

Reproduction and Development

Zelmay Jan

July 2023

Table of Contents

1	Introduction	3
2	Asexual and Sexual Reproduction	3
2.1	Asexual Reproduction	3
2.2	Variations in Sexual Reproduction	4
2.3	Reproductive Cycles	4
3	Fertilization	5
4	Development	6
4.1	General Development	6
4.2	Accessory Sex Organs and External Genitalia Development	7
5	Endocrine Regulation	8
6	Male Reproductive System	8
6.1	Anatomy	8
6.2	Gonadotropin Secretion	10
6.3	Spermatogenesis	10
6.4	Accessory Sex Organs	12
6.5	Erection, Emission, and Ejaculation	13
7	Female Reproductive System	13
7.1	Anatomy	13
7.2	Ovarian Cycle	14
7.3	Menstrual Cycle	16
7.4	Changes in the Endometrium	17
7.5	Menopause	18
8	Fertilization, Lactation, and Birth	19
8.1	Capacitation	19
8.2	Fertilization	19
8.3	Birth and Pregnancy	20
8.4	Lactation	22

9 Disorders	23
9.1 Hermaphroditism and Pseudohermaphroditism	23
9.2 Endometriosis	23
9.3 Ectopic Pregnancy	23
9.4 Genetic Screening	24
10 Contraceptives	24
10.1 Rhythm Method	24
10.2 Coitus Interrupts	25
10.3 Oral Contraceptives	25
10.4 Sterilization	25
10.5 Abortion	26
11 Development	26
11.1 Cleavage	26
11.2 Gastrulation in Frogs	26
11.3 Gastrulation in Chicks	27
11.4 Gastrulation in Humans	28
11.5 Organogenesis	29
11.6 Cytoskeleton in Morphogenesis	30
11.7 Fate Mapping	30
11.8 Axis Formation	31
11.9 Induction in Pattern Formation	31
12 Conclusion	32

1 Introduction

We would like to issue a gentle warning that the content discussed within this handout may be sensitive and explicit. Reproduction is a fundamental biological process that allows living organisms to perpetuate their species, but it involves topics that might be uncomfortable or triggering for some individuals.

Welcome to our guide on reproduction and development, a fundamental mechanism that perpetuates life across all forms of organisms inhabiting our planet. In the following pages, we delve into the intricacies of this biological phenomenon, shedding light on its diverse processes and underlying principles.

2 Asexual and Sexual Reproduction

2.1 Asexual Reproduction

Asexual reproduction is a form of reproduction that does not involve fertilization; it usually relies entirely on mitotic cell division and produces a genetically identical individual. This simple form of reproduction is generally exclusive to invertebrates.

- In **budding**, a section of the child forms from an outgrowth of the parent organism. However, the outgrowth remains attached to the parent organism. This form of reproduction occurs in stony corals.
- **Fission** splits the parent organism into two individuals of even size.
- In **fragmentation**, part of the parent organism breaks off. The lost body parts that form from fragmentation then undergo **regeneration**. This mechanism is found in annelids, corals, cnidarians, and tunicates.

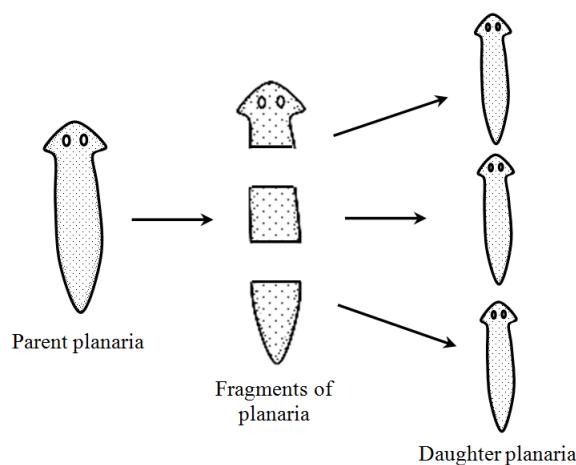


Figure 1: Fragmentation in Planarians. (Source: funscience)

- In **parthenogenesis**, the egg develops without being fertilized. This occurs in bees, wasps, and ants.
 - In bees, unfertilized queen eggs form haploid males via parthenogenesis.

- Parthenogenesis is also known to occur in vertebrates in response to low population density. Komodo dragons, hammerhead sharks, and even sawfish have been observed to undergo parthenogenesis.

2.2 Variations in Sexual Reproduction

Sexual reproduction, in most animals, requires the mating of a male and female. The rest of the handout will be dedicated to describing this process in humans. However, in many animals, finding a partner for reproduction is difficult; animals solve this problem by removing the distinction between the two genders, creating a **hermaphrodite**, for which the organism has reproductive systems of both genders.

- In **hermaphrodites**, which include corals and sea slugs among many other species, any two individuals can mate since they both have male and female reproductive systems. In certain corals, self-fertilization can also occur.
- In the **blue-headed Wrasse**, males live in a harem with several females. When the lone male dies, the biggest female undergoes sex reversal to replace the male. This form of reproduction is known as **protogyny** (gyno = female, proto = first).
- **Oysters**, much like the blue-headed wrasse, undergo sex reversal. However, oysters begin as males and transition to females as they gain size. This mechanism is called **protandry**.
 - This strategy is favored due to the positive correlation between a female's size and the number of gametes she produces. In other words, it is more favorable to be female when bigger.

2.3 Reproductive Cycles

Most animals display cycles in their reproductive activity. This way, animals can produce offspring only when their chances of survival are optimal.

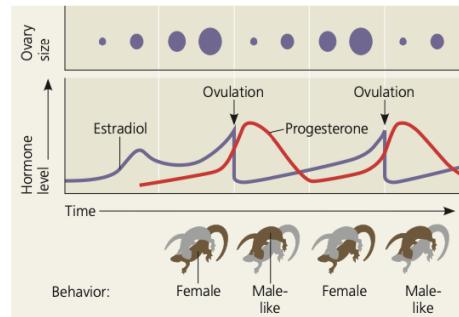
- In some animals, climate change has resulted in a large reduction in reproductive success, such as in caribou.
 - Caribou migrate to birthing grounds in the spring to eat and to birth calves. They determine the time of their visit based on the length of the day in order to optimize the amount of grass available when they visit. As a result of climate change, there is a mismatch between the length of day and the time of grass sprouting, resulting in less food available to caribou and thus a decline in their population.
- Reproductive cycles can also be linked to asexual reproduction. In *Daphnia*, for example, asexual reproduction (i.e. parthenogenesis) occurs during times of adverse environmental conditions.

- *Aspidoscelis* is a genus of asexual whiptail lizards that still observe mating rituals despite being fully asexual.
 - When estrogen levels are high in an individual, it mimics female mating patterns. However, if progesterone levels are high, it mimics male mating patterns. One female of each mating pair has to mimic a male.

This leads to an interesting discussion on the evolutionary background behind sexual reproduction. One may argue that asexual reproduction is superior when considering the number of offspring an individual can have compared to a sexually reproducing individual. However, sexual reproduction allows for genetic diversity and thus greater adaptation compared to asexual reproduction. Thus, it can be generalized that asexual reproduction is more valuable in stable, favorable environments with little change.



(a) Both lizards in this photograph are *A. uniparens* females. The one on top is playing the role of a male. Individuals switch sex roles two or three times during the breeding season.



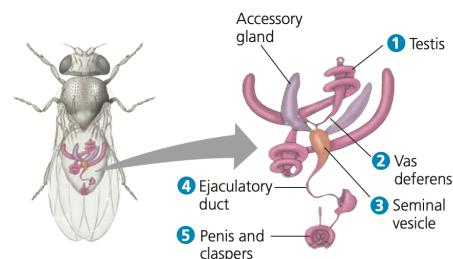
(b) The changes in sexual behavior of *A. uniparens* individuals are correlated with the cycles of ovulation and changing levels of the sex hormones estradiol and progesterone. These drawings track the changes in ovary size, hormone levels, and sexual behavior of one female lizard (shown in brown).

Figure 2: Reproductive cycle of *Aspidoscelis*. (Source: Campbell)

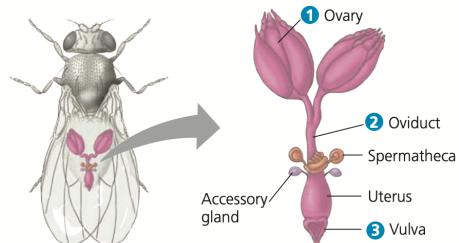
3 Fertilization

- In **external fertilization**, the female releases eggs into its surroundings, then the male releases its sperm into the environment to fertilize the eggs. This form of fertilization requires a moist environment.
 - In **spawning**, for example, a population of individuals in the same area release their gametes at the same time. Spawning is synchronized via environment cues, such as day length, temperature, or pheromones.
 - The **palolo worm** coordinates the release of gametes with the phase of the moon. During the last quarter of the moon in the spring, palolo worms break off their gamete-filled tails, which rise to the surface and release the egg. The eggs are then quickly fertilized by sperm.
 - Asynchronous fertilization is also possible. However, courtship behaviors encourage the release of sperm and egg at the same time between two animals, leading to higher fertilization success.
- **Internal fertilization** is more common in dry areas and requires complementary reproductive systems.
 - Internal fertilization systems also make use of **pheromones**, small molecules similar to hormones that alter the physiology of other organisms.

- Animals that rely on internal fertilization often have fewer offspring, but those offspring generally have higher survival rates. This is because zygotes and embryos are better protected from potential predators, as they are kept safe from the external environment.
- Most animals have **gonads** in order to produce sperm and eggs for reproduction.
- Those lacking gonads, like palolo worms and other annelids, instead produce gametes from undifferentiated cells lining the coelom.
- Many species also have accessory ducts and other structures that nourish the sperm and optimize fertilization.
 - Fruit flies and other insects have complex reproductive systems that include **spermathecae**, which store sperm in females for months until release during optimal conditions. It is also worth noting that female fruit flies expel old sperm and replace it with new sperm when a second male mates with them. As such, males may transfer chemicals to the female after mating with her, making her less receptive to mating again.
 - In non-mammalian vertebrates, the digestive, excretory, and reproductive systems all exit to one opening, the **cloaca**. In these species, males often turn their cloaca inside out to release their sperm.
 - In mammalian vertebrates, males often share openings between the digestive and excretory systems, while females often share openings between the excretory and reproductive systems.



(a) **Male fruit fly.** Sperm form in the testes, pass through a sperm duct (vas deferens), and are stored in the seminal vesicles. The male ejaculates sperm along with fluid from the accessory glands. (Males of some species of insects and other arthropods have appendages called claspers that grasp the female during copulation.)



(b) **Female fruit fly.** Eggs develop in the ovaries and then travel through the oviducts to the uterus. After mating, sperm are stored in the spermathecae, which are connected to the uterus by short ducts. The female uses a stored sperm to fertilize each egg as it enters the uterus before she passes the egg out through the vulva.

Figure 3: Sex organs in flies.
(Source: Campbell Biology)

4 Development

4.1 General Development

- Although the Y chromosome is much smaller than the X chromosome, it is vital to male development. The genes found on the Y chromosome are located in palindromic sequences, allowing for local recombination, which protects the Y chromosome against mutations.
- The **SRY** gene on the Y chromosome codes for **tissue-determining factor (TDF)**, which begins the development of the male reproductive system around 40 days of development. The lack of *SRY* results in the development of the ovaries instead.
- Within the testes are the **seminiferous tubules**, which appear early in the development of the male reproductive system and consist of both non-germ and germ cells.

