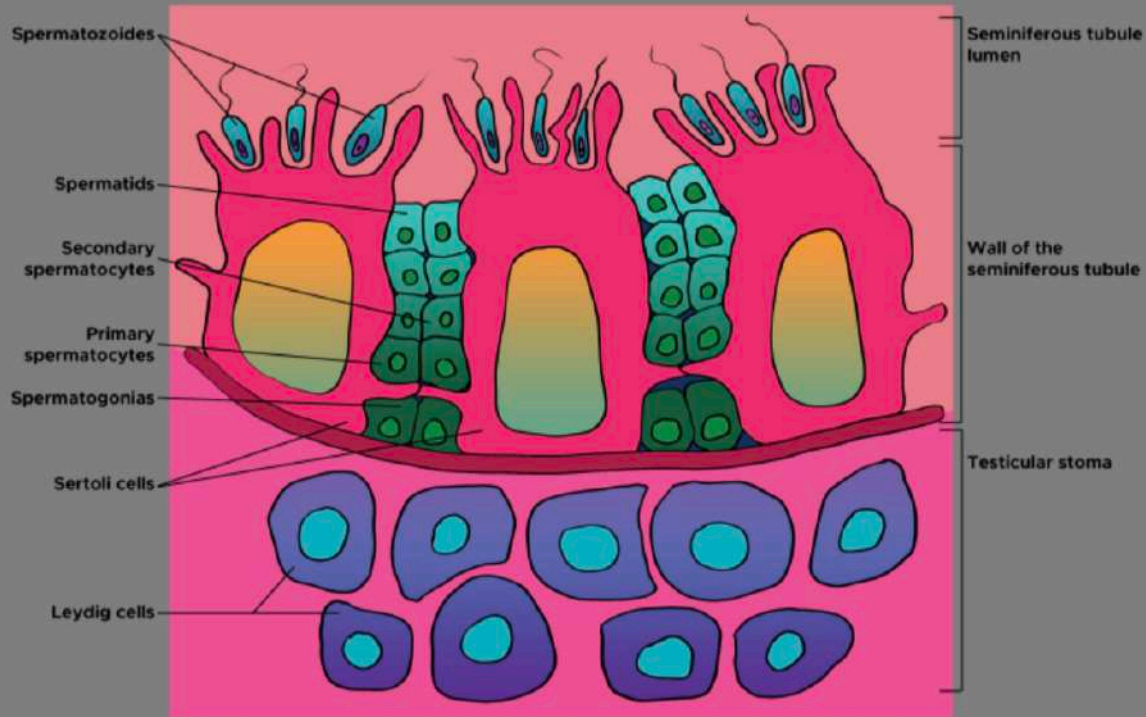


Spermatogenesis



<https://unibo.smartzoom.com/s1241/course1776/f1815/i1823/>

Testes



Major Functions of Sertoli Cells

- Maintenance of the blood-testis barrier
- Secretion of tubular fluid (10 to 20 $\mu\text{L/g}$ of testis/h)
- Secretion of androgen-binding protein
- Secretion of estrogen and inhibin
- Secretion of a wide variety of other proteins (e.g., growth factors, transferrin, retinal-binding protein, metal-binding proteins)
- Maintenance and coordination of spermatogenesis
- Phagocytosis of residual bodies of sperm cells

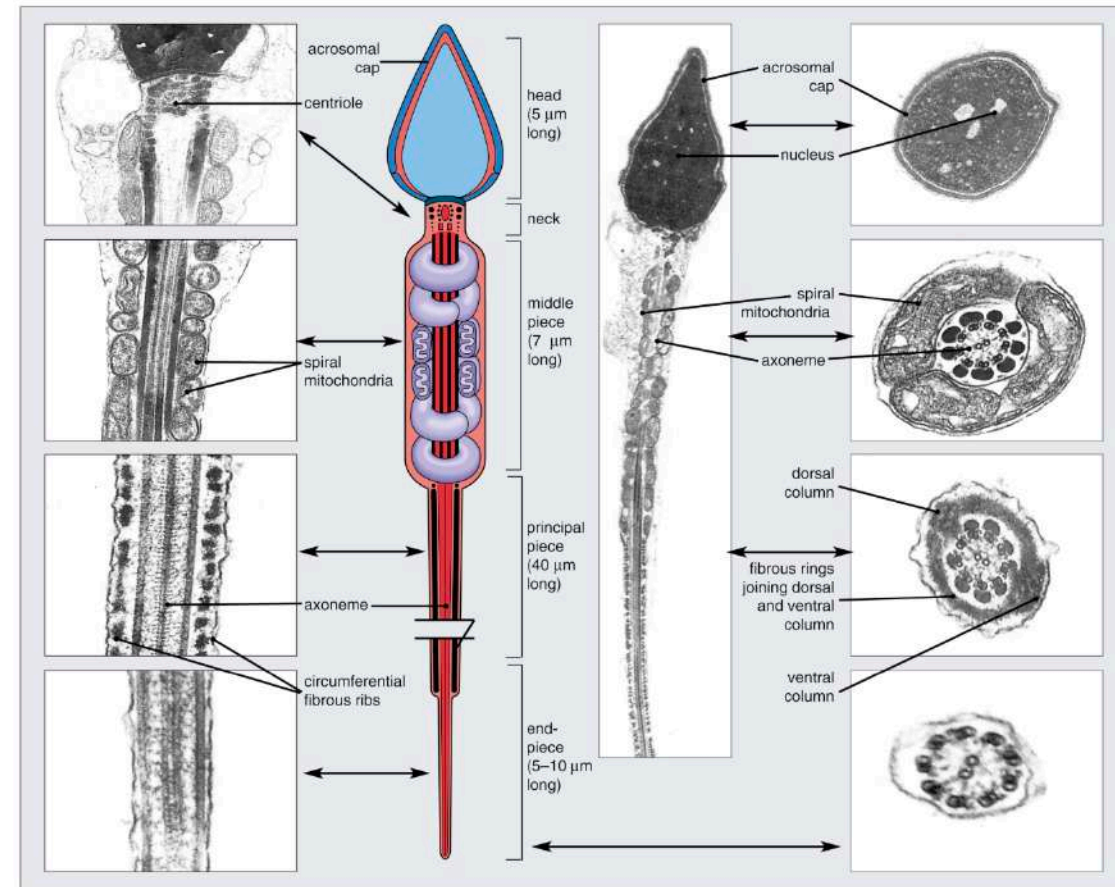
Spermatogenesis

The anterior two thirds of the head are covered by the **acrosome**, a cap-like organelle containing enzymes that facilitate sperm penetration during fertilization.

The tail provides the motility of the sperm, assisting with its transport to the site of fertilization in the ampulla of the uterine tube.

*The **tail of the sperm** consists of three parts: middle piece, principal piece, end piece.*

The middle piece contains the energy-producing **mitochondria**, which fuel the lashing movements of the tail. *Hox genes influence microtubul dynamics at the molecular level in shaping the head of the sperm and in the formation of the tail.*



A

Spermatozoa Undergo Terminal Step of Functional Maturation Called Capacitation

Capacitation, the final step of sperm maturation, consists mainly of changes in the acrosome that prepare it to release the enzymes required to penetrate the zona pellucida, a shell of glycoprotein surrounding the oocyte.

Capacitation takes place within the female genital tract and is thought to require contact with secretions of the oviduct. Spermatozoa used in in vitro fertilization (IVF) procedures are artificially capacitated. Spermatozoa with defective acrosomes may be injected directly into oocytes to assist reproduction in humans

Media of calcium ions, bicarbonate, and serum albumin to help the capacitation *in vitro*

What would be a direct consequence of a single point mutation that modifies the proteins inside the acrosome?

