CHAPTER 38

The Female Reproductive System

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CHAPTER OUTLINE

KEY CONCEPTS

1. Pulses of hypothalamic GnRH regulate the secretion of LH and FSH, which enhance follicular development, steroidogenesis, ovulation, and formation of the corpus luteum.
2. LH and FSH, in coordination with ovarian theca and granulosa cells, regulate the secretion of follicular estradiol.
3. Ovulation occurs as the result of a positive feedback of follicular estradiol on the hypothalamic-pituitary axis that induces LH and FSH surges.
4. Follicular development occurs in distinct steps: primordial, primary, secondary, tertiary, and graafian follicle stages.
5. Follicular rupture (ovulation) requires the coordination of appropriately timed LH and FSH surges that induce inflammatory reactions in the graafian follicle, leading to dissolution at midcycle of the follicular wall by several ovarian enzymes.
6. Follicular atresia results from the withdrawal of gonadotropin support.
7. The formation of a functional corpus luteum requires the presence of an LH surge, adequate numbers of LH receptors, sufficient granulosa cells, and significant progesterone secretion.
8. The uterine cycle is regulated by estradiol and progesterone, such that estradiol induces proliferation of the uterine endometrium, whereas progesterone induces differentiation of the uterine endometrium and the secretion of distinct products.
9. During puberty, the hypothalamus begins to secrete increasing quantities of GnRH, which increases LH and FSH secretion, enhances ovarian function, and leads to the first ovulation.
10. Menopause ensues from the loss of numerous oocytes in the ovary and the subsequent failure of follicular development and estradiol secretion. LH and FSH levels rise from the lack of negative feedback by estradiol.

The fertility of the mature human female is cyclic. The release from the ovary of a mature female germ cell or ovum occurs at a distinct phase of the menstrual cycle. The secretion of ovarian steroid hormones, estradiol and progesterone, and the subsequent release of an ovum during the menstrual cycle are controlled by cyclic changes in LH and FSH from the pituitary gland, and estradiol and progesterone from the ovaries. The cyclic changes in steroid hormone secretion cause significant changes in the structure and function of the uterus in preparing it for the reception of a fertilized ovum. At different stages of the menstrual cycle, progesterone and estradiol exert negative- and positive-feedback effects on the hypothalamus and on pituitary gonadotrophs, generating the cyclic pattern of LH and FSH release characteristic of the female reproductive system. Since the hormonal events during the menstrual cycle are delicately synchronized, the menstrual cycle can be readily affected by stress and by environmental, psychological, and social factors.

The female cycle is characterized by monthly bleeding, resulting from the withdrawal of ovarian steroid hormone support of the uterus, which causes shedding of the superficial layers of the uterine lining at the end of each cycle. The first menstrual cycle occurs during puberty. Menstrual

cycles are interrupted during pregnancy and lactation and cease at menopause. Menstruation signifies a failure to conceive and results from regression of the corpus luteum and subsequent withdrawal of luteal steroid support of the superficial endometrial layer of the uterus.

AN OVERVIEW OF THE FEMALE REPRODUCTIVE SYSTEM

An overview of the interactions of hormonal factors in female reproduction is shown in Figure 38.1. The female hormonal system consists of the brain, pituitary, ovaries, and reproductive tract (oviduct, uterus, cervix, and vagina). In the brain, the hypothalamus produces gonadotropin-releasing hormone (GnRH), which controls the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH).

The mature ovary has two major functions: the maturation of germ cells and steroidogenesis. Each germ cell is ultimately enclosed within a follicle, a major source of steroid hormones during the menstrual cycle. At ovulation, the ovum or egg is released and the ruptured follicle is transformed into a corpus luteum, which secretes progesterone as its main product. FSH is primarily involved in stimulating the growth of ovarian follicles, while LH induces ovulation. Both LH and FSH regulate follicular steroidogenesis and androgen and estradiol secretion, and LH regulates the secretion of progesterone from the corpus luteum. Ovarian steroids inhibit the secretion of LH and FSH with one exception: Just prior to ovulation (at midcycle), estradiol has a positive-feedback effect on the hypothalamic-pituitary axis and induces significant increases in the secretion of GnRH, LH, and FSH. The ovary also produces three polypeptide hormones. **Inhibin** suppresses the secretion of FSH. **Activin** (an inhibin-binding protein) increases the secretion of FSH, and **follistatin** (an activin-binding protein) reduces the secretion of FSH.

Shortly after fertilization, the embryo begins to develop placenta cells, which attach to the uterine lining and unite with the maternal placental cells. The **placenta** produces several pituitary-like and ovarian steroid-like hormones. These hormones support placental and fetal development throughout pregnancy and have a role in parturition. The mammary glands are also under the control of pituitary hormones and ovarian steroids, and provide the baby with immunological protection and nutritional support through lactation. Lactation is hormonally controlled by **prolactin** (PRL) from the anterior pituitary, which regulates milk production, and **oxytocin** from the posterior pituitary, which induces milk ejection from the breasts.

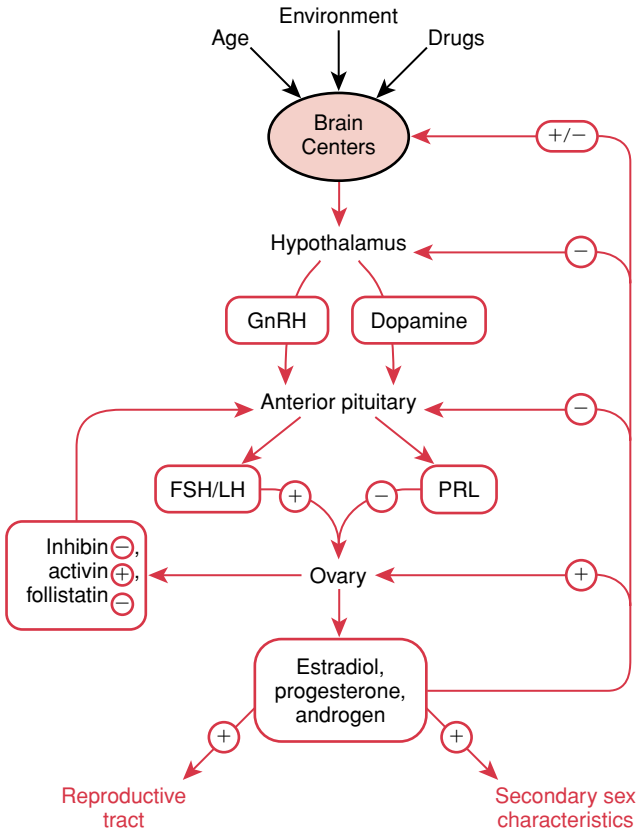

FIGURE 38.1 Regulation of the reproductive tract in the female. The main reproductive hormones are shown in boxes. Positive and negative regulations are depicted by plus and minus signs.

THE HYPOTHALAMIC-PITUITARY AXIS

The hypothalamic-pituitary axis has an important role in regulating the menstrual cycle. GnRH, a decapeptide produced in the hypothalamus and released in a pulsatile manner, controls the secretion of LH and FSH through a portal vascular system (see Chapter 32). Blockade of the portal system reduces the secretion of LH and FSH and leads to ovarian atrophy and a reduction in ovarian hormone secretion. The secretion of GnRH by the hypothalamus is regulated by neurons from other brain regions. Neurotransmitters, such as epinephrine and norepinephrine, stimulate the secretion of GnRH, whereas dopamine and serotonin inhibit secretion of GnRH. In addition, ovarian steroids and peptides and hypothalamic neuropeptides can regulate the secretion of GnRH. GnRH stimulates the pituitary gonadotrophs to secrete LH and FSH. GnRH binds to high-affinity receptors on the gonadotrophs and stimulates the secretion of LH and FSH through a phosphoinositide-protein kinase C-mediated pathway (see Chapter 1).

A graph of LH release throughout the female life span is shown in Figure 38.2. During the neonatal period, LH is released at low and steady rates without pulsatility; this period coincides with lack of development of mature ovarian follicles and very low to no ovarian estradiol secretion. Pulsatile release begins with the onset of puberty and for several years is expressed only during sleep; this period coincides with increased but asynchronous follicular development and with increased secretion of ovarian estradiol. Upon the establishment of regular functional menstrual cycles associated with regular ovulation, LH pulsatility prevails throughout the 24-hour period, changing in a monthly cyclic manner. In postmenopausal women whose ovaries lack sustained follicular development and exhibit

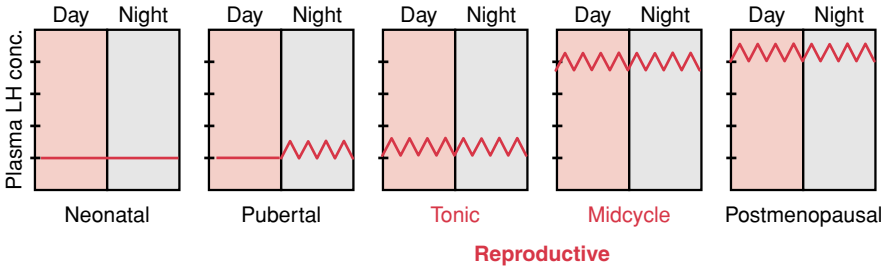

FIGURE 38.2 **Relative levels of LH release in human females throughout life.** (Modified from Yen SSC, et al. In: Ferin M, et al., eds. Biorhythms and Human Reproduction. New York: Wiley, 1974.)

low ovarian estradiol secretion, mean circulating LH levels are high and pulses occur at a high frequency.

THE FEMALE REPRODUCTIVE ORGANS

The female reproductive tract has two major components: the ovaries, which produce the mature ovum and secrete progestins, androgens, and estrogens; and the ductal system, which transports ovum, is the place of the union of the sperm and egg, and maintains the developing conceptus until delivery. The morphology and function of these structures change in a cyclic manner under the influence of the reproductive hormones.

