

16

Puberty in the Female and Its Disorders

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Puberty is the stage of development during which secondary sexual characteristics appear and there is a transition from the sexually immature to the sexually mature stage. Adolescence is widely used as a generally synonymous term for puberty, but it is often used to convey an added cultural connotation as a psychosocial coming of age.

By the mid-1960s a general concept of the major factors involved in the initiation of puberty was established (Fig. 16.1).^{1,2} A decrease in sensitivity of the brain "gonadostat" to sex hormone negative feedback was thought to be the primary event. This signaled the hypothalamus to discharge neurohumors (then unidentified), which in turn stimulated the pituitary to release gonadotropins. The resultant rise in secretion of the gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), was thought to account directly for increased estrogen production by the ovary. A mature relationship was thought to develop in which the blood levels of estrogen and gonadotropins were regulated reciprocally via the gonadostat,³ much as a furnace is regulated by a thermostat. The pineal was identified as having gonadal suppressive properties. The increased adrenocortical secretion of 17-ketosteroids (17-KS), which becomes apparent at about the time of puberty ("adrenarche"), was thought to be caused by a pituitary factor stimulating adrenal androgens in synergism with adrenocorticotropic hormone (ACTH).⁴

The rapid scientific advances since 1965 have permitted this concept to be tested in increasingly sophisticated ways. In the

subsequent decade, radioimmunoassay (RIA), originally developed by Yalow and Berson, was applied to the measurement of gonadotropins and sex steroids; the gonadotropin-releasing hormone (GnRH) for both LH and FSH was isolated, identified, and synthesized by Guillemin's and Schally's groups. Cyclic adenosine-3',5'-monophosphate (cAMP), postulated by Sutherland to mediate the action of peptide hormones, was found to mediate gonadotropin effects on the ovarian follicle. The initial steps in the mechanism of action of steroid hormones were defined by Jensen, Gorski, and their groups. The landmark nature of many of these discoveries was recognized by the awarding of Nobel Prizes in Medicine to Sutherland in 1971 and to Yalow, Schally, and Guillemin in 1977.

Our present view of the mechanisms controlling puberty is more refined and complex than it once was, although the earlier schema is correct in a general sense. The gonadostat is a patently oversimplistic concept for a complex system that regulates the activity of the hypothalamic GnRH pulse generator, a functionally interconnected and synchronized network of GnRH neurons.⁵ The gonadostat setting seems to change throughout childhood in a biphasic manner. This concept is illustrated in Fig. 16.2.^{6,7} During most of fetal and perinatal life, the gonadostat is insensitive to negative feedback by sex steroid hormones; at this time the nascent neuroendocrine-gonadal axis functions at a pubertal level. The gonadostat becomes increasingly sensitive to negative feedback during infancy but does not become highly sensitive until midchildhood, at which time GnRH pulse generator activity is minimal. During late prepuberty, the gonadostat begins to relinquish its inhibition. This permits the onset of puberty. The changing set-point initially permits increasing, episodic secretion of GnRH. Increasing sensitivity of the pituitary gonadotropic cells to GnRH follows. The change in LH and FSH secretion is first detectable during sleep. Gradually, the gonads become increasingly sensitized to gonadotropin stimulation, grow at an increased rate, and bring about sustained rises in plasma sex steroid hormone levels. Some of these phenomena synergize with others, so that autoamplification occurs and the pace of change accelerates. Eventually, the set-point for gonadotropin release comes to vary sufficiently to encompass a positive feedback mechanism.

The data on which this model is based are presented later. The most recent data on the hormonal milieu and accompanying physical stages of normal puberty are then presented. Abnormal puberty is subsequently discussed: the causes, differential diagnosis, and management.

