

# Hypothalamic–Pituitary– Ovarian Axis and Control of the Menstrual Cycle

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## 1.1 Introduction

The menstrual cycle is the result of an orchestra of hormones. It involves the interaction of many endocrine glands as well as a responsive uterus. The menstrual cycle remains a complex process where many aspects are still not well understood. In this chapter we will examine the control of the menstrual cycle through the interaction of the central nervous system, namely, the hypothalamus and pituitary, and the ovaries, resulting in the cyclic and ordered sloughing of the uterine endometrial lining. The first section of this chapter, *The Menstrual Cycle*, will review the phases of the menstrual cycle. In the second section, *Anatomy of the Menstrual Cycle*, the hypothalamic, pituitary, ovarian, and uterine activities will be reviewed. The key hormones that play a role in the control of the menstrual cycle include gonadotropin-releasing hormone (GnRH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol, and progesterone (Table 1.1). In addition to these key hormones, there are other peptide and non-peptide hormones that play a role in the menstrual cycle that will also be discussed. These hormones will be discussed in the third section, *Endocrinology of the Menstrual Cycle*.

## ■ ■ Clinical Case

A 25-year-old woman comes to the office with report of unpredictable cycles. She has always had irregular and unpredictable menstrual cycles since her first menses. She is planning to get pregnant in the near future. She inquires as to why she has these irregularities and would like to know what goes into controlling and ultimately regulating her cycle.

## 1.2 The Menstrual Cycle

The menstrual cycle can be divided into three phases: proliferative (follicular), ovulation, and secretory (luteal). The menstrual cycle is also described based on its length (number of days between onset of menstrual bleeding in one cycle and the onset of bleeding of the next cycle). The median duration of a menstrual cycle is 28 days [1–3]. Most individuals will describe a cycle length between 25 and 30 days [1–3]. The variability in length of a menstrual cycle is based on the variable length of the follicular phase. The luteal phase is

■ **Table 1.1** Major hormones of the hypothalamic–pituitary–ovarian axis<sup>a</sup>

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 $\alpha$ - and $\beta$ -subunits	$\alpha$ : 6q12.21 $\beta$ : 11p13	Gonadotrophs of anterior pituitary	1.5–4 h	5–25 mIU/mL
LH	Glycoprotein with $\alpha$ - and $\beta$ -subunits	$\alpha$ : 6q12.21 $\beta$ : 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 $\alpha$ - and $\beta$ -subunits Inhibin A = $\alpha$ + $\beta$ A Inhibin B = $\alpha$ + $\beta$ B	$\alpha$ : 2q33 $\beta$ A: 2q13 $\beta$ B: 7p15	Granulosa cells	30–60 min	A: 10–60 B: 10–150 pg/mL

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constant in most women and is 14 days in length. Polymenorrhea is described as menstrual cycles that occur at intervals less than 21 days. Conversely, oligomenorrhea is described as menstrual cycles that occur at intervals more than 35 days. During menstruation, blood loss is typically 30 mL [4], and amounts greater than 80 mL (menorrhagia) are considered abnormal [4].

The *proliferative phase* begins at the onset of menses until ovulation takes place. Folliculogenesis takes place during this phase of the menstrual cycle. A dominant follicle is selected from a pool of growing follicles that will be destined to ovulate. The growth of follicles in this stage will depend on pituitary hormones such as FSH. The growth of the follicle also leads to production of estradiol from the layers of granulosa cells surrounding it. Estradiol is responsible for the proliferation of the endometrial lining of the uterus.

*Ovulation* happens at the peak of follicular growth in response to an LH surge [5]. Prior to ovulation, follicles grow to sizes greater than 20 mm in average diameter [6]. LH is then released in a positive-feedback manner from the anterior pituitary due to prolonged exposure to estradiol. For this positive feedback to take place, levels of estradiol above 200 pg/mL for approximately 50 h are necessary [7] (■ Fig. 1.1). Approximately 12 h after the LH peak, the oocyte is released [8, 9]. In order for the oocyte to release from the follicle, several proteolytic enzymes and prostaglandins are activated, leading to the digestion of the follicle wall collagen [10]. Once an oocyte is released, the fallopian tube is responsible for picking it up where it will await fertilization.

The *secretory phase* starts after ovulation. During this phase, the remaining granulosa cells that are not released with the oocyte during the ovulation process enlarge and acquire lutein (carotenoids), which is yellow in color. These granulosa cells are now called the corpus luteum and predominantly secrete progesterone. Peak progesterone production is noted 1 week after ovulation takes place (see ■ Fig. 1.1). Progesterone is required to convert the endometrial lining of the uterus from a proliferative one into a secretory endometrium in preparation for embryo implantation. The life span of the corpus luteum and, hence, progesterone production will depend on continued LH support from the anterior pituitary. If a pregnancy takes place, hCG (human

chorionic gonadotropin) of pregnancy will maintain the corpus luteum. However, if a pregnancy fails to happen, luteolysis takes place and the corpus luteum is converted to a white scar called the corpus albicans. The loss of the corpus luteum and the subsequent loss of progesterone lead to the instability of the endometrium and the sloughing of the endometrium, signaling a new menstrual cycle.