- a. Inability to dissolve zona pellucida's glycocalyx
- b. Impairment of sperm capacitation
- c. None of them
- d. Impairment of sperm motility
- e. Failure to complete spermatogenesis

Oogenesis: from Oogonia to oocytes

Fetal period: oogonia proliferate by mitosis and enlarge to form primary oocytes

At birth all primary oocytes completed the prophase of the first meiotic division until puberty

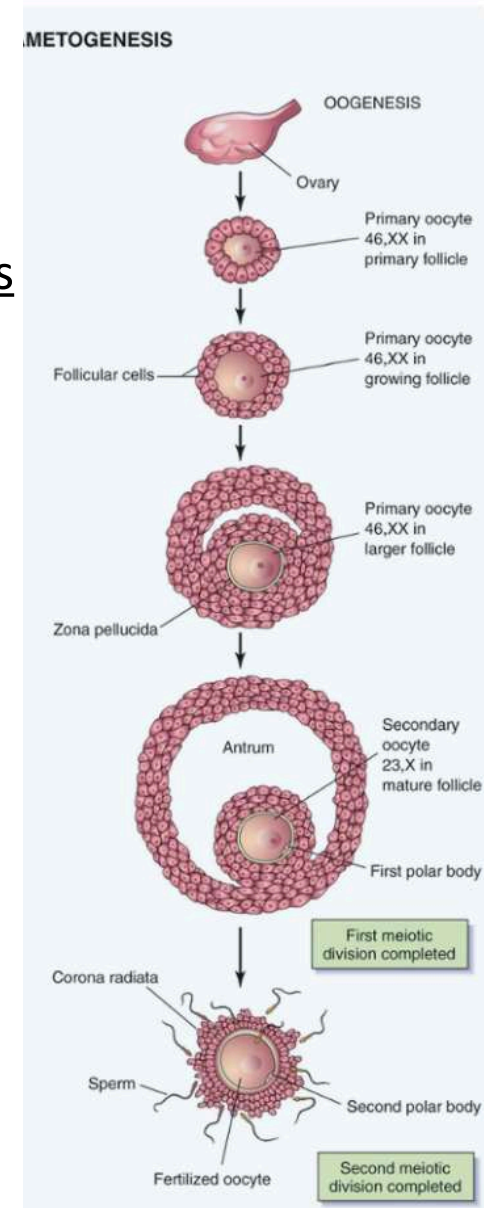
Before ovulation a primary oocyte completes the first meiotic division, with unequal cytoplasm division (1 polar body, that will soon degenerate)

The secondary oocyte receive most of the cytoplasm

At ovulation the nucleus of the secondary oocyte begins the II meiotic division.

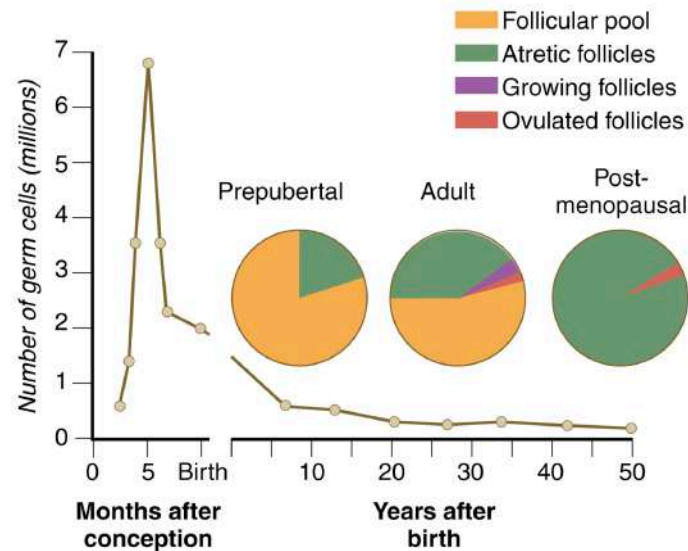
If there is fertilization the second meiotic division is completed and a II polar body is formed.

The secondary oocyte is large and visible by unaided eyes.



- Up to 2.000.000 primary oocytes are usually present in the ovaries of a neonate.
- Most of them regress during childhood so that by puberty no more than **40.000** remain.
- Of these about **400 oocytes mature** into secondary oocytes and reach ovulation

How many years does a woman typically ovulate for?



Comparison between male and female gametes

Compared to sperms, the oocytes are massive, immotile and have abundant cytoplasm

Two kind of sperms: 22 autosomes + X or Y: 23, X or 23, Y

Only one type of secondary oocytes: 23, X

The difference in sex hormones complement forms the basis of primary sex determination

Gametogenesis differences in males and females

After the Primordial Germinal Cells (PGCs) enter the genital ridge, they stop migrating and undergo 2 or 3 mitosis and enter the premeiotic stage.

- In male: PGCs arrest soon-at 6th week of embryonal development
- In female: PGCs enter the meiotic prophase as primary oocytes at the fifth month of fetal development

If male Primordial Germinal Cells (XY) are transplanted into female (XX) embryos, they follow the course of female PGC - regardless of their chromosome constitution

- Also PGCs that fail to reach the gonads also progresses through meiosis as oocytes-regardless of their genotype.
- All PGC are programmed to be oocytes— cell autonomous - Tet 1 dependent (TF erasing epigenetic marks in the DNA)
- In male genital ridge there is a male meiosis inhibitor produced by the Sertoli cells

Male vs Female

- In males, PGCs remain dormant from the sixth week of embryonic development until puberty. At **puberty, seminiferous tubules** mature and PGCs differentiate into **spermatogonia**. Successive waves of spermatogonia undergo **meiosis** and mature into spermatozoa. Spermatozoa are produced continuously from puberty until death.
- In females, PGCs undergo a few more mitotic divisions to differentiate into **oogonia**. By the fifth month of fetal development, all oogonia begin meiosis, after which they are called **primary oocytes**, which enter a state of dormancy, and they remain in meiotic arrest as primary oocytes until sexual maturity. Starting at puberty, each month a few ovarian follicles resume development in response to the monthly surge of pituitary gonadotropic hormones, but usually only one primary oocyte matures into a secondary oocyte and is ovulated. This oocyte enters a second phase of meiotic arrest and does not actually complete meiosis unless it is fertilized. These monthly cycles continue until the onset of menopause at approximately fifty years of age.