- **Leydig cells** are the endocrine portion of the testes. In the presence of luteinizing hormone (LH), they produce **testosterone**.
- **Sertoli cells** provide structural support and aid the development of sperm. They help produce testosterone in the growing embryo, resulting in the masculinization of embryonic tissues and contributing to the varying levels of testosterone throughout growth.
 - * Testosterone increases 8 weeks after conception, falls in the second trimester, rises in the third month after birth and falls in the 7-12 months after birth until adolescence.
- **Germ cells** become sperm (eggs in females) through meiosis and specialization.

4.2 Accessory Sex Organs and External Genitalia Development

- Male accessory organs develop from the **Wolffian duct**, while female accessory organs develop from the **Müllerian duct**. Both of these ducts are present at birth.
 - In males, Sertoli cells secrete **Müllerian Inhibiting Factor (MIF)**, causing the Müllerian duct to degenerate. Leydig cells also secrete testosterone, causing the Wolffian duct to form the epididymis, vas deferens, seminal vesicles, and ejaculatory duct.
 - In females, the lack of MIF results in degeneration of the Wolffian duct and the resulting differentiation of the Müllerian duct into the accessory sex organs.
- In the first six weeks of development, males and females share the same external genitalia. Testosterone is converted to a derivative called **dihydrotestosterone (DHT)**, which masculinizes the tissue and results in the external genitalia.

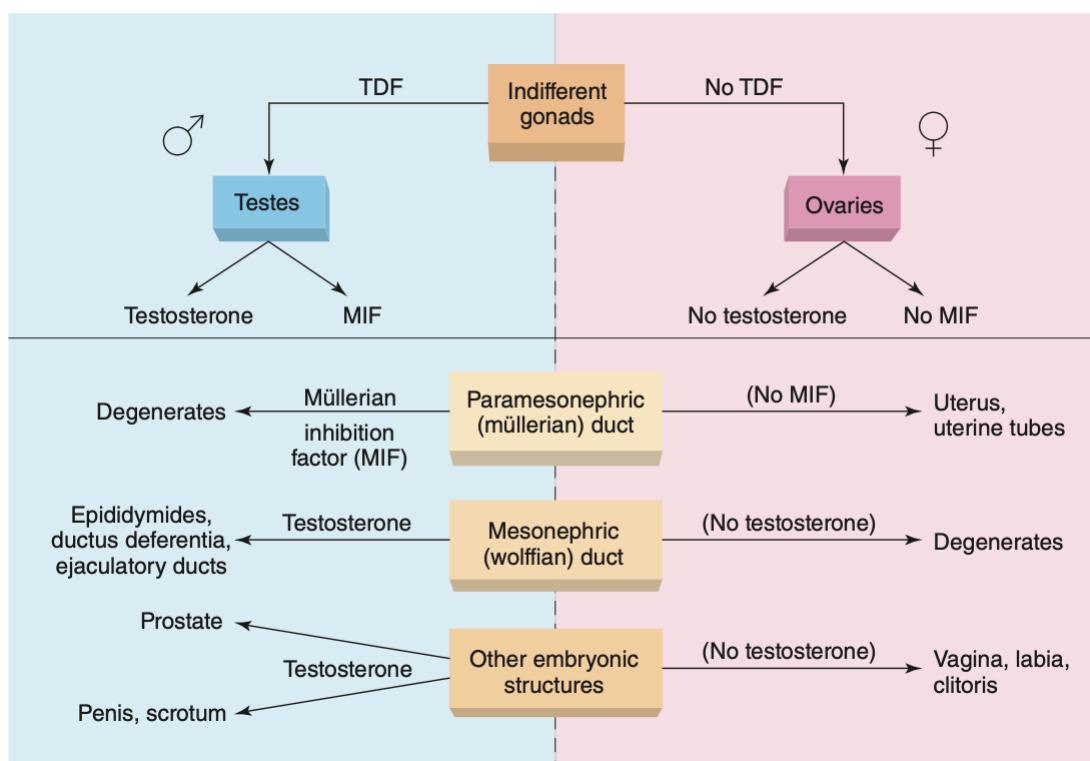


Figure 4: Pathway for development of sex organs. (Source: Fox Human Physiology)

5 Endocrine Regulation

Puberty, gamete production, and maintenance of the sexual structures depends on the secretions of the gonadotropins, **luteinizing hormone (LH)** and **follicle-stimulating hormone (FSH)**.

- The production and secretion of gonadotropins depends on **gonadotropin-releasing hormone (GnRH)**.
- The ovaries and testes secrete **inhibin**, which inhibits FSH secretion. Furthermore, FSH and LH induce sex steroid secretion, which in turn inhibits gonadotropin and GnRH secretion. This is known as **negative feedback**.
- Gonadotropin secretion is pulsatile, thus preventing the desensitization of receptors.
- **Kisspeptin**, a neurotransmitter found in the hypothalamus, increases GnRH secretion. In females, it changes the brain's structure to induce cyclical GnRH secretion. In males, androgens suppress kisspeptin secretion, allowing for noncyclical secretion.
- In many animals, puberty begins with the weakening of inhibitory receptors on the hypothalamus. In humans, it is likely a result of decreased inhibition by GABAergic neurons, along with increased kisspeptin secretion.
- In females, increased estradiol secretion results in the stimulation of the epiphyseal growth plate and, thus, a growth spurt. Estrogen stimulates chondrocytes to divide, causing the cartilage matrix to grow and calcify. Estrogen also stimulates breast development and causes **menarche**, the first menstrual flow.
 - Puberty is inhibited by exercise in females because leptin, secreted by adipose tissue, is required for puberty.
- In males, testosterone results in the growth of the penis, as well as the development of other sex characteristics. In bone, testosterone is converted to estrogen, stimulating growth. Testosterone also directly stimulates bone growth under the periosteum, the membrane covering the bone. This results in wider bones.
- Hair growth in both males and females is promoted by steroids secreted by the adrenal cortex.

6 Male Reproductive System

6.1 Anatomy

- The scrotum and the penis constitute the external genitalia of the male.
- The internal male reproductive system consists of cells that secrete hormones and sperm. It provides ducts for the movement of gametes and houses glands important for the production of fluids necessary for the movement of sperm.

- The testis consists of highly coiled seminiferous tubules involved in the production of sperm. It must be kept at a temperature around two centigrade lower than the core body temperature. This is attained through the lowering of the testis and the surrounding scrotum during embryonic development such that they hang away from the body. **Cremaster muscles** also travel through the spermatic cord to reflexively move the testis up and down in order to maintain temperature. In rodents, the testis ascends after mating season to prevent sperm production.

- Sperm moves from the seminiferous tubules to the epididymis, where it can stay for a few weeks. During ejaculation, the sperm moves through a muscular vas deferens, which connects secretions from the seminal vesicle to the ejaculatory duct. The ejaculatory duct opens into the urethra, which opens the tip of the penis.

• Accessory Glands

- The seminal vesicle, bulbourethral gland, and prostate gland all secrete fluids that combine with sperm to form semen. Before ejaculation, the bulbourethral glands release a clear mucus that neutralizes any acidic urine in the way. The accessory glands' functions will be further described later in this handout.

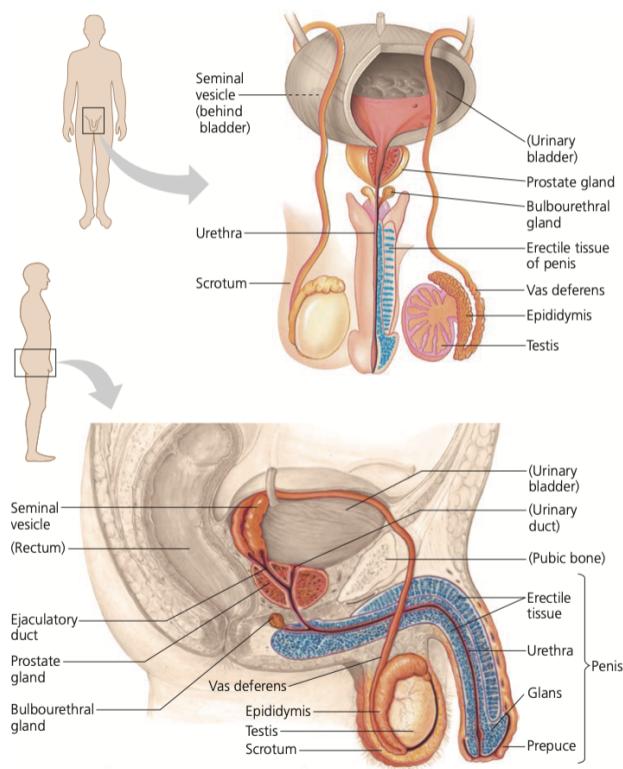


Figure 5: Diagram of sperm's pathway.
(Source: Campbell Biology)

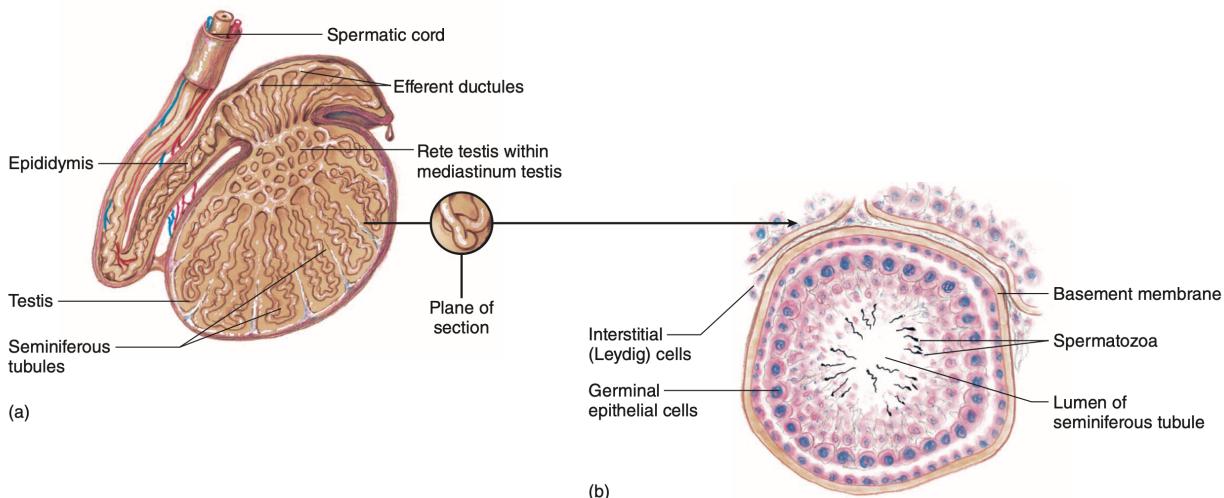


Figure 6: Diagram of where sperm are formed. (Source: Fox Human Physiology)

6.2 Gonadotropin Secretion

- Sertoli cells produce inhibin to inhibit FSH secretion. The latter is done when FSH itself binds to receptors on Sertoli cells. This binding also stimulates spermatogenesis.
- Leydig cells produce testosterone to inhibit gonadotropin secretion. They are found in the interstitium, which is heavily inundated with blood vessels in order to allow testosterone to easily travel throughout the testis.
- Testosterone is converted to many different compounds within cells to function in different tasks. Testosterone is converted to DHT in the prostate and skin and to estrogen in the skeletal system. In males, estrogen is important in adipose tissue, while testosterone is important in muscles.

