Hypothalamic-Pituitary-Ovarian Axis and Control of the Menstrual Cycle

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Contents

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1

Key Points

- The menstrual cycle is divided into follicular and luteal phases, with the follicular phase starting with onset of menses and ending with onset of the LH surge, and the luteal phase starting with onset of the LH surge and ending with onset of menses.
- The hypothalamic-pituitary-ovarian (HPO) axis is a tightly regulated system controlling the menstrual cycle and female reproduction through multiple positive and negative feedback loops.
- Pulsatile GnRH release into hypophyseal portal circulation stimulates pituitary LH and FSH production. LH and FSH are gonadotropins that stimulate ovarian follicular development and hormone production, leading to a slow rise in estradiol production during the follicular phase and the onset of high progesterone production in the luteal phase.
- The LH surge brings about resumption of meiosis in the oocyte, production of prostaglandins to facilitate follicular rupture, and the formation of the corpus luteum, which produces the hormones required to prepare the endometrium for implantation.

1.1 Introduction

The menstrual cycle is the product of a cascade of hormones from many interacting endocrine glands coordinating a cyclic ovarian and uterine response. Ovarian production of estrogens and progestins is largely regulated by the hypothalamus and anterior pituitary gland, both of which are also regulated by serum hormone levels. The interplay between these endocrine systems forms the basis of the hypothalamic-pituitary-ovarian (HPO) axis.

This chapter will cover how the time-, location-, and dose-dependent interactions between the central nervous system, endocrine, and pelvic organs coordinate to reliably grow and release one egg each month while simultaneously preparing the endometrium for a pregnancy. The first section details the stages of the menstrual cycle. The second section covers the functional anatomy of the hypothalamus, anterior pituitary, ovaries, and uterus. The third section discusses neuroendocrine regulation of the menstrual cycle. The last section also reviews the key hormones driving the menstrual cycle, with a particular focus on gonadotropin-releasing hormone (GnRH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol, and progesterone (Table 1.1).

1.2 The Menstrual Cycle

The menstrual cycle is conventionally divided into two different stages of ovarian activity as well as two phases of uterine response. The ovarian cycle includes the follicular phase and the luteal phase, separated by ovulation. The uterine cycle includes the proliferative phase and the secretory phase. Each menstrual cycle spans from the first full day of menstrual bleeding to the first full day in the following menstrual period. On average this cycle lasts 28 days, though a cycle length between 24 and 38 days is considered within normal range [1, 2]. This range is based on variation on the length of the follicular/proliferative phase, with the luteal/secretory phase remaining relatively constant at 14 days. Normal menses last 3–8 days [1]. Bleeding quantity on average during menses is 30 mL, and blood loss greater than 80 mL is considered heavy menstrual bleeding [3]. While a wide variety of conditions may lead to abnormal uterine bleeding, deviations from these parameters in the menstrual cycle may suggest a breakdown in regulation of the hypothalamic-pituitary-ovarian axis or a structural abnormality involving the pelvic organs [1, 4].

Table 1.1 Major hormones of the hypothalamic-pituitary-ovarian axis[a]

Hormone	Structure	Gene location	Major site(s) of production	Half-life	Serum concentration
GnRH	Decapeptide	8p21–8p11.2	Arcuate nucleus of hypothalamus	2–4 min	N/A
FSH	Glycoprotein with α- and β-subunits	α: 6q12.21 β: 11p13	Gonadotrophs of anterior pituitary	1.5–4 h	5–25 mIU/mL
LH	Glycoprotein with α- and β-subunits	α: 6q12.21 β: 19q12.32	Gonadotrophs of anterior pituitary	20–30 min	5–25 mIU/mL
Estradiol	18 carbon steroid	NA	Granulosa cells	2–3 h	20–400 pg/mL
Progesterone	21 carbon steroid	NA	Theca-lutein cells	5 min	0.1–30 ng/mL
Inhibin	Peptide with α- and β-subunits Inhibin A = α + βA Inhibin B = α + βB	α: 2q33 βA: 2q13 βB: 7p15	Granulosa cells	30–60 min	A: 10–60 B: 10–150 pg/mL

[a]Reproduced with permission from Falcone and Hurd [110]

1.2.1 The Follicular Phase

During the follicular (proliferative) phase of the menstrual cycle, the dominant hormone, FSH, stimulates folliculogenesis. Folliculogenesis involves recruitment of a cohort of immature antral follicles in the ovary, one of which becomes the dominant follicle for the menstrual cycle. Antral follicles are visible on transvaginal ultrasound around menses as homogeneous hypoechoic cysts measuring 2–10 mm in mean diameter across two dimensions. After receiving appropriate signals, one or more follicles from the cohort of antral follicles grow to maturation. The follicle that goes on to ovulate is often recognizable several days prior to ovulation as the so-called dominant follicle and is typically the largest diameter follicle, growing to 17–25 mm prior to ovulation [5]. The dominant follicle is the most responsive to FSH, owing to its expression of the highest concentration of FSH receptors on its surface among the growing antral follicles [6]. Surrounding the dominant follicle is a bed of granulosa cells expressing aromatase, the final enzyme involved in estradiol biosynthesis. Increased estradiol production influences uterine response in the follicular phase by stimulating proliferation of the endometrial glands. Rising estradiol also provides negative feedback to reduce GnRH production from the hypothalamus and decrease FSH production from the pituitary in the early follicular phase, thereby leading to apoptosis of the other more FSH-sensitive follicles recruited in that cycle and limiting multifollicular ovulation.

1.2.2 Early Luteal Phase

The luteinizing hormone surge marks the beginning of the luteal phase and triggers ovulation. The LH surge is mediated by a switch from negative pituitary feedback to estradiol levels during the early follicular phase to positive feedback in response to rising estradiol levels in the late follicular phase, with a level > 200 pg/mL for over 50 hours being sufficient to promote the LH surge [4]. It is also accompanied by a shift from inactive, glycosylated to biologically active, non-glycosylated

LH isoforms as estradiol levels rise [7]. For granulosa cells to achieve this level of estradiol, the dominant follicle typically has grown to >15 mm [5, 8]. Animal studies on the nature of the switch from negative to positive feedback of estradiol on LH production point to increased estradiol inducing increased transmission of glutamate and gamma-aminobutyric acid (GABA) in GnRH neurons [9, 10]. The LH surge promotes the first meiotic division of the oocyte in the dominant follicle, previously arrested at the diplotene phase, and the ultimate rupture of the follicle leading to oocyte release. Meiosis is not completed until fertilization. Rupture occurs secondary to LH-influenced production of prostaglandins and eicosanoid signaling molecules that culminate in an ordered release of proteolytic enzymes [11]. Ovulation occurs about 36 hours after the start of the LH surge and 12 hours after the peak of the LH surge, leading to follicular rupture and oocyte release [12]. The fallopian tube, the typical site of fertilization, will then sweep up the oocyte.

1.2.3 Mid- to Late Luteal Phase

Estrogen levels fall and progesterone levels dramatically rise during the luteal phase. While progesterone production from the follicle begins to rise late in the follicular phase, it is predominantly produced following the LH surge, which transforms the granulosa cells in the basement membrane into progesterone-producing luteal cells.

The combined cystic structure following follicular rupture is called the corpus luteum, literally "yellow body" in Latin. These luteal cells are vacuolated and filled with lutein, a straw-colored fluid. By day 7 or 8 of the luteal phase, peak progesterone levels and vascularization of the corpus luteum are reached (Fig. 1.1) [13].