The ovaries are in the pelvic portion of the abdominal cavity on both sides of the uterus and are anchored by ligaments (Fig. 38.3). An adult ovary weighs 8 to 12 g and consists of an outer cortex and an inner medulla, without a sharp demarcation. The cortex is surrounded by a fibrous tissue, the tunica albuginea, covered by a single layer of surface epithelium continuous with the mesothelium covering the other organs in the abdominal cavity. The cortex contains oocytes enclosed in **follicles** of various sizes, **corpora lutea**, **corpora albicantia**, and **stromal cells**. The medulla contains connective

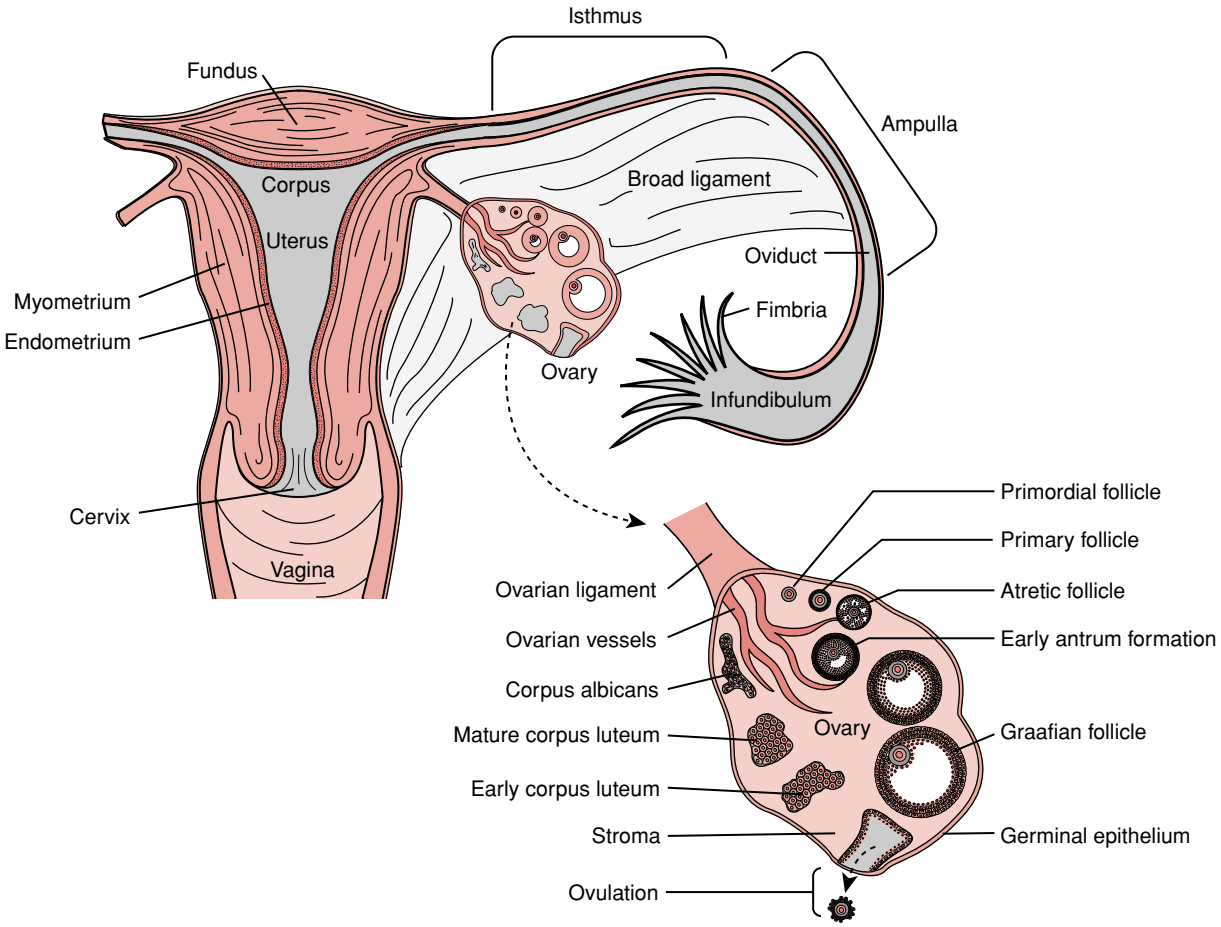

FIGURE 38.3 **The female reproductive organs.** (Modified from Patton BM. Human Embryology. New York: McGraw-Hill, 1976.)

and interstitial tissues. Blood vessels, lymphatics, and nerves enter the medulla of the ovary through the hilus.

On the side that ovulates, the **oviduct** (fallopian tube) receives the ovum immediately after ovulation. The oviducts are the site of fertilization and provide an environment for development of the early embryo. The oviducts are 10 to 15 cm long and composed of sequential regions called the **infundibulum**, **ampulla**, and **isthmus**. The infundibulum is adjacent to the ovary and opens to the peritoneal cavity. It is trumpet-shaped with finger-like projections called **fimbria** along its outer border that grasp the ovum at the time of follicular rupture. Its thin walls are covered with densely ciliated projections, which facilitate ovum uptake and movement through this region. The ampulla is the site of fertilization. It has a thin musculature and well-developed mucosal surface. The isthmus is located at the uterotubal junction and has a narrow lumen surrounded by smooth muscle. It has sphincter-like properties and can serve as a barrier to the passage of germ cells. The oviducts transport the germ cells in two directions: sperm ascend toward the ampulla and the zygote descends toward the uterus. This requires coordination between smooth muscle contraction, ciliary movement, and fluid secretion, all of which are under hormonal and neuronal control.

The **uterus** is situated between the urinary bladder and rectum. On each upper side, an oviduct opens into the uterine lumen, and on the lower side, the uterus connects to the vagina. The uterus is composed of two types of tissue. The outer part is the **myometrium**, composed of multiple layers of smooth muscle. The inner part, lining the lumen of the uterus, is the **endometrium**, which contains a deep **stromal layer** next to the myometrium and a superficial epithelial layer. The stroma is permeated by spiral arteries and contains much connective tissue. The epithelial layer is interrupted by uterine glands, which also penetrate the stromal layer and are lined by columnar secretory cells. The uterus provides an environment for the developing fetus, and eventually, the myometrium will generate rhythmic contractions that assist in expelling the fetus at delivery.

The **cervix** (neck) is a narrow muscular canal that connects the vagina and the body (corpus) of the uterus. It must dilate in response to hormones to allow the expulsion of the fetus. The cervix has numerous glands with a columnar epithelium that produces mucus under the control of estradiol. As more and more estradiol is produced during the follicular phase of the cycle, the cervical mucus changes from a scanty viscous material to a profuse watery and highly elastic substance called **spinnbarkeit**. The viscosity of the spinnbarkeit can be tested by touching it with a piece of paper and lifting vertically. The mucus can form a thread up to 6 cm under the influence of elevated estradiol. If a drop of the cervical mucus is placed on a slide and allowed to dry, it will form a typical **fern**ing pattern when under the influence of estradiol.

The **vagina** is well innervated, and has a rich blood supply. It is lined by several layers of epithelium that change histologically during the menstrual cycle. When estradiol levels are low, as during the prepubertal or postmenopausal periods, the vaginal epithelium is thin and the secretions are scanty, resulting in a dry and infection-susceptible area. Estradiol induces proliferation and **cornification** (**keratinization**) of the vaginal epithelium, whereas progesterone opposes those actions and induces the influx of polymorphonuclear leukocytes into the vaginal fluids. Estradiol also activates vaginal glands that produce lubricating fluid during coitus.

FOLLICULOGENESIS, STEROIDOGENESIS, ATRESIA, AND MEIOSIS

Most follicles in the ovary will undergo atresia. However, some will develop into mature follicles, produce steroids, and ovulate. As follicles mature, oocytes will also mature by entering meiosis, which produces the proper number of chromosomes in preparation for fertilization.

The Primordial Follicle Contains an Oocyte Arrested in Meiosis

Female germ cells develop in the embryonic yolk sac and migrate to the genital ridge where they participate in the development of the ovary (Table 38.1). Without germ cells, the ovary does not develop. The germs cells, called **oogonia**, actively divide by mitosis. Oogonia undergo mitosis only during the prenatal period. By birth, the ovaries contain a finite number of oocytes, estimated to be about 1 million. Most of them will die by a process called atresia. By puberty, only 200,000 oocytes remain; by age 30, only 26,000 remain; and by the time of menopause, the ovaries are essentially devoid of oocytes.

When oogonia cease the process of mitosis, they are called **oocytes**. At that time they enter the meiotic cycle (or meiosis, to prepare for the production of a haploid ovum), become arrested in prophase of the first meiotic division, and remain arrested in that phase until they either die or grow into mature oocytes at the time of ovulation. The **primordial follicle** (Fig. 38.4) is 20 μm in diameter and contains an oocyte, which may or may not be surrounded by a single layer of flattened (squamous) **pregranulosa cells**. When pregranulosa cells surround the oocyte, a basement membrane develops, separating the granulosa from the ovarian stroma.

A Graafian Follicle Is the Final Stage of Follicle Development

Folliculogenesis (also called follicular development) is the process by which follicles develop and mature (see Fig. 38.3). Follicles are in one of the following physiological states: resting, growing, degenerating, or ready to ovulate. During each menstrual cycle, the ovaries produce a group of growing follicles of which most will fail to grow to maturity and will undergo follicular atresia (death) at some stage of development. However, one **dominant follicle** generally emerges from the cohort of developing follicles and it will ovulate, releasing a mature haploid ovum.

Primordial follicles are generally considered the nongrowing resting pool of follicles, which gets progressively depleted throughout life; by the time of menopause, the ovaries are essentially devoid of all follicles. Primordial follicles are located in the ovarian cortex (peripheral regions of the ovary) beneath the tunica albuginea.

TABLE 38.1 Different Stages in the Development of an Ovum and Follicle

Stage	Process	Ovum	Follicle
Fetal life	Migration	Primordial germ cells	
	Mitosis	Oogonia	Primordial follicle
	First meiotic division begins	Primary oocyte	Primary follicle
Birth	Arrest in prophase		
	Growth of oocyte and follicle		
Puberty	Follicular maturation		Secondary follicle
Cycle			Antral follicle
Ovulation	Resumption of meiosis	Secondary oocyte	Graafian follicle
	Emission of first polar body		
	Arrest in metaphase		
			Corpus luteum
Fertilization	Second meiotic division complete	Zygote	
	Emission of second polar body		
Implantation	Mitotic divisions	Embryo	
	Blastocyst		
Parturition	Body Patterning	Fetus	Corpus albicans

Progression from primordial to the next stage of follicular development, the primary stage, occurs at a relatively constant rate throughout fetal, juvenile, prepubertal, and adult life. Once primary follicles leave the resting pool, they are committed to further development or atresia. Most become atretic, and typically only one fully developed follicle will ovulate. The conversion from primordial to primary follicles is believed to be independent of pituitary gonadotropins. The exact signal that recruits a follicle from a resting to a growing pool is unknown; it could be programmed by the cell genome or influenced by local ovarian growth regulators.

The first sign that a primordial follicle is entering the growth phase is a morphological change of the flattened pregranulosa cells into cuboidal granulosa cells. The cuboidal granulosa cells proliferate to form a single continuous layer of cells surrounding the oocyte, which has enlarged from 20 μm in the primordial stage to 140 μm in diameter. At this stage, a glassy membrane, the **zona pellucida**, surrounds the oocyte and serves as means of attachment through which the granulosa cells communicate with the oocyte. This is the **primary follicular stage** of development, consisting of one layer of cuboidal granulosa cells and a basement membrane.