View the table below to see the various actions of androgens (e.g., testosterone).

Category	Action
Sex Determination	Growth and development of wolffian ducts into epididymis, ductus deferens, seminal vesicles, and ejaculatory ducts Development of urogenital sinus into prostate Development of male external genitalia (penis and scrotum)
Spermatogenesis	At puberty: Completion of meiotic division and early maturation of spermatids After puberty: Maintenance of spermatogenesis
Secondary Sex Characteristics	Growth and maintenance of accessory sex organs Growth of penis Growth of facial and axillary hair Body growth
Anabolic Effects	Protein synthesis and muscle growth Growth of bones Growth of other organs (including larynx) Erythropoiesis (red blood cell formation)

Figure 7: Synthesis pathway (left) and actions of (right) androgens.
(Source: Fox Human Physiology)

6.3 Spermatogenesis

- During embryonic development, diploid cells migrate to the embryonic testes and become **spermatagonia**, stem cells that will produce sperm by mitosis and meiosis. These cells are located by the basement membrane, closest to the nourishing blood vessels.
- The diploid cells undergo mitosis to form a primary spermatocyte while also regenerating the original stem cell. This prevents the stem cell supply from being exhausted.
- The primary spermatocyte then undergoes meiosis to form secondary spermatocytes.
- The secondary spermatocytes undergo a second meiotic division to form spermatids.

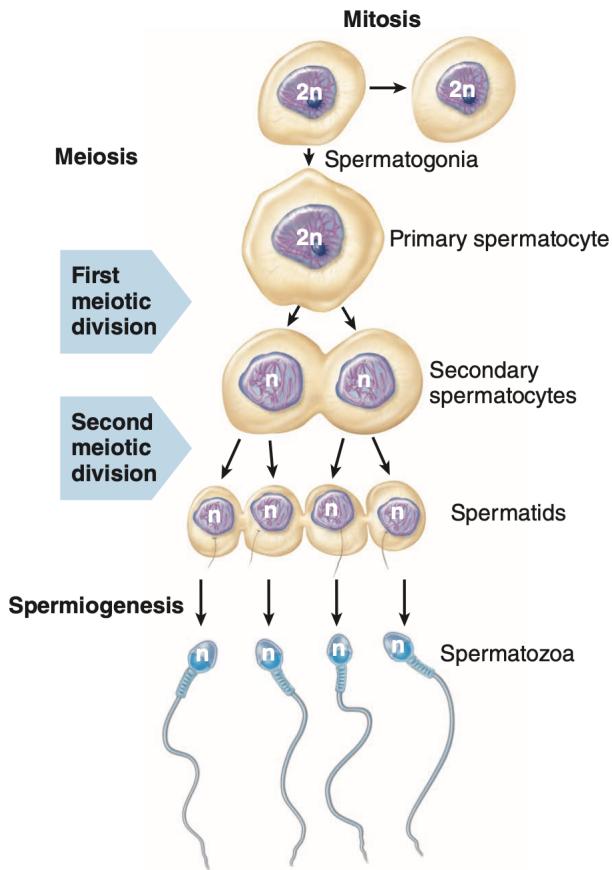


Figure 8: Spermatogenesis. (Source: Fox Human Physiology)

- The development of the spermatids into functional spermatozoa (i.e., sperm cells) requires a process called **spermatogenesis**.
 - During spermatogenesis, histones are modified and replaced by proteins called **protamines**. This allows for the compaction of the spermatozoa nucleus, followed by the development of the flagella.
 - **Sertoli cells** are located on the basement membrane and form a ring around the tubules connected by tight junctions, a **blood-testis barrier**. This barrier prevents autoimmune reactions, only allowing certain molecules to pass. In other words, it creates an immunologically privileged site, with little to no immune cells.
 - Sertoli cells consist of large amounts of cytoplasm from the basement to the lumen. They are connected via tight junctions between them, and spermatogenesis occurs in the spaces between the tight junctions. As the products slowly move through these spaces, the tight junctions constantly break and reform to accommodate this change.
 - During spermatogenesis, the Sertoli cells phagocytize the bulges of cytoplasm off the side of the sperm. During this process, the Sertoli cells also provide chemicals and products required by the sperm, such as products of the X chromosome not found in some sperm.
 - Sertoli cells secrete androgen-binding protein (ABP) into the lumen of the tubule, allowing for the concentration of testosterone within the tubule, where it aids the function of sperm.

- A spermatozoa consists of a head, with a nucleus, DNA, and an overlying cap called an **acrosome**, as well as a three-part 9+2 flagella called an **axoneme**. The midpiece (superior) contains mitochondria and a fibrous sheath. The principal piece only contains the fibrous sheath. The endpiece (inferior) lacks both of these.

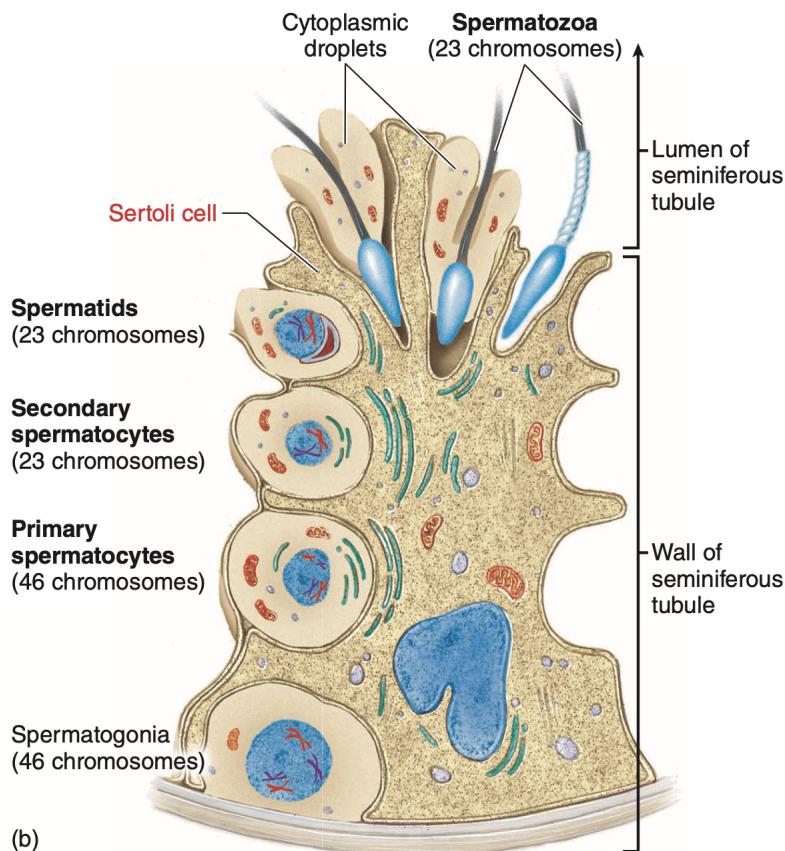


Figure 9: Spermatogenesis. (Source: Fox Human Physiology)

- In spermatogenesis, the division of primary spermatocytes past prophase I requires testosterone, which is converted to its derivatives and stimulates the completion of division. Testosterone secreted by Leydig cells also stimulates spermatogenesis in the seminiferous tubules.
- FSH is not required for spermatogenesis, but it makes for a more optimal environment and allows for greater fertility.

6.4 Accessory Sex Organs

- Following sperm production in the seminiferous tubules, sperm is moved into the rete testis, where it drains via the efferent ductules into the epididymis, which is then drained by the vas deferens.
- When sperm enter the epididymis they are immotile due to the low pH. However, as they move through the epididymis, they obtain resistance to this low pH.
 - In the female reproductive tract, the pH is neutralized by the alkaline secretions of the prostate, so sperm becomes fully active and motile.

- The seminal vesicles are the first to add their fructose-rich fluid that composes 60% of the volume in semen.
- Next, pores in the prostate gland allow citric acid, calcium, and coagulation proteins to enter the semen.
 - Coagulation proteins allow the semen to coagulate after ejection before being decoagulated in the female reproductive system.

6.5 Erection, Emission, and Ejaculation

- The two **corpora cavernosa** consist of erectile tissue in the ventral portion of the penis. The corpora cavernosa are innervated by the deep artery of the penis.
- Nitric oxide (NO), produced by parasympathetic axons, is released to the corpora cavernosa, activating guanylyl cyclase (GC). This increases cyclic guanosine monophosphate (cGMP) levels and causes Ca²⁺ outflow. This then results in vasodilation, which causes an erection. Endothelial cells of the corpora cavernosa also produce NO, allowing for further vasodilation.
- The **corpus spongiosum** is a mass of erectile tissue surrounding the urethra in the dorsal portion of the penis.
- **Emission** is the movement of semen into the urethra, and **ejaculation** is the expulsion of semen from the urethra. Emission and ejaculation are stimulated by sympathetic nerves that cause the contraction of muscles and glands.

7 Female Reproductive System

7.1 Anatomy

- The two **ovaries**, analogous to testes in males, enter into the **Fallopian tube**.
- The ova released into the uterine tubes by ovulation are drawn in by cilia into the **uterus**, a muscular, pear-shaped organ. There are three layers in the uterus, the outer perimetrium, middle myometrium, and the inner endometrium.
 - The endometrium consists of nonkeratinized, stratified squamous epithelium with a stratum basale and an upper stratum functionalis. The stratum functionalis grows cyclically throughout the menstrual cycle.
- The uterus opens into the **vagina**, which is separated from the uterus by a cervical mucus.

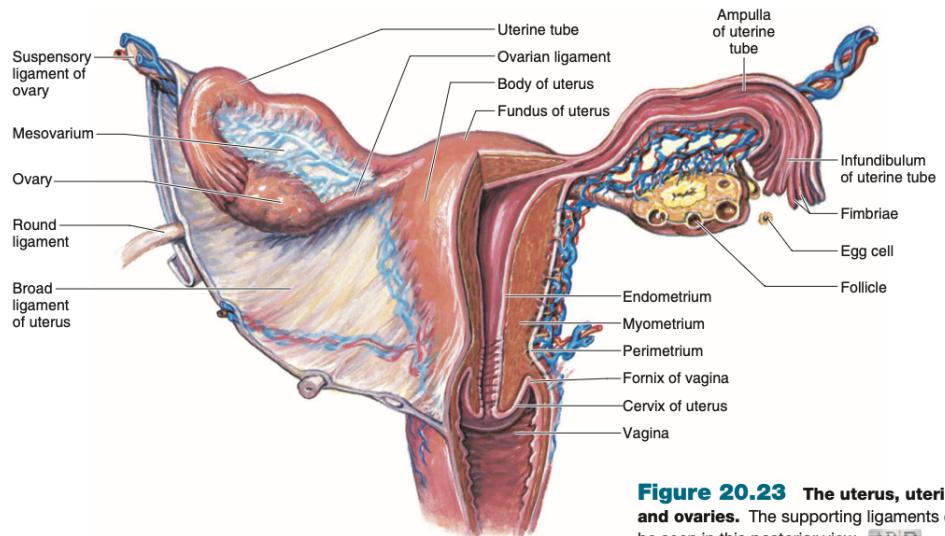


Figure 20.23 The uterus, uterine tubes, and ovaries. The supporting ligaments can also be seen in this posterior view.