DEVELOPMENT OF THE FEMALE REPRODUCTIVE SYSTEM

Maturation of the Neuroendocrine-Ovarian Axis Fetus

Neuroendocrine Unit. The anterior lobe of the pituitary gland, of stromal ectodermal origin, and the posterior lobe, of neural origin, differentiate by 11 weeks' gestational age.⁸ By this time, GnRH neurons have migrated from the olfactory placode into place in the medial basal hypothalamus.⁹ Hypothalamic GnRH subsequently rises in parallel with fetal pituitary and serum LH and FSH.¹⁰ All peak at about 20 to

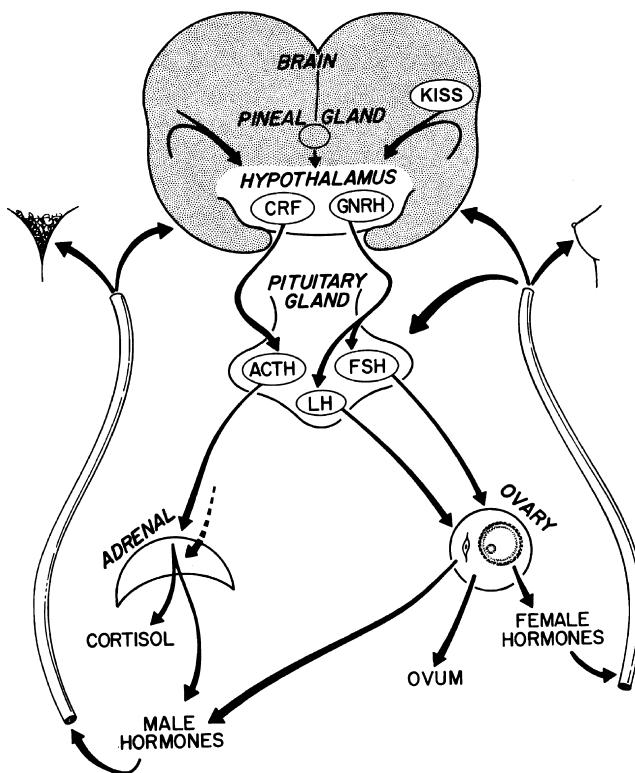


Fig. 16.1 Schematic representation of the neuroendocrine-ovarian axis involved in normal pubertal development. ACTH, Adrenocorticotrophic hormone; CRF, corticotropin-releasing factor; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; KISS, kisspeptin; LH, luteinizing hormone.

24 weeks, as the connections of the pituitary portal system become complete, to levels not again seen until menopause.¹¹

Serum LH and FSH levels are higher in human female than male fetuses.¹¹ In rats, GnRH-containing neurons develop earlier in females than in males,¹² and there are sexual dimorphisms in the degree of synapsing of specific tracts with

dendritic spines in the preoptic nucleus, one of the major GnRH-containing areas of the hypothalamus.^{13,14} These differences may be determined by gonadal sex steroid hormone output. In all species studied, fetal secretion of LH, particularly LH pulse frequency, is permanently desensitized to estradiol-progesterone negative feedback by fetal virilization.¹⁵ In the rat, this has been demonstrated to be mediated by permanent impairment of estradiol-induced progesterone receptor (PR) gene expression.¹⁶

In late gestation, fetal hypothalamic GnRH and pituitary gonadotropin secretion fall to low levels. These changes are likely explicable by the negative feedback effect of the high sex steroids produced by the fetoplacental unit. Meanwhile, maturation of the central nervous system (CNS) tracts that inhibit hypothalamic GnRH secretion and mediate gonadal negative feedback signals appears to progress throughout gestation.^{17,18}

The production of gonadotropins by the fetal pituitary seems to facilitate normal ovarian development. Hypophysectomy of rhesus fetuses has been reported to reduce the number of germ cells and oocytes, as well as the integrity of the rete ovarii.¹⁹ Therefore it seems that survival of gametes depends upon the secretions of the fetal pituitary.