Progesterone induces dramatic changes on the endometrium, including cessation of proliferation of endometrial glands, leading to increased glandular tortuosity, and appearance of vacuoles in the endometrial glands that soon secrete a range of proteins and peptides into the endometrial cavity. These processes prepare the endometrium for embryo implantation. If embryo implantation occurs, human chorionic gonadotropin (hCG), produced by the early embryo as early as 8–9 days after fertilization, will cause the corpus luteum to continue its production of progesterone via its action on the LH/hCG receptor [14].

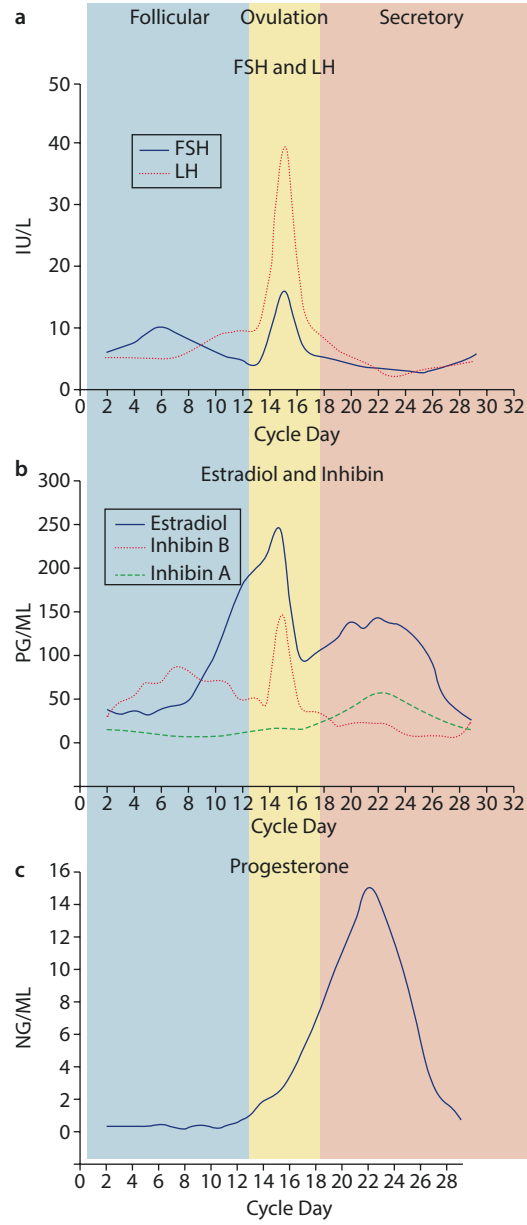

Fig. 1.1 Hormone fluctuations during the menstrual cycle. **a** Mean values of FSH and LH throughout the cycle. **b** Mean values of estradiol and inhibin. **c** Mean values of progesterone during the menstrual cycle. (Reproduced with permission from Falcone and Hurd [110])

If implantation does not occur, decreasing LH levels late in the luteal phase, induced by negative pituitary feedback, will cause a drop in progesterone levels. Declining estrogen and progesterone levels lead to enzymatic degradation of the functionalis layer of the endometrium, inflammatory infiltration, and vasoconstriction, thus beginning the process of menses. During the late luteal phase, the declining levels of estrogen and progesterone reduce their negative feedback on FSH production, enabling FSH levels to rise and begin the menstrual cycle again.

1.3 Anatomy of the Menstrual Cycle

This section will cover the anatomy of the hypothalamus, pituitary, ovary, and uterus and highlight the endocrinological connections between them that underpin the HPO axis.

1.3.1 Hypothalamus

The central nervous system is the primary regulator of the menstrual cycle. Menstrual cycle control originates from the hypothalamus and pituitary gland. The hypothalamus is located at the base of the brain, below the third ventricle and above the pituitary gland (Fig. 1.2) [15]. It is bordered anteriorly by the optic chiasm and inferiorly by the mammillary bodies. The hypothalamus is a master regulator of the menstrual cycle via its signals to the anterior pituitary gland, but it also controls many other essential functions, including homeostasis, management of emotion and behavior in connection with the limbic system, circadian rhythms, the sleep-wake cycle, electrolyte balance, and food intake.

The hypothalamus consists of three zones – lateral, medial, and periventricular. Each of these zones contains several nuclei. The arcuate nucleus, located in the periventricular zone, produces GnRH and is the hypothalamic regulator of the HPO axis and reproduction. During development, GnRH neurons originating at the olfactory area migrate to the arcuate nucleus along olfactory axon fibers, at which point they become hormonally active [15, 16]. The GnRH neurons synthesize GnRH from a larger 92 amino-acid precursor [17]. GnRH, which is released into the hypophyseal portal vessels, reaches the hypothalamus, thus allowing the arcuate nucleus of the hypothalamus to control the pituitary gland [18].

The hypothalamus also transmits several other hormones via the hypophyseal portal system to communicate with the anterior pituitary. These hypothalamic hormones include growth hormone-releasing hormone (GHRH), which prompts the anterior pituitary to release growth hormone (GH); prolactin-releasing hormone (PRH), which prompts the anterior pituitary to stimulate milk production through release of prolactin; thyrotropin-releasing hormone (TRH), which modulates thyroid function by stimulating the anterior pituitary to release thyroid-stimulating hormone (TSH); and corticotropin-releasing hormone (CRH), which regulates adrenal function via anterior pituitary production of adrenocorticotropic hormone (ACTH).

1.3.2 Pituitary

The pituitary gland is a small ovular structure suspended in the underside of the brain by the pituitary stalk (infundibulum). It sits in a bony cradle in the sphenoid bone called the sella turcica (Figs. 1.2 and 1.3) [16]. Due to the location of the pituitary in the anatomically confined sella turcica, significant enlargement of the pituitary, as may occur with pituitary tumors such as prolactinomas, may cause headache and compression of adjacent cranial nerves involved in vision, leading to bilateral hemianopsia, though most patients with pituitary tumors are initially asymptomatic.

There are two parts of the pituitary gland (anterior and posterior) which have separate embryonic origins and functions. The anterior lobe, the relevant pituitary component of the hypothalamic-pituitary-ovarian axis, derives from an outpouching of the pharynx known as Rathke's pouch. The anterior lobe of the pituitary can be further divided into three smaller parts – the pars anterior, pars intermedia, and pars tuberalis [18]. The pars ante-