The follicle continues to grow, mainly through proliferation of its granulosa cells, so that several layers of granulosa cells exist in the **secondary follicular stage** of development (see Fig. 38.4). As the secondary follicle grows deeper into the cortex, stromal cells, near the basement membrane, begin to differentiate into cell layers called **theca interna** and **theca externa**, and a blood supply with lymphatics and nerves forms within the thecal component. The granulosa layer remains avascular.

The theca interna cells become flattened, epithelioid, and steroidogenic. The granulosa cells of secondary follicles acquire receptors for FSH and start producing small amounts of estrogen. The theca externa remains fibroblastic and provides structural support to the developing follicle.

Development beyond the primary follicle is gonadotropin-dependent, begins at puberty, and continues in a cyclic manner throughout the reproductive years. As the follicle continues to grow, theca layers expand, and fluid-filled spaces or **antra** begin to develop around the granulosa cells. This early antral stage of follicle development is referred to as the **tertiary follicular stage** (see Fig. 38.4). The critical hormone responsible for progression from the preantral to the antral stage is FSH. Mitosis of the granulosa cells is stimulated by FSH. As the number of granulosa cells increases, the production of estrogens, the binding capacity for FSH, the size of the follicle, and the volume of the follicular fluid all increase significantly.

As the antra increase in size, a single, large, coalesced antrum develops, pushing the oocyte to the periphery of the follicle and forming a large 2- to 2.5-cm-diameter **graafian follicle** (preovulatory follicle; see Fig. 38.4). Three distinct granulosa cell compartments are evident in the graafian follicle. Granulosa cells surrounding the oocyte are **cumulus granulosa cells** (collectively called cumulus oophorus). Those cells lining the antral cavity are called **antral granulosa cells** and those attached to the basement membrane are called **mural granulosa cells**. Mural and antral granulosa cells are more steroidogenically active than cumulus cells.

In addition to bloodborne hormones, antral follicles have a unique microenvironment in which the follicular fluid contains different concentrations of pituitary hormones, steroids, peptides, and growth factors. Some are present in the follicular fluid at a concentration 100 to 1,000 times higher than in the circulation. Table 38.2 lists some parameters of human follicles at successive stages of development in

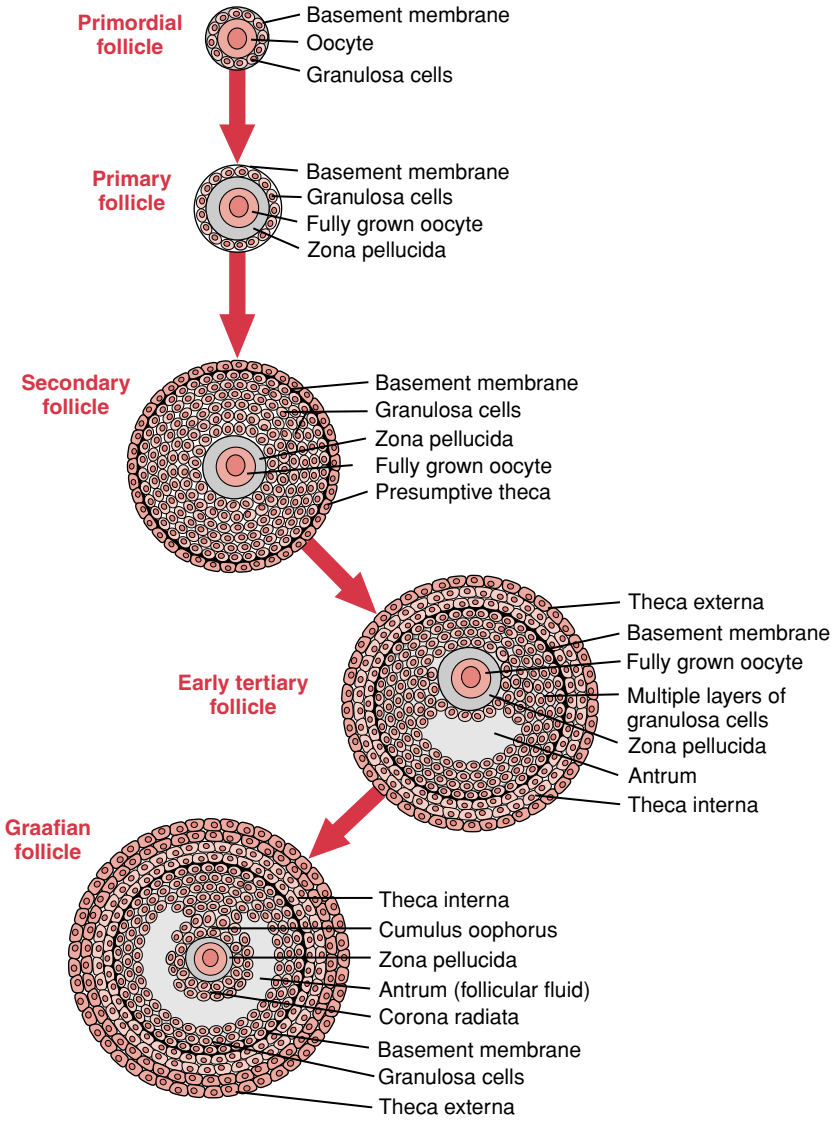

FIGURE 38.4 The developing follicle, from primordial through graafian. (Modified from Erickson GF. In: Sciarra JJ, Speroff L, eds. Reproductive Endocrinology, Infertility, and Genetics. New York: Harper & Row, 1981.)

the follicular phase. There is a 5-fold increase in follicular diameter and a 25-fold rise in the number of granulosa cells. As the follicle matures, the intrafollicular concentration of FSH does not change much, whereas that of LH increases and that of PRL declines. Of the steroids, the concentrations of estradiol and progesterone increase 20-fold, while androgen levels remain unchanged.

The follicular fluid contains other substances, including inhibin, activin, GnRH-like peptide, growth factors, opioid peptides, oxytocin, and plasminogen activator. Inhibin and activin inhibit and stimulate, respectively, the release of FSH from the anterior pituitary. Inhibin is secreted by granulosa cells. In addition to its effect on FSH secretion, inhibin also has a local effect on ovarian cells.

TABLE 38.2 Different Parameters of Follicles During the First Half of the Menstrual Cycle

Cycle (day)	Diameter (mm)	Volume (mL)	Granulosa Cells ($\times 10^6$)	FSH ng/mL	LH	PRL	A	E2	P4
1	4	0.05	2	2.5	—	60	800	100	—
4	7	0.15	5	2.5	—	40	800	500	100
7	12	0.50	15	3.6	2.8	20	800	1,000	300
12	20	0.50	50	3.6	2.8	5	800	2,000	2,000

FSH, follicle-stimulating hormone; LH, luteinizing hormone; PRL, prolactin; A, androstenedione; E2, estradiol; P4, progesterone. (Modified from Erickson GF. An analysis of follicle development and ovum maturation. Semin Reprod Endocrinol 1986;4:233–254.)

Granulosa and Theca Cells Both Participate in Steroidogenesis

The main physiologically active steroid produced by the follicle is **estradiol**, a steroid with 18 carbons. **Steroidogenesis**, the process of steroid hormone production, depends on the availability of cholesterol, which originates from several sources and serves as the main precursor for all of steroidogenesis. Ovarian cholesterol can come from plasma lipoproteins, *de novo* synthesis in ovarian cells, and cholesterol esters within lipid droplets in ovarian cells. For ovarian steroidogenesis, the primary source of cholesterol is low-density lipoprotein (LDL).

The conversion of cholesterol to **pregnenolone** by **cholesterol side-chain cleavage enzyme** is a rate-limiting step regulated by LH using the second messenger cAMP (Fig. 38.5). LH binds to specific membrane receptors on theca cells, activates adenylyl cyclase through a G protein, and increases the production of cAMP. cAMP increases LDL receptor mRNA, the uptake of LDL cholesterol, and cholesterol ester synthesis. cAMP also increases the transport of cholesterol from the outer to the inner mitochondrial membrane, the site of pregnenolone synthesis, using a unique protein called **steroidogenic acute regulatory protein** (StAR). Pregnenolone, a 21-carbon steroid of the progestin family, diffuses out of the mitochondria and enters the ER, the site of subsequent steroidogenesis.

Two steroidogenic pathways may be used for subsequent steroidogenesis (see Fig. 37.9). In theca cells, the **delta 5 pathway** is predominant; in granulosa cells and the corpus luteum, the **delta 4 pathway** is predominant. Pregnenolone gets converted to either **progesterone** by 3β-hydroxysteroid dehydrogenase in the delta 4 pathway or to **17α-hydroxypregnenolone** by 17α-hydroxylase in the delta 5 pathway. In the delta 4 pathway, progesterone gets converted to **17α-hydroxyprogesterone** (by 17α-hydroxylase), which is subsequently converted to **androstenedione** and **testosterone** by 17,20-lyase and 17β-hydroxysteroid dehydrogenase (17-ketosteroid reductase), respectively. In the delta 5 pathway, 17α-hydroxypregnenolone gets converted to **dehydroepiandrosterone** (by 17,20-lyase), which is subsequently converted to androstenedione by 3β-hydroxysteroid dehydrogenase. The androgens contain 19 carbons. Testosterone and androstenedione diffuse from the thecal compartment, cross the basement membrane, and enter the granulosa cells.

In the granulosa cell, under the influence of FSH, with cAMP as a second messenger, testosterone and androstenedione are then converted to estradiol and **estrone**, respectively, by the enzyme aromatase, which aromatizes the A ring of the steroid and removes one carbon (see Fig. 38.5; see Fig. 37.9). Estrogens typically have 18 carbons. Estrone can then be converted to estradiol by 17β-hydroxysteroid dehydrogenase in granulosa cells.

In summary, estradiol secretion by the follicle requires cooperation between granulosa and theca cells and coordination between FSH and LH. An understanding of this **two-cell, two-gonadotropin hypothesis** requires recognition that the actions of FSH are restricted to granulosa cells because all other ovarian cell types lack FSH receptors. LH actions, on the other hand, are exerted on theca, granulosa, and stromal (interstitial) cells and the corpus luteum. The expression of LH receptors is time-dependent because theca cells acquire LH receptors at a relatively early stage, whereas LH receptors on granulosa cells are induced by FSH in the later stages of the maturing follicle.