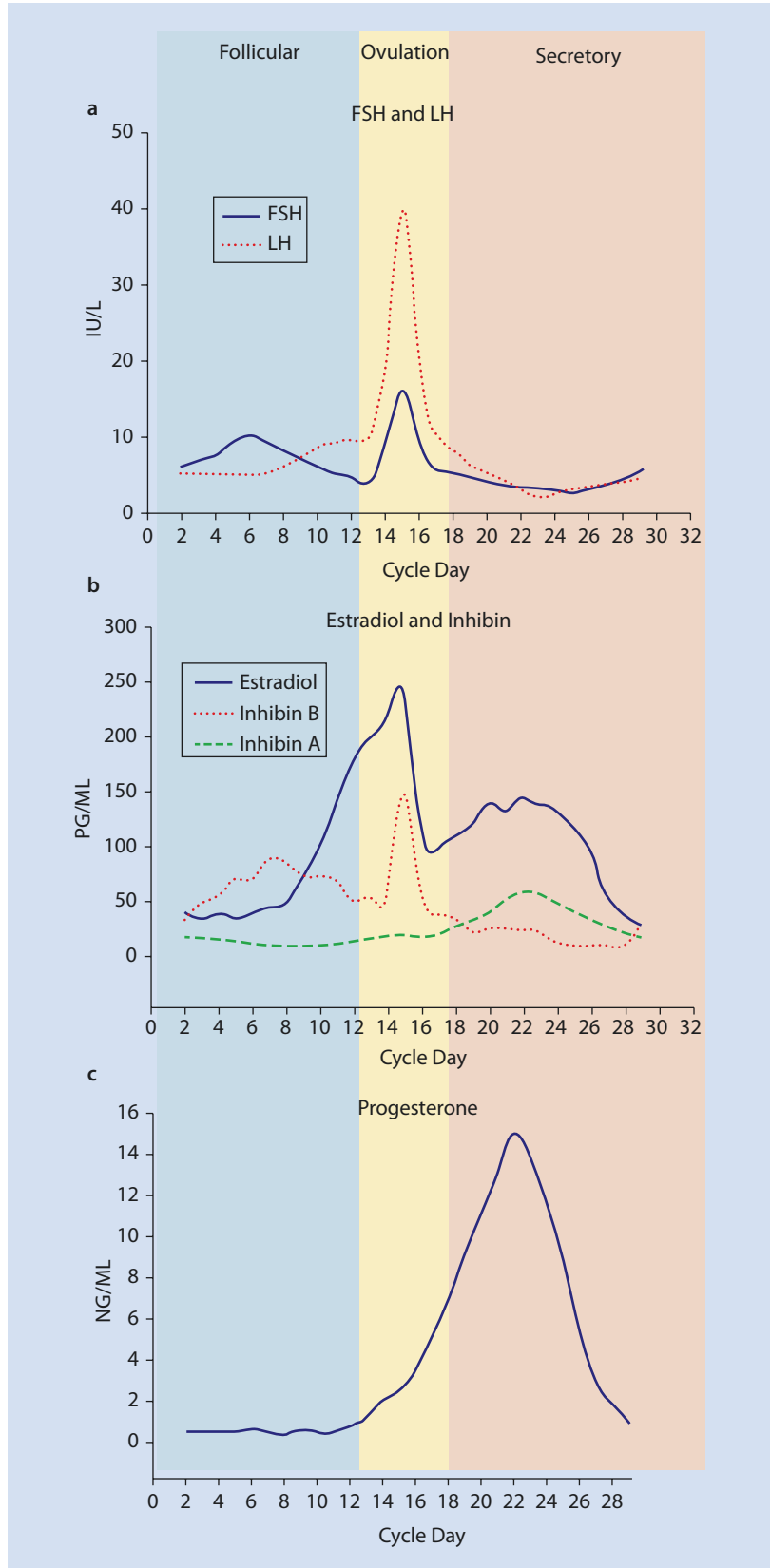
### 1.3 Anatomy of the Menstrual Cycle

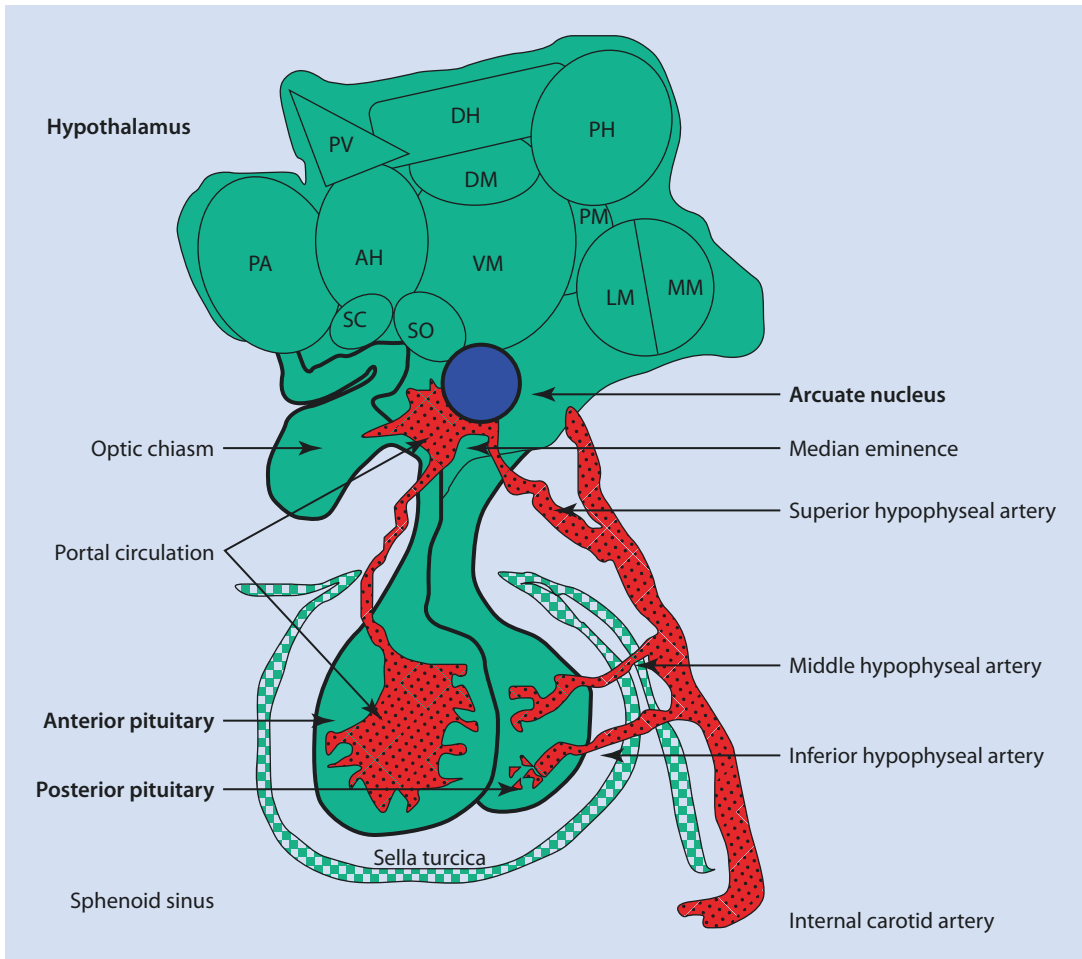
The initial signals for a menstrual cycle are initiated from the central nervous system. The pertinent endocrine portion of the central nervous system consists of the hypothalamus and the pituitary gland.