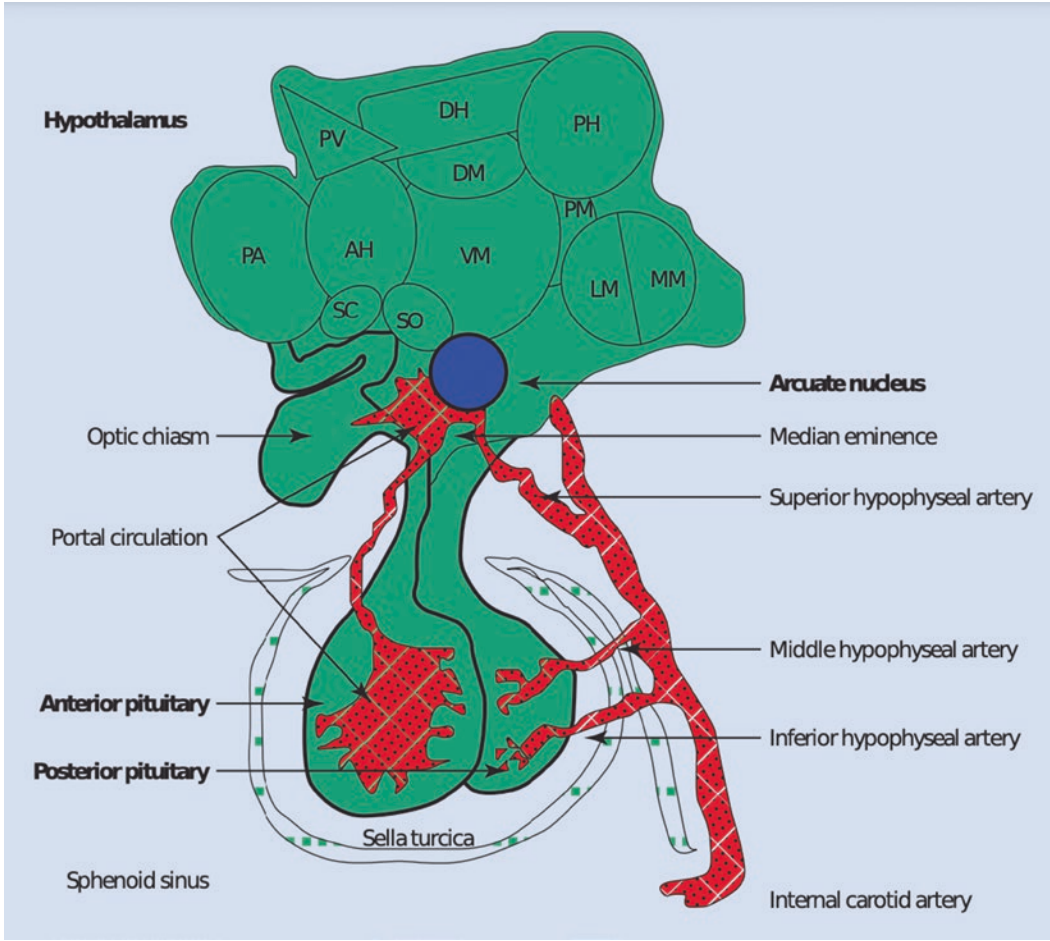

Fig. 1.2 Illustration of the hypothalamus, pituitary, sella turcica, and portal system. The arcuate nucleus is the primary site of GnRH-producing neurons. GnRH is released from the median eminence into the portal system. The blood supply of the pituitary gland derives from the internal carotid arteries. In addition to the arcuate nucleus, the other hypothalamic nuclei are *SO* supraoptic nucleus, SC suprachiasmatic nucleus, PV paraventricular nucleus, DM dorsal-medial nucleus, VM ventromedial nucleus, PH posterior hypothalamic nucleus, PM premammillary nucleus, LM lateral mammillary nucleus, MM medial mammillary nucleus. The three hypothalamic areas are PA preoptic area, AH anterior hypothalamic area, and DH dorsal hypothalamic area. (Reproduced with permission from Falcone and Hurd [110])

rior lobe consists of glandular epithelium and secretes six major hormones: prolactin, GH, ACTH, FSH, LH, and TSH.

The secretion of these hormones is controlled by the hypothalamus via hormones that travel through the hypophyseal portal vessels to act on the specialized cells responsible for secretion of each of the six hormones. The hypophyseal portal vessels originate with a branch of the internal carotid artery, known as the superior hypophyseal artery. The hypophyseal artery forms a capillary plexus around the hypothalamus, where it picks up neurotransmitters, such as GnRH, and transports them to the next capillary plexus that surrounds the anterior pituitary [18].

In the case of the HPO axis, GnRH produced by the hypothalamus reaches pituitary gonadotropes to stimulate FSH and LH release. The other hypothalamic hormones transmitted through the hypophyseal portal system act on their own specific cell types in the anterior pituitary, including thyrotropes, which produce TSH; somatotrophs, which produce GH; lactotrophs, which secrete prolactin; and corticotrophs, which secrete ACTH (Table 1.2).

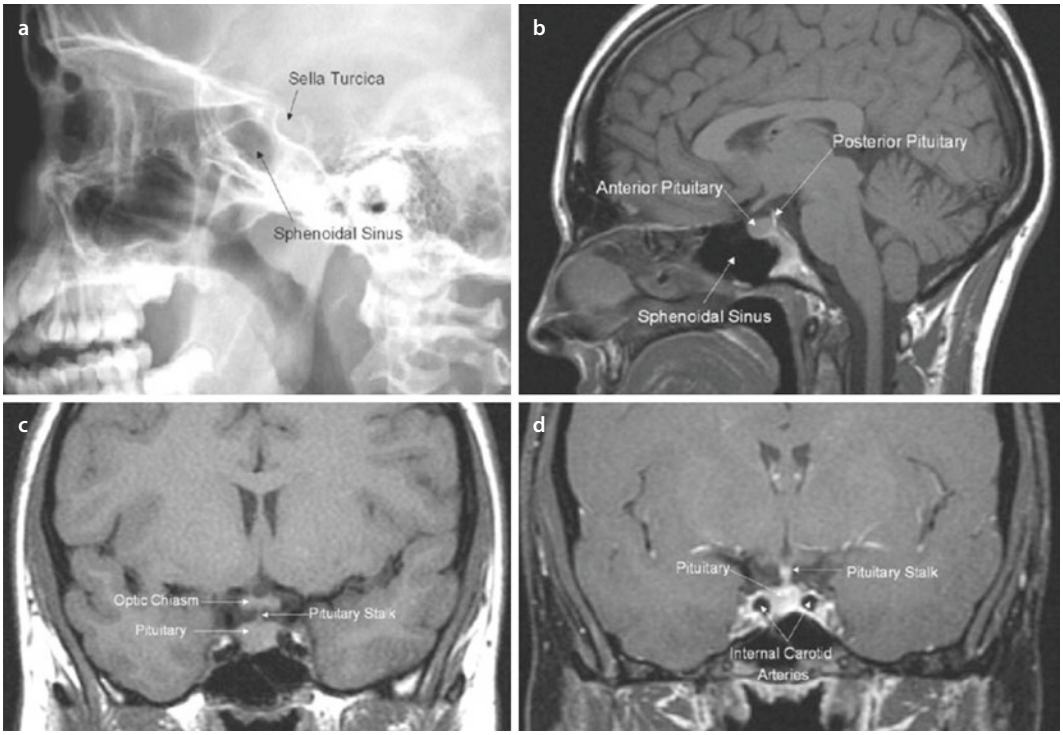

Fig. 1.3 X-ray and T1-weighted MRI images of the pituitary gland. **a** Lateral skull film with the sphenoidal sinus and sella turcica. **b** Sagittal section demonstrating the relationship between the sphenoidal sinus and the pituitary gland. The normal posterior pituitary is brighter on MRI compared to the anterior pituitary. The sella turcica is not well seen on MRI. **c** Coronal section demonstrating the relationship of the pituitary to the optic chiasm and the pituitary stalk. **d** Coronal section after gadolinium contrast, demonstrating the close proximity of the pituitary to the internal carotid arteries. (Reproduced with permission from Falcone and Hurd [110])

Table 1.2 Major cell types of the anterior pituitary gland[a]

Cell type	Appearance on light microscopy	Cellular frequency (%)	Hormone products
Somatotrophs	Acidophilic	50	Growth hormone
Lactotrophs	Acidophilic	20	Prolactin
Corticotrophs	Basophilic	20	Adrenocorticotrophic hormone (ACTH)
Thyrotrophs	Basophilic	5	Thyroid-stimulating hormone (TSH) and free α-subunit
Gonadotrophs	Basophilic	5	Follicle-stimulating hormone (FSH), luteinizing hormone (LH), and free α-subunit

[a]Reproduced with permission from Falcone and Hurd [110]

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The posterior lobe of the pituitary is embryonically derived from the forebrain as an extension of the hypothalamus. The posterior lobe secretes two hormones – antidiuretic hormone (responsible for regulation of blood osmolarity) and oxytocin (secreted during labor and with breastfeeding and involved in milk release into lactiferous ducts, uterine contractions, and social bonding).