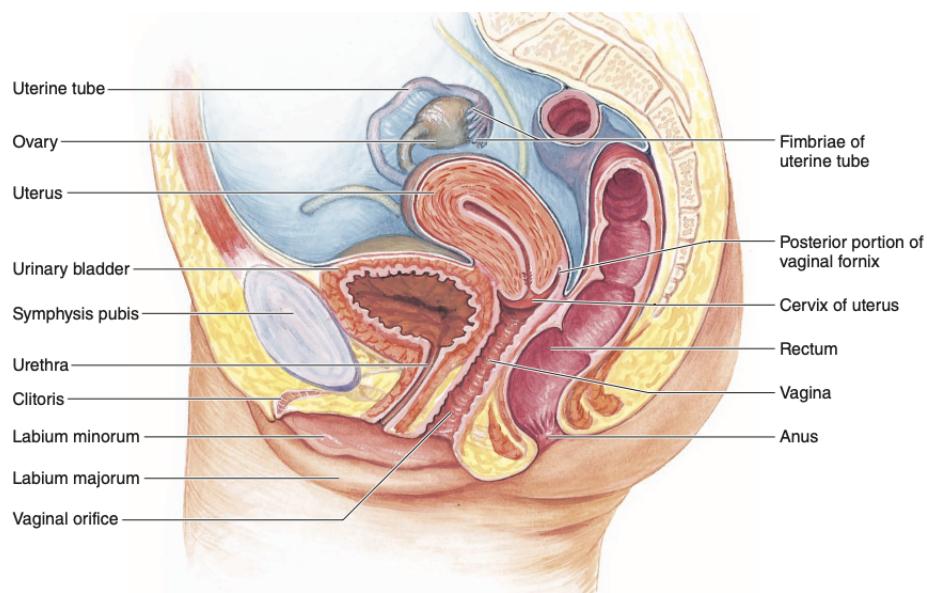


Figure 10: Diagram of female reproductive system. (Source: Fox Human Physiology)

7.2 Ovarian Cycle

- Germ cells migrate from the embryo and multiply via mitosis to form **oogonia**, most of which undergo apoptosis. The remaining oogonia undergo meiosis and are arrested at prophase I to form diploid **primary oocytes**, just as in spermatogenesis.
- Throughout the lifetime of a woman, only 400 oocytes will likely ovulate. This contrasts with spermatogenesis, in which stem cells are regenerated. Primary oocytes that are not stimulated to divide are contained in primary follicles.
- Primary follicles contain a single layer of surrounding follicle cells. FSH stimulation results in division to form layers of **granulosa cells** that surround the oocyte.

- The oocyte develops vesicles containing fluid, becoming a **secondary follicle**. The vesicles eventually fuse and create a cavity called the **antrum**. This follicle is known as a **Graafian follicle**.
- To form a secondary oocyte, the primary oocyte completes its meiotic division, forming a polar body, which later degenerates, and a secondary oocyte, which retains most of the cytoplasm. The secondary oocyte continues meiosis until it stops at metaphase II.
- The theca interna of the ovarian follicles produces testosterone in response to LH, which diffuses into granulosa cells, where aromatase converts it into estradiol. Thus, estradiol is produced as the follicles grow bigger.

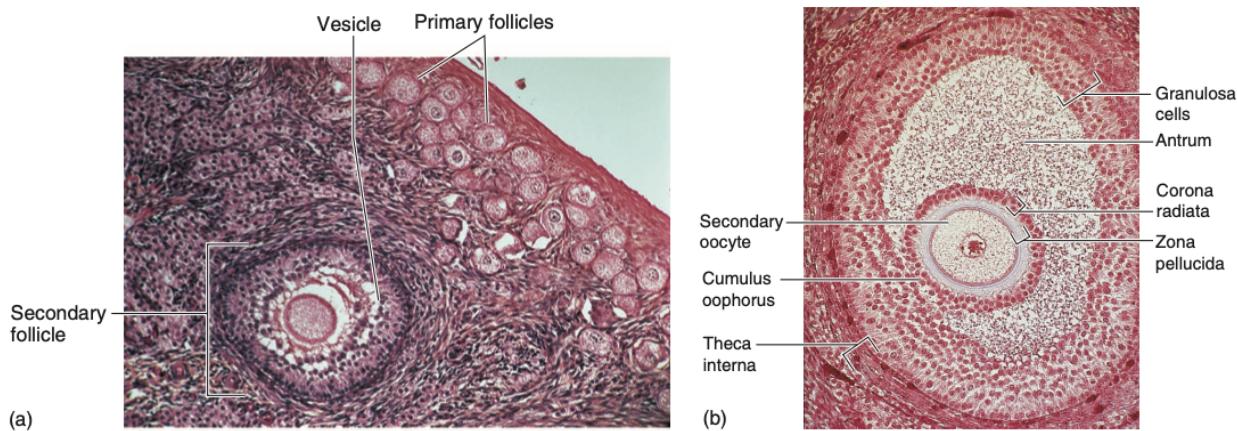


Figure 11: Labeled cross-section of follicles. (Source: Fox Human Physiology)

- Usually, only one follicle becomes dominant and ovulates, while the rest are **atretic** and fail to rupture. FSH and LH promote ovulation and protect the follicles from atresia, while androgens and certain proteins promote atresia.
- The mature follicle ruptures via ovulation, still surrounded by the corona radiata and zona pellucida. The sperm must pass through these layers to fertilize the egg and complete the last meiotic division, which produces a polar body and developing zygote.
- Following ovulation, LH promotes the formation of the **corpus luteum** out of the empty follicle, which secretes estradiol and progesterone. At the end of menstruation, it regresses to form a non-functional **corpus albicans**.

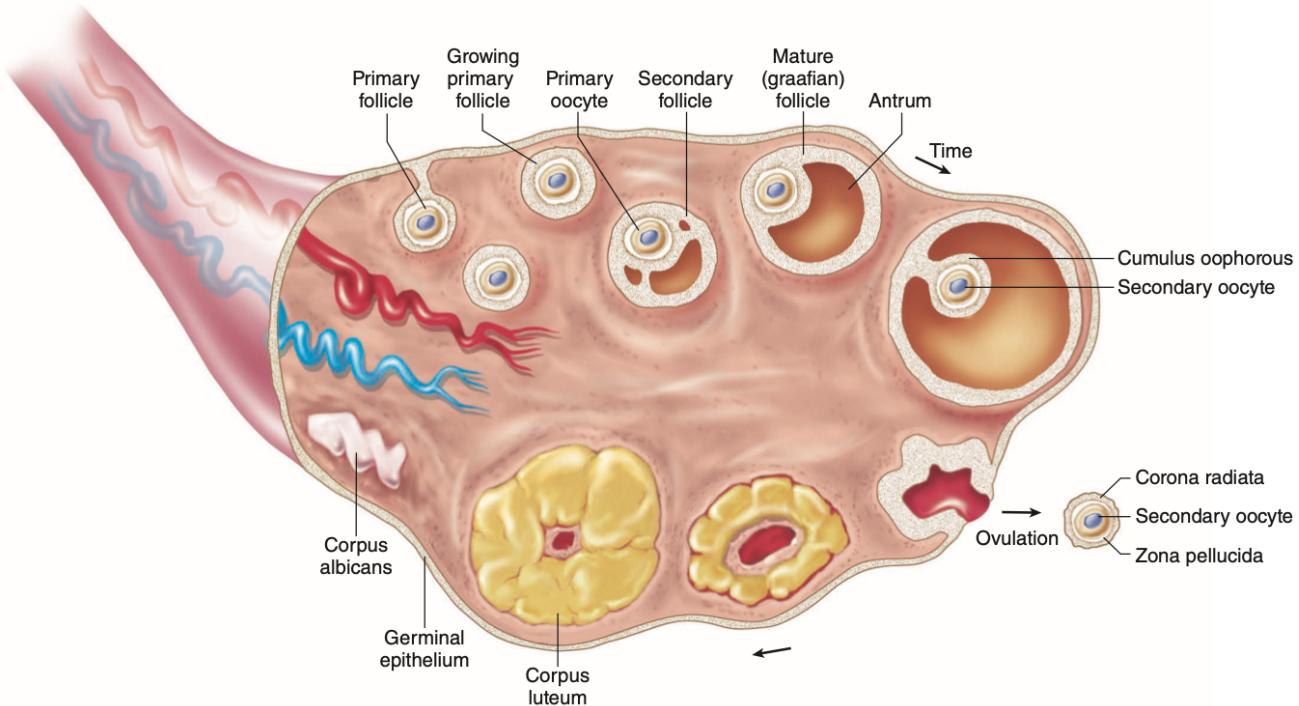


Figure 12: Diagram of follicle development. (Source: Fox Human Physiology)

7.3 Menstrual Cycle

- In females, cyclic changes in gonadotropin hormones result in ovarian changes that follow monthly cycles. The resulting hormonal changes are accompanied by changes in the endometrium.
- Only primates have a menstrual cycle. In other mammals, the endometrium is simply reabsorbed without fluid flow. Mammals with estrous cycles only undergo sexual activity during the period surrounding ovulation in order to maximize pregnancy chances, while those with menstrual cycles can copulate throughout the year. Bears and wolves have a single estrous cycle, while elephants have several. Cats ovulate only upon mating.
- The **follicular phase** lasts from the first day of menstruation until ovulation (i.e., days 1-13). Menstruation lasts from days 1-5 of an average cycle.
 - As follicles grow, becoming Graafian follicles, estradiol levels begin to rise, reaching their peak at about day 13. Low levels of estradiol inhibit LH. However, high levels of estradiol stimulate the secretion of LH.
 - FSH stimulates the production of FSH receptors on granulosa cells, increasing their sensitivity. FSH and estradiol also stimulate the production of LH receptors.
 - FSH levels decline around the middle of the menstrual cycle, causing atresia of non-dominant follicles. However, the increased sensitivity of FSH receptors and the increased number of FSH receptors allow dominant follicles to survive this fall in FSH.
 - The rise in estradiol during the follicular phase increases GnRH pulses via positive feedback. As a result, LH levels increase rapidly, generating an **LH surge** that triggers ovulation.

- The **luteal phase** lasts from the first day of ovulation until the first day of menstruation.
 - After ovulation, LH stimulates the empty follicle to develop into the corpus luteum. This structure secretes progesterone and estradiol, allowing for negative feedback on FSH and LH secretion. This prevents the development of new follicles in order to avoid multiple simultaneous pregnancies. Note that the collective action of estradiol and progesterone results in negative feedback, while estradiol by itself results in positive feedback.
 - The late luteal phase sees a drop in estrogen and progesterone levels as the corpus luteum becomes the corpus albicans. This transition is mediated by a uterine hormone called luteolysin. Normal function of this hormone is prevented by LH, but the corpus luteum lowers LH levels, leading to its own degradation.
 - At the end of the cycle, estrogen and progesterone levels fall, allowing a new cycle to begin.