The *hypothalamus* consists of only 0.3% of the total brain, measuring 4 cm<sup>3</sup>, and weighing approximately 10 g. Despite its small size, it contains many nuclei that are responsible for endocrine regulation, reproduction, metabolism, temperature regulation, emotional responses, and electrolyte balance [11] (■ Fig. 1.2). The hypothalamus lays beneath the thalamus, hence, the nomenclature. Laterally, it is bordered by the anterior part of the subthalamus, the internal capsule, and the optic tract [11]. The hypothalamus forms the lateral wall and floor of the third ventricle. The median eminence of the hypothalamus extends to the anterior pituitary and contains neurosecretory neurons that affect hormone production from the anterior pituitary. The hypothalamus is comprised of three zones: lateral, medial, and periventricular. Within each zone lie several nuclei, where the arcuate nucleus is pertinent to reproduction. The arcuate nucleus is responsible for the production of GnRH. GnRH is secreted into the portal pituitary circulation, reaching the anterior pituitary to affect FSH and LH release from the anterior pituitary. The hypothalamus also influences thyroid function via TRH (thyrotropin-releasing hormone), adrenal function via CRH (corticotropin-releasing hormone), and growth and metabolic homeostasis via GHRH (growth hormone-releasing hormone) [11].

The *pituitary gland* is a pea-sized gland, also known as the master endocrine gland. It measures 12 × 8 mm and weighs approximately 500 mg [11].

**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 Mahutte NG, Ouhilal S. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007



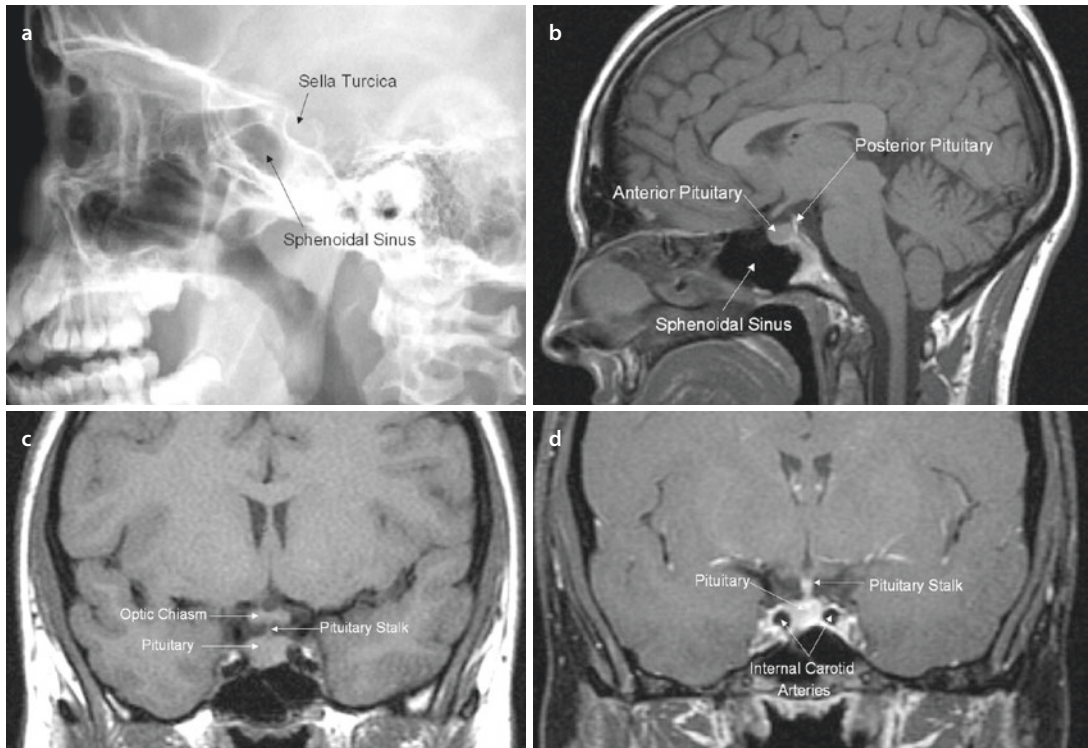


**Fig. 1.2** Illustration of the hypothalamus, pituitary, sella turcica, and portal system. The arcuate nucleus is the primary site of GnRH-producing neurons. GnRH is released from the median eminence into the portal system. The blood supply of the pituitary gland derives from the internal carotid arteries. In addition to the arcuate nucleus, the other hypothalamic nuclei are *SO* supraoptic nucleus, *SC* suprachiasmatic nucleus, *PV* paraventricular nucleus; *DM*

*dorsalmedial 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, *DH* dorsal hypothalamic area. Reproduced with permission from Mahutte NG, Ouhilal S. In: Hurd WW, Falcone T, eds. *Clinical reproductive medicine and surgery*. St. Louis, MO: Mosby/Elsevier; 2007

It is located beneath the third ventricle and above the sphenoidal sinus in a bony cavity called the sella turcica (see [Figs. 1.2](#) and [1.3](#)). The adult pituitary gland contains two major parts: the adenohypophysis and the neurohypophysis. The neurohypophysis is a diencephalic downgrowth connected with the hypothalamus, while the adenohypophysis is an ectodermal derivative of the stomatodeum [11]. The pituitary gland can also be divided into two major lobes: anterior and posterior. The anterior lobe is equivalent to the adenohypophysis, while the posterior lobe is equivalent

to the neurohypophysis. The difference is that the nomenclature of anterior and posterior lobes does not include the infundibulum, which extends from the hypothalamus to the pituitary gland, which contains neural hypophysial connections and is continuous with the median eminence [11]. The anterior pituitary contains several cell types: gonadotropes (responsible for secretion of FSH and LH), thyrotropes (responsible for the secretion of thyroid-stimulating hormone [TSH]), adrenocorticotropes (responsible for the secretion of ACTH), somatomammotropes (responsible for