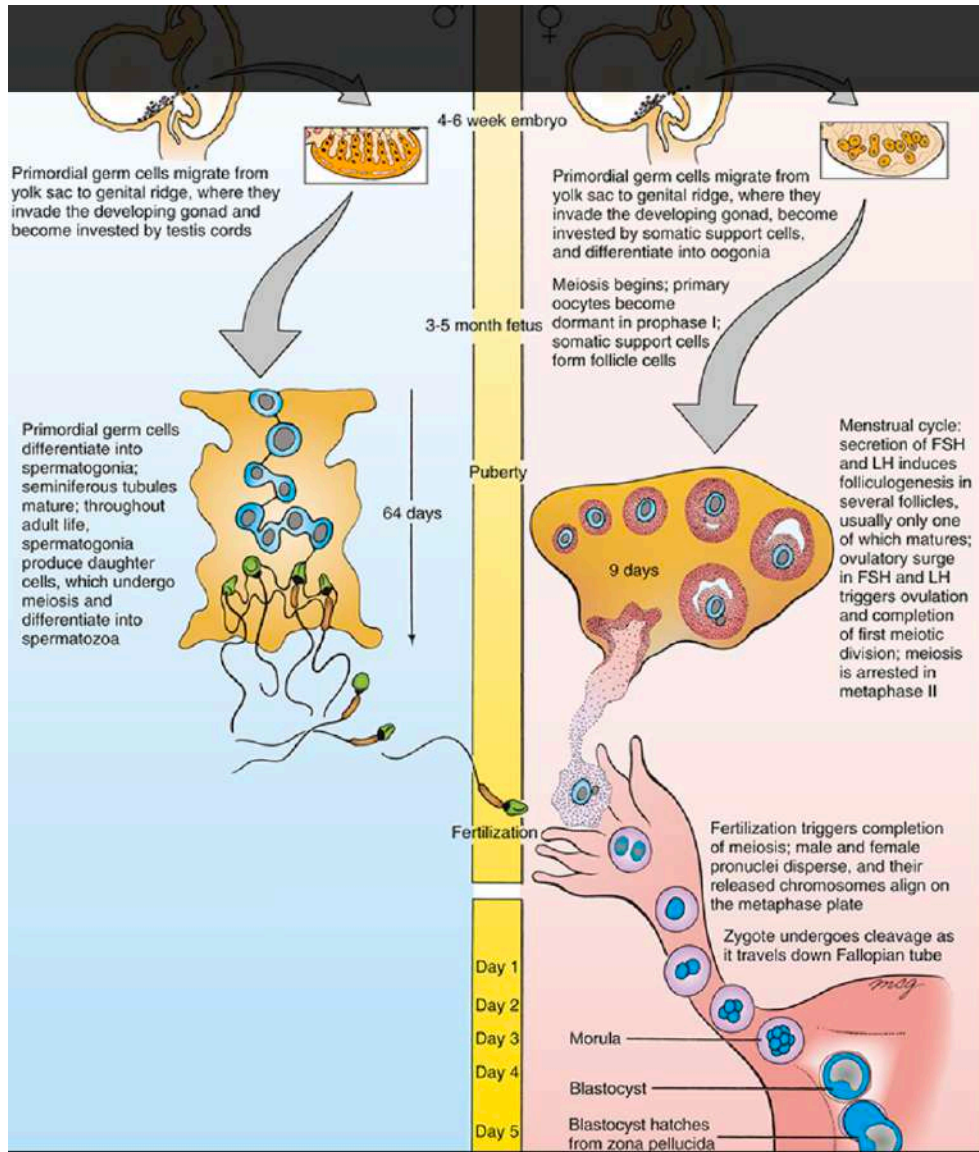
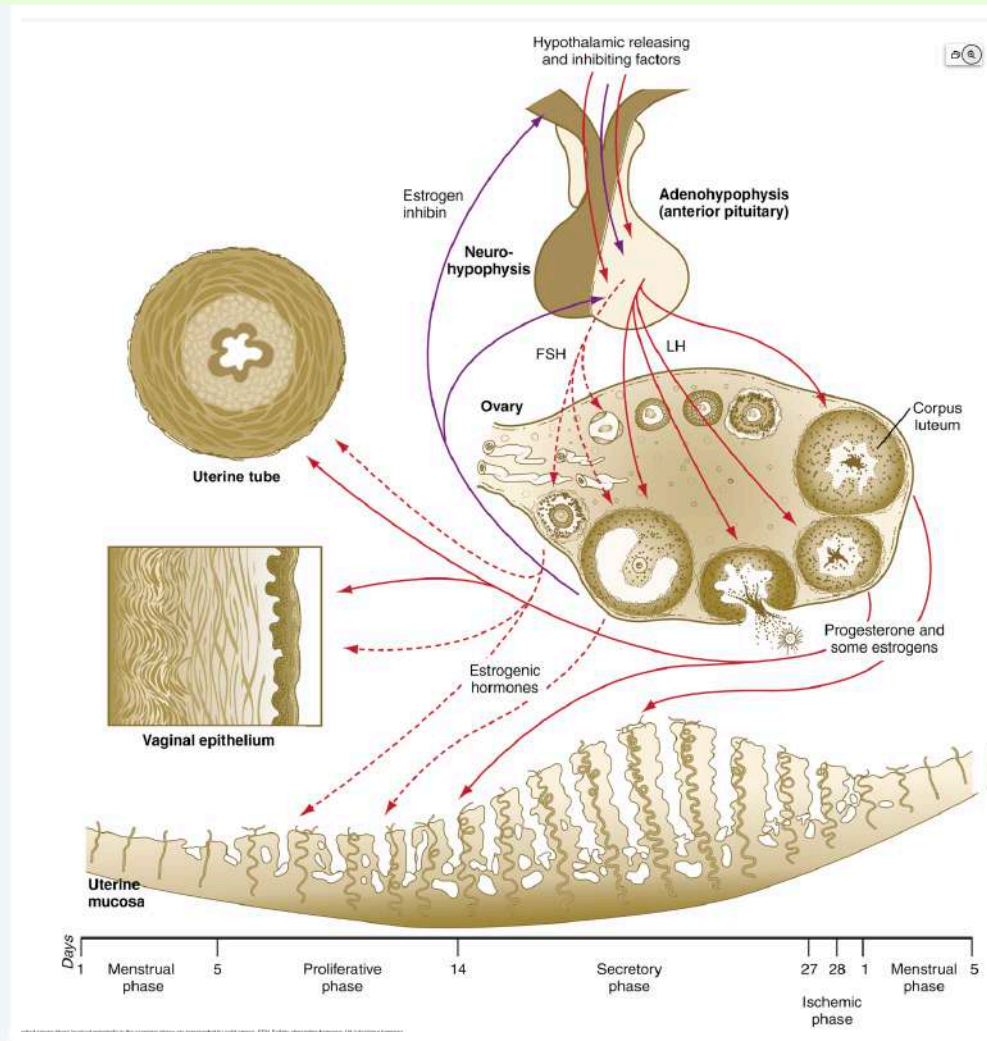
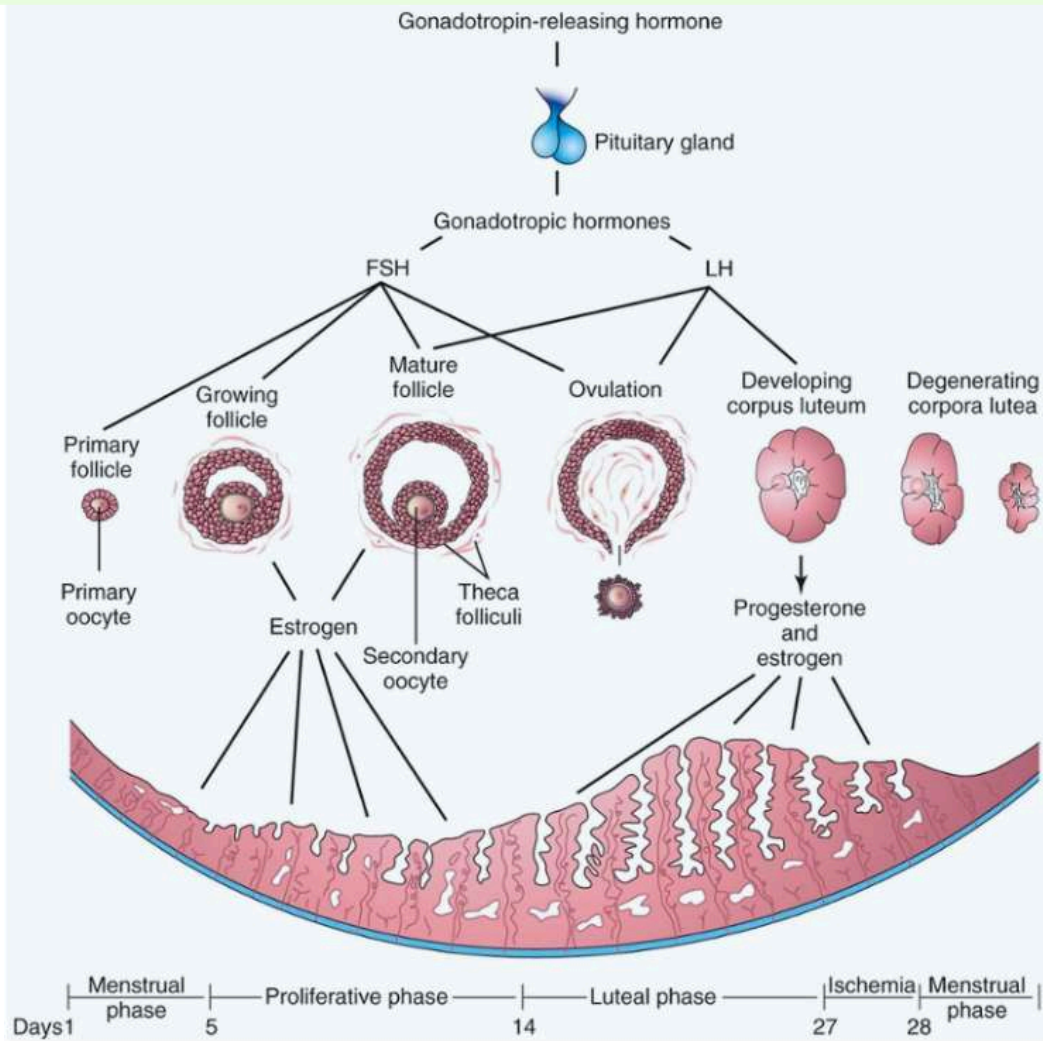