Ovary. The ovaries differentiate in the urogenital ridge adjacent to the anlage of the adrenal cortex and the kidney. The granulosa cells are the homologues of the Sertoli cells of the testes. The theca, interstitial, and hilus cells are the homologues of the Leydig cells; hilus cells may even contain crystalloids like Leydig cells. Adrenocortical rests occasionally have been found in the hilus of the ovary.²⁰ Conversely, ovarian rests have been identified in the adrenal glands.²¹

The primitive germ cells migrate into the ovary from the yolk sac endoderm during the first month of gestation. The testes become histologically discernable by 8 weeks' gestation.²² The ovaries develop²³ in the absence of testicular development being switched on by the signaling cascade initiated by the SRY gene on the Y chromosome.^{24,25} Activation of the β -catenin signaling pathway by Wnt-4 and R-spondin1 permit forkhead (Fox) L2 transcription factor expression by germ cells to activate ovarian differentiation by sustaining oocyte and granulosa cell development and suppressing Sertoli and Leydig cell differentiation; they also support later aspects of follicle development.^{26,27} Steroidogenic factor-1 (SF-1) WT-1, LIM-1, and

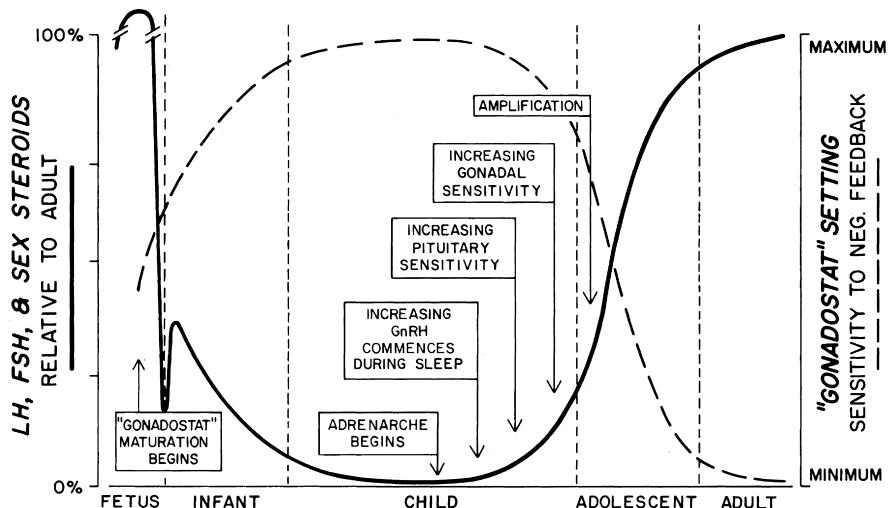


Fig. 16.2 The changing pattern of serum gonadotropins and sex hormones from fetal life to maturity in relationship to the apparent sensitivity of the central nervous system "gonadostat" to the negative feedback effect of sex hormones and the underlying hormonal events. FSH, Follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone. (Modified from Grumbach, M., Grave, C., Mayer, F. (Eds.) (1974), The Control of the Onset of Puberty. New York, John Wiley & Sons.)

possibly *DAX-1* genes play roles in the formation of the ovaries.²⁸ Germ cell bone morphogenetic proteins (BMPs) are necessary for primordial germ cell proliferation.

Primitive germ cells undergo mitotic division to become oogonia, a process that is maximum at 8 to 12 weeks. Oogonia then undergo oogenesis, entering the prophase of meiosis to become primary oocytes beginning at 12 to 16 weeks.²⁹ The number of oocytes reaches a peak at 20 weeks when there are 6.8 million germ cells, of which 80% appear to be viable (Fig. 16.3).³⁰ When oocytes enter the diplotene stage of meiotic prophase they must be furnished with granulosa cells to form a primordial follicle, or else they undergo atresia.³¹

The ovary remains histologically undifferentiated until primordial follicles appear at about 16 weeks, when the epithelium of the secondary sex cords provides granulosa cells to the oocytes.²² However, the fetal ovary has the capacity for androgen and estrogen formation and signaling, although at a far lower level than the testes, by 12 weeks.^{22,32–35} Primordial follicles become primary follicles when the encircling granulosa cell layer becomes cuboidal. Primordial and small primary follicles (Fig. 16.4)^{36,37} are resting follicles, which are the major repository of germ cells.³⁸ This stock of germ cells is depleted only very slowly during childhood (see Fig. 16.3). Residence of primordial follicles in the ovarian cortex restrains their progression partly because of cortex mechanical rigidity.³⁹ Mechanical effects are mediated by the growth-restrictive Hippopotamus signaling pathway and by vascular permeability⁴⁰ via the vascular endothelial growth factor signaling pathway.