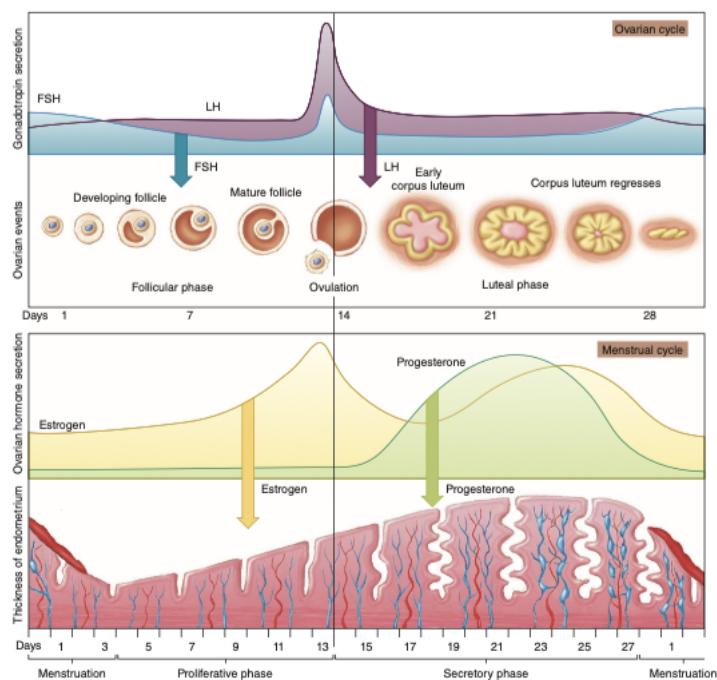


Figure 20.33 The cycle of ovulation and menstruation. The downward arrows indicate the effects of the hormones. APR

Figure 13: Hormone levels throughout the menstrual cycle. (Source: Fox Human Physiology)

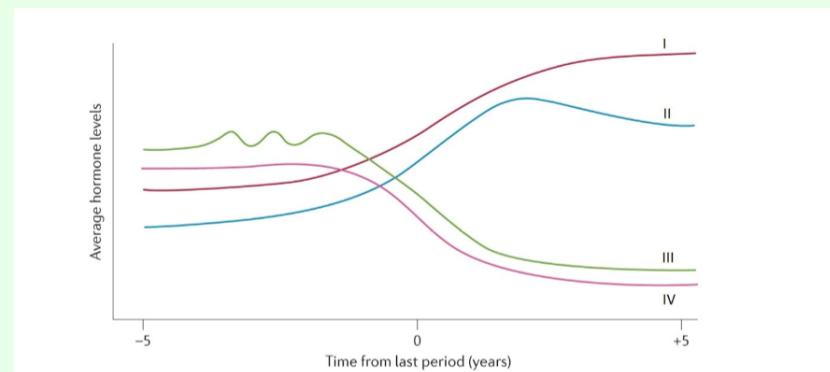
7.4 Changes in the Endometrium

- The **proliferative phase** coincides with the follicular phase of menstruation. Increased estradiol levels stimulate the growth of the stratum functionalis, along with coiled blood vessels in the endometrium, found only in primates, called spiral arteries.
- The **secretory phase** coincides with the luteal phase of menstruation. Increased progesterone levels stimulate the development of uterine glands, resulting in a thick and spongy endometrium. This phase also sees an increase in glycogen levels in the endometrium.
- The **menstrual phase** sees necrosis of the stratum functionalis. Furthermore, vasoconstriction of arteries results in bleeding and shedding of the endometrium.

7.5 Menopause

- The depletion of follicles results in a decrease in estrogen and inhibin secretion, which increases FSH and LH secretion due to the loss of negative feedback. The lack of estrogen often leads to osteoporosis in postmenopausal women.
- Postmenopausal women have small amounts of estrone, a type of estrogen that is formed by androgens in adipose tissue.
- Women with higher amounts of adipose later in life, therefore, have a lower chance of developing osteoporosis.
- Menopause causes many symptoms, including loss of lubrication in the vaginal wall associated with atrophy, increased risk of atherosclerosis, osteoporosis, and hot flashes (i.e., falls in core body temperature, followed by sudden feelings of heat and sweating).

Example 7.1: (USABO Semifinals Exam 2020) With the onset of menopause, ovarian follicles gradually stop releasing eggs. A corresponding change in reproductive hormone levels occurs. The figure shows the changes in estradiol, FSH, hormone X, and LH during this pre- to post-menopausal transition. Hormone X is a marker for developing follicles. Which choice correctly labels the four hormones?



- I - Estradiol; II - Hormone X; III - FSH; IV - LH
- I - FSH; II - LH; III - Estradiol; IV - Hormone X
- I - FSH; II - Estradiol; III - LH; IV - Hormone X
- I - Hormone X; II - LH; III - Estradiol; IV - FSH
- I - Estradiol; II - Hormone X; III - LH; IV - FSH

Solution: During menopause, follicles become depleted, and estrogen and inhibin secretion also fall. This results in increased FSH and LH secretion, meaning they correspond to I and II on the graph. Since follicles are depleted, hormone X must be either III or IV, leaving estradiol to also be III or IV. Thus, the **answer must be B**.

8 Fertilization, Lactation, and Birth

8.1 Capacitation

- The male ejaculates around 300 million sperm into the female vagina. Only around 100 survive, of which about 10 gain the ability to fertilize an ovum, known as **capacitance**.
- The female reproductive tract is alkaline. In conjunction with the removal of hydrogen from the sperm, this allows for a raise in the pH of a sperm cytoplasm and thus the activation of dynein, a motor protein, in the flagellum.
- The rise in sperm pH also activates a calcium channel in the flagellum's principal piece, called a **CatSper channel**. The subsequent rise in calcium results in hyperactivation of the flagellum, with a resulting increase in motility.
- The sperm moves along the oviduct via chemotaxis and thermotaxis, attracted toward specific chemicals and warmer temperatures.

8.2 Fertilization

- Sperm binds to carbohydrates on the glycoprotein-rich zona pellucida. Through this binding, sperm is exposed to progesterone, which is secreted by the corona radiata around the ovum.
- The progesterone activates CatSper channels in the head of the sperm, inducing the **acrosome reaction**, in which the acrosome in the sperm fuses with the outer membrane, resulting in the release of acrosomal enzymes. These enzymes, which are proteases and hyaluronidases, digest the hyaluronic acid surrounding the extracellular matrix of the egg, allowing the sperm to dig through the zona pellucida to fuse with the egg.
- When the sperm binds to the egg, a calcium wave is triggered, in which cytoplasmic calcium increases, starting from one pole of the egg and traveling to the opposite pole. This wave prevents other sperm from fertilizing the egg and also restarts the second meiotic division.
- The sperm contributes DNA and a centrosome, necessary for microtubule organization during mitosis. Mitochondria brought in by the sperm degenerate. Thus, all mitochondrial DNA is maternal.
- In **in vitro fertilization (IVF)**, the mother receives injections of FSH to stimulate the growth of ovarian follicles, along with other hormones to prevent ovulation. The follicles are removed from the ovary, and sperm is injected into the egg through the zona pellucida. The embryo is then grown until it reaches the 8-cell stage, in which it is transferred to the uterus to restart growth.

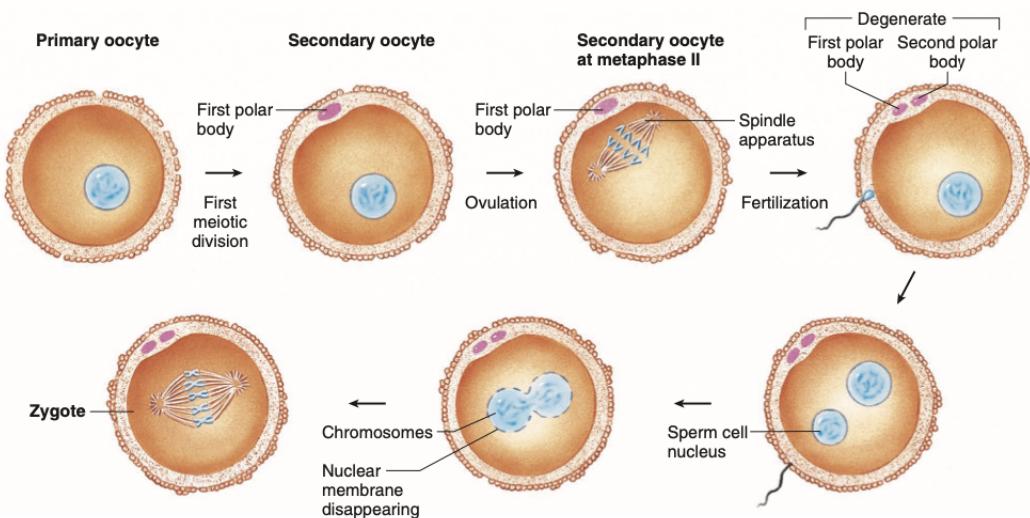


Figure 14: Diagram of fertilization. (Source: Fox Human Physiology)

8.3 Birth and Pregnancy

- After ovulation, a sperm fertilizes the oocyte and restarts meiosis. Cleavage occurs as the egg travels through the oviduct. By the time the embryo reaches the uterus, it is a ball of cells. The embryo then implants itself into the uterus about seven days after conception.
- In a typical menstrual cycle, the corpus luteum would degenerate, causing a drop in hormones that makes the endometrium slough off. In order to prevent this in a pregnant female, the implanted embryo secretes human **chorionic gonadotropin (hCG)**, similar to LH, in order to maintain the corpus luteum so that it continues to secrete progesterone and estrogen.
- Carrying an embryo in the stomach is known as **pregnancy** or **gestation**.
- During the **first trimester**, which lasts for three months, the embryo secretes hCG to maintain the secretion of progesterone and estrogen by the corpus luteum. Like other gonadotropins, hCG can be detected in or extracted from the urine.
 - The embryo obtains its nutrients from the endometrium, while the outer trophoblast forms outgrowths and associates with the endometrium to form the **placenta**.
 - The placenta provides nutrients and immune protection to the embryo and helps it dispose of metabolic wastes. The umbilical cord connects the placenta with the fetus, allowing blood to travel between the two.
 - If an embryo splits during the first trimester, it can form a monozygotic (i.e., identical) twin. If two follicles mature and are both fertilized and implanted, then dizygotic (i.e., fraternal) twins can be born.
 - During **organogenesis**, the development of body organs, the embryo is very susceptible to alcohol and other damage. The heart begins to beat by the 4th week and can be detected by the 8th-10th week using a handheld Doppler.