Fig. 1.1 Origin and migration of primordial germ cells in the human embryo. A, Location of primordial germ cells in the 16-somite human embryo (midsagittal view). B, Pathway of migration (arrow) through the dorsal mesentery. C, Cross section showing the pathway of migration (arrows) through the dorsal mesentery and into the gonad.



Fig. 1.2 A, Sacrococcygeal teratoma in a fetus. B, Massive oropharyngeal teratoma. (Courtesy of M. Barr, Ann Arbor, Mich.)

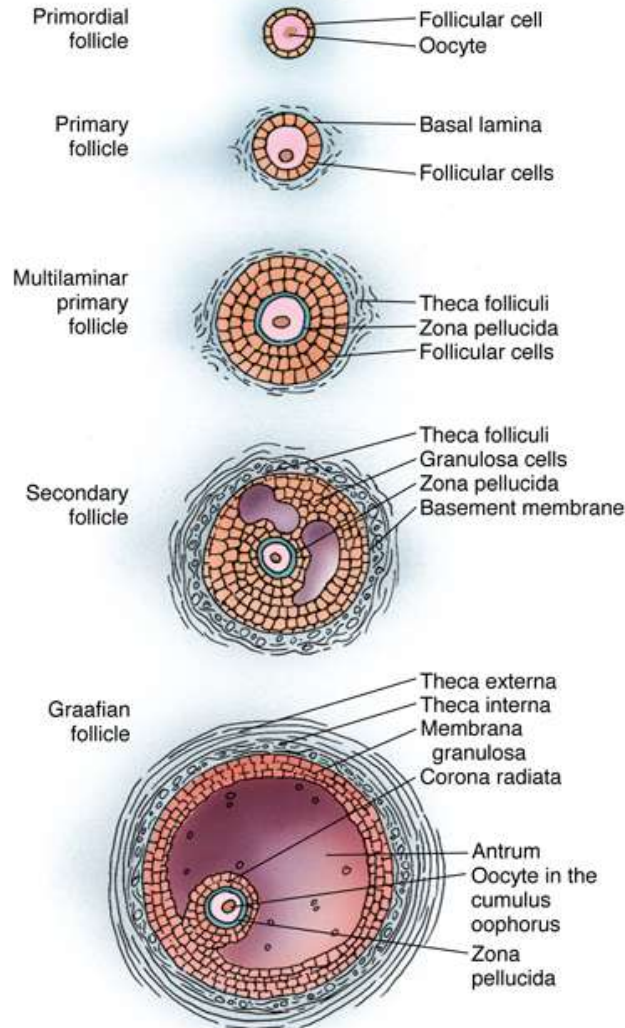
Female reproductive cycles



Ovarian Cycle

Is the process that leads to the development of a mature follicle, followed by ruptures and oocyte expelling, ready for the fertilization

Ovarian Follicles



B

Before the onset of puberty, all the follicles of the ovarian **cortex** are in the **primordial follicle** stage. The pulsatile release of GnRH from the hypothalamus results in a similar, pulsatile, release of gonadotropins (follicle-stimulating hormone [FSH], and luteinizing hormone [LH]). Follicular development usually culminates in the release of a **single oocyte** (ovulation).

Ovarian follicles: consist of a primary oocyte and its associated follicular cells.

4 differentiation stages of follicular development from **Primordial follicles**,

- 1- **Unilaminar**
- 2- **Multilaminar primary follicles**
- 3- **Secondary (antral) follicles**
- 4- **Graafian (mature) follicles.**

The development of the primordial and primary follicles is independent on FSH; instead, the differentiation and proliferation of the follicular cells are triggered by as yet uncharacterized local factors secreted by cells of the ovary.

Secondary and later follicles, however, are under the influence of FSH.

Follicular development usually culminates in the release of a single oocyte (ovulation).

<https://unibo.smartzoom.com/s1241/course1776/f1815/i2437/>

- *Name the 4 differentiation stages of follicular development from Primordial follicles*
- Which of the following is not a differentiation stages of follicular development from Primordial follicles?

Ovarian Follicles — Primary Follicles

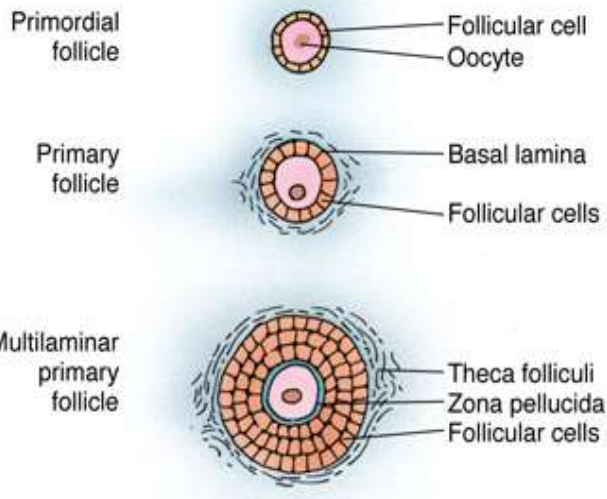
The **primary oocyte** grows to about 100 to 150 μm in diameter with an enlarged nucleus.

Follicular cells become cuboidal in shape, It is called a **unilaminar primary follicle**.