1.3.3 Ovaries

The female gonads, the ovaries, are paired ovular structures which embryologically originate in the mesonephric ridge and descend into the pelvis [19]. The ovaries are the site of development and maturation of oocytes, the female gametes, and production of estradiol (E2) and progesterone in response to LH and FSH. The ovary can be divided histologically into three components:

1. Surface – comprised of a single layer of cuboidal epithelium
2. Cortex – comprised of connective tissue stroma and ovarian follicles containing oocytes
3. Medulla – comprised of connective tissue and a neurovascular network

In the ovarian cortex, granulosa and theca cells surround each follicle. Prior to birth, these follicles are initially referred to as primordial follicles, each containing an oogonium, which is derived from a primary germ cell. All primary oocytes in these primordial follicles remain arrested in the prophase stage of meiosis I until puberty. Immature follicles, not yet recruited, are surrounded by a thin layer of granulosa cells [20]. With the onset of puberty and a rise in FSH and LH during each menstrual cycle, a cohort of about 20 primordial follicles are recruited to undergo the first stage of meiosis and become primary follicles.

Once the first meiotic division is completed, the follicle is termed a secondary follicle (Fig. 1.4) [20]. During this stage of early follicular maturation, stromal cells surrounding the follicle differentiate into theca cells to produce androgens under the influence of LH signals.

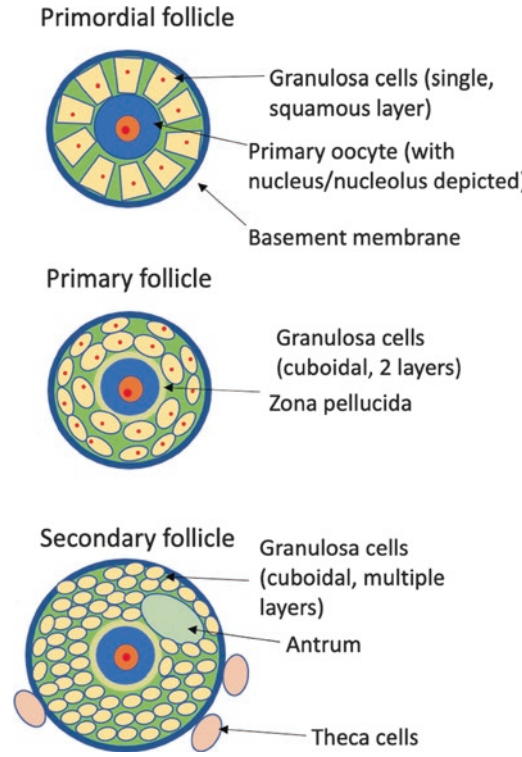

Fig. 1.4 Stages of follicular development. Each primordial follicle contains an oogonium arrested in the first meiotic division in prophase. The primordial follicle is surrounded by a single layer of squamous granulosa cells. With each menstrual cycle, a small number of primordial follicles are recruited to become primary follicles. Granulosa cells develop a second layer and become cuboidal. The zona pellucida, a layer of glycoproteins separating the oocyte and granulosa cells, develops. Upon completion of the first meiotic division, the primary follicle becomes a secondary follicle. During this transition, a pool of follicular fluid known as the antrum coalesces and is surrounded by androgen-producing theca cells

The androgens, androstenedione, and testosterone, produced by the theca cells, diffuse to the nearby granulosa cells, which express the aromatase enzyme under the influence of FSH signals and convert the androgens to estrogens (Table 1.3). Theca cells express LH receptors but not FSH receptors, while granulosa cells express FSH receptors but not LH receptors [21]. For this reason, although FSH is the only signal required for early folliculogenesis, LH is essential for producing the androgens that form the substrate of estrogen biosynthesis to promote follicle maturation.

Table 1.3 Site of synthesis of major steroidogenic products of the ovary

Cell type	Major steroid hormone products
Theca cells	Androgens (androstenedione, DHEA, testosterone)
Granulosa cells	Estrogens (estradiol, estrone, inhibin, AMH)
Theca-lutein cells	Progestogens (progesterone, 17-hydroxyprogesterone)
Granulosa-lutein cells	Estrogens (estradiol, estrone)

Granulosa cells within each follicle are responsible for the final stages of estrogen production, primarily estradiol and, to a lesser extent, estrone. Granulosa cells are also the source of anti-Mullerian hormone (AMH) and inhibin. AMH levels in reproductive-aged women reflect granulosa cell quantity, which is itself correlated with the primordial follicle pool. [22, 23] Thus, AMH is used clinically in the prediction of ovarian reserve for women undergoing infertility evaluation [24].

Once ovulation has occurred, the corpus luteum secretes estradiol and progesterone initially under support of luteal-phase LH. If implantation occurs, embryonic hCG allows the corpus luteum to continue producing these hormones. Progesterone is primarily produced by the corpus luteum during early pregnancy until around 10 weeks gestational age and is essential for maintaining the pregnancy through around 7 weeks gestational age, after which placental production is sufficient to maintain the pregnancy [25]. If there is no pregnancy or hCG to rescue the corpus luteum, it will develop into a white fibrous streak in the ovary known as the corpus albicans [19].

Each ovary has two peritoneal attachments. The ovarian ligament attaches the ovary to the uterus and supplies secondary blood supply to the ovary. The suspensory ligament of the ovary (infundibulopelvic ligament) contains the primary neurovascular structures and connects the hilum of the ovary to the pelvic sidewall. Additionally, the ovary is attached to the broad ligament via the mesovarium [26].

The ovarian arteries, which directly branch from the abdominal aorta, provide the primary vascular supply to the ovaries. Anastomotic contribution from the uterine arteries, which branch from the anterior division of the internal iliac artery, provides collateral ovarian blood supply. Venous return occurs directly to the inferior vena cava from the right ovarian vein and via the left renal vein from the left ovarian artery [26].

Identification of the hilum at the anterolateral aspect of the ovary is particularly important in surgical planning, for example, during excision of ovarian cysts or endometriomas adjacent to the hilum. Surgical injury to the hilum or thermal injury from use of electrosurgery can disrupt ovarian blood supply and jeopardize remaining healthy ovarian tissue [27]. Ovarian vascular anatomy is also particularly important during ovarian transposition surgery, in which the ovary is relocated with its suspensory ligament blood supply in order to move the ovaries out of the pelvis and protect them from damage during therapeutic pelvic radiation.

1.3.4 Uterus

While it does not directly regulate the HPO axis, the uterus cyclically responds to the fluctuating hormones produced by it. The hormonal response of the endometrium is critical for normal menstrual function and to prepare the endometrium for embryonic implantation. The uterus lies in the pelvis, between the rectum and bladder. It consists of two parts, the corpus (body) and the cervix. The uterine wall contains three distinct layers:

1. The perimetrium – the outermost layer consisting of connective tissue.
2. The myometrium – the middle smooth muscular layer. The myometrium distends during pregnancy and contracts secondary to hormonal stimuli.
3. Endometrium – the inner mucosal layer constituting the primary hormonally responsive tissue affected by the menstrual cycle (see ► Sect. 1.2).