The biosynthetic enzymes are differentially expressed in the two cells. Aromatase is expressed only in granulosa cells, and its activation and induction are regulated by FSH. Granulosa cells are deficient in 17α-hydroxylase and cannot proceed beyond the C-21 progestins to generate C-19 androgenic compounds (see Fig. 38.5). Consequently, estrogen production by granulosa cells depends on an adequate supply of exogenous aromatizable androgens, provided by theca cells. Under LH regulation, theca cells produce androgenic substrates, primarily androstenedione and testosterone, which reach the granulosa cells by diffusion. The androgens are then converted to estrogens by aromatization.

In follicles, theca and granulosa cells are exposed to different microenvironments. Vascularization is restricted to the theca layer because blood vessels do not penetrate the

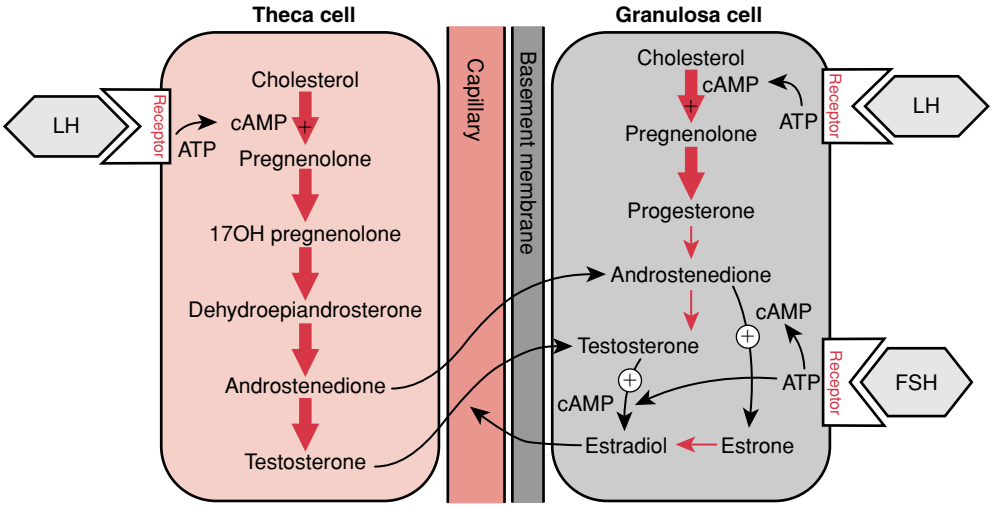

FIGURE 38.5 The two-cell, two-gonadotropin hypothesis. The follicular theca cells, under control of LH, produce androgens that diffuse to the follicular granulosa cells, where they are converted to estrogens via an FSH-supported aromatization reaction. The dashed line indicates that granulosa cells cannot convert progesterone to androstenedione because of the lack of the enzyme 17α-hydroxylase.

basement membrane. Theca cells, therefore, have better access to circulating cholesterol, which enters the cells via LDL receptors. Granulosa cells, on the other hand, primarily produce cholesterol from acetate, a less efficient process than uptake. In addition, granulosa cells are bathed in follicular fluid and exposed to autocrine, paracrine, and juxtacrine control by locally produced peptides and growth factors. "Juxtacrine" describes the interaction of a membrane-bound growth factor on one cell with its membrane-bound receptor on an adjacent cell.

FSH acts on granulosa cells by a cAMP-dependent mechanism and produces a broad range of activities, including increased mitosis and cell proliferation, the stimulation of progesterone synthesis, the induction of aromatase, and increased inhibin synthesis. As the follicle matures, the number of receptors for both gonadotropins increases. FSH stimulates the formation of its own receptors and induces the appearance of LH receptors. The combined activity of the two gonadotropins greatly amplifies estrogen production.

Androgens are produced by theca and stromal cells. They serve as precursors for estrogen synthesis and also have a distinct local action. At low concentrations, androgens enhance aromatase activity, promoting estrogen production. At high concentrations, androgens are converted by 5α-reductase to a more potent androgen, such as dihydrotestosterone (DHT). When follicles are overwhelmed by androgens, the intrafollicular androgenic environment antagonizes granulosa cell proliferation and leads to apoptosis of the granulosa cells and subsequent follicular atresia.

Follicular Atresia Probably Results From a Lack of Gonadotropin Support

Follicular **atresia**, the degeneration of follicles in the ovary, is characterized by the destruction of the oocyte and granulosa cells. Atresia is a continuous process and can occur at any stage of follicular development. During a woman's lifetime approximately 400 to 500 follicles will ovulate; those are the only follicles that escape atresia, and they represent a small percentage of the 1 to 2 million follicles present at birth. The cause of follicular atresia is likely due to lack of gonadotropin support of the growing follicle. For example, at the beginning of the menstrual cycle, several follicles are selected for growth but only one follicle, the dominant follicle, will go on to ovulate. Because the dominant follicle has a preferential blood supply, it gets the most FSH (and LH). Other reasons for the lack of gonadotropin support of nondominant follicles could be a lack of FSH and LH receptors or the inability of granulosa cells to transduce the gonadotropin signals.

During atresia, granulosa cell nuclei become **pyknotic** (referring to an apoptotic process characterized by DNA laddering), and/or the oocyte undergoes **pseudomaturation**, characteristic of meiosis. During the early stages of oocyte death, the nuclear membrane disintegrates, the chromatin condenses, and the chromosomes form a metaphase plate with a spindle; the term *pseudomaturation* is appropriate because these oocytes are not capable of successful fertilization. During atresia of follicles containing theca cells, the theca layer may undergo hyperplasia and hypertrophy and may remain in the ovary for extended periods of time.

Meiosis Resumes During the Periovulatory Period

All healthy oocytes in the ovary remain arrested in prophase of the first meiosis. When a graafian follicle is subjected to a surge of gonadotropins (LH and/or FSH), the oocyte within undergoes the final stages of meiosis, resulting in the production of a mature gamete. This maturation is accomplished by two successive cell divisions in which the number of chromosomes is reduced, producing haploid gametes. At fertilization, the diploid state is restored.

Primary oocytes arrested in meiotic prophase 1 (of the first meiosis) have duplicated their centrioles and DNA (4n DNA) so that each chromosome has two identical **chromatids**. Crossing over and chromatid exchange occur during this phase, producing genetic diversity. The resumption of meiosis, ending the first meiotic prophase and beginning of meiotic metaphase 1, is characterized by disappearance of the nuclear membrane, condensation of the chromosomes, nuclear dissolution (germinal vesicle breakdown), and alignment of the chromosomes on the equator of the spindle. At meiotic anaphase 1, the homologous chromosomes move in opposite directions under the influence of the retracting meiotic spindle at the cellular periphery. At meiotic telophase 1, an unequal division of the cell cytoplasm yields a large **secondary oocyte** (2n DNA) and a small, nonfunctional cell, the **first polar body** (2n DNA). Each cell contains half the original 4n number of chromosomes (only one member of each homologous pair is present, but each chromosome consists of two unique chromatids).

The secondary oocyte is formed several hours after the initiation of the LH surge but before ovulation. It rapidly begins the second meiotic division and proceeds through a short prophase to become arrested in metaphase. At this stage, the secondary oocyte is expelled from the graafian follicle. The second arrest period is relatively short. In response to penetration by a spermatozoon during fertilization, meiosis 2 resumes and is rapidly completed. A second unequal cell division soon follows, producing a small **second polar body** (1n DNA) and a large fertilized egg, the **zygote** (2n DNA, 1n from the mother and 1n from the father). The first and second polar bodies either degenerate or divide, yielding small nonfunctional cells. If fertilization does not occur, the secondary oocyte begins to degenerate within 24 to 48 hours.

FOLLICLE SELECTION AND OVULATION

The number of ovulating eggs is species-specific and is influenced by genetic, nutritional, and environmental factors. In humans, normally only one follicle will ovulate, but multiple ovulations in a single cycle (superovulation) can be induced by the timed administration of gonadotropins or antiestrogens. The mechanism by which one follicle is selected from a cohort of growing follicles is poorly understood. It occurs during the first few days of the cycle, immediately after the onset of menstruation. Once selected,

the follicle begins to grow and differentiate at an exponential rate and becomes the dominant follicle.

In parallel with the growth of the dominant follicle, the rest of the preantral follicles undergo atresia. Two main factors contribute to atresia in the nonselected follicles. One is the suppression of plasma FSH in response to increased estradiol secretion by the dominant follicle. The decline in FSH support decreases aromatase activity and estradiol production and interrupts granulosa cell proliferation in those nondominant follicles. The dominant follicle is protected from a fall in circulating FSH levels because it has a healthy blood supply, FSH accumulated in the follicular fluid, and an increased density of FSH receptors on its granulosa cells. Another factor in selection is the accumulation of atretogenic androgens, such as DHT, in the nonselected follicles. The increase in DHT changes the intrafollicular ratio of estrogen to androgen and antagonizes the actions of FSH.

As the dominant follicle grows, vascularization of the theca layer increases. On day 9 or 10 of the cycle, the vascularity of the dominant follicle is twice that of the other antral follicles, permitting a more efficient delivery of cholesterol to theca cells and better exposure to circulating gonadotropins. At this time, the main source of circulating estradiol is the dominant follicle. Since estradiol is the primary regulator of LH and FSH secretion by positive and negative feedback, the dominant follicle ultimately determines its own fate.

The midcycle LH surge occurs as a result of rising levels of circulating estradiol, and it causes multiple changes in the dominant follicle, which occur within a relatively short time. These include the resumption of meiosis in the oocyte (as already discussed); granulosa cell differentiation and transformation into luteal cells; the activation of proteolytic enzymes that degrade the follicle wall and surrounding tissues; increased production of prostaglandins, histamine, and other local factors that cause localized hyperemia; and an increase in progesterone secretion. Within 30 to 36 hours after the onset of the LH surge, this coordinated series of biochemical and morphological events culminates in follicular rupture and ovulation. The midcycle FSH surge is not essential for ovulation because an injection of either LH or human chorionic gonadotropin (hCG) before the endogenous gonadotropin surge can induce normal ovulation. However, only follicles that have been adequately primed with FSH will ovulate because they contain sufficient numbers of LH receptors for ovulation and subsequent luteinization.

Four ovarian proteins are essential for ovulation: the progesterone receptor, the cyclooxygenase enzyme (which converts arachidonic acid to prostaglandins), cyclin D2 (a cell cycle regulator), and a transcription factor called C/EBPβ (CCAAT/enhancer binding protein). The mechanisms by which these proteins interact to regulate follicular rupture are largely unknown. However, mice with specific disruption of genes for any of these proteins fail to ovulate, and these proteins are likely to have a functional role in human ovulation.