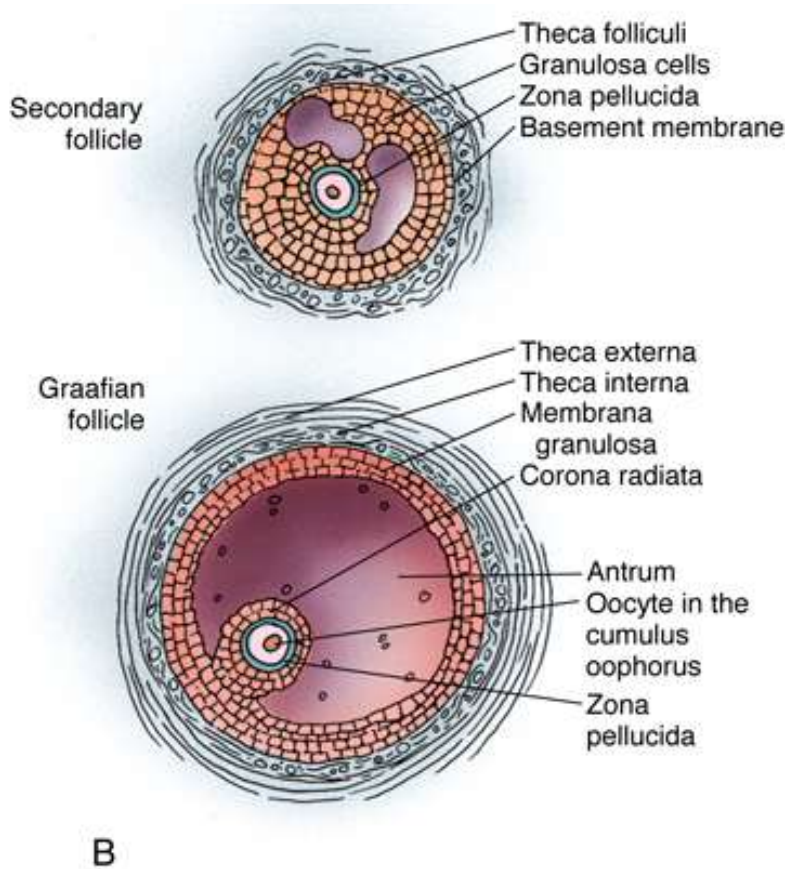
When the follicular cells proliferate and stratify, forming several layers of cells around the primary oocyte, the follicle is called a **multilaminar primary follicle**, and the follicular cells are more commonly referred to as **granulosa cells**.

The theca interna cells produce the male sex hormone **androstenedione**, which enters the granulosa cells, where it is converted by the enzyme **aromatase** into the estrogen - **estradiol**. During this stage, an amorphous substance (the **zona pellucida**) appears, separating the oocyte from the surrounding follicular cells.

Stromal cells or granulosa cells form an inner **theca interna**, composed mostly of a richly vascularized cellular layer, and an outer **theca externa**, composed mostly of fibrous connective tissue.



Ovarian Follicles — Secondary Follicles



Ovarian structure (A) and follicular development (B). Note the corpus luteum and corpus albicans. All the stages of follicular development, from the primordial follicle stage to the graafian follicle stage, are presented.

Secondary follicles are similar to primary follicles except for the presence of accumulations of liquor folliculi among the granulosa cells.

Continued proliferation of the granulosa cells of the secondary follicle depends on **FSH** released by basophil cells of the anterior pituitary.

During ovulation a surge in LH production from the basophil cells of the anterior pituitary will allow the discharge of the mature oocyte, which is ready for the fertilization.

The follicle will undergo atresia, and the granulosa cells will change to granulosa lutein and theca lutein: thus forming the corpus luteum, which will produce **Progesterone**

Follicles Development

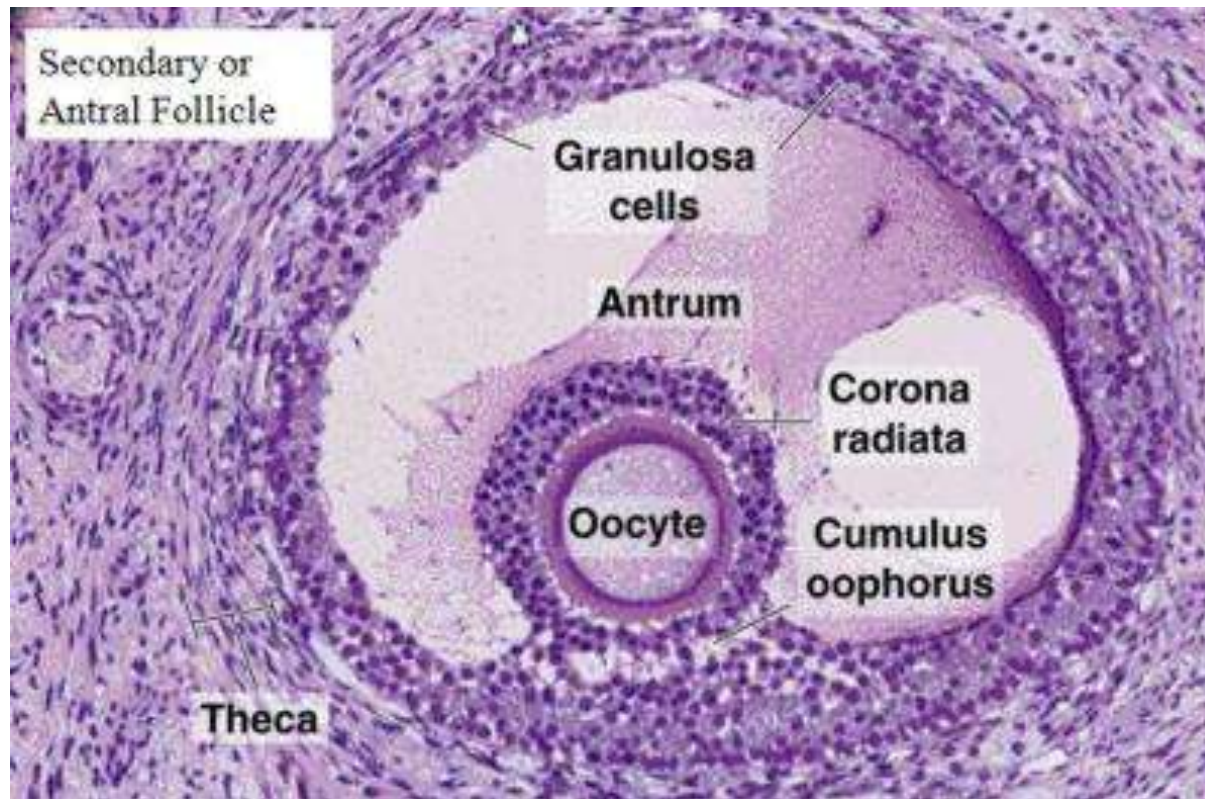
Development of an ovarian follicle is characterized by:

1. Growth and differentiation of a primary oocyte
2. Proliferation of follicular cells
3. Formation of the zona pellucida
4. Development of a connective tissue capsule surrounding the follicle — ***theca folliculi***.