Secondary follicles and preantral follicles, characterized, respectively, by organization of a distinct theca cell layer and proliferation of granulosa cell layers, then appear successively. Preantral follicles develop at 24 to 26 weeks.^{22,31} Antral (graafian) follicles appear near term, and those granulosa cells enveloping the oocyte to become the cumulus.^{37,41,42} Ovarian estrogen production appears to be virtually unresponsive to gonadotropins until early antral follicles develop at near term gestational age.^{18,43,44} One or two antral follicles of 1 to 2 mm in diameter are present in the ovary at term.^{18,22,31} At this time, ovarian follicle development is complete,^{37,41,42} and the complement of ova is greater than at any other time during postnatal life (see Fig. 16.3), totaling 2 million, of which half appear atretic.^{30,45}

Both X-chromosomes are active in oocytes,⁴⁶ and the oocytes secrete factors, such as growth differentiation factor-9 (GDF9),⁴⁰ necessary for the induction of the granulosa cell layer that is necessary for oocyte survival.^{41,47} Oocyte-specific chemokines and transcription factors then coordinately direct the formation of primordial follicles and their subsequent development to primary follicles.⁴⁰ GDF9 interaction with growth factors, such as BMP 9 and transforming growth factor beta (TGF- β), is then critical for primary follicle granulosa cell proliferation. Then preantral follicles develop when GDF9, in coordination with other growth factors, induces the theca cell layer from fibroblast-like stem cells.^{48,49} A host of local factors then regulate further follicle growth and development; for example, the forkhead transcription factor FOXL2, expressed specifically in granulosa cells, restrains GDF9 from prematurely activating follicle growth.⁵⁰

Estrogen receptor (ER) expression is critical for development of the granulosa cell layer.⁴⁰ Insulin and androgen promote the primordial-primary follicle transition. Only upon reaching the early antral follicle stage does further follicle development become strictly dependent on FSH action.

Follicle number is determined by the balance between survival and atresia of ovarian germ cells. The endowment of ovarian germ cells has been thought to be determined during fetal life since the germ cells of the ovary, unlike those of the testes, seem to be a nonrenewing population. However, female germline stem cells can replicate,⁵¹ which suggests that local environmental factors extrinsic to the oocyte hold it in a state of suspended animation.⁵² The endowment of follicles may also be influenced by circulating factors, such as toxins⁵³ and placental insufficiency.⁵⁴ Some clinical evidence suggests that fetal undernutrition slows the rate of atresia.⁵⁵ Studies in mice indicate that puberty appears to be a critical developmental window for the regulation of the follicle population because a wave of primordial follicle depletion is triggered by gonadotropin action on the intrinsic apoptotic pathway.⁵⁶

Placenta. The fetoplacental unit becomes the major source of sex hormones in the female fetus in the latter half of pregnancy: the fetal adrenal gland provides 17-KS as substrate for the formation of potent sex steroids by the placenta. Excess androgen, from any source, in the female fetus masculinizes genital