**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 Mahutte NG, Ouhilal S. In: Hurd WW, Falcone T, eds. *Clinical reproductive medicine and surgery*. St. Louis, MO: Mosby/Elsevier; 2007

the secretion of GH), and lactotropes (responsible for the secretion of prolactin) (Table 1.2). In addition to these hormones, the anterior pituitary secretes activin, inhibin, and follistatin, which play a role in menstrual cycle regulation. The posterior pituitary lobe contains two cell types that secrete ADH (antidiuretic hormone) and oxytocin. The communication between the hypothalamus and the anterior pituitary is vascular; however, it is a neuronal connection between the hypothalamus and the posterior pituitary.

The gonads in the female consist of the bilateral *ovaries*. The ovaries are located in the pelvis along the sides of the uterus. In reproductive-age women, ovaries measure approximately  $2.5 \times 3 \times 1.5$  cm in size. Laterally, the ovary is attached to the pelvic sidewall by the infundibulopelvic ligament, which contains the vascular supply to the ovary (ovarian artery and vein). The ovary consists of an outer cortex

and an inner medulla. The ovarian follicles are found in the cortex, while the medulla mainly contains fibromuscular tissue and vasculature. Each ovarian follicle consists of an oocyte surrounded by layers of granulosa and theca cells. These layers will vary depending on the maturation stage of the oocyte contained within the follicle. Within the ovarian cortex, follicles can be found in different stages of development. Earlier stages of follicular development are independent of central nervous system hormone production, while later stages of follicular development will depend on reproductive hormones produced by the central nervous system. The growing ovarian follicle will produce estradiol from the granulosa cells (Table 1.3). After ovulation, the remnant cells of the follicle luteinize and start secreting progesterone. The granulosa cells are also responsible for the secretion of inhibin as well as anti-Müllerian hormone (AMH).

**Table 1.2** Major cell types of the anterior pituitary gland<sup>a</sup>

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 $\alpha$ -subunit
Gonadotrophs	Basophilic	5	Follicle-stimulating hormone (FSH), luteinizing hormone (LH), and free $\alpha$ -subunit

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

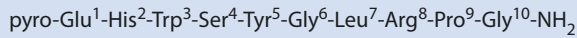
Cell type	Major steroid hormone products
Theca cells	Androgens (androstenedione, DHEA, testosterone)
Granulosa cells	Estrogens (estradiol, estrone, inhibin, AMH)
Theca-lutein cells	Progestogens (progesterone, 17-hydroxyprogesterone)
Granulosa-lutein cells	Estrogens (estradiol, estrone)

The *uterus* is largely a receptive organ to all the steroid hormones that emanate from the endocrine glands. The uterus is a fibromuscular organ that is bordered anteriorly by the urinary bladder and posteriorly by the rectum. The uterus can be divided into two major portions: an upper body (corpus) and a lower cervix. The hollow portion of the uterus contains a mucosal lining called the endometrium. The endometrium contains several layers of cells: the basal layer and the superficial layer. The basal layer is responsible for the regeneration of the endometrial cells. The superficial layers undergo the cyclic changes of the menstrual cycle. The endometrium normally proliferates in response to the rising estradiol levels in the first half of the menstrual cycle and in the second half of the menstrual cycle it is converted to a

secretory layer in response to progesterone produced by the corpus luteum. If the cycle does not result in a pregnancy, where there is lack of hCG, progesterone production is not maintained by the corpus luteum, and the endometrium becomes unstable and sloughs in preparation for a new cycle and another attempt for pregnancy.

## 1.4 Endocrinology of the Menstrual Cycle

*GnRH* is a decapeptide synthesized in the hypothalamus and first described in the 1970s by Schally [12–14] and Guillemin [15] for which they received the Nobel Prize [14–18] (■ Fig. 1.4). GnRH neurons can be detected in the fetal hypothalamus as early as 9–10 weeks of gestation [19]. GnRH neurons originate from the olfactory area [20], later migrating to the olfactory placode to rest in the arcuate nucleus of the hypothalamus [21]. The hypothalamic GnRH neurons then send projections to the pituitary. The association of GnRH neurons and the olfactory system can be demonstrated in a condition called Kallmann syndrome, where GnRH deficiency is coupled with anosmia [22]. Pheromones, small airborne molecules secreted by one individual and perceived by another individual, also suggest the common origin of GnRH molecules and the olfactory system. Pheromones may explain why women living or working in close proximity may develop synchrony in their menstrual cycles [23, 24].



**Fig. 1.4** Structure of GnRH-1. Reproduced with permission from Mahutte NG, Ouhilal S. In: Hurd WW, Falcone T, eds. *Clinical reproductive medicine and surgery*. St. Louis, MO: Mosby/Elsevier; 2007

To date, three types of GnRH (GnRH-I, GnRH-II, and GnRH-III) have been detected in humans [25, 26]. Many other GnRH types have been described in fish, amphibians, and protochordates [27, 28]. GnRH-I is the classic hypothalamic hormone responsible for the regulation, synthesis, and secretion of the pituitary gonadotropins FSH and LH [29]. GnRH-II was first described in brain tissue and has since been found in many other peripheral tissues, such as the endometrium, breast, and ovaries [30–33]. GnRH-III was first identified in Lamprey in 1993 [34], and Yahalom et al. described the presence of GnRH-III in neurons from the hypothalamus [25]. The role of GnRH-III in humans is unclear. GnRH-III does not have strong LH and FSH releasing potency, but it has been shown to exert a direct antiproliferative effect on cancer cells and is being studied for use as an antitumor agent [35].