The earliest responses of the ovary to the midcycle LH surge are the release of vasodilatory substances, such as histamine, bradykinin, and prostaglandins, which mediate increased ovarian and follicular blood flow. The highly vascularized dominant follicle becomes hyperemic and edematous and swells to a size of at least 20 to 25 mm in diameter. There is also an increased production of follicular fluid, disaggregation of granulosa cells, and detachment of the oocyte-cumulus complex from the follicular wall, moving it to the central portion of the follicle. The basement membrane separating theca cells from granulosa cells begins to disintegrate, granulosa cells begin to undergo luteinization, and blood vessels begin to penetrate the granulosa cell compartment.

Just prior to follicular rupture, the follicular wall thins by cellular deterioration and bulges at a specific site called the **stigma**, the point on the follicle that actually ruptures. As ovulation approaches, the follicle enlarges and protrudes from the surface of the ovary at the stigma. In response to the LH surge, **plasminogen activator** is produced by theca and granulosa cells of the dominant follicle and converts plasminogen to **plasmin**. Plasmin is a proteolytic enzyme that acts directly on the follicular wall and stimulates the production of **collagenase**, an enzyme that digests the connective tissue matrix. The thinning and increased distensibility of the wall facilitates the rupture of the follicle. The extrusion of the oocyte-cumulus complex is aided by smooth muscle contraction. At the time of rupture, the oocyte-cumulus complex and follicular fluid are ejected from the follicle.

The LH surge triggers the resumption of the first meiosis. Up to this point, the primary oocyte has been protected by unknown factors within the follicle from premature cell division. The LH surge also causes transient changes in plasma estradiol and a prolonged increase in plasma progesterone concentrations. Within a couple of hours after the initiation of the LH surge, the production of progesterone, androgens, and estrogens begins to increase. Progesterone, acting through the progesterone receptor on granulosa cells, promotes ovulation by releasing mediators that increase the distensibility of the follicular wall and enhance the activity of proteolytic enzymes. As LH levels reach their peak, plasma estradiol levels plunge because of down-regulation by LH of FSH receptors on granulosa cells and the inhibition of granulosa cell aromatase. Eventually, LH receptors on luteinizing granulosa cells escape the down-regulation, and progesterone production increases.

FORMATION OF THE CORPUS LUTEUM FROM THE POSTOVULATORY FOLLICLE

In response to the LH and FSH surges and after ovulation, the wall of the graafian follicle collapses and becomes convoluted, blood vessels course through the luteinizing granulosa and theca cell layers, and the antral cavity fills with blood. The granulosa cells begin to cease their proliferation and begin to undergo hypertrophy and produce progesterone as their main secretory product. The ruptured follicle develops a rich blood supply and forms a solid structure called the **corpus luteum** (yellow body). The mature corpus luteum develops as the result of numerous biochemical and morphological changes, collectively referred to as **luteinization**. The granulosa cells and theca cells in the corpus luteum are called **granulosa-lutein cells** and **theca-lutein cells**, respectively.

Continued stimulation by LH is needed to ensure morphological integrity (healthy luteal cells) and functionality (progesterone secretion). If pregnancy does not occur, the

corpus luteum regresses, a process called **luteolysis** or **luteal regression**. Luteolysis occurs as a result of apoptosis and necrosis of the luteal cells. After degeneration, the luteinized cells are replaced by fibrous tissue, creating a nonfunctional structure, the **corpus albicans**. Therefore, the corpus luteum is a transient endocrine structure formed from the postovulatory follicle. It serves as the main source of circulating steroids during the luteal (postovulatory) phase of the cycle and is essential for maintaining pregnancy during the first trimester (see Case Study) as well as maintaining menstrual cycles of normal length.

The process of luteinization begins before ovulation. After acquiring a high concentration of LH receptors, granulosa cells respond to the LH surge by undergoing morphological and biochemical transformation. This change involves cell enlargement (hypertrophy) and the development of smooth ER and lipid inclusions, typical of steroid-secreting cells. Unlike the nonvascular granulosa cells in the follicle, luteal cells have a rich blood supply. Invasion by capillaries starts immediately after the LH surge and is facilitated by the dissolution of the basement membrane between theca and granulosa cells. Peak vascularization is reached 7 to 8 days after ovulation.

Differentiated theca and stroma cells, as well as granulosa cells, are incorporated into the corpus luteum, and all three classes of steroids—androgens, estrogens, and progestins—are synthesized. Although some progesterone is secreted before ovulation, peak progesterone production is reached 6 to 8 days after the LH surge. The life span of the corpus luteum is limited. Unless pregnancy occurs, it degenerates within about 13 days after ovulation. During the menstrual cycle, the function of the corpus luteum is maintained by LH; therefore, LH is referred to as a **luteotropic hormone**. Lack of LH can lead to luteal insufficiency (see Clinical Focus Box 38.1).

Regression of the corpus luteum at the end of the cycle is not understood. Luteal regression is thought to be induced by locally produced luteolytic agents that inhibit LH action. Several ovarian hormones, such as estrogen, oxytocin, prostaglandins, and GnRH, have been proposed, but their role as **luteolysins** is controversial. The corpus luteum is rescued from degeneration in the late luteal phase by the action of human chorionic gonadotropin (hCG), an LH-like hormone that is produced by the embryonic trophoblast during the implantation phase (see Chapter 39). This hormone binds the LH receptor and increases cAMP and progesterone secretion.

THE MENSTRUAL CYCLE

Under normal conditions, ovulation occurs at timed intervals. Sexual intercourse may occur at any time during the cycle, but fertilization occurs only during the postovulatory period. Once pregnancy occurs, ovulation ceases, and after parturition, lactation also inhibits ovulation. The first **menstrual cycle** occurs in adolescence, usually around age 12. The initial period of bleeding is called the **menarche**. The first few cycles are usually irregular and anovulatory, as the result of delayed maturation of the positive feedback by estradiol on a hypothalamus that fails to secrete significant GnRH. During puberty, LH secretion occurs more during periods of sleep than during periods of being awake, resulting in a diurnal cycle.

CLINICAL FOCUS BOX 38.1

Luteal Insufficiency

Occasionally, the corpus luteum will not produce sufficient progesterone to maintain pregnancy during its very early stages. Initial signs of early spontaneous pregnancy termination include pelvic cramping and the detection of blood, similar to indications of menstruation. If the corpus luteum is truly deficient, then fertilization may occur around the idealized day 14 (ovulation), pregnancy will terminate during the deficient luteal phase, and menses will start on schedule. Without measuring levels of hCG, the pregnancy detection hormone, the woman would not know that she is pregnant because of the continuation of regular menstrual cycles. **Luteal insufficiency** is a common cause of infertility. Women are advised to see their physician if pregnancy does not result after 6 months of unprotected intercourse.

Analysis of the regulation of progesterone secretion by the corpus luteum provides insights into this clinical problem. There are several reasons for luteal insufficiency. First, the number of luteinized granulosa cells in the corpus luteum may be insufficient because of the ovulation of a small follicle or the premature ovulation of a follicle that was not fully developed. Second, the number of LH receptors on the luteinized granulosa cells in the graafian follicle and developing corpus luteum may be insufficient. LH receptors mediate the action of LH, which stimulates progesterone secretion. An insufficient number of LH receptors could be due to insufficient priming of the developing follicle with FSH. It is well known that FSH increases the number of LH receptors in the follicle. Third, the LH surge could have been inadequate in inducing full luteinization of the corpus luteum, yet there was sufficient LH to induce ovulation. It has been estimated that only 10% of the LH surge is required for ovulation, but the amount required for full luteinization and adequate progesterone secretion to maintain pregnancy is not known.

If progesterone values are low in consecutive cycles at the midluteal phase and do not match endometrial biopsies, exogenous progesterone may be administered in order to prevent early pregnancy termination during a fertile cycle. Other options include the induction of follicular development and ovulation with clomiphene and hCG. This treatment would likely produce a large, healthy, estrogen-secreting graafian follicle with sufficient LH receptors for luteinization. The exogenous hCG is given to supplement the endogenous LH surge and to ensure full stimulation of the graafian follicle, ovulation, adequate progesterone, and luteinization of the developing corpus luteum.

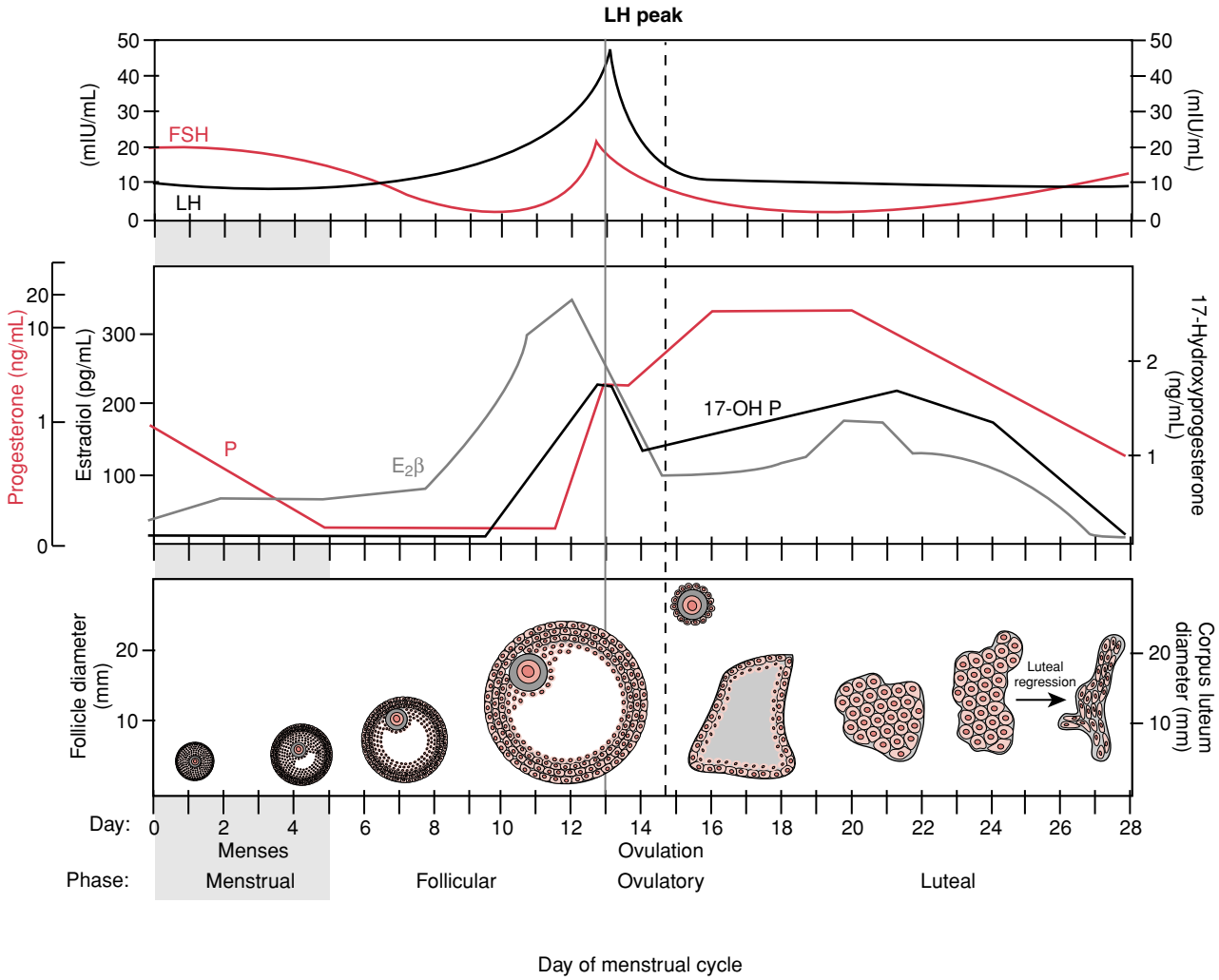

FIGURE 38.6 **Hormonal and ovarian events during the menstrual cycle.** P, progesterone; $E_2\beta$, estradiol; 17-OH P, 17-hydroxyprogesterone.