- Hormonal changes such as high levels of progesterone result in changes in the mother, including the formation of a mucus plug in the cervix, enlargement of the breasts and uterus, and interruption of menstrual cycling. The mother may also experience nausea and morning sickness.
- By the end of the first trimester, the embryo is about 5 centimeters long.
- The **second and third trimesters** see the formation of fingernails, external genitalia, and the outer ears, as well as the beginning of fetal movements.
 - The corpus luteum degenerates, and the placenta takes over the production of gonadotropins.
 - By the end of the second trimester, the embryo grows to about 30 centimeters long.
 - In the third trimester, the fetus grows to about 50 centimeters long, and activity slows down as the fetus fills the amniotic sac.
 - **Labor** follows 3 stages: dilation of the cervix, delivery of the baby, and delivery of the placenta. During labor, contractions are induced in the uterus by local regulators and hormones to push the fetus and placenta out of the body. Uterine contractions stimulate oxytocin secretion, providing positive feedback to continue contractions.
 - In the fetus, ACTH is released by the posterior pituitary in response to CRH from the hypothalamus, resulting in the secretion of cortisol and dehydroepiandrosterone (DHEA), an androgen, from the adrenal cortex.
 - Cortisol results in surfactant secretion to prepare the fetal lungs to breathe.
 - DHEA is converted to estriol, which activates the myometrium to express more receptors for oxytocin and prostaglandins. Typically, progesterone inhibits this function of estriol. In addition, estriol stimulates gap junction formation between myometrial cells to allow for synchronous contraction.

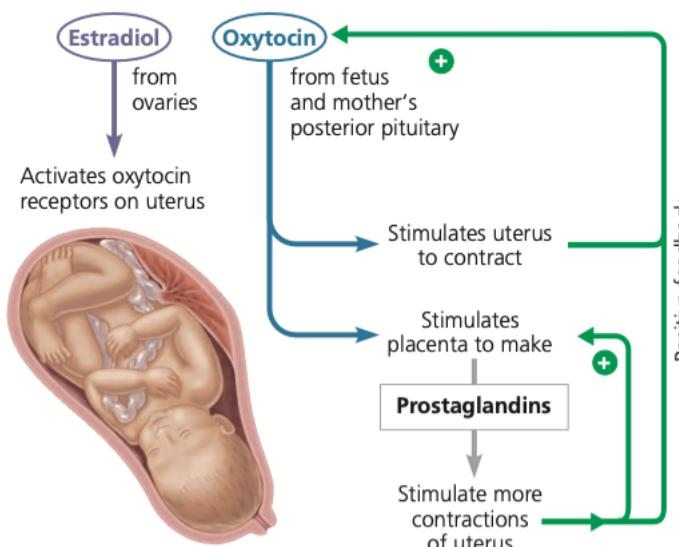


Figure 15: Positive feedback loop during birth. (Source: Campbell Biology)

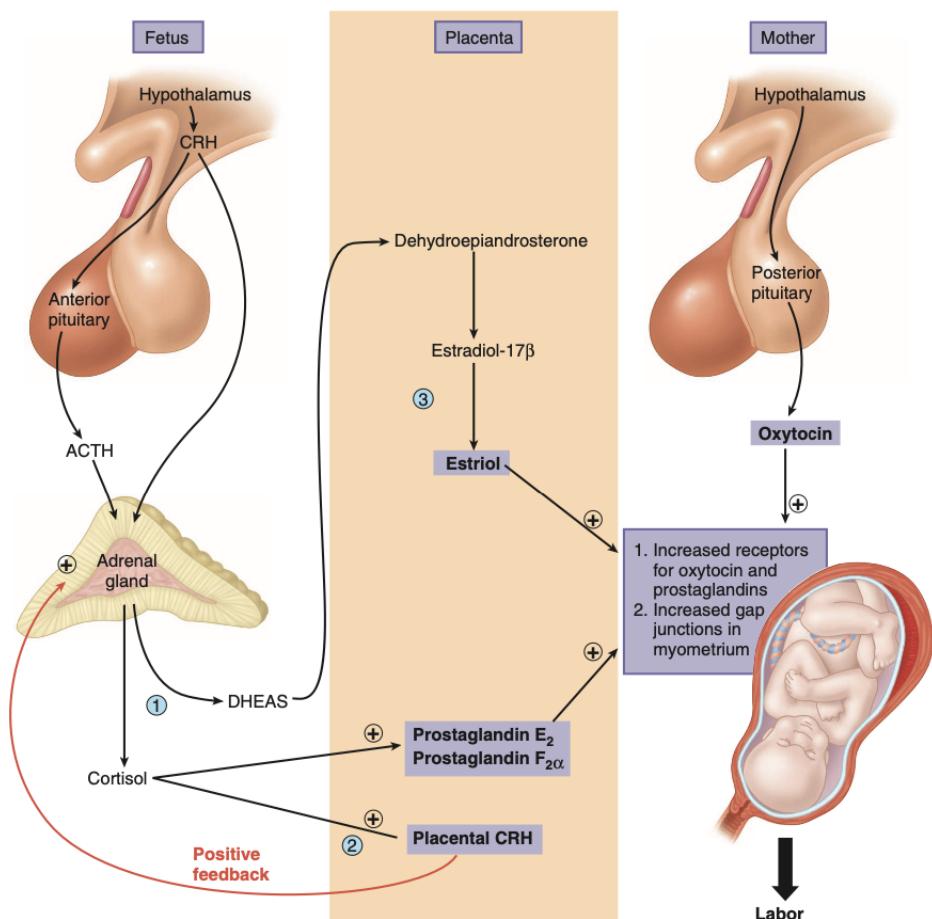


Figure 16: Detailed pathway of the positive feedback in birth. (Source: Fox Human Physiology)

8.4 Lactation

- **Mammary glands** are composed of multiple lobules, within which lies around 15 lobes in each breast. The lobules contain structures known as alveoli, different from those of the lungs, that secrete milk into ducts that lead up to the nipple.
- Prolactin stimulates the production of milk by the mammary glands. Dopamine inhibits prolactin production, and estrogen stimulates dopamine production.
- When the placenta is expelled after labor, the rapid drop in estrogen results in an increase in prolactin that stimulates milk production.
- Breastfeeding results in the **nursing reflex**, which inhibits dopamine secretion and results in oxytocin secretion. Oxytocin stimulates **milk ejection** via **myoepithelial cells**, which are involved in the propulsion of milk through the ducts to the nipple. This reflex also inhibits GnRH secretion and is thus a natural contraceptive.

9 Disorders

9.1 Hermaphroditism and Pseudohermaphroditism

- **Hermaphroditism** occurs when some embryonic cells obtain the short arm of the Y chromosome while others do not, this sometimes results in half ovary half testis or ovotestis (ovary and testis combined).
- **Pseudohermaphroditism** is characterized by underdeveloped or incorrect external genitalia that does not match with their gender.
 - **Congenital Adrenal Hyperplasia** occurs when there is a mutation in cortisol synthesis resulting in loss of negative feedback (due to reduced cortisol) and thus large amounts of androgen secretion from the adrenal cortex. Females will still develop a uterus but the external genitalia will be ambiguous and masculinized.
 - **Testicular Feminization Syndrome** occurs when testosterone receptors are faulty leading to feminized genitalia. Furthermore, there is a lack of negative feedback leading to large amounts of circulating testosterone. This testosterone gets converted to estrogen in adipose tissues which results in a female appearance without menstruation.
 - **5-alpha Reductase Deficiency** occurs when the enzyme **5--reductase** is deficient which leads to normal internal genitalia but underdeveloped external genitalia. This is because 5--reductase is used to make DHT from testosterone, and DHT is required for external genitalia formation.

9.2 Endometriosis

- Endometriosis results from the back flow of endometrial tissue through the uterine tubes into the pelvic cavity where it responds to cycles in hormones and undergoes normal menstruation and bleeding. This results in scarring, inflammation, infertility and intense pain.
- Treated with a drug called **nafarelin** that acts as a GnRH analogue resulting in continuous hormonal secretion which as opposed to normal pulsatile secretion results in downregulation of GnRH receptors and thus decreased LH and FSH secretion. This allows for menopause like conditions that prevents menstruation and thus abnormal bleeding.

9.3 Ectopic Pregnancy

- An ectopic pregnancy occurs when a fertilized egg implants within the Fallopian tube resulting in internal bleeding via Fallopian tube rupture.
 - The chance of this condition occurring becomes more likely due to bacterial scarring from, for example, an STI (sexually transmitted infection).

9.4 Genetic Screening

- **Chorionic Villus Sampling**, a needle is inserted through the cervix and a portion of the placenta (composed of the fetus' cells) is removed. These cells proliferate fast, so karyotype and molecular tests can occur immediately. Can be done after the 10th week of pregnancy.
- **Amniocentesis**, a needle is inserted through the uterus and a sample of amniotic fluid is taken and tested, an ultrasound monitors this process. This fluid must be centrifuged to isolate the cells and then can be tested for certain molecules indicative of a disease, otherwise they must be cultured for several weeks before performing a karyotype on them. Only can be done after the 15th week of pregnancy.

• Ultrasound

- Reflected sound waves are used to project an image of the fetus, noninvasive and allows for the imaging of anatomical abnormalities.

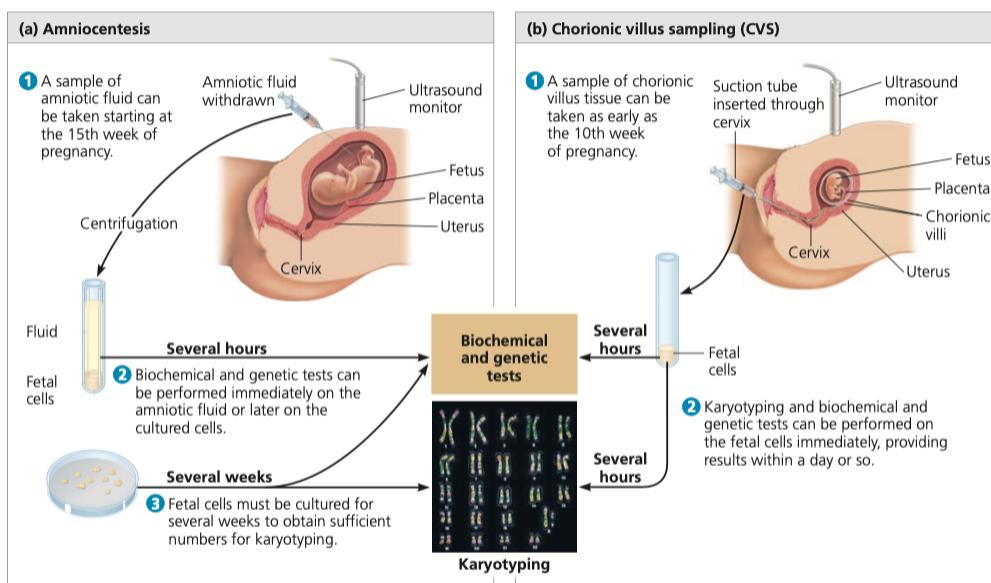


Figure 17: (Source: Campbell Biology)

10 Contraceptives

Contraception is the prevention of pregnancy. There are many contraceptives and many opportunities for the prevention of pregnancy. The most effective contraceptives are intrauterine devices (IUDs) and hormonal contraceptives.

10.1 Rhythm Method

- Conception is most likely to occur one to two days before ovulation. Therefore **natural family planning, the rhythm method, or temporary abstinence** involves planned sexual intercourse times to lower the likelihood of pregnancy. More than 6 days before and one day after ovulation are the safest times to have coitus.

- Temperature changes during the menstrual cycle can be tracked. There is a lowered temperature during the LH surge due to low estradiol levels. However, temperature increases after ovulation as progesterone increases. Monitoring body temperature is thus useful for a woman who is planning to have kids but not to prevent pregnancy, as it cannot be used to predict menstruation.