GnRH-I is synthesized from a larger, 92 amino acid precursor [36]. After synthesis, GnRH-I travels to the median eminence of the hypothalamus and is released in the portal circulation in a pulsatile fashion. The GnRH-I molecule lifespan is very short, as it is cleaved rapidly, with a half-life of 2–4 min. Because of this rapid cleavage, peripheral levels of GnRH-I are difficult and do not correlate well to pituitary action.

GnRH-I acts on the anterior pituitary leading to the synthesis and storage of gonadotropins, movement of the gonadotropins from the reserve pool to a readily released point, and finally the secretion of gonadotropins. For this action to take place appropriately, pulsatile GnRH release is necessary [37, 38]. Continuous GnRH secretion will lead to the suppression of FSH and LH release as well as suppression of FSH and LH gene transcription by the anterior pituitary [39, 40]. This is the basis of use of GnRH agonists such as Lupron for the suppression of gonadotropin secretion. The pulse frequency of GnRH will vary depending on the menstrual cycle phase. LH pulse frequency is used to indicate GnRH pulse secretion (Table 1.4).

**Table 1.4** Menstrual cycle variation in LH pulse frequency and amplitude<sup>a</sup>

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

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GnRH-II differs from GnRH-I by three amino acids at positions 5, 7, and 8 [26, 41]. Also, in contrast to GnRH-I, GnRH-II is mainly expressed outside the brain [26, 42, 43], including the human placenta [44]. Similar to GnRH-I release from the hypothalamus, GnRH-II is released from the placenta in a pulsatile fashion [44, 45].

Various factors are believed to play a role in GnRH secretion. Estrogen has been shown to have a positive as well as a negative effect on GnRH-I secretion. Estrogen suppresses GnRH-I secretion in a negative-feedback fashion [46]. In addition, estrogen has a differential regulation on GnRH-I and GnRH-II mRNA levels. Estrogen increased GnRH-II mRNA levels while it decreased GnRH-I mRNA levels [47]. Progesterone is also noted to play a stimulatory role on GnRH-I mRNA, which was decreased by the progesterone receptor antagonist RU48 [48]. However, no difference in the expression level of GnRH-II was seen with progesterone or the anti-progestin mifepristone [48].

Two types of GnRH receptors have been described in humans: GnRH-I receptor (GnRH-IR)



**Table 1.5** Properties of commercially available GnRH agonists<sup>a</sup>

	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: Nal for Gly	3–4 h	200	Intranasal
Triptorelin	Decapeptide 6: Trp for Gly	3–4 h	36–144	SC, IM depot
Leuprolide	Nonapeptide 6: Leu for Gly 10: NH <sub>2</sub> Et for Gly	1.5 h	50–80	SC, IM depot
Buserelin	Nonapeptide 6: Ser(O <sup>t</sup> Bu) for Gly 10: NH <sub>2</sub> Et for Gly	1.5 h	20–40	SC, intranasal
Goserelin	Decapeptide 6: Ser(O <sup>t</sup> 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

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and the GnRH-IIR (GnRH-IIR). The GnRH-IR is a G protein-coupled transmembrane receptor (GPCR) [49, 50]. However, the mammalian GnRH-IR lacks the carboxyl-terminal tail [50, 51]. Activation of the GnRH-IR leads to the activation of phospholipase C, which in turn generates the second messengers inositol triphosphate and diacyl glycerol, stimulating protein kinase, cyclic adenosine monophosphate (cAMP), and release [52] of calcium ions. In addition to the brain, GnRH-IR can be found in the human placenta [53, 54], ovarian follicles [33, 55], in myometrium and leiomyomata [56, 57], as well as human pancreas, liver, heart, skeletal muscle, kidney, and peripheral blood [58–61]. GnRH-IIR is also a GPCR, but unlike the GnRH I-R, it has a C-terminal cytoplasmic tail [62]. GnRH-IIR can be found in the pituitary, placenta, ovary, uterus, prostate, mature sperm, pancreas, small and large intestines, kidney, and liver [26, 33, 63–65].

GnRH analogues have been developed by changes made to the amino acid sequence of the GnRH molecule. These changes result in the extension of the GnRH half-life as well as its biologic activity. There are two major groups of GnRH

analogues: GnRH agonists and GnRH antagonists (Table 1.5) In the case of GnRH agonist use, the continuous activation of the GnRH receptor results in desensitization due to a conformational change of the receptor, uncoupling from G proteins, internalization of the receptor as well as reduced synthesis of the receptor [66, 67]. Prior to the desensitization by GnRH agonists, there is an initial flare where there is increased gonadotropin secretion. Desensitization then takes place 7–14 days later. Unlike GnRH agonists, GnRH antagonists do not cause a flare effect upon initial administration; instead, GnRH antagonists cause an immediate suppression of gonadotropin secretion that is rapid and is reversible [68]. Currently, GnRH analogues are available in injectable form in the treatment of many reproductive conditions, such as precocious puberty, endometriosis, and uterine leiomyomata; they are also being used in in vitro fertilization treatment cycles. Oral forms of GnRH analogues are under investigation. Elagolix is an orally active GnRH antagonist under investigation for use in reproductive conditions [69, 70].

GnRH acts on the anterior pituitary to secrete gonadotropins: FSH and LH. FSH is a glycoprotein

dimer consisting of two subunits:  $\alpha$  (alpha)- and  $\beta$  (beta)-subunits. The  $\alpha$ -subunit is common in FSH and LH as well as TSH and hCG. The  $\beta$ -subunit is distinct and hormone-specific, which allows the differential function of each hormone. The  $\alpha$ -subunit consists of 92 amino acids, while the FSH  $\beta$ -subunit consists of 118 amino acids and five sialic acid residues. Sialic acid residues are responsible for the half-life of the hormone, where the higher the sialic acid content the longer the half-life of that molecule [71]. FSH has a half-life of several hours. The addition of sialic acid to urinary obtained or recombinant FSH products leads to their longer half-life. The rate-limiting step in gonadotropin production is the availability of  $\beta$ -subunits. In addition to GnRH stimulation of FSH  $\beta$ -subunit synthesis, FSH  $\beta$ -subunit synthesis is dependent on the presence of activin [72, 73].