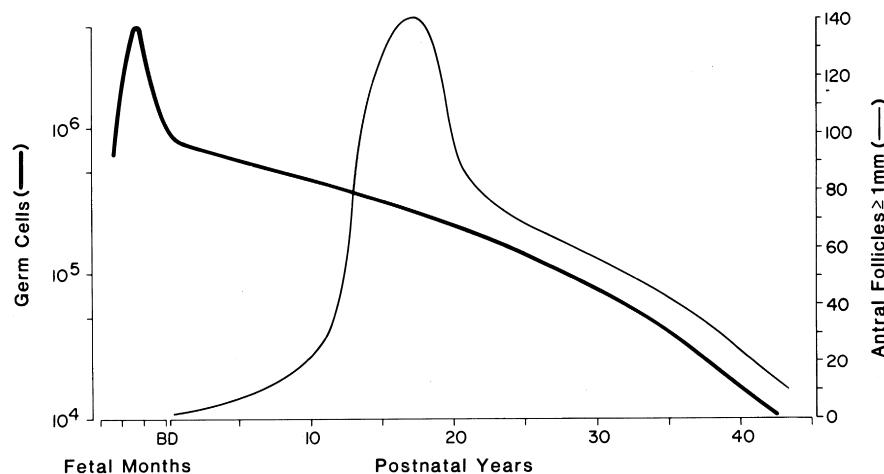


Fig. 16.3 The development of ovarian follicles from fetal life to maturity. Curves for total number of viable germ cells (thick line) and large antral follicles (thin line) smoothed from the data of Baker and Block. The number of germ cells is maximal at the fifth month of fetal life. The loss of germ cells is exponential throughout postnatal life. At puberty, a marked shift occurs in the pattern of development of follicles. An increased fraction grows to large antral size.