The average menstrual cycle length in adult women is 28 days, with a range of 25 to 35 days. The interval from ovulation to the onset of menstruation is relatively constant, averaging 14 days in most women and is dictated by the fixed life span of the corpus luteum. In contrast, the interval from the onset of menses to ovulation (the follicular phase) is more variable and accounts for differences in cycle lengths among ovulating women.

The menstrual cycle is divided into four phases (Fig. 38.6). The **menstrual phase**, also called **menses** or **menstruation**, is the bleeding phase and lasts about 5 days. The ovarian **follicular phase** lasts about 10 to 16 days; follicle development occurs, estradiol secretion increases, and the uterine endometrium undergoes proliferation in response to rising estrogen levels. The **ovulatory phase** lasts 24 to 48 hours, and the **luteal phase** lasts 14 days. In the luteal phase, progesterone is produced, and the endometrium secretes numerous proteins in preparation for implantation of an embryo.

The cycles become irregular as menopause approaches around age 50, and cycles cease thereafter. During the reproductive years, menstrual cycling is interrupted by conception and lactation and is subjected to modulation by physiological, psychological, and social factors.

The Menstrual Cycle Requires Synchrony Among the Ovary, Brain, and Pituitary

The menstrual cycle requires several coordinated elements: hypothalamic control of pituitary function, ovarian follicular and luteal changes, and positive and negative feedback of ovarian hormones at the hypothalamic-pituitary axis. We have discussed separately the mechanisms that regulate the synthesis and release of the reproductive hormones; now we put them together in terms of sequence and interaction. For this purpose, we use a hypothetical cycle of 28 days (see Fig. 38.6), divided into four phases as follows: menstrual (days 0 to 5), follicular (days 0 to 13), ovulatory (days 13 to 14), and luteal (days 14 to 28).

During menstruation, estrogen, progesterone, and inhibin levels are very low as a result of the luteal regression that has just occurred and the low estrogen synthesis by immature follicles. The plasma FSH levels are high while LH

levels are low in response to the removal of negative feedback by estrogen, progesterone, and inhibin. A few days later, however, LH levels slowly begin to rise. FSH acts on a cohort of follicles recruited 20 to 25 days earlier from a resting pool of smaller follicles. The follicles on days 3 to 5 average 4 to 6 mm in diameter, and they are stimulated by FSH to grow into the preantral stages. In response to FSH, the granulosa cells proliferate, aromatase activity increases, and plasma estradiol levels rise slightly between days 3 and 7. The designated dominant follicle is selected between days 5 and 7, and increases in size and steroidogenic activity. Between days 8 and 10, plasma estradiol levels rise sharply, reaching peak levels above 200 pg/mL on day 12, the day before the LH surge.

During the early follicular phase, LH pulsatility is of low amplitude and high frequency (about every hour). Coinciding pulses of GnRH are released about every hour. As estradiol levels rise, the pulse frequency in GnRH further increases, without a change in amplitude. The mean plasma LH level increases and further supports follicular steroidogenesis, especially since FSH has increased the number of LH receptors on growing follicles. During the midfollicular to late follicular phase, rising estradiol and inhibin from the dominant follicle suppress FSH release. The decline in FSH, together with an accumulation of nonaromatizable androgens, induces atresia in the nonselected follicles. The dominant follicle is saved by virtue of its high density of FSH receptors, the accumulation of FSH in its follicular fluid (see Table 38.2), and the acquisition of LH receptors by the granulosa cells.

The midcycle surge of LH is rather short (24 to 36 hours) and is an example of positive feedback. For the LH surge to occur, estradiol must be maintained at a critical concentration (about 200 pg/mL) for a sufficient duration (36 to 48 hours) prior to the surge. Any reduction of the estradiol rise or a rise that is too small or too short eliminates or reduces the LH surge. In addition, in the presence of elevated progesterone, high concentrations of estradiol do not induce an LH surge. Paradoxically, although it exerts negative feedback on LH release most of the time, positive feedback by estradiol is required to generate the midcycle surge.

Estrogen exerts its effects directly on the anterior pituitary, with GnRH playing a permissive, albeit mandatory, role. This concept is derived from experiments in monkeys whose medial basal hypothalamus, including the GnRH-producing neurons, was destroyed by lesioning, resulting in a marked decrease in plasma LH levels. The administration of exogenous GnRH at a fixed frequency restored LH release. When estradiol was given at an optimal concentration for an appropriate time, an LH surge was generated, in spite of maintaining steady and unchanging pulses of GnRH.

The mechanism that transforms estradiol from a negative to a positive regulator of LH release is unknown. One factor involves an increase in the number of GnRH receptors on the gonadotrophs, increasing pituitary responsiveness to GnRH. Another factor is the conversion of a storage pool of LH (perhaps within a subpopulation of gonadotrophs) to a readily releasable pool. Estrogen may also increase GnRH release, serving as a fine-tuning or failsafe mechanism. A small but distinct rise in progesterone occurs before the LH surge. This rise is important for augmenting the LH surge and, together with estradiol, promotes a concomitant surge in FSH. There are indications that the midcycle FSH surge is important for inducing enough LH receptors on granulosa cells for luteinization, stimulating plasminogen activator for follicular rupture, and activating a cohort of follicles destined to develop in the next cycle.

The LH surge reduces the concentration of 17α-hydroxylase and subsequently decreases androstenedione production by the dominant follicle. Estradiol levels decline, 17-hydroxyprogesterone increases, and progesterone levels plateau. The prolonged exposure to high LH levels during the surge down-regulates the ovarian LH receptors, accounting for the immediate postovulatory suppression of estradiol. As the corpus luteum matures, it increases progesterone production and reinitiates estradiol secretion. Both reach high plasma concentrations on days 20 to 23, about 1 week after ovulation.

During the luteal phase, circulating FSH levels are suppressed by the elevated steroids. The LH pulse frequency is reduced during the early luteal phase, but the amplitude is higher than that during the follicular phase. LH is important at this time for maintaining the function of the corpus luteum and sustaining steroid production. In the late luteal phase, both LH pulse frequency and amplitude are reduced by a progesterone-dependent, opioid-mediated suppression of the GnRH pulse generator.

After the demise of the corpus luteum on days 24 to 26, estradiol and progesterone levels plunge, causing the withdrawal of support of the uterine endometrium, culminating within 2 to 3 days in menstruation. The reduction in ovarian steroids acts centrally to remove feedback inhibition. The FSH level begins to rise and a new cycle is initiated.

Estradiol and Progesterone Influence Cyclic Changes in the Reproductive Tract

The female reproductive tract undergoes cyclic alterations in response to the changing levels of ovarian steroids. The most notable changes occur in the function and histology of the oviduct and uterine endometrium, the composition of cervical mucus, and the cytology of the vagina (Fig. 38.7). At the time of ovulation, there is also a small but detectable rise in basal body temperature, caused by progesterone. All of the above parameters are clinically useful for diagnosing menstrual dysfunction and infertility.

The oviduct is a muscular tube lined internally with a ciliated, secretory, columnar epithelium with a deeper stromal tissue. Fertilization occurs in the oviduct, after which the zygote enters the uterus; therefore, the oviduct is involved in transport of the gametes and provides a site for fertilization and early embryonic development. Estrogens maintain the ciliated nature of the epithelium, and ovariectomy causes a loss of the cilia. Estrogens also increase the motility of the oviducts. Exogenous estrogen given around the time of fertilization can cause premature expulsion of the fertilized egg, whereas extremely high doses of estrogen can cause "tube locking," the entrapment of the fertilized

egg and an ectopic pregnancy. Progesterone opposes these actions of estrogen.

The endometrium (also called uterine mucosa) is composed of a superficial layer of epithelial cells and an underlying stromal layer. The epithelial layer contains glands that penetrate the stromal layer. The glands are lined by a secretory columnar epithelium.

The **endometrial cycle** consists of four phases. The **proliferative phase** coincides with the midfollicular to late follicular phase of the menstrual cycle. Under the influence of the rising plasma estradiol concentration, the stromal and epithelial layers of the uterine endometrium undergo hyperplasia and hypertrophy and increase in size and thickness. The endometrial glands elongate and are lined with columnar epithelium. The endometrium becomes vascularized, and more spiral arteries, a rich blood supply to this region, develop. Estradiol also induces the formation of progesterone receptors and increases myometrial excitability and contractility.

The **secretory phase** begins on the day of ovulation and coincides with the early to midluteal phase of the menstrual cycle. The endometrium contains numerous progesterone receptors. Under the combined action of progesterone and estrogen, the endometrial glands become coiled, store glycogen, and secrete large amounts of carbohydrate-rich mucus. The stroma increases in vascularity and becomes edematous, and the spiral arteries become tortuous (see Fig. 38.7). Peak secretory activity, edema formation, and overall thickness of the endometrium are reached on days 6 to 8 after ovulation in preparation for implantation of the blastocyst. Progesterone antagonizes the effect of estrogen on the myometrium and reduces spontaneous myometrial contractions.

The **ischemic phase**, generally not depicted graphically, occurs immediately before the menses and is initiated by the declining levels of progesterone and estradiol caused by regression of the corpus luteum. Necrotic changes and abundant apoptosis occur in the secretory epithelium as it collapses. The arteries constrict, reducing the blood supply to the superficial endometrium. Leukocytes and macrophages invade the stroma and begin to phagocytose the ischemic tissue. Leukocytes persist in large numbers throughout menstruation, providing resistance against infection to the denuded endometrial surface.