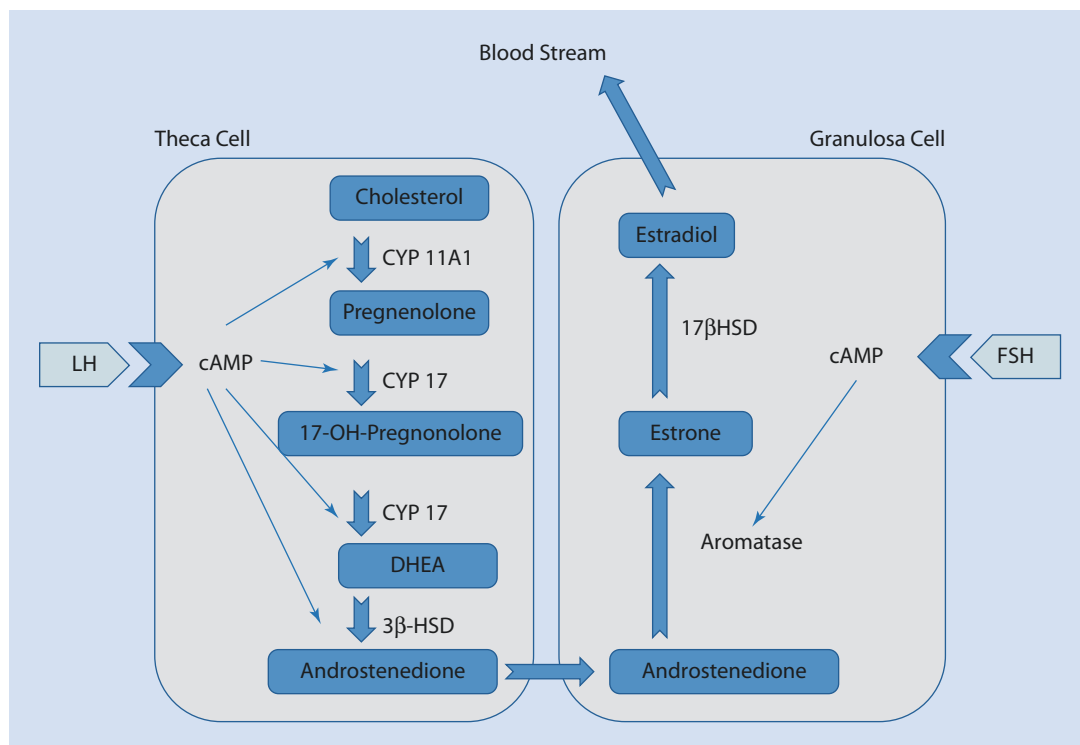
FSH starts to rise a few days prior to the onset of menses and is responsible for the recruitment of a cohort of ovarian follicles as well as a selection of the dominant follicle (see [■ Fig. 1.1](#)). FSH induces granulosa cell growth and activates aromatase activity, which converts androgens into estrogens. FSH levels then start to decline owing to estrogen and inhibin B production by the growing follicular granulosa cells. Despite this drop in the FSH level, the dominant follicle continues to grow as it acquires the highest concentration of FSH receptors (secondary to increase in surrounding granulosa cell number), making it more resistant to the drop in FSH level [74]. In addition, the drop in FSH level causes a higher androgenic microenvironment in the non-dominant follicles. FSH then declines after ovulation of the dominant follicle.

LH is also a glycoprotein dimer consisting of two subunits:  $\alpha$  (alpha)- and  $\beta$  (beta)-subunits. The  $\beta$ -subunit of LH consists of 121 amino acids and one to two sialic acid residues, giving it its shorter half-life of approximately 20 min. Because of this shorter half-life, LH needs to be rapidly synthesized and typically has pulses higher in amplitude than FSH. As with FSH, LH also starts to rise prior to the onset of menses. The LH increase throughout the follicular phase of the cycle is gradual. Immediately prior to ovulation, LH surges in response to estradiol production by the dominant follicle in a positive-feedback fashion. LH levels then decline in the secretory phase of the cycle (see [■ Fig. 1.1](#)). Little is known as to why LH initially responds negatively to estrogen,

while later the feedback relationship becomes positive. Many researchers have looked into this, with some researchers describing possible synaptic transmission speed as the reason for the positive feedback [75], while others suggest that the continued estrogen presence causes an increase in Glutamate [76] and Gamma Amino Butyric Acid (GABA) transmission in GnRH neurons [77, 78]. However, these studies have been performed in animals and no equivalent studies have been shown in humans.

FSH and LH receptors both belong to the GPCR family. FSH receptors exist exclusively on the membrane of granulosa cells, while LH receptors are found on membranes of theca cells. In the presence of estradiol, FSH induces LH receptors on granulosa cells. LH receptor activity primarily stimulates androstenedione production from theca cells, which is transported to neighboring granulosa cells, aromatized to estrone, and eventually converted to estradiol. This is the basis of the two-cell theory of the ovary ([■ Fig. 1.5](#)).

Endogenous opiates (*opioids*) are naturally occurring narcotics produced by the brain. There are three classes of opiates: enkephalin, endorphin, and dynorphin. Endorphin levels increase throughout the menstrual cycle; they are at their lowest at the time of menses and at their highest in the luteal phase. Sex steroids appear to play a role in endorphin secretion. Estradiol has been shown to increase endorphin secretion, while the sequential addition of progesterone to estradiol showed a higher endorphin secretion in ovariectomized monkeys [79]. An increase in endorphin release has been shown to decrease LH pulse frequency [80], while opioid receptors blockers, such as naltrexone, have been shown to increase LH pulse frequency [81]. The suppression of gonadotropin secretion by endogenous opiates is secondary to suppression of hypothalamic GnRH release [82]; thus, opiates appear to play a role in hypothalamic amenorrhea ([■ Table 1.6](#)). Treatment of women with hypothalamic amenorrhea with opioid receptor antagonists appears to correct the problem, causing a return of ovulation and menstrual cyclicity [83, 84]. It is also believed that stress-related amenorrhea is the result of GnRH suppression by endogenous opiates. Women suffering from stress-related amenorrhea demonstrate higher hypothalamic corticotropin-releasing hormone. Proopiomelanocortin, the precursor to endorphins, is controlled mainly by corticotropin-releasing hormone [79]. In addition, hypothalamic amenorrhea that develops in athletes



**Fig. 1.5** 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 stim-

ulates 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-hydroxy pregnenolone

**Table 1.6** Neurotransmitter effects on GnRH release<sup>a</sup>

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

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may also be secondary to opioid rise during exercise [85, 86].