The endometrium consists of two anatomic layers – the functionalis and basalis. The functionalis layer constitutes the cellular interface with the endometrial cavity and undergoes cyclic change with the menstrual cycle. The basalis layer is primarily responsible for regenerating the functionalis layer in each cycle [28]. Increasing estrogen levels lead to glandular proliferation, increase in stromal matrix, and elongation of terminal arterioles within the functionalis of the endometrium.

Progesterone, which rises most dramatically following the LH surge, causes the endometrium to undergo secretory changes characterized by terminal artery lengthening, superficial stromal edema, and glandular tortuosity. If implantation does not occur, falling estrogen/progesterone levels lead to cessation of glandular activity and stromal development, ultimately ending with enzymatic degradation and vasoconstriction within the functionalis [29]. The functionalis layer becomes unstable and sloughs off with menses.

Inappropriately coordinated hormonal influences on the endometrium and lack of cyclic hormonal response will result in pathological endometrial findings. For example, in cases of unopposed estrogen due to anovulatory cycles, as frequently seen in patients with obesity, polycystic ovarian syndrome, or other metabolic disorders, endometrial tissue can respond with continuous proliferation, and potentially progress to hyperplasia or malignancy [29].

1.4 Endocrinology of the Menstrual Cycle

Human reproduction hinges on an intact HPO axis demanding an ordered response across multiple organ systems. First, GnRH-secreting neurons direct gonadotrophs to produce LH and FSH. These hormones primarily act on ovarian tissue to promote synthesis of the major female sex hormones estradiol and progesterone. Finally, the sex hormones act on steroid receptors throughout the body to produce dramatic changes in gene transcription, including dramatic effects on the endometrium.

pyro-Glu^{1}-His^{2}-Trp^{3}-Ser^{4}-Tyr^{5}-Gly^{6}-Leu^{7}-Arg^{8}-Pro^{9}-Gly^{10}-NH_2

Fig. 1.5 Structure of GnRH-1. (Reproduced with permission from Falcone and Hurd [110])

First sequenced and characterized in 1971, GnRH is a decapeptide (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH2) and is the key regulator in the pituitary-gonadal axis (Fig. 1.5) [30]. It is located as a single gene copy on the short arm of chromosome 8 and is secreted into the hypophyseal portal circulation to act chiefly on the anterior pituitary gland. GnRH synthesis occurs in a small subset of hypothalamic neurons which secrete it in pulsatile fashion into the hypophyseal portal blood system, through which it is transported to the anterior pituitary gland. Binding and activation of GnRH receptors triggers LH and FSH synthesis and release, ultimately leading to gametogenesis and gonadal steroidogenesis for sexual maturation and reproductive function [31].

The approximately 1000–3000 GnRH-producing cells found in the arcuate nucleus of the hypothalamus arrive there via neuroendocrine cell migration during embryogenesis from the olfactory placode. Abnormalities in this migratory pathway have clinical ramifications: Kallmann syndrome (KS) represents a failure in olfactory and GnRH neuronal migration from the olfactory placode to the hypothalamus resulting in GnRH deficiency. A number of distinct genetic causes for defects in GnRH migration have been identified, including variants with X-linked, autosomal dominant, and autosomal recessive inheritance patterns, though the X-linked form is the most common. Because KS genetic mutations impair cell migration in nasal areas, patients experience both hypothalamic amenorrhea and anosmia (inability to smell) [32, 33]. The clinical spectrum of other isolated gonadotrophic-release hormone deficiencies also includes constitutional delay of puberty and adult-onset hypothalamic hypogonadism.

At least two GnRH isoforms have been identified in humans and additional GnRH isoforms exist in fish, amphibians, and protochordates [31, 34]. In humans, GnRH-I is

the "classical" GnRH isoform that regulates synthesis and secretion of FSH and LH [35]. The other main GnRH isoform in humans is the "chicken-GnRH/GnRH-II" isoform. GnRH-II is conserved from fish to mammals and differs from the classical GnRH-I by only three amino acids. However, its expression is localized to specific nuclei (supraoptic, paraventricular, suprachiasmatic) of the central nervous system and peripheral tissues (medial basal hypothalamus and female reproductive tissues including ovaries/placenta/endometrium) but has a limited role in the menstrual cycle. Its role appears to be in regulating sexual behavior [36–38].

GnRH pulse control is regulated by multiple hormones and neurotransmitters. Among them, dopamine, secreted directly into portal blood via the hypothalamic tuberoinfundibular pathway, suppresses arcuate GnRH production. In contrast, norepinephrine cell bodies located in the mesencephalon and lower brainstem stimulate GnRH production.

1.4.1 GnRH Agonists and Antagonists

The half-life of GnRH is approximately 2–4 minutes, due to rapid cleavage of the bonds between amino acids 5–6, 6–7, and 9–10. Chemical alteration of the amino acids in GnRH at these positions, combined with carboxyl- and amino-terminal modifications, has enabled the synthesis of an array of GnRH peptide analogues with distinct properties, including longer half-life, pure agonist activity, and pure antagonist activity (Table 1.4). A number of these peptide analogues have found clinical applications. Continuous use of GnRH agonists leads to high-affinity binding to and occupancy of the GnRH receptor. GnRH agonist binding to its receptor induces initial gonadotroph activation and FSH/LH synthesis (the so-called "flare" effect), followed by desensitization and downregulation of the GnRH receptor, leading to suppressed FSH and LH levels within 1–3 weeks [15, 39–41]. GnRH antagonists do not produce this initial

Table 1.4 Properties of commercially available GnRH agonists[a]

	Structure and substitutions at positions 6 and 10	Half-life	Relative potency	Route of administration
GnRH	Native decapeptide	2–4 min	1	IV, SC
Nafarelin	Decapeptide 6: D-Naphthylalanine for Gly	3–4 h	200	Intranasal
Triptorelin	Decapeptide 6: D-Trp for Gly	3–4 h	36–144	SC, IM depot
Leuprolide	Nonapeptide 6: D-Leu for Gly 10: NHEt for Gly	1.5 h	50–80	SC, IM depot
Buserelin	Nonapeptide 6: Ser(O^{t}Bu) for Gly 10: NHEt for Gly	1.5 h	20–40	SC, intranasal
Goserelin	Decapeptide 6: Ser(O^{t}Bu) for Gly 10: AzaGly for Gly	4.5 h	50–100	SC implant
Histrelin	Decapeptide 6: DHis for Gly 10: AzaGly for Gly	50 min	100	SC

[a]Reproduced with permission from Falcone and Hurd [110]

agonistic activity, and instead lead to immediate suppression of LH and FSH production via competitive inhibition [42–44].

Non-peptide, small molecular GnRH antagonists have also been developed. These drugs mechanistically work as peptide GnRH antagonists do but offer oral bioavailability, which is not possible with peptides. Elagolix, a competitive non-peptide GnRH receptor antagonist, has FDA approval for treatment of moderate to severe endometriosis [45, 46]. Another oral GnRH antagonist, relugolix, has been approved in Japan for treatment of uterine myomas.