10.2 Coitus Interrupts

- This method involves the withdrawal of the penis prior to ejaculation. This method is highly unreliable, as the bulbourethral gland, also known as Cowper's gland, often releases sperm when clearing the urethra of urine.

10.3 Oral Contraceptives

- Oral contraceptives increase estradiol and progesterone levels using a synthetic estrogen and a synthetic progesterone known as progestin. This results in negative feedback of GnRH and thus lowered FSH and LH secretion, preventing ovulation. Progestin by itself results in the thickening of cervical mucus to block sperm from entering the uterus.
- To prevent abnormal endometrium growth, these pills are stopped on the third week to allow for menstruation, then restarted with a placebo pill for the fourth week before continuing.
- Patches and oral contraceptives prevent drug metabolism by the liver and include the transdermal patch, vaginal ring, and injections of medroxyprogesterone acetate (DMPA). IUDs and subcutaneous patches are more long-acting than the contraceptives listed above. Furthermore, these contraceptives may increase the chance of heart attack.

10.4 Sterilization

- Sterilization is the prevention of gamete release. **Tubal ligation**, one method of this, is the tying of the oviduct to prevent eggs from traveling. **Vasectomy**, another method, is the cutting and knotting of the vas deferens to prevent sperm from entering during ejaculation. In a vasectomized male, sperm does not build up; it is reabsorbed when it reaches the knot. These methods do not affect sex hormone secretion.

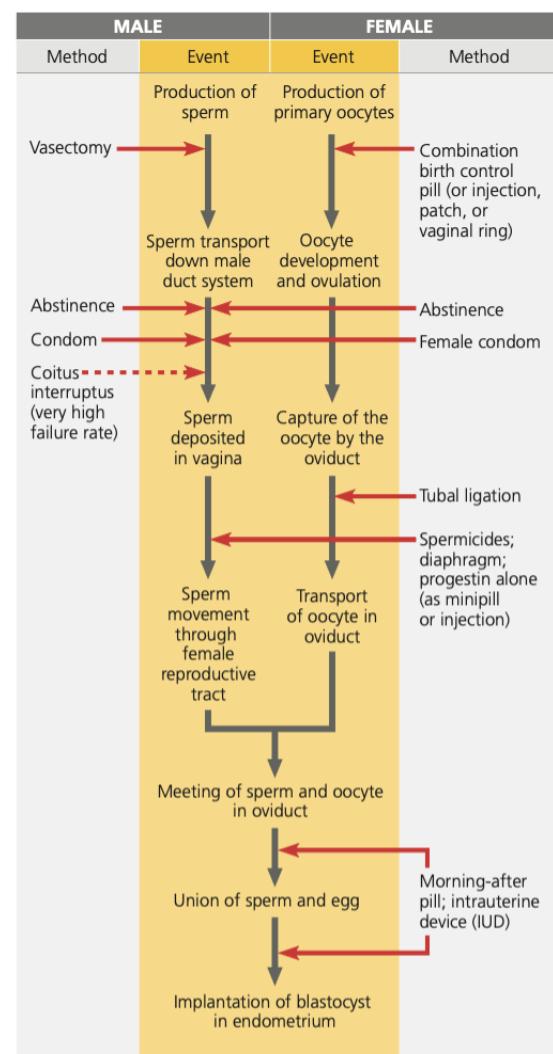


Figure 18: Points of contraception.
(Source: Campbell Biology)

10.5 Abortion

- **Abortion** is the termination of a pregnancy in progress. **Mifepristone (RU486)** blocks progesterone receptors in the uterus, preventing the maintenance of pregnancy. It is often taken with prostaglandins to induce uterine contraction.

11 Development

11.1 Cleavage

- **Cleavage** is the rapid cell division that occurs in a newly fertilized egg. It consists of mainly the S and M phases without growth during the G1 and G2 phases.
- Development of a fertilized egg is carried out by preexisting mRNA and proteins, as the DNA in the egg is insufficient to sustain such a large cytoplasm.
- There is no increase in the size of the cells. Rather, the cytoplasm is partitioned into **blastomeres**, each of which consists of a **blastula** surrounding a fluid-filled cavity called a **blastocoel**.
- In species such as sea urchins, cleavage is uniform across embryos. In frogs and other species, it is asymmetric.

11.2 Gastrulation in Frogs

- Each germ layer corresponds to a different set of structures in the adult animal. For example, the ectoderm forms the outer nervous system, while the mesoderm gives rise to the muscle and skeleton.

ECTODERM	MESODERM	ENDODERM
<ul style="list-style-type: none"> • Epidermis of skin and its derivatives (including sweat glands, hair follicles) • Epithelial lining of mouth and anus • Cornea and lens of eye • Nervous system • Sensory receptors in epidermis • Adrenal medulla • Tooth enamel • Epithelium of pineal and pituitary glands 	<ul style="list-style-type: none"> • Notochord • Skeletal system • Muscular system • Muscular layer of stomach and intestine • Excretory system • Circulatory and lymphatic systems • Reproductive system (except germ cells) • Dermis of skin • Lining of body cavity • Adrenal cortex 	<ul style="list-style-type: none"> • Epithelial lining of digestive tract • Epithelial lining of respiratory system • Lining of urethra, urinary bladder, and reproductive system • Liver • Pancreas • Thymus • Thyroid and parathyroid glands

Figure 19: The products of the three germ layers. (Source: stemcellthailand.org)

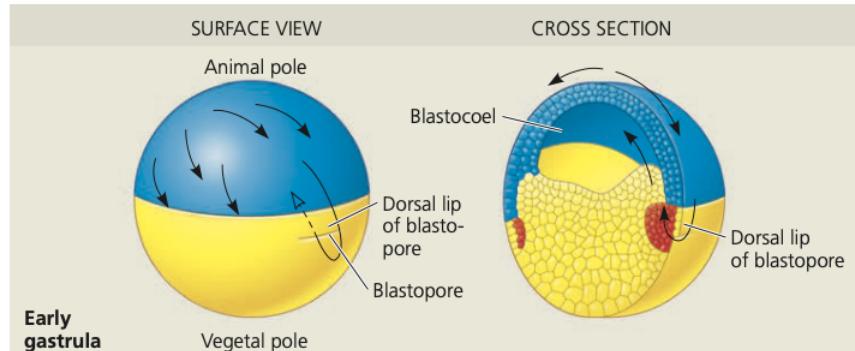
- In frogs, gastrulation begins on the dorsal side, opposite to the entry of sperm. Gastrulation generates three germ layers. In other words, frogs, like vertebrates and other bilaterally symmetric animals, are **triploblasts**. **Diploblasts** only have two germ layers. The extra germ layer in a triploblast is known as the **mesoderm**.
- First, cells on the dorsal side **invaginate** and form a crease known as the **blastopore**. Cells from the animal pole begin to roll into the hollow interior, forming the mesoderm and endoderm of the gastrula.

- Next, the blastospore spreads along the cell, and the two ends connect to form a circle that becomes smaller as the ectoderm moves downward.
- finally, the germ layers have been created and now begin development. The blastospore has become a **yolk plug**

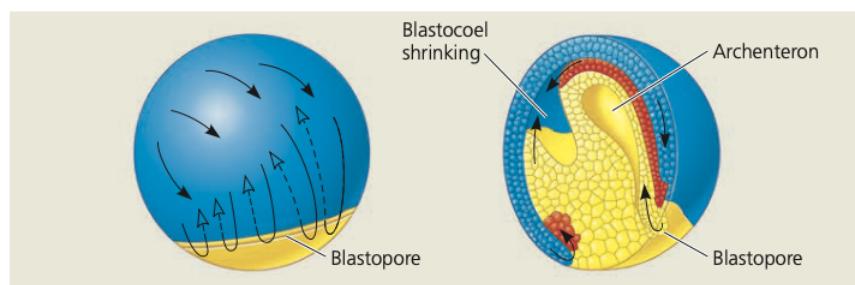


▼ Figure 47.10 Gastrulation in a frog embryo. In the frog blastula, the blastocoel is displaced toward the animal pole and is surrounded by a wall several cells thick.

1 Gastrulation begins when cells on the dorsal side invaginate to form a small indented crease, the blastopore. The part above the crease is called the **dorsal lip**. As the blastopore is forming, a sheet of cells begins to spread out of the animal hemisphere, rolls inward over the dorsal lip (involution), and moves into the interior (shown by the dashed arrow). In the interior, these cells will form endoderm and mesoderm, with the endodermal layer on the inside. Meanwhile, cells at the animal pole change shape and begin spreading over the outer surface.



2 The blastopore extends around both sides of the embryo as more cells invaginate. When the ends meet, the blastopore forms a circle that becomes smaller as ectoderm spreads downward over the surface. Internally, continued involution expands the endoderm and mesoderm; an archenteron forms and grows as the blastocoel shrinks and eventually disappears.



3 Late in gastrulation, the cells remaining on the surface make up the ectoderm. The endoderm is the innermost layer, and the mesoderm lies between the ectoderm and endoderm. The circular blastopore surrounds a plug of yolk-filled cells.

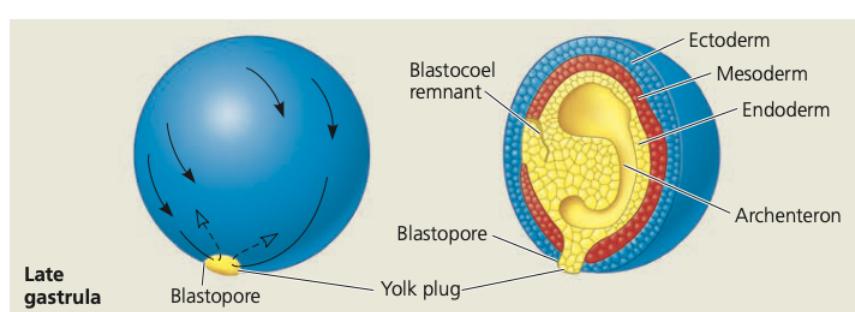


Figure 20: Diagram of steps in gastrulation. (Source: Campbell Biology)

11.3 Gastrulation in Chicks

- There are many similarities between chicken and frog gastrulation. As you read on, try to make connections between them as a result of the conserved evolution of development.
- A chicken egg is composed of the yellow that we commonly call yolk. A small spot on top of this yolk is the animal pole, which will actually form the embryo.

- The cells of the **epiblast** are the ones that eventually form the embryo of the chicken. These cells move towards the midline of the animal pole in a process analogous to the dorsal lip in amphibians, forming a furrow called the **primitive streak**.
- The cells that form the primitive streak push the lower hypoblast cells to form the endoderm, while the rest form the mesoderm. The cells left at the top form the ectoderm.
- The cells of the **hypoblast** connect the yolk mass to the embryo and form a sac around the yolk.

Example 11.1: (USABO Open Exam 2017) Select all of the following choices that correctly match the tissue to the embryonic germ layer from which it is primarily formed (Select ALL that apply):

- A. Ectoderm and spinal cord.
- B. Mesoderm and heart.
- C. Endoderm and thyroid.
- D. Ectoderm and epidermis.
- E. Endoderm and liver.