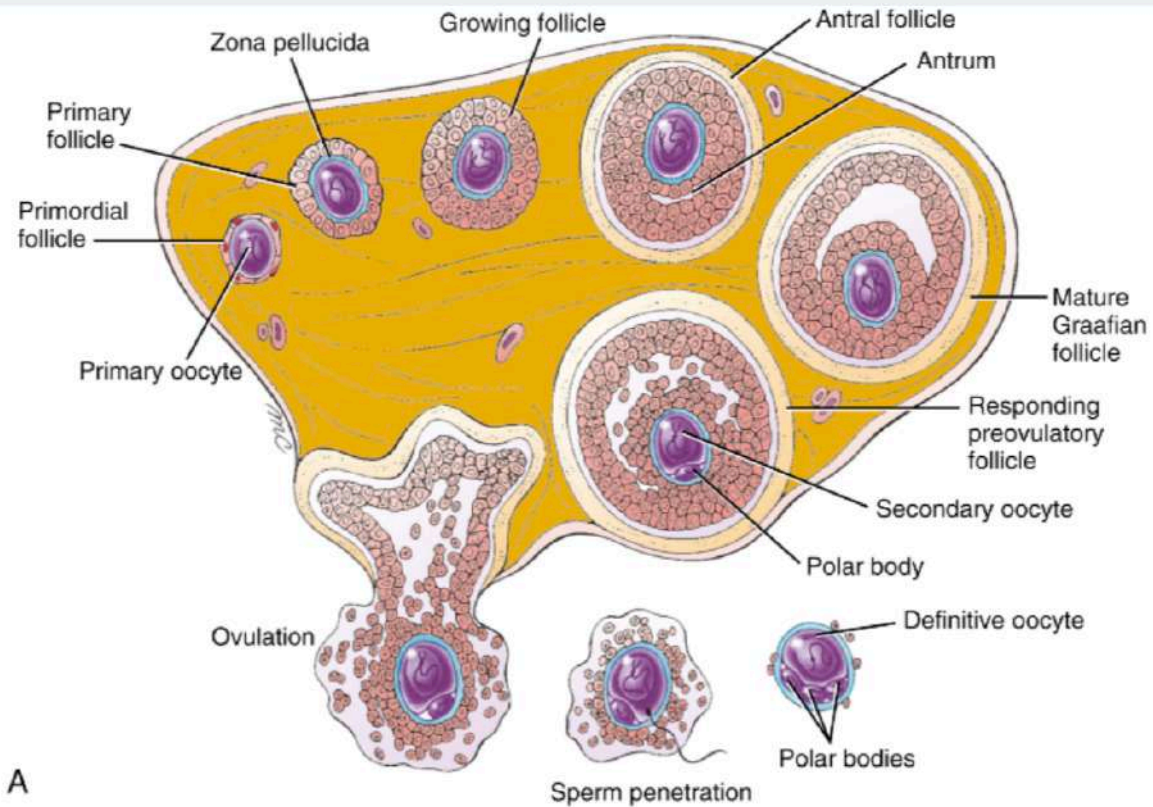
Thecal cells are believed to produce an *angiogenic factor* that promotes growth of blood vessels that provide nutritive support for follicular development.

Ovulation

The **follicular cells** divide actively and forming the **antrum**, containing **follicular fluid**. When the antrum forms, the ovarian follicle is called a **secondary follicle**. The primary oocyte is surrounded by follicular cells—corona radiata—that project into the enlarged antrum.

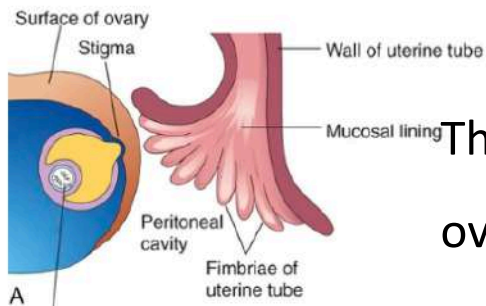


The Ovarian Cycle

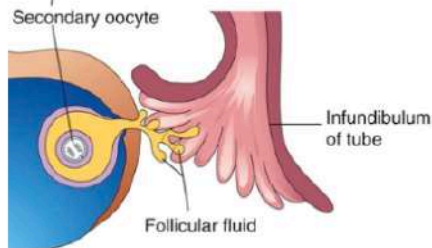


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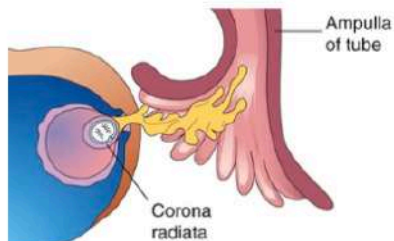
Ovulation



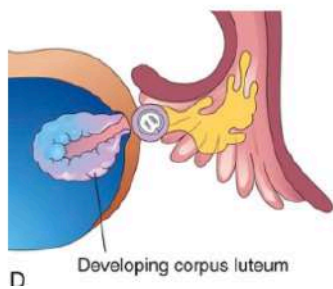
The follicle continues to enlarge and soon forms a bulge on the surface of the ovary.



A small oval, avascular spot, the **stigma**, soon appears on this bulge.

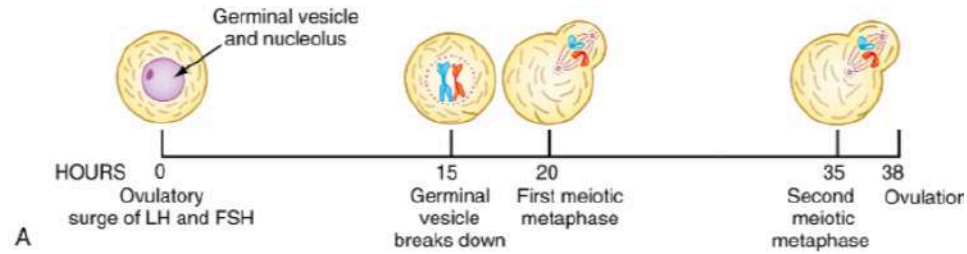


Before ovulation, the secondary oocyte and some cells of the cumulus oophorus detach from the interior of the distended follicle.

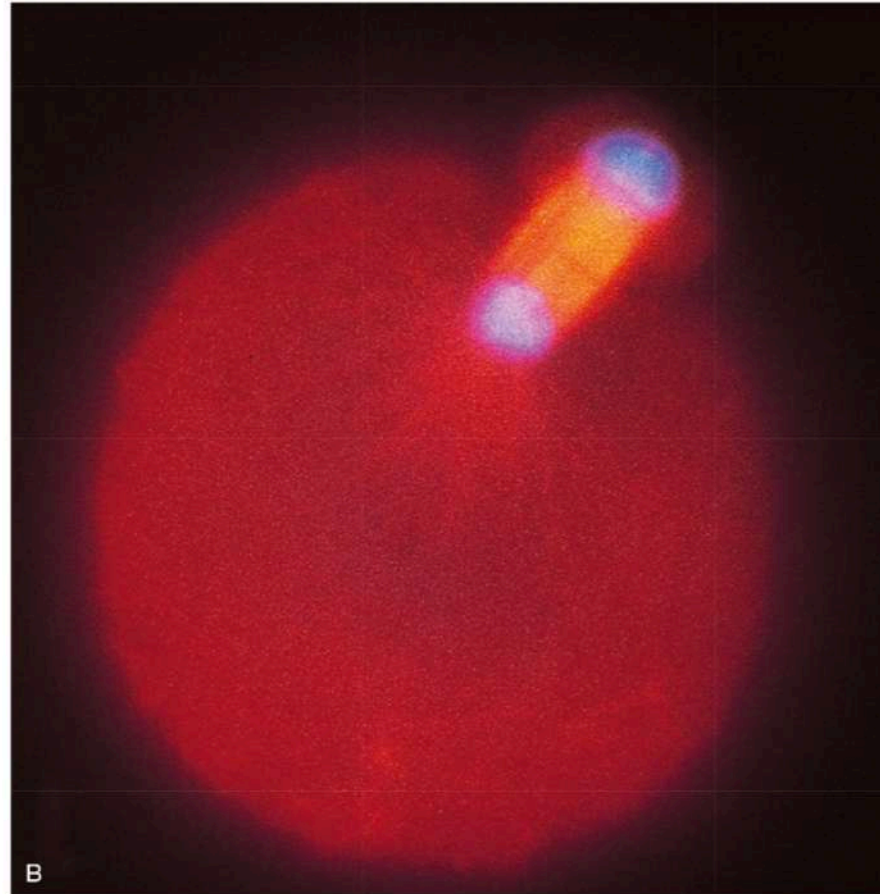


The expelled secondary oocyte is surrounded by the **zona pellucida**, an acellular glycoprotein coat, and one or more layers of follicular cells, which are radially arranged to form the **corona radiata** and cumulus oophorus