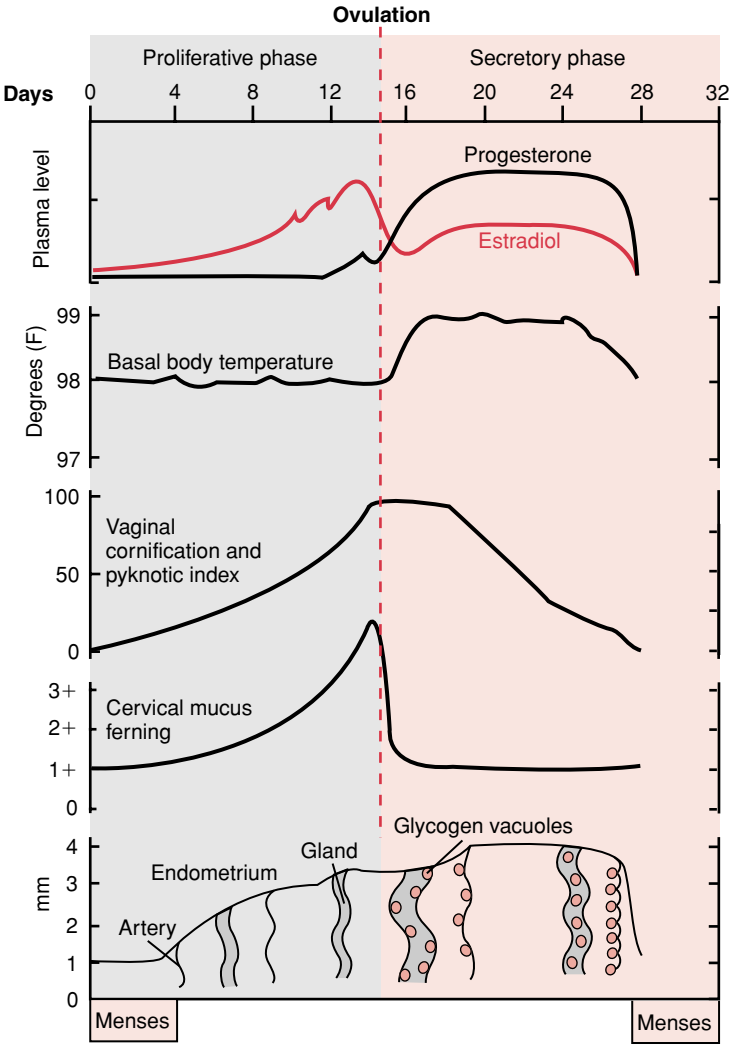

FIGURE 38.7 Cyclic changes in the uterus, cervix, vagina, and body temperature in relationship to estradiol, progesterone, and ovulation during the menstrual cycle. (Modified from Odell WD. The reproductive system in women. In: Degroot LJ, et al, eds. Endocrinology. Vol 3. New York: Grune & Stratton, 1979.)

Desquamation and sloughing of the entire functional layer of the endometrium occurs during the **menstrual phase** (menses). The mechanism leading to necrosis is only partly understood. The reduction in steroids destabilizes lysosomal membranes in endometrial cells, resulting in the liberation of proteolytic enzymes and increased production of vasoconstrictor prostaglandins (e.g., $PGF_{2\alpha}$). The prostaglandins induce vasospasm of the spiral arteries, and the proteolytic enzymes digest the tissue. Eventually, the blood vessels rupture and blood is released, together with cellular debris. The endometrial tissue is expelled through the cervix and vagina, with blood from the ruptured arteries. The menstrual flow lasts 4 to 5 days and averages 30 to 50 mL in volume. It does not clot because of the presence of fibrinolysin, but the spiral arteries constrict, resulting in a reduction in bleeding.

Changes in the properties of the cervical mucus promote the survival and transport of sperm and, thus, can be important for normal fertility. The cervical mucus undergoes cyclic changes in composition and volume. During the follicular phase, estrogen increases the quantity, alkalinity, viscosity, and elasticity of the mucus. The cervical muscles relax, and the epithelium becomes secretory in response to estrogen. By the time of ovulation, elasticity of the mucus or spinnbarkeit is greatest. Sperm can readily pass through the estrogen-dominated mucus. With progesterone rising either after ovulation, during pregnancy, or with low-dose progestogen administration during the cycle, the quantity and elasticity of the mucus decline; it becomes thicker (low spinnbarkeit) and does not form a ferning pattern when dried on a microscope slide. With these conditions, the mucus provides better protection against infections and sperm do not easily pass through.

The vaginal epithelium proliferates under the influence of estrogen. Basophilic cells predominate early in the follicular phase. The columnar epithelium becomes cornified (keratinized) under the influence of estrogen and reaches its peak in the periovulatory period. During the postovulatory period, progesterone induces the formation of thick mucus, the epithelium becomes infiltrated with leukocytes, and cornification decreases (see Fig. 38.7).

ESTROGEN, PROGESTIN, AND ANDROGEN: TRANSPORT AND METABOLISM

The principal sex steroids in the female are estrogen, progestin, and androgen. Three **estrogens** are present in significant quantities—estradiol, estrone, and estriol. Estradiol is the most abundant and is 12 and 80 times more potent than estrone and estriol, respectively. Much of estrone is derived from peripheral conversion of either androstenedione or estradiol (see Fig 37.9). During pregnancy, large quantities of estriol are produced from dehydroepiandrosterone sulfate after 16α-hydroxylation by the fetoplacental unit (see Chapter 39). Most estrogens are bound to either **albumin** (~60%) with a low affinity or to **sex hormone-binding globulin** (SHBG) (~40%) with high affinity. Estrogens are metabolized in the liver through **oxidation** or conversion to **glucuronides** or **sulfates**. The metabolites are then excreted in the urine.

The most important **progestin** is progesterone. It is secreted in significant amounts during the luteal phase of the menstrual cycle. During pregnancy, the corpus luteum secretes progesterone throughout the first trimester, and the placenta continues progesterone production until parturition. Small amounts of 17-hydroxyprogesterone are secreted along with progesterone. Progesterone binds equally to albumin and to a plasma protein called **corticosteroid-binding protein** (**transcortin**). Progesterone is metabolized in the liver to **pregnanediol** and, subsequently, excreted in the urine as a glucuronide conjugate.

Circulating **androgens** in the female originate from the ovaries and adrenals and from peripheral conversion. Androstenedione and dehydroepiandrosterone (DHEA) originate from the adrenal cortex (see Chapter 34), and ovarian theca and stroma cells. Peripheral conversion from androstenedione provides an additional source of testosterone. Testosterone can also be converted in peripheral tissues to **dihydrotestosterone** (DHT) by **5α-reductase**. However, the primary biologically active androgen in women is testosterone. Androgens bind primarily to SHBG and bind to albumin by about half as much. Androgens are also metabolized to water-soluble forms by oxidation, sulfation, or glucuronidation and excreted in the urine.

PUBERTY

During the prepubertal period, the hypothalamic-pituitary-ovarian axis becomes activated—an event known as **gonadarche**—and gonadotropins increase in the circulation and stimulate ovarian estrogen secretion. The increase in gonadotropins is a direct result of increased secretion of GnRH. Factors stimulating the secretion of GnRH include glutamate, norepinephrine, and neuropeptide Y emanating from synaptic inputs to GnRH-producing neurons. In addition, a decrease in γ-aminobutyric acid (GABA), an inhibitor of GnRH secretion, may occur at this time. It is also known that the response of the pituitary to GnRH increases at the time of **puberty**. Collectively, numerous factors control the rise in ovarian estradiol secretion that triggers the development of physical characteristics of sexual maturation.

Estradiol induces the development of **secondary sex characteristics**, including the breasts and reproductive tract, and increased fat in the hips. Estrogens also regulate the growth spurt at puberty, induce closure of the epiphyses, have a positive effect in maintaining bone formation, and can antagonize the degrading actions of parathyroid hormone on bone. Therefore, estrogens have a positive effect on bone maintenance, and later in life, exogenous estrogens oppose the osteoporosis often associated with menopause.

As mentioned earlier, the first menstruation is called menarche and occurs around age 12. The first ovulation does not occur until 6 to 9 months after menarche because the hypothalamic-pituitary axis is not fully responsive to the feedback effects of estrogen. During the pubertal period, the development of breasts, under the influence of estrogen, is known as **thelarche**. At this time, the appearance of axillary and pubic hair occurs, a development known as **pubarche**, controlled by adrenal an-

drogens. The adrenals begin to produce significant amounts of androgens (dehydroepiandrosterone and androstenedione) 4 to 5 years prior to menarche, and this event is called **adrenarche**. The adrenal androgens are responsible in part for pubarche. Adrenarche is independent of gonadarche.

MENOPAUSE

Menopause is the time after which the final menses occurs. It is associated with the cessation of ovarian function and reproductive cycles. Generally, menstrual cycles and bleeding become irregular, and the cycles become shorter from the lack of follicular development (shortened follicular phases). The ovaries atrophy and are characterized by the presence of few, if any, healthy follicles.

The decline in ovarian function is associated with a decrease in estrogen secretion and a concomitant increase in LH and FSH, which is characteristic of menopausal women (Table 38.3). It is used as a diagnostic tool. The elevated LH stimulates ovarian stroma cells to continue producing androstenedione. Estrone, derived almost entirely from the peripheral conversion of adrenal and ovarian androstenedione, becomes the dominant estrogen (see Fig. 37.9). Because the ratio of estrogens to androgens decreases, some women exhibit hirsutism, which results from androgen excess. The lack of estrogen causes atrophic changes in the breasts and reproductive tract, accompanied by vaginal dryness, which often causes pain and irritation. Similar changes in the urinary tract may give rise to urinary disturbances. The epidermal layer of the skin becomes thinner and less elastic.

Hot flashes, as a result of the loss of vasomotor tone, osteoporosis, and an increased risk of cardiovascular disease are not uncommon. Hot flashes are associated with episodic increases in upper body and skin temperature, peripheral vasodilation, and sweating. They occur concurrently with LH pulses but are not caused by the gonadotropins because they are evident in hypophysectomized women. Hot flashes, consisting of episodes of sudden warmth and sweating, reflect temporary disturbances in the hypothalamic thermoregulatory centers, which are somehow linked to the GnRH pulse generator.

Osteoporosis increases the risk of hip fractures and estrogen replacement therapy reduces the risk. Estrogen antagonizes the effects of PTH on bone but enhances its effect on kidney, i.e., it stimulates retention of calcium. Estrogen also promotes the intestinal absorption of calcium through 1,25-dihydroxyvitamin D_3.