Solution: The spinal cord forms from the neural tube, which is derived from ectodermal tissue. The heart is part of the circulatory system, which is derived from the mesoderm. Remember that in the previous diagram, the thyroid was described to be derived from the endoderm. The epidermis is the top layer of the skin, while the dermis is below it. Thus, the epidermis is derived from the ectoderm, while the dermis is derived from the mesoderm. Finally, the liver is part of the digestive system, which is derived from the endodermal germ layer. The **answer is ABCDE**.

11.4 Gastrulation in Humans

- In humans, fertilization occurs in the oviduct, and development begins as the egg makes its way down the oviduct.
- Before implantation, the embryo has an inner cell mass that eventually develops into the fetus, as well as a surrounding chorion composed of **trophoblast** cells.
- The chorion develops into an outer syncytiotrophoblast and an inner cytotrophoblast. The inner cell mass that gives rise to the 3 germ layers is separated from the chorion by the **amniotic cavity**.
- The syncytiotrophoblast secretes enzymes that digest the endometrium, creating blood-filled cavities into which the cytotrophoblast extend villi. This forms the **chorion frondosum**, which eventually forms the placenta. The placenta consists of the fetal frondosum and the maternal **decidua basalis** in contact with the frondosum.
- The trophoblast forms the 4 extraembryonic membranes that enclose structures outside of the embryo. Gastrulation begins and a primitive streak forms, just as in chicks, with some epiblast cells forming the mesoderm and endoderm and other cells forming the ectoderm.

- The **extraembryonic mesoderm** and the 4 extraembryonic membranes surround the embryo. In mammals, reptiles, and birds, these 4 extraembryonic membranes are the chorion, allantois, amnion, and yolk sac. All vertebrate embryos require an aqueous environment to allow for development.
 - In **amniotes**, which include mammals, reptiles, and birds, a container shell or uterus allows for developing embryos to be surrounded by a fluid called the **amnion**. Fish and amphibians do not require an amnion because their eggs are surrounded by water.
 - The chorion functions in gas exchange. The amnion protects the embryo. The allantois disposes of waste in reptile eggs, and it does the same as part of the umbilical cord in mammals. The yolk sac encloses the yolk in reptile eggs.

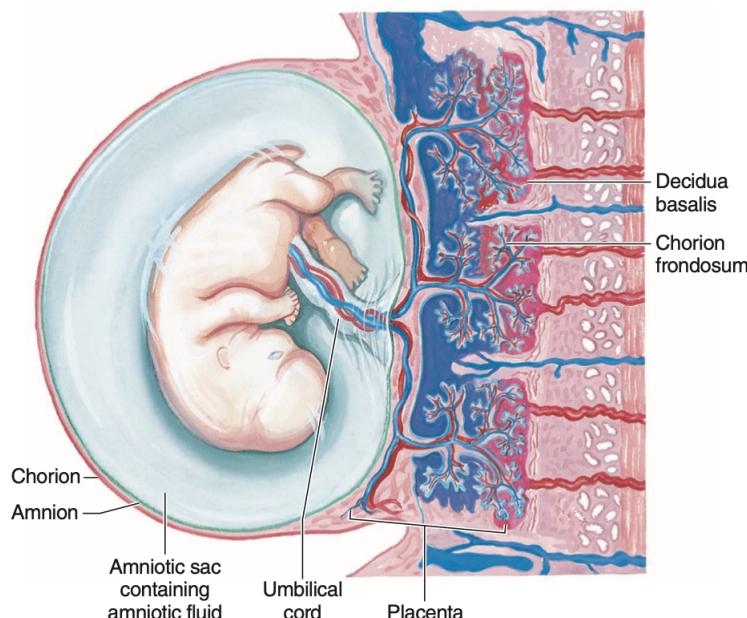


Figure 21: Implanting of the embryo into the placenta. (Source: Fox Human Physiology)

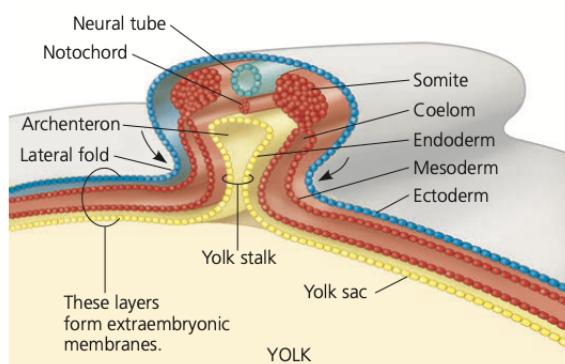
11.5 Organogenesis

- During organogenesis, the embryonic germ layers develop into the organs. Sometimes, multiple germ layers form different parts of a single organ.
- In **neurulation**, cells from the mesoderm form the notochord, which runs down the dorsal side of the embryo. These cells secrete signaling molecules that cause the ectoderm above to form the **neural plate**. The cells of the neural plate curve the structure inward to form a **neural tube**. The anterior end develops into the brain, while the posterior end forms the spinal cord. The notochord degenerates except in certain parts that form the disks in the spine.
 - Spina bifida** is the failure of the neural tube to close properly. It can result in nerve damage and thus leg paralysis.

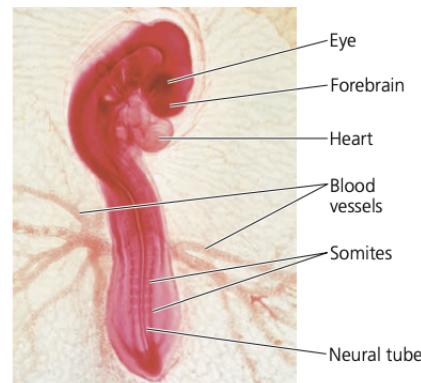
- In **cell migration**, some cells develop on the edges where the neural tube pinches off from the neural fold. These cells migrate to the periphery of the embryo forming nerves, teeth, and skull bones. Cells lateral to the notochord form blocks known as somites, which organize the segmentation of the body. Some form vertebrae, skeletal muscles, and mesenchyme, the latter of which consists of stem cells in the skeletal system.



Figure 47.15
Organogenesis in a chick embryo.



(a) Early organogenesis. The archenteron forms when lateral folds pinch the embryo away from the yolk. The embryo remains open to the yolk, attached by the yolk stalk, as shown in this cross section.



(b) Late organogenesis. Rudiments of most major organs have already formed in this 3-day old chick embryo. Blood vessels extending from the embryo supply the extraembryonic membranes, as seen in this light micrograph (LM).

Figure 22: Organogenesis in chicks. (Source: Campbell Biology)

11.6 Cytoskeleton in Morphogenesis

- In neurulation, microtubules orient themselves laterally at the ventral surface of the cuboidal ectoderm and horizontally at the dorsal surface. Actin contraction creates a wedge shape that bends the ectoderm inward.
- In **convergent extension**, cells elongate in one direction and crawl between each other. Thus, the sheet becomes long and narrow. This process occurs in the formation of the primitive streak of chick eggs and the archenteron of sea urchin embryos.
- In **cell migration**, cells of the neural crest and somites migrate throughout the embryo via the cytoskeleton, similar to amoeboid movement. Cell adhesion molecules (i.e., glycoproteins) are important in this process, as well as the extracellular matrix (ECM).

11.7 Fate Mapping

- In fate mapping, specific regions of a blastula are marked with a dye, and the location of the cells in those regions is tracked to study the origin of adult tissues.
- Microscopic analysis can also be used to track cells, along with mutating or destroying cells with a focused laser and observing its effect on development.
- Fate mapping was used to determine the location of **P granules** in *C. elegans*. They were found to move posteriorly during the first mitotic cell division and become concentrated toward the end of cleavage, eventually giving rise to the germline.

11.8 Axis Formation

- In frogs, the anterior-posterior (AP) axis is formed during oogenesis. However, the dorsal-ventral (DV) axis is formed after fertilization. When the sperm binds to the animal pole, the overlying cytoplasm rotates toward the point of entry. This activates certain regulatory proteins that allow the dorsal side to form in the **gray crescent**, an area formed by the rotation that is opposite to where the sperm entered.
- In humans, the orientation of the sperm's nucleus with respect to the egg's nucleus determines the orientation of cleavage. Both axes form in the blastula and gastrula stages.
- In chickens, the pull of gravity as the egg travels down the oviduct determines the AP axis.
- In zebrafish, signals across the embryo determine axial patterning.
- In insects, gradients of transcription factors determine axial patterning.
- Once the AP and DV axes have been determined, the left-right (LR) axis is easy to determine. In the **two-cilia hypothesis** for vertebrates, motile cilia rotate clockwise to drive fluid to flow to the left, while non-motile cilia detect this flow. This allows for left-right asymmetry.

11.9 Induction in Pattern Formation

- Pattern formation is the arrangement of organs in the place they should be. This is brought upon by positional information, a cell's location in relation to the defined axes.
- A scientist and his student (i.e., Spemann and Mangold) transplanted a dorsal lip from one frog onto another part of a different frog, triggering gastrulation. Later, the **Spemann organizer** was named, a group of cells that inhibit bone morphogenic protein 4 (BMP4), which normally prevents neural tube and notochord formation.
- In chickens, wings and limbs form as limb buds, which is composed of a mesoderm tissue covered by overlying ectoderm. The anterior side of the limb is toward the thumb, while the posterior side is toward the little finger. The dorsal side is the back of the hand, while the ventral side is the palm side. The proximal side is toward the shoulder, while the distal side is toward the hand.
- The **apical ectodermal ridge (AER)** on the ectoderm of the limb bud secretes fibroblast growth factor (FGF), which promotes limb outgrowth along the proximal-distal axis.
- The **zone of polarizing activity (ZPA)** is found on the posterior side of the mesoderm of the limb bud. It secretes a protein called sonic hedgehog (SHH), whose gradient determines the formation of the digits. At high concentrations nearest the ZPA, SHH induces the formation of the posterior digits (e.g., the pinky). At low concentrations farthest from the ZPA, SHH induces the formation of the anterior digits (e.g., the thumb).

Example 11.2 (USABO Semifinal Exam 2018) Which of the following correctly lists the steps required for the formation of neural tubes in vertebrates?

- I. Cuboidal ectodermal cells form a continuous sheet.

- II. Microtubules help elongate the cells of the neural plate.
- III. Actin filaments at the dorsal end of the cells may contract, deforming the cells into wedge cells.
- IV. Cells wedging in the opposite direction causes the ectoderm to form a hinge. It pinches off of the neural plate and forms the neural tube.
- A. I → II → III → IV
- B. I → III → II → IV
- C. II → I → III → IV
- D. I → II → IV → III
- E. III → II → I → IV

Solution: Looking through the choices, we know that IV must be last, as it includes "forms the neural tube." We now look at the rest of the answer choices. During neurulation, the ectoderm is composed of cuboidal cells that overlie the epithelium. Thus, I has to be the first step. As discussed in this handout, actin contraction leads to the wedging of the neural plate to form the neural tube. Therefore, III must be the second to last step, and II must be the second step. Logically speaking, this also makes sense, as the cell must be elongated and have more microtubules to contract. Thus, **the answer is A.**

12 Conclusion

As we conclude this comprehensive journey through the realms of development and reproduction, we know now more about life's most intricate and enduring processes. From the inception of life through the miracle of fertilization to the intricate dance of cellular divisions, and the orchestration of growth, we have uncovered the underlying mechanisms that govern existence. I hope this handout served you well and aids you on your USABO endeavors.

Peace Out - Zelmay Jan