1.4.2 Control of GnRH Pulsatility

GnRH neurons retain the ability to synchronize GnRH release into the hypothalamic-hypophyseal portal vessels as pulses. Variations in the GnRH pulse frequency and amplitude lead to differential production of FSH and LH, thus allowing one hormone to simultaneously regulate the levels of its two primary targets. Direct measurement of GnRH production is technically challenging because the hormone has a 2–4-minute half-life and is restricted to the hypophyseal portal vessels, though direct measurement via portal blood collection under anesthesia has been performed in animal models [47, 48]. Because LH has a much shorter half-life than FSH (20 minutes versus 3 hours), serum LH levels primarily reflect recent LH production under GnRH control. Thus, human studies attempting to measure GnRH levels have used serial measurements of LH levels as surrogate for GnRH production. Such studies have demonstrated that low-frequency GnRH pulses (<1 pulse/2–3 hours) preferentially stimulate FSH, while high-frequency GnRH pulses (1 pulse/60–90 min) preferentially stimulate LH production (Table 1.5). GnRH pulsatility appears to be controlled by GnRH-I gene promoter activity in GnRH-producing hypothalamic tissue [49]. In the absence of ovarian regulation, GnRH pulses occur approximately once per hour [50]. The follicular phase is characterized by higher frequency, lower amplitude GnRH pulses favoring LH production, while the luteal phase is characterized by lower frequency, higher amplitude pulses favoring the FSH production that is necessary to recruit a cohort of follicles for the next cycle [51].

Table 1.5 Menstrual cycle variation in LH pulse frequency and amplitude[a]

Cycle phase	Mean frequency (min)	Mean amplitude (mIU/mL)
Early follicular	90	6.5
Mid-follicular	50	5
Late follicular	60–70	7
Early luteal	100	15
Mid-luteal	150	12
Late luteal	200	8

[a]Reproduced with permission from Falcone and Hurd [110]

1.4.3 Structure of Gonadotropins

FSH, LH, TSH, and hCG are all glycoprotein heterodimers composed of a shared 92 amino-acid alpha subunit and a unique beta subunit. The beta subunits in these hormones enable their differential functions and pharmacology. FSH and LH beta subunit biosynthesis and availability, controlled by GnRH, is the rate-limiting step of gonadotropin production.

1.4.4 Biological Sources of Sex Steroids

Estradiol (E2), the most potent biologically produced estrogen, is the main secretory product of the follicle. Additional estrogen steroid hormones include estrone (E1) and estriol (E3). Estrone is formed principally by peripheral conversion of androstenedione, though smaller amounts are made from hepatic conversion of estradiol via 17-beta-hydroxysteroid dehydrogenase activity. Estriol is the main form of estrogen formed by the placenta in pregnancy and is only produced at biologically active doses during pregnancy,

though some hepatic production outside of pregnancy occurs via 16α-hydroxylation of estradiol or estrone.

Estrogens, including estradiol, are largely bound to carrier proteins in the bloodstream. Albumin and sex hormone-binding globulin bind 98% of total estradiol, leaving only 2% circulating freely in the bloodstream and bioavailable. Unbound estradiol, like all sex steroid hormones, is fat-soluble and freely enters cells. Estrogens are only active in cells expressing estrogen receptors. Inside the cytoplasm estradiol binds to its cognate steroid receptors, estrogen receptor-alpha and estrogen receptor-beta, before undergoing nuclear translocation and facilitating transcription of a wide range of genes [52, 53].

In preovulatory follicles, androgen and estrogen synthesis is accomplished preferentially via the "delta-5" steroidogenic pathway, involving the conversion of pregnenolone to 17-hydroxypregnenolone. Theca cells then mediate conversion of 17-hydroxypregnenolone to androgens, which are then shuttled to neighboring granulosa cells for aromatization to estrogens (Fig. 1.6). Androgens, including testosterone, androstenedione, dehydroepiandrosterone (DHEA), are produced by the theca cells, with androstenedione being the major product. Testosterone is primarily derived from peripheral conversion of androstenedione via 17β-hydroxysteroid dehydrogenase. Androstenedione and testosterone undergo aromatization in granulosa cells, at which point they are converted to estradiol (Fig. 1.7).

1.4.5 Follicular Phase Endocrinology

The early follicular phase is marked by serum estradiol levels typically <50 pg/mL. FSH begins rising a couple of days prior to menses

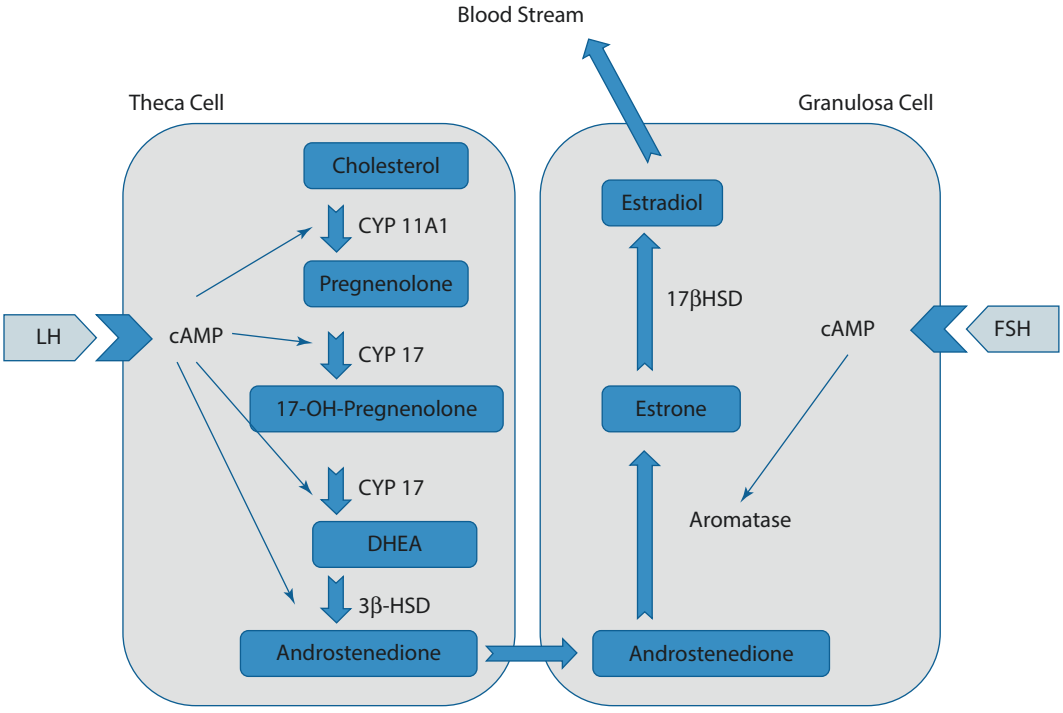

Fig. 1.6 The two-cell theory of ovarian steroidogenesis. Binding of luteinizing hormone (LH) to its receptor on ovarian theca cells stimulates the conversion of cholesterol to androstenedione. Binding of follicle-stimulating hormone (FSH) to its receptor on ovarian granulosa cells stimulates the aromatization of androgens to estrogens. cAMP cyclic adenosine monophosphate, CYP11A1 side-chain cleavage enzyme, CYP17 17-hydroxylase, HSD hydroxysteroid dehydrogenase, 17-OH pregnenolone 17-hydroxypregnenolone