38 h onset to Ovulation



By twenty hours, the chromosomes are lined up in metaphase - Cell division to form the secondary oocyte and the first polar body rapidly



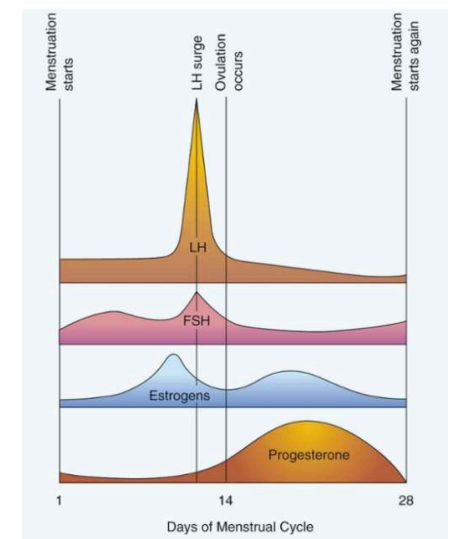
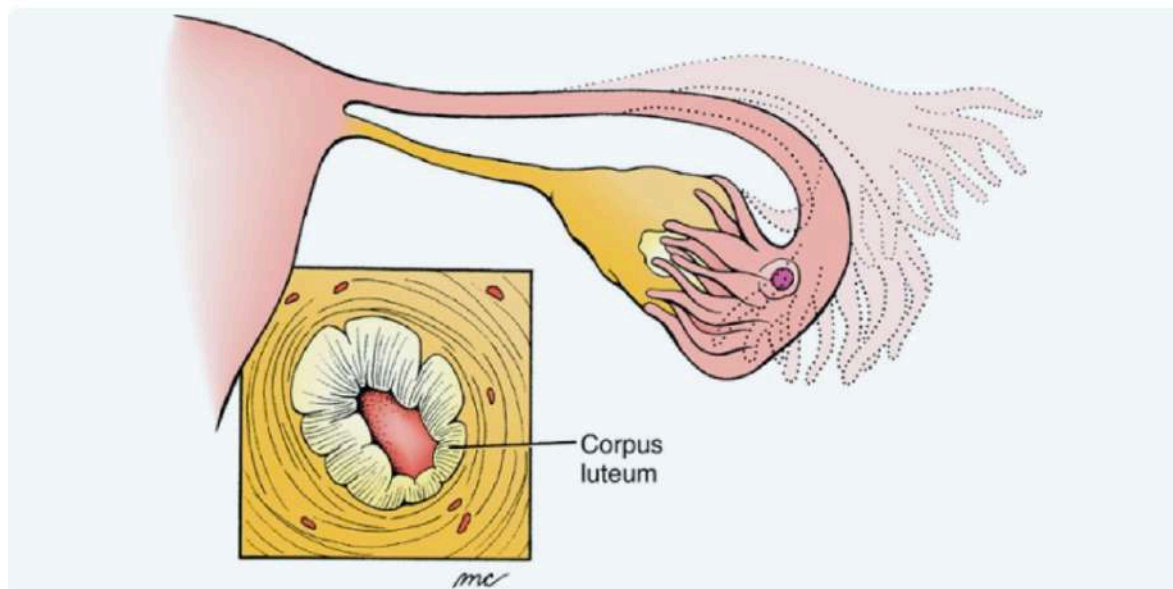
The secondary oocyte promptly begins the meiotic division but about three hours before ovulation is arrested at the second meiotic metaphase.

Ovulation follows within 24-36 hours of a surge of LH

which appears to be the result of signaling molecules from the granulosa cells (feedback).

This surge, elicited by a high estrogen level in blood, appears to cause the stigma to rupture, expelling the secondary oocyte along with the follicular fluid.

Plasmins and matrix metalloproteinases (MMPs) also appear to have some control over stigma rupture



Corpus Luteum

Under the influence of LH, the walls of the follicle develop into a endocrine glandular structure, the **corpus luteum**, which secretes primarily progesterone and some estrogen.

If the oocyte is fertilized, the corpus luteum enlarges to form a ***corpus luteum of pregnancy*** and increases its hormone production. Degeneration of the corpus luteum is prevented by *human chorionic gonadotropin* (hCG).

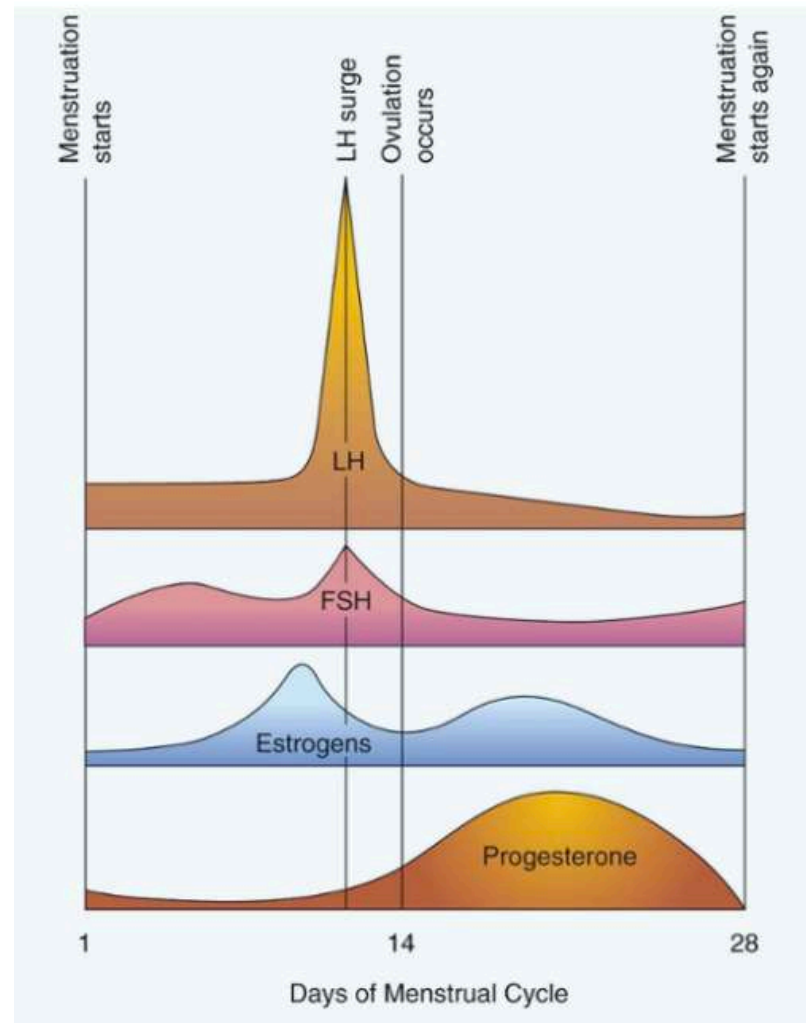
If the oocyte is not fertilized, the corpus luteum degenerates 10 to 12 days after ovulation. It is then called a ***corpus luteum of menstruation***.