Ovarian peptide hormones such as inhibin, activin, and AMH also play a role in the menstrual cycle by modulating central nervous system

hormone release. Inhibin, activin, and AMH all belong to the transforming growth factor- $\beta$  (beta) superfamily (TGF- $\beta$ ) of ligands.

*Inhibin* is a polypeptide mainly secreted by granulosa cells, but has also been found in pituitary gonadotropes [87, 88]. Inhibin is comprised of a  $\alpha$  (alpha)- and  $\beta$  (beta)-subunits. Two forms of inhibin have been identified: inhibin-A and inhibin-B, each containing an identical  $\alpha$ -subunit but a unique  $\beta$ -subunit. Inhibin-A is predominantly secreted in the luteal phase of the menstrual cycle, while inhibin-B is predominantly secreted in the follicular phase of the menstrual cycle [89]. Inhibin is released by granulosa cells in response to FSH [90] and selectively inhibits FSH secretion from the anterior pituitary [91], thus creating a negative-feedback loop (see Fig. 1.1).

In contrast, *activin*, which is also secreted by the granulosa cells, augments the secretion of FSH by enhancing GnRH receptor formation [92, 93]. The effects of activin are blocked by inhibin and follistatin [94].

*Follistatin* is a peptide secreted by pituitary gonadotropes [95]. Follistatin inhibits FSH synthesis and secretion by sequestering activin [96, 97]. Inhibin inhibits follistatin production, while activin stimulates its production.

*AMH* is a product of the granulosa cells of small antral and pre-antral follicles and is reflective of their quantity [98]. It may be reflective of the ovarian reserve which is often a clinical term for the size of the primordial follicle pool. Although the role of AMH has been well described for causing Müllerian duct regression in the male fetus, its role in females in the post-fetal life period has not been well defined. It is believed that AMH, through a paracrine effect in the ovary, inhibits FSH-stimulated follicle growth, contributing to the emergence of the dominant follicle [79]. The relationship among AMH, the follicular pool, and recruitment throughout the reproductive life cycle is complex and is dependent on the stage of sexual development [99]. Clinically AMH has been used in the prediction of ovarian reserve in women undergoing fertility evaluation and treatment [100]. However, the dichotomy of poor reserve vs. normal reserve is not evident [100]. AMH levels are elevated in patients with polycystic ovary syndrome and decreased in women exposed to antineoplastic drugs.

*Leptin* is a protein cytokine secreted by adipocytes. It consists of 167 amino acids and is secreted by adipose tissue, reflecting amounts of body fat [101]. Leptin's most significant role is energy homeostasis. It is regulated by many factors, such as obesity, glucose, and insulin, which promote its secretion, whereas fasting, androgens, and thyroid hormone inhibit its secretion. Its role in reproduction is not well understood. As mentioned earlier, CRH is increased in stress-related amenorrhea and is also increased in weight-loss amenorrhea. It is not understood why CRH increases. The reduction in leptin level in these clinical scenarios may play a role in this CRH increase in the brain [102]. Leptin has also been shown to indirectly affect pituitary FSH and LH secretion in gonadotropin-stimulated fertility treatment cycles [103].

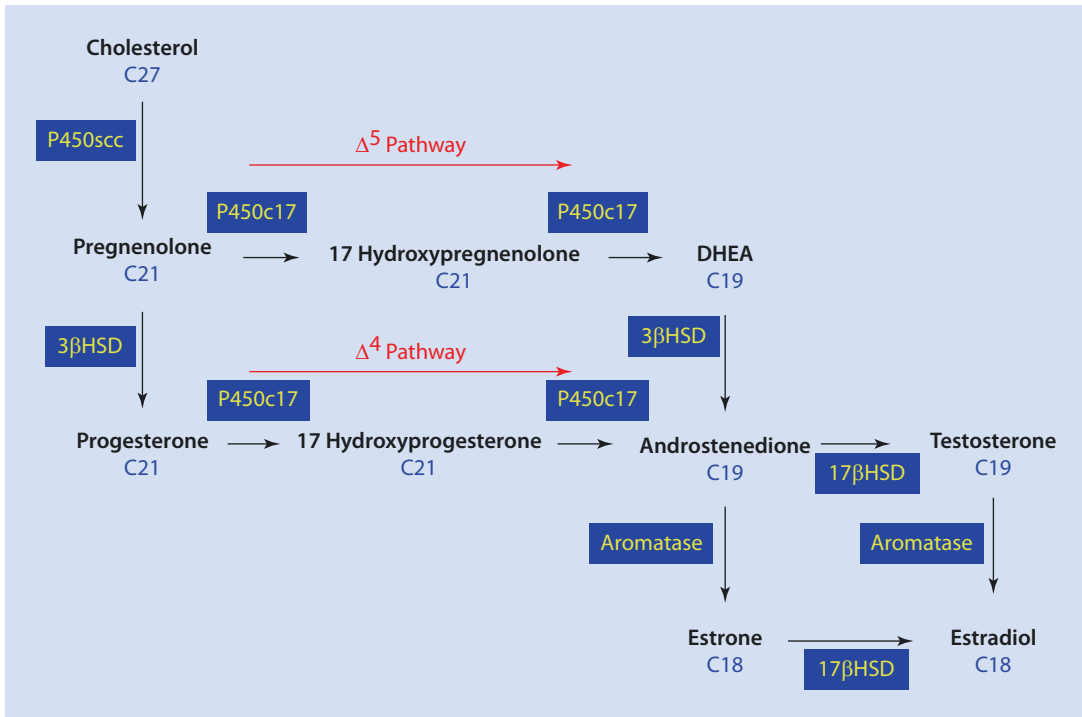
Estrogens are 18-carbon steroid hormones and include estrone (E1), estradiol (E2), and estriol (E3). The most potent estrogen is *estradiol* and is the product of the ovary. Estrone is mainly the product of peripheral androstenedione conversion. Estrone is also generated in the liver via 17 $\beta$  (beta)-hydroxysteroid dehydrogenase conversion