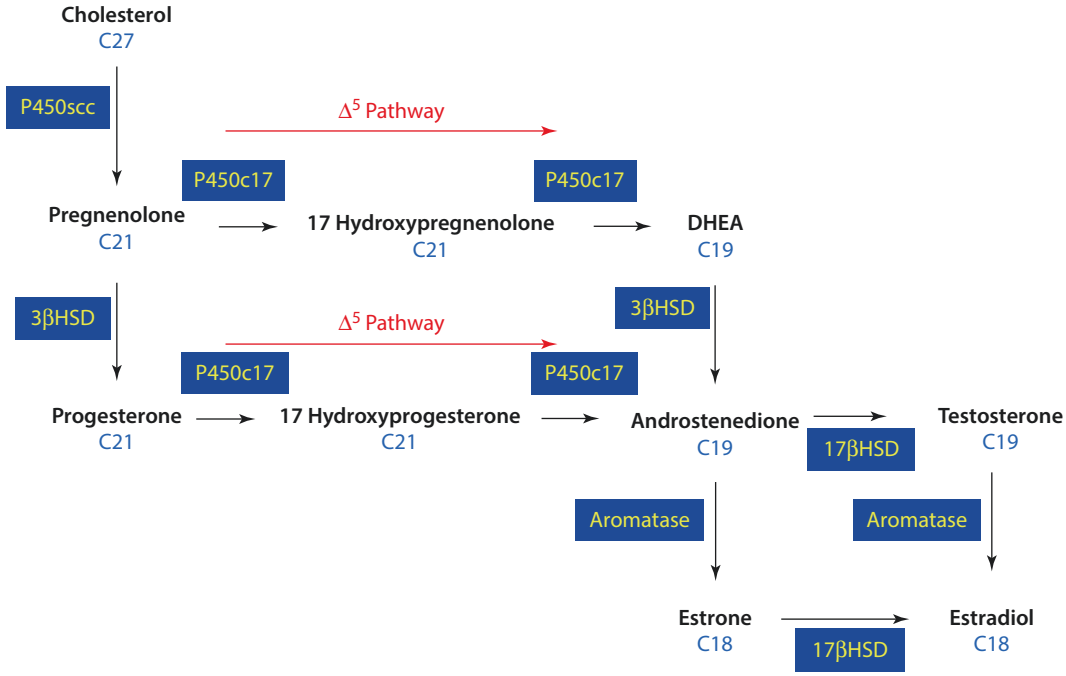

Fig. 1.7 The Δ(delta)^5 and Δ(delta)^4 pathways. The rate-limiting step in steroidogenesis is the conversion of cholesterol to pregnenolone via side-chain cleavage (P450scc). In the follicular phase, pregnenolone is preferentially converted to androstenedione via the Δ^5 pathway involving 17-hydroxypregnenlone and dehydroepiandrosterone (DHEA). In contrast, the corpus luteum preferentially converts pregnenolone to progesterone (Δ^4 pathway) via 3β(beta) hydroxysteroid dehydrogenase (3βHSD). (Reproduced with permission from Falcone and Hurd [110])

and is critical for the recruitment of ovarian follicles (Fig. 1.1). Increasing FSH levels lead to antral follicle growth of 2–6 mm/day. Each granulosa cell has approximately 1500 FSH receptors enabling rapid E2 production [6]. In the presence of rising estradiol levels, FSH stimulates formation of LH receptors on granulosa cells permitting small progesterone and 17-hydroxyprogesterone production, initially providing positive feedback to the pituitary to augment LH release and supporting the biosynthesis of the androgen precursors required for further estradiol biosynthesis. However, rising estrogen levels during early follicular development induce downregulation of pituitary synthesis of the FSH beta subunit [54].

The follicle destined for ovulation (the so-called dominant follicle) is selected at approximately cycle day 7. This follicle expresses the greatest concentration of FSH receptors on the surface of its granulosa cells, allowing it to continue expressing aromatase and synthesize estrogens locally to further enhance FSH action despite the declining pituitary FSH production during the late follicular phase triggered by the rising serum estrogen. Thus, the dominant follicle can continue to grow while the other follicles undergo apoptosis. Estradiol levels, produced by aromatase in the granulosa cells in the dominant follicle, continue to rise during the follicular phase until they peak at 200–250 pg/mL around the time of ovulation during a monofollicular ovulatory cycle.

1.4.6 Luteal-Phase Endocrinology

The LH surge mediates the transition to the luteal phase. LH levels can fluctuate more dramatically than FSH in part because LH has a shorter half-life, approximately 20 minutes compared with several hours for FSH

(Table 1.1). Although various sugars comprise the carbohydrate components of these glycoproteins, sialic acid is the critical regulator of their half-life [55–57]. LH has fewer glycosylation sites and therefore fewer sialic acid residues on average on its beta subunit than FSH, enabling more rapid elimination [58].

LH levels begin to rise just prior to the onset of menses and rise gradually throughout the follicular phase before peaking prior to ovulation. This LH surge occurs secondary to positive feedback from high levels of circulating estrogen. LH levels then decline during the secretory phase (Fig. 1.1). The LH surge triggers resumption of meiosis in the oocyte, a switch toward increased progesterone production from the theca-granulosa cells, production of eicosanoids in the follicle, and finally follicular rupture to release the oocyte.

Following ovulation, remaining granulosa cells that are not released with the oocyte enlarge and eventually vacuolate and luteinize. These luteinized granulosa cells combine with theca-lutein cells and surrounding stroma to compose the corpus luteum. Intensive neovascularization stimulated by pituitary LH is central to corpus luteum formation and is mediated by VEGF, fibroblast growth factors, angiopoietins, and insulin-like growth factors [59, 60]. The corpus luteum is the critical source of progesterone and estradiol, both of which are essential for preparing the endometrium for pregnancy and then supporting it through the early first trimester. Progesterone levels are typically <2 ng/mL in the follicular phase, while exceeding 5 ng/mL during its peak in the luteal phase, thanks to active production by the corpus luteum. The individual corpus luteum can survive for 11–17 days (mean 14.2) independent of LH secretion [61].

Estradiol levels drop following ovulation but rise again in the mid-luteal phase due to increased production by the corpus luteum. Progesterone production from the corpus luteum depends on availability of circulating cholesterol substrate. The granulosa cells previously surrounding the follicle become luteal cells, which have significant steroidogenic capacity but lack LH receptors. Luteal cells remain in close contact with theca cells, which contain LH receptors. Gap junctions between these two cell types enable rapid response to LH stimulation and efficient progesterone production [62]. Luteal-phase progesterone secretion causes a decline in GnRH pulse frequency from the hypothalamus, thereby terminating the LH surge.

There is large natural variation in luteal-phase plasma progesterone levels, ranging from 2.3 to 40.1 pg/mL over 24 hours within the same healthy patient [57]. However, the essential role of luteal progesterone is demonstrated clearly in assisted reproduction cycles complicated by luteal-phase deficiency (LPD), a condition in which the corpus luteum produces insufficient progesterone to support a necessary secretory endometrium for embryo implantation and survival. It is speculated that this is due to hypothalamic/pituitary downregulation or disruption of granulosa cells during oocyte retrieval. Use of either progesterone or hCG (to promote endogenous progesterone production) during assisted reproduction cycles in the luteal phase improves live birth rates [63]. The ideal luteal progesterone level to support a pregnancy and the optimal method for luteal support in assisted reproduction cycles remains unknown.

Progesterone, a steroid hormone largely bound in the bloodstream to albumin and corticosteroid-binding globulin, exerts its effects by binding of its unbound form to progesterone receptors (PRs). There are two predominant PR isoforms, PR-A and PR-B, which are transcribed from alternate start sites in the same gene. PR-A is the predominant form in the uterus and the main mediator of decidualization in humans. A shorter, N-terminally truncated receptor isoform, PR-C, has been detected by biochemical means. However, it lacks the ability to initiate transcription and does not appear to be a naturally occurring isoform [64]. PR-A and PR-B are induced in preovulatory follicles in as few as 4–8 hrs in response to LH stimulation and are required for LH-dependent follicular rupture [65]. PR-A is transcriptionally less active than PR-B and can function to inhibit PR-B. [66] However, PR-A alone is necessary and suf-

ficient to regulate ovulatory function [67]. PR-A knockout mice develop mature functional follicles with a severe impairment in the ability to rupture (ovulate), while PR-B knockout mice remain largely unaffected during superovulation [68–70].