The degenerated corpus luteum is subsequently transformed into white scar tissue in the ovary, forming the ***corpus albicans***.

Why Is Folliculogenesis Selectively Stimulated in Only a Few Follicles Each Month?

- Uncertain
- One possibility is that follicles become progressively more sensitive to the stimulating effects of FSH as they advance in development
- Another possibility is that the selection process is regulated by a complex system of feedback between pituitary and ovarian hormones and growth factors.

Hormonal Profile

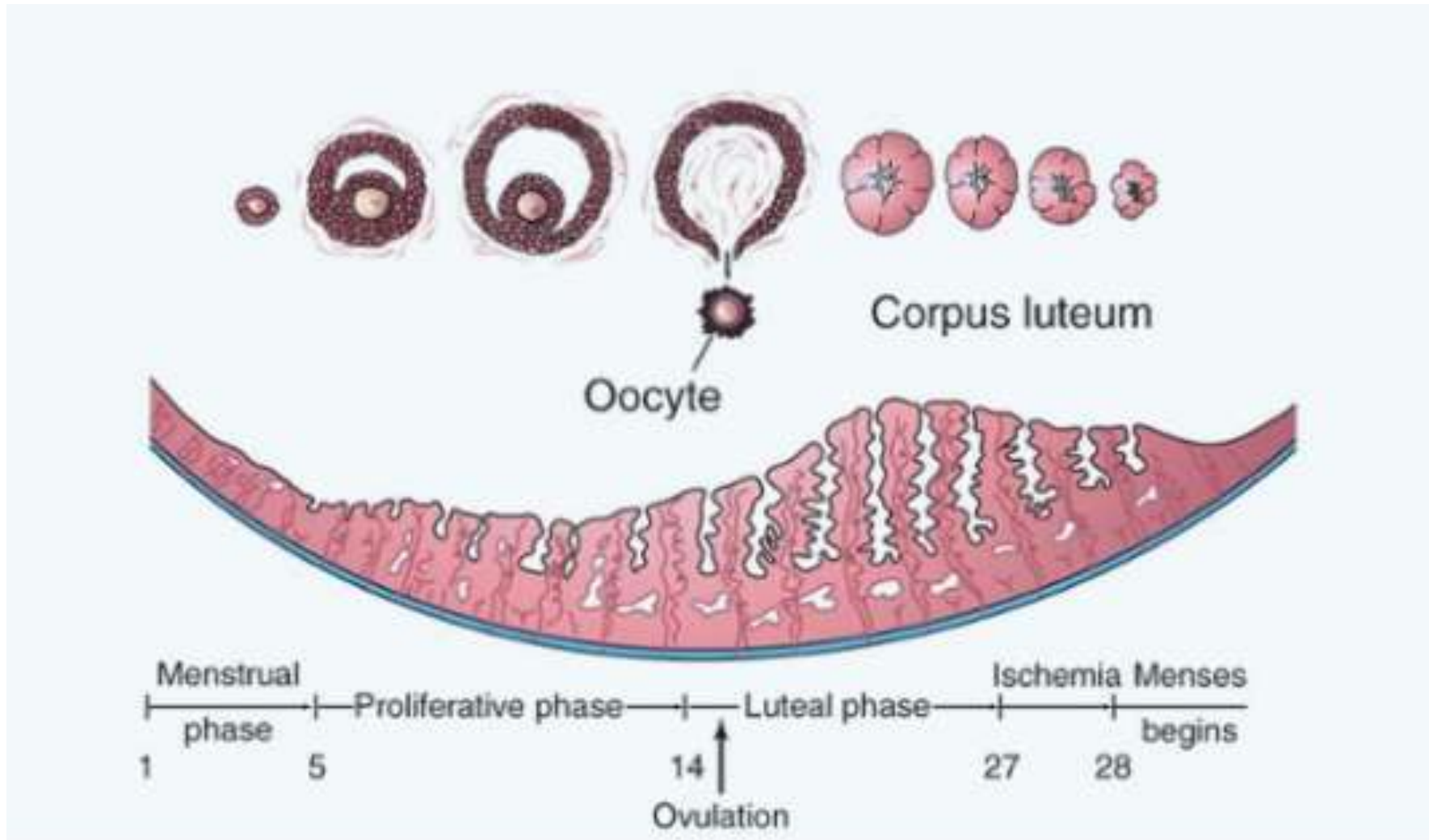


Menstrual Cycle

The cycle is the period during which the oocyte matures, is ovulated, and enters the uterine tube.

Estrogen and progesterone produced by the ovarian follicles and corpus luteum cause cyclic changes in the endometrium of the uterus. These monthly changes in the uterine lining constitute the menstrual cycle.

UTERINE CYCLE



Viability of Oocytes and Sperms

Oocytes in the uterine tube are usually fertilized within 12 hours of ovulation.

In vitro observations have shown that oocytes cannot be fertilized after 24 hours, and they degenerate shortly thereafter. Most sperms do not survive for more than 24 hours in the female genital tract. Some sperms are captured in folds of the mucosa of the cervix and are gradually released into the cervical canal and pass through the body of the uterus into the uterine tubes.

Semen and oocytes can be frozen and stored for many years to be used in assisted reproduction.

Summary

- Gametogenesis
- Ovarian Cycle

<https://studentconsult.inkling.com/read/larsen-human-embryology-schoenwolf-5/videos/animation-1-1>

- **Question 1:** Spermatogonia, derived from primordial germ cells, divide by mitosis during which period(s)?
- All postnatal periods
- Early fetal life
- Continuously throughout postpuberty life x
- The first 2 weeks of embryonic development
- During the process of spermiogenesis

- **Question 2:** Most oocytes become atretic and degenerate during which period(s)?
- At menopause
- Between birth and puberty
- During early postnatal periods
- Following ovulation during each menstrual cycle
- Between the fifth and seventh fetal months x

- **Question 3:** In oocytes, the first meiotic division is completed during which period(s)?
- During fetal development
- During the proliferative phase of the menstrual cycle
- In response to the peak of FSH and LH during the menstrual cycle x
- Following fertilization
- Between birth and puberty

Question 4: During the average menstrual cycle, LH and FSH levels are highest during which period?

- The proliferative phase
- On day 1 of the cycle
- The luteal (secretory) phase
- Immediately prior to ovulation x
- The menstrual phase

Question: Following ovulation, the corpus luteum is formed from which structure?

- The corpus atreticum
- The cumulus oophorus
- The theca externa, theca interna, and granulosa x
- The zona pellucida
- Both A and D

Question 9: The “definitive oocyte” (Haploid, 1N) is generated as a result of which of the following?

- In response to LH and FSH at the time of ovulation
- In response to fertilization following ovulation
- In response to gonadal hormones at puberty
- In response to progesterone secreted by the corpus luteum
- In response to hCG secreted by the conceptus

Question 10: Binding of sperm to integrin α 6 β 1 mediates which of the following?

- Fertilization reaction
- Acrosome reaction
- Fusion of sperm and oocyte plasma membranes
- Capacitation of sperm
- Release of sperm from zona pellucida