Menopausal symptoms are often treated with hormone replacement therapy (HRT), which includes estrogens and progestins. HRT is not an uncommon treatment to improve the quality of life. In some patients, treatment with estrogen can cause adverse effects, such as vaginal bleeding, nausea, and headache. Estrogen therapy is contraindicated in cases of existing reproductive tract carcinomas or hypertension and other cardiovascular disease. The prevailing opinion is that the benefit of treating postmenopausal women with estrogens for limited periods outweighs any risk of developing breast or endometrial carcinomas.

INFERTILITY

One of five women in the United States will be affected by infertility. A thorough understanding of female endocrinology, anatomy, and physiology are critical to gaining insights into solving this major health problem. Infertility can be caused by several factors. Environmental factors, disorders of the central nervous system, hypothalamic disease, pituitary disorders, and ovarian abnormalities can interfere with follicular development and/or ovulation. If a normal ovulation occurs, structural, pathological, and/or endocrine problems associated with the oviduct and/or uterus can prevent fertilization, impede the transport or implantation of the embryo, and, ultimately, interfere with the establishment or maintenance of pregnancy.

Amenorrhea Is Caused by Endocrine Disruption

Menstrual cycle disorders can be divided into two categories: **amenorrhea**, the absence of menstruation, and **oligomenorrhea**, infrequent or irregular menstruation. **Primary amenorrhea** is a condition in which menstruation has never occurred. An example is **Turner's syndrome**, also called gonadal dysgenesis, a congenital abnormality caused by a nondisjunction of one of the X chromosomes, resulting in a 45 X0 chromosomal karyotype. Because the two X chromosomes are necessary for normal ovarian development, women with this condition have rudimentary gonads and do not have a normal puberty. Because of ovarian steroid deficiency (lack of estrogen), secondary sex characteristics remain prepubertal, and plasma LH and FSH are elevated. Other abnormalities include short stature, a webbed neck, a coarctation of the aorta, and renal disorders.

Another congenital form of primary amenorrhea is **hypogonadotropism** with anosmia, similar to Kallmann's syn-

TABLE 38.3 Serum Gonadotropin and Steroid Levels in Premenopausal and Postmenopausal Women

		Menstrual Cycle			
Hormone	Units	Follicular	Preovulatory	Luteal	Postmenopausal
LH	mIU/mL	2.5–15	15–100	2.5–15	20–100
FSH	mIU/mL	2–10	10–30	2–6	20–140
Estradiol	pg/mL	70–200	200–500	75–300	
Progesterone	ng/mL	≤0.5	≤1.5	4–20	≤0.5

drome in males (see Chapter 37). Patients do not progress through normal puberty and have low and nonpulsatile LH and FSH levels. However, they can have normal stature, female karyotype, and anosmia. The disorder is caused by a failure of olfactory lobe development and GnRH deficiency. Primary amenorrhea can also be caused by a congenital malformation of reproductive tract structures originating from the müllerian duct, including the absence or obstruction of the uterus, cervix, or upper vagina.

Secondary amenorrhea is the cessation of menstruation for longer than 6 months. Pregnancy, lactation, and menopause are common physiological causes of secondary amenorrhea. Other causes are premature ovarian failure, polycystic ovarian syndrome, hyperprolactinemia, and hypopituitarism.

Premature ovarian failure is characterized by amenorrhea, low estrogen levels, and high gonadotropin (LH and FSH) levels before age 40. The symptoms are similar to those of menopause, including hot flashes and an increased risk of osteoporosis. The etiology is variable, including chromosomal abnormalities; lesions resulting from irradiation, chemotherapy, or viral infections; and autoimmune conditions.

Polycystic ovarian syndrome, also called **Stein-Leventhal syndrome**, is a heterogeneous group of disorders characterized by amenorrhea or anovulatory bleeding, an elevated LH/FSH ratio, high androgen levels, hirsutism, and obesity. Although the etiology is unknown, the syndrome may be initiated by excessive adrenal androgen production, during puberty or following stress, that deranges the hypothalamic-pituitary axis secretion of LH. Androgens are converted peripherally to estrogens and stimulate LH release. Excess LH, in turn, increases ovarian stromal and thecal androgen production, resulting in impaired follicular maturation. The LH-stimulated ovaries are enlarged and contain many small follicles and hyperplastic and luteinized theca cells (the site of LH receptors). The elevated plasma androgen levels cause hirsutism, increased activity of sebaceous glands, and clitoral hypertrophy, which are signs of virilization in females.

Hyperprolactinemia is also a cause of secondary amenorrhea. **Galactorrhea**, a persistent milk-like discharge from the nipple in nonlactating individuals, is a frequent symptom and is due to the excess prolactin (PRL). The etiology of hyperprolactinemia is variable. Pituitary prolactinomas account for about 50% of cases. Other causes are hypothalamic disorders, trauma to the pituitary stalk, and psychotropic medications, all of which are associated with a reduction in dopamine release, resulting in an increased PRL secretion. Hypothyroidism, chronic renal failure, and hepatic cirrhosis are additional causes of hyperprolactinemia. In some forms of hypothyroidism, increased hypothalamic thyrotropin-releasing hormone (TRH) is thought to contribute to excess PRL secretion, as experimental studies reveal that exogenous TRH increases the secretion of PRL. The mechanism by which elevated PRL levels suppress ovulation is not entirely clear. It has been postulated that PRL may inhibit GnRH release, reduce LH secretion in response to GnRH stimulation, and act directly at the level of the ovary by inhibiting the action of LH and FSH on follicle development.

Oligomenorrhea can be caused by excessive exercise and by nutritional, psychological, and social factors. **Anorexia nervosa**, a severe behavioral disorder associated with the lack of food intake, is characterized by extreme malnutrition and endocrine changes secondary to psychological and nutritional disturbances. About 30% of patients develop amenorrhea that is not alleviated by weight gain. Strenuous exercise, especially by competitive athletes and dancers, frequently causes menstrual irregularities. Two main factors are thought to be responsible: a low level of body fat, and the effect of stress itself through endorphins that are known to inhibit the secretion of LH. Other types of stress, such as relocation, college examinations, general illness, and job-related pressures, have been known to induce some forms of oligomenorrhea.

Female Infertility Is Caused by Endocrine Malfunction and Abnormalities in the Reproductive Tract

The diagnosis and treatment of amenorrhea present a challenging problem. The amenorrhea must first be classified as primary or secondary, and menopause, pregnancy, and lactation must be excluded. The next step is to determine whether the disorder originates in one of the following areas: the hypothalamus and central nervous system, the anterior pituitary, the ovary, and/or the reproductive tract.

Several treatments can alleviate infertility problems; for example, some success has been achieved in hypothalamic disease with pulsatile administration of GnRH. When hypogonadotropism is the cause of infertility, sequential administration of FSH and hCG is a common treatment for inducing ovulation, although the risk of ovarian hyperstimulation and multiple ovulations is increased. Hyperprolactinemia can be treated surgically by removing the pituitary adenoma containing numerous lactotrophs (prolactin-secreting cells). It can also be treated pharmacologically with bromocriptine, a dopaminergic agonist that reduces the size and number of the lactotrophs and PRL secretion. Treatment with clomiphene, an antiestrogen that binds to and blocks estrogen receptors, can induce ovulation in women with endogenous estrogens in the normal range. Clomiphene reduces the negative feedback effects of estrogen and thus increases endogenous FSH and LH secretion. When reproductive tract lesions are the cause of infertility, corrective surgery or *in vitro* fertilization is the treatment of choice.

REVIEW QUESTIONS

DIRECTIONS: Each of the numbered items or incomplete statements in this section is followed by answers or by completions of the statement. Select the ONE lettered answer or completion that is BEST in each case.

1. Estradiol synthesis in the graafian follicle involves
   (A) Activation of LH-stimulated granulosa production of androgen
   (B) Stimulation of aromatase in the granulosa cell by FSH
   (C) Decreased secretion of progesterone from the corpus luteum, resulting in increased LH
   (D) Inhibition of the LH surge during the preovulatory period
   (E) Synergy between FSH and progesterone
2. Granulosa cells do not produce estradiol from cholesterol because they do not have an active
   (A) 17α-Hydroxylase
   (B) Aromatase
   (C) 5α-Reductase
   (D) Sulfatase
   (E) Steroidogenic acute regulatory protein
3. A clinical sign indicating the onset of the menopause is
   (A) The onset of menses near age 50
   (B) An increase in plasma FSH levels
   (C) An excessive presence of corpora lutea
   (D) An increased number of cornified cells in the vagina
   (E) Regular menstrual cycles
4. Increased progesterone during the postovulatory period is associated with
   (A) Proliferation of the uterine endometrium
   (B) Enhanced development of graafian follicles
   (C) Luteal regression
   (D) An increase in basal body temperature by 0.5 to 1.0°C
   (E) Increased secretion of FSH
5. The theca interna cells of the graafian follicle are distinguished by
   (A) Their capacity to produce androgens from cholesterol
   (B) The lack of cholesterol side-chain cleavage enzyme
   (C) Aromatization of testosterone to estradiol
   (D) The lack of a blood supply
   (E) The production of inhibin
6. Disruption of the hypothalamic-pituitary portal system will lead to
   (A) High circulating levels of PRL, low levels of LH and FSH, and ovarian atrophy
   (B) Enhanced follicular development as a result of increased circulating levels of PRL
   (C) Ovulation, followed by increased circulating levels of progesterone
   (D) A reduction of ovarian inhibin levels, followed by increased circulating FSH
   (E) Excessive androgen production by the ovaries
7. Inhibin is an ovarian hormone that
   (A) Inhibits the secretion of LH and PRL
   (B) Is produced by granulosa cells and inhibits the secretion of FSH
   (C) Only has local ovarian effects and no effect on the secretion of FSH
   (D) Has two forms, A and B, with the same β subunits but distinct α subunits
   (E) Binds activin and increases FSH secretion
8. Spinnbarkeit formation is induced by
   (A) Secretory endometrium
   (B) Progesterone action on the uterus
   (C) Androgen production from the ovaries
   (D) Estrogen action on the vaginal secretions
   (E) Prolactin secretion
9. Successful fertilization is most likely to occur when the oocyte is in
   (A) The oviduct and has entered the second meiotic division
   (B) The uterus and has completed the first meiotic division
   (C) Metaphase of mitosis
   (D) The graafian follicle, which then enters the oviduct
   (E) The uterus, extruding the second polar body and implanting
10. The enzyme, 5α-reductase is responsible for
    (A) Conversion of cholesterol to pregnenolone and enhancing steroidogenesis
    (B) Conversion of testosterone to dihydrotestosterone
    (C) Aromatization of testosterone to estradiol
    (D) Increasing the synthesis of LH
    (E) Female secondary sex characteristics

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