1.4.7 Additional Mediators

Follistatin is a highly glycosylated monomeric protein found in follicular fluid that binds activins with high affinity for important endocrine regulation at the level of the pituitary [71, 72]. The activin-inhibin-follistatin triad together directs the synthesis and secretion of FSH and thereby folliculogenesis. The biphasic release of FSH (in the late luteal/early follicular phase and then in the late follicular phase/periovulation) is attributed to the balance of activin and follistatin.

Activins and inhibins are structurally related protein heterodimers that, despite structural similarity, function antagonistically. Activin is derived from ovarian granulosa cells and contains two subunits identical to the beta subunits of inhibin A and B. Activin functions to augment FSH secretion and block prolactin, ACTH, and GH secretion [73, 74]. It also increases FSH receptor number and binding in the granulosa cell and augments stimulation of aromatization [75].

Additionally, the ovarian granulosa cells, as well as pituitary gonadotropes, also produce inhibins, which function to block FSH secretion by the pituitary gland [73, 76, 77]. There are two forms of inhibin, inhibin A and inhibin B, differing only by their beta subunit. Inhibin B predominates in the early follicular phase before decreasing and giving way for inhibin A production before ovulation, which then dominates during luteal phase [76, 78–80]. Inhibin B production declines as follicles grow above 10–14 mm, while inhibin A remains actively produced. Inhibin B is secreted by growing antral follicle granulosa cells during the early follicular phase and has been used historically as a marker for follicular health and number [81, 82]. Inhibin B production is positively regulated by FSH: levels peak early in the follicular phase, decline until they peak again midcycle, and then remain low during the luteal phase. FSH secretion from the anterior pituitary is therefore regulated by negative feedback from FSH-promoted inhibin release by granulosa cells [83, 84]. Inhibin A, secreted by the corpus luteum, dominates the luteal phase of the menstrual cycle and may be involved in the gradual release of ovarian negative feedback on FSH secretion during the final days of the menstrual cycle during the luteal-follicular transition [85].

The selective inhibitory role of inhibin on FSH production was largely established from experimental animal models. Administration of serum containing anti-inhibin antibodies in rats and hamsters led to increased serum FSH-beta mRNA but no change in LH-beta mRNA levels [86]. While the clinical utility of monitoring inhibin levels during assisted reproduction cycles is limited, higher serum inhibin A and B levels are noted on the day of ovulation trigger and overall in patients with cycles complicated by ovarian hyperstimulation syndrome [87].

Anti-Mullerian hormone (AMH), a member of the transforming growth factor-beta family of glycoprotein differentiation factors, comes from the same gene family as activins and inhibins. AMH functions chiefly during embryogenesis to inhibit Mullerian duct formation [88, 89]. Its functions later in life are less clear, though AMH produced by granulosa cells during adulthood has autocrine and paracrine effects on oocyte maturation and development [90]. AMH paracrine activity inhibits FSH-stimulated follicle growth, permitting selection of a dominant follicle [91]. Circulating AMH levels are highest in the late follicular phase, peaking simultaneously with inhibin A levels just prior to ovulation, though these findings are of limited clinical relevance and random AMH levels are routinely obtained to measure ovarian reserve [81, 92].

Leptin, an adipokine produced by adipose tissue, reflects levels of body fat and is primarily involved in energy homeostasis [93]. Leptin levels also regulate the reproductive cycle and fluctuate in reproductive-age women during a menstrual cycle [94]. Higher leptin concentrations are noted in the luteal

phase, and studies in goat model systems suggest that leptin upregulates angiogenic factors to support early corpus luteum formation [95, 96]. Moreover, leptin deficiency under various pathological conditions such as hypothalamic amenorrhea or lipodystrophy leads to anovulation, and replacement with recombinant leptin can restore menstrual cycle regularity and enable fertility [97, 98].

Kisspeptin, encoded by the KISS1 gene, plays a crucial role as a regulator of normal reproductive function, gonadotropin secretion, sex hormone-mediated feedback, and adult fertility [99]. Kisspeptin signals directly to GnRH neurons to release GnRH into portal circulation, enabling pulsatile LH secretion, implying that it also regulates ovulation. Kisspeptin may support reproductive function by maintaining LH-dependent expression of ovarian matrix metalloproteinases [68]. Loss-of-function mutations of the KISS1R (kisspeptin receptor) have been linked to pubertal failure disorders, implying that kisspeptin is required for puberty onset and maintenance of normal reproductive functions [100, 101].

More recent studies have centered on arcuate nuclear co-expression of neurokinin, dynorphin, and kisspeptin, forming the so-called KNDy neurons, known to play a role in the tonic control of gonadotropin secretion by modulating GnRH release. The KNDy paradigm proposes that neurokinin stimulates and dynorphin inhibits kisspeptin secretion onto GnRH neurons [102]. Additional support for this model derives from studies indicating neuronal hypertrophy in postmenopausal mammalian animal models and hypogonadotropic hypogonadism in comparable species during their reproductive periods [103].

Endogenous opioids are found widely throughout the body, though they are found in greatest concentrations within the arcuate nucleus of the medial basal hypothalamus and in hypophyseal portal blood [104, 105]. The three major endogenous opioid peptide classes – endorphins, enkephalins, and dynorphins – are derived from three precursor proteins, respectively: pre-opiomelanocortin (POMC), preproenkephalin A, and preproenkephalin B. These opioids retain multiple endocrine functions, including mediating secretion of FSH, LH, and prolactin. An increase in endorphin release results in a decrease in LH pulse frequency [106]. Consequently, an increase in endogenous opioids is implicated in the pathogenesis of hypothalamic amenorrhea in female athletes [107]. Conversely, opioid receptor blockers increase LH pulse frequency [108]. For women with hypothalamic amenorrhea, treatment with opioid receptor antagonists can enable return of cyclical menses [109]. Opioids affect gonadotropin secretion primarily by modulating GnRH and in feedback pathways with gonadal sex steroid hormones (Table 1.6).

Table 1.6 Neurotransmitter effects on GnRH release[a]

Neurotransmitter	Effect
Dopamine	Inhibits GnRH release
Endorphin	Inhibits GnRH release
Serotonin	Inhibits GnRH release
Norepinephrine, epinephrine	Stimulates GnRH release

[a]Reproduced with permission from Falcone and Hurd [110]

1.5 Review Questions

1. What is the effect of progesterone on the endometrium?
   A. Cessation of proliferation of endometrial glands, leading to increased glandular tortuosity
   B. Hypertrophy and proliferation of glands
   C. Enzymatic degradation and vasoconstriction
   D. Increase in stromal matrix and elongation of terminal arterioles

1

2. Which ligament provides the primary neurovascular supply to the ovary?
   A. Broad ligament
   B. Infundibulopelvic ligament
   C. Ovarian ligament
   D. Uterosacral ligament

3. What is the effect of continuous GnRH administration on long-term LH and FSH release?
   A. No effect
   B. Increase
   C. Decrease
   D. Loss of cyclic release without net change

1.6 Answers

1. A. Cessation of proliferation of endometrial glands, leading to increased glandular tortuosity

2. B. Infundibulopelvic ligament

3. C. Decrease

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