of estradiol. Estriol is the principal estrogen formed by the placenta during pregnancy. Serum estradiol levels rise during the follicular phase of the menstrual cycle and are in parallel to the growth of the follicle. Estradiol is mainly found bound in the bloodstream to carrier proteins. Albumin carries approximately 60% of estradiol, while sex hormone-binding globulin binds 38% of estradiol, with 2% remaining as free in the bloodstream. This free hormone is active and capable of entering target cells. In the early follicular phase, serum estradiol levels do not exceed 50 pg/mL. At peak follicular growth, the level rises to approximately 200–250 pg/mL. Estradiol levels drop with ovulation, but a second rise is seen in the mid-luteal phase, reflecting estrogen secretion from the corpus luteum (see ■ Fig. 1.1). Circulating estrogens are conjugated in the liver to form sulfates and glucuronides; 80% are excreted in the urine and the remaining 20% in bile.

There are two known estrogen receptors: estrogen receptor-alpha (ER- $\alpha$ ) and estrogen receptor-beta (ER- $\beta$ ) [104, 105]. Both receptors contain DNA-binding and hormone-binding domains, a hinge region, and a transcriptional activation function (TAF) domain. Estrogen will enter any cell, but only cells containing the estrogen receptor will respond. The receptor is typically nuclear in location, but can be shuttled to the cytoplasm via a process called nucleocytoplasmic shuttling [79]. Once estrogen binds to its receptor, activation of gene transcription then takes place.

It is also known that estradiol has a negative-feedback effect on FSH secretion. This negative-feedback effect is the direct effect of estradiol coupled to its receptor, causing repression of FSH- $\beta$  (beta) subunit transcription [106].

Similar to estrogen, *progesterone* is a steroid hormone. Progesterone is a 21-carbon molecule and is the main steroid of the corpus luteum. In the follicular phase, progesterone levels are typically <2 ng/mL. Progesterone reaches its peak in the mid-luteal phase, with levels exceeding 5 ng/mL (see ■ Fig. 1.1). The majority of progesterone in the bloodstream is bound to albumin (80%) and corticosteroid-binding globulin (18%). A very small amount of progesterone is bound to SHBG (0.5%). The remaining progesterone is free in the circulation. The liver is responsible for clearing progesterone from the circulation by converting progesterone to pregnanediol, which is conjugated to glucuronic acid and excreted in the urine.



**Fig. 1.6** The  $\Delta(\delta)^5$  and  $\Delta(\delta)^4$  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  $\Delta^5$  pathway involving 17-hydroxypregnenolone and dehydroepiandrosterone (DHEA). In contrast, the corpus luteum preferentially converts pregnenolone to progesterone ( $\Delta^4$  pathway) via  $3\beta$ (beta) hydroxysteroid dehydrogenase ( $3\beta$ HSD). Reproduced with permission from Mahutte NG, Ouhilal S. In: Hurd WW, Falcone T, eds. Clinical reproductive medicine and surgery. St. Louis, MO: Mosby/Elsevier; 2007

Similar to estrogen, there are several progesterone receptors: progesterone receptor-A (PR-A), progesterone receptor-B (PR-B), and progesterone receptor-C (PR-C). PR-B is the positive regulator of progesterone effects, while PR-A and PR-C antagonize PR-B.

At high concentrations, progesterone inhibits FSH and LH secretion through effects on both the hypothalamus and pituitary [107]. The presence of progesterone in the luteal phase also causes the decline in GnRH pulse frequency in the hypothalamus. At low concentrations, progesterone can stimulate LH release only after exposure to estrogen and progesterone [108]. Progesterone also causes a depletion of estrogen receptors, which is the mechanism of protection against endometrial hyperplasia by progesterone.

*Androgens* are the major products of theca cells. Androgens are 19-carbon steroids and include: androstenedione, testosterone, and dehydroepiandrosterone (DHEA). The principal secreted androgen by theca cells is androstenedione. Most of the

testosterone is the product of peripheral conversion of androstenedione through the actions of  $17\beta$ -hydroxysteroid dehydrogenase. Under the effect of FSH, androstenedione and testosterone are then further aromatized in granulosa cells and converted to estrogens (Fig. 1.6).

The androgen receptor exists in a full-length B form and a shorter A form [109]. Androgens and progestins can cross-react to their receptor but only when present in high concentration.

In preovulatory follicles, the preferred steroid pathway for androgen and estrogen synthesis is the  $\Delta(\delta)^5$  pathway, which involves the conversion of pregnenolone to 17-hydroxypregnenolone. In the theca cell, 17-hydroxypregnenolone is converted to androgens. Due to the lack of ability of theca cells to metabolize androgens, they are carried to the neighboring granulosa cells for aromatization (see Figs. 1.5 and 1.6). In contrast, in the corpus luteum the preferred pathway is the  $\Delta(\delta)^4$  pathway of steroidogenesis, which deals with the conversion of pregnenolone to progesterone. The rate-limiting

step in steroidogenesis is the side-chain cleavage of cholesterol to pregnenolone. In the ovary, this step is regulated by LH. LH stimulation leads to increased cAMP production and increased low-density lipoprotein (LDL) receptor mRNA and subsequent increased LDL intake. LDL is the major form of cholesterol used for steroidogenesis. cAMP-activated steroidogenic acute regulatory protein (StAR) causes an increase in the transport of cholesterol across the mitochondrial membrane, where side-chain cleavage can take place [110]. From there, all the remaining ovarian hormones can be produced.

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