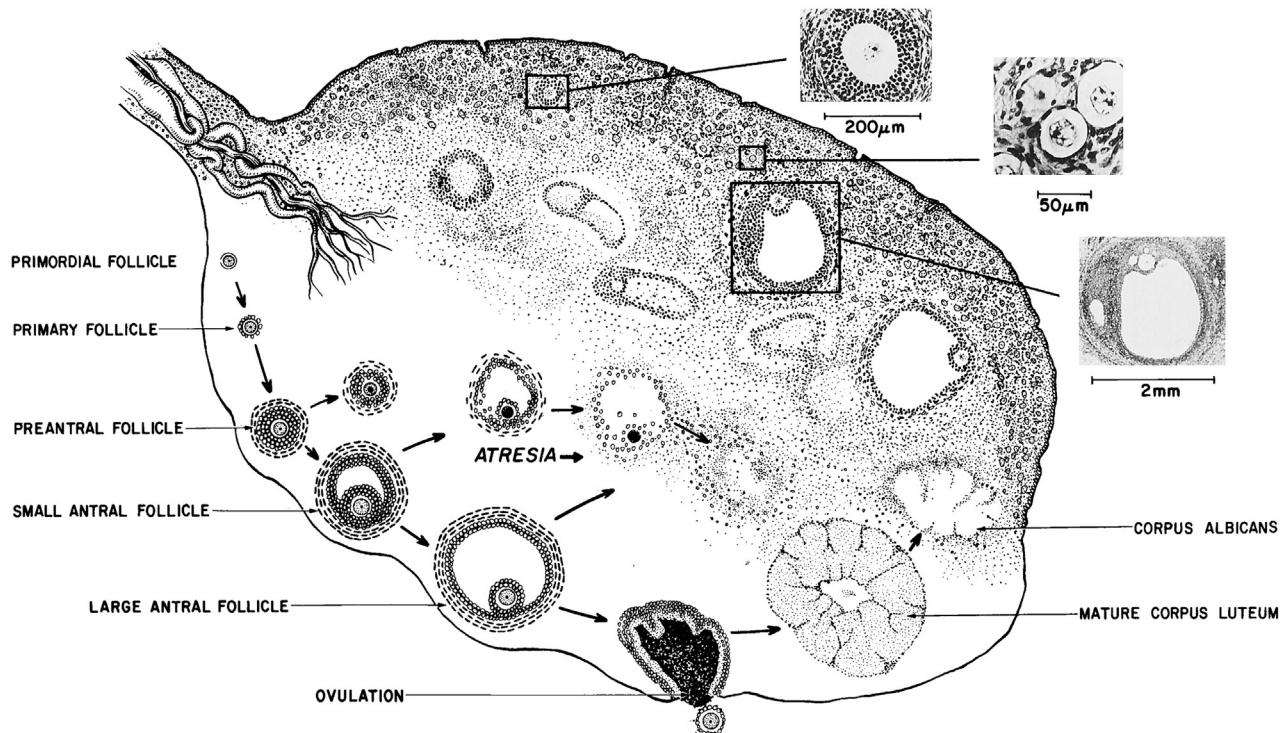


Fig. 16.4 The human ovary. The lower portion of the figure shows the classification of follicles. Preantral follicles contain as many as 300 granulosa cells, and their diameter ranges from 50 to 200 μm. The oocyte diameter increases from 25 or less to 80 μm. Antral (graafian, tertiary, or vesicular) follicles have a fluid-filled antrum and a full-grown oocyte, are lined with more than 300 granulosa cells, and have a well-developed theca. They are greater than 200 μm in diameter. The dimensions of the mature ovary are approximately 1.25 × 2.75 × 4 cm. The upper portion of the figure illustrates the histological appearance of the perimenarcheal ovary. (Photomicrographs from Peters, H. (1979). The human ovary in childhood and early maturity. *Eur J Obstet Gynecol Reprod Biol*, 9(3), 137; modified from Ross, G.T., Schreiber, J.R. (1978). The ovary. In: Yen, S.S.C., Jaffe, R. (eds.), *Reproductive Endocrinology*. Philadelphia, WB Saunders, p. 63.)

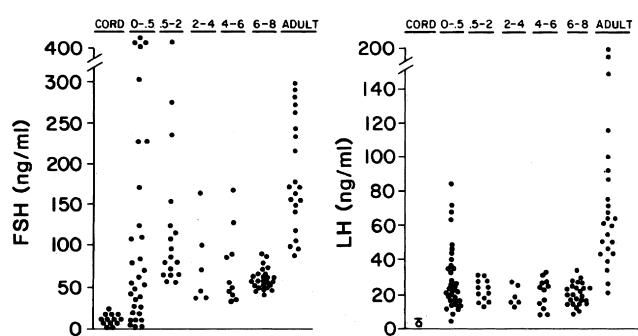


Fig. 16.5 Left: The distribution of serum gonadotropin levels according to early generation radioimmunoassays from birth to adulthood (age in years). Left: follicle-stimulating hormone (FSH). Right: luteinizing hormone (LH) levels. Umbilical cord level of LH measured by beta-subunit-specific radioimmunoassay. Standard LER-907: 100 ng equivalent to 2 mIU FSH and 6 mIU LH of the First International Reference Preparation of human pituitary gonadotropin for bioassay. (Data from Winter, J., Faiman, C., Hobson, W., Prasad, A., Reyes, F. (1975). Pituitary-gonadal relations in infancy: I. Patterns of serum gonadotropin concentrations from birth to four years of age in man and chimpanzee. *J Clin Endocrinol Metab*, 40, 545; Kaplan, S., Grumbach, M., Aubert, M. (1976). The ontogenesis of pituitary hormones and hypothalamic factors in the human fetus. *Recent Prog Horm Res*, 32, 161.)

differentiation, as discussed in other chapters. This also programs for LH elevation and insulin resistance in adult life.¹⁵ Another factor predisposing to postnatal insulin resistance is placental insufficiency, via hypoxemia and resultant overactivation of fetal prostaglandin production and cortisol secretion.⁵⁷

Adolescent

The endocrinological changes of puberty actually begin in late preadolescence before secondary sex characteristics appear, as just reviewed. The underlying basic event is increasing secretion of hypothalamic GnRH. Puberty is the consequence of the hypothalamus releasing GnRH with increasing frequency and amplitude, first only at night, then gradually throughout the day.

Increased GnRH secretion in man was initially deduced when Kastin, Job, Grumbach and their collaborators demonstrated that preadolescent children had GnRH-releasable pituitary stores of LH and FSH (Figs. 16.6 and 16.9).⁸⁷ Subsequently, it was reported that in man, the output of an immunoreactive fragment of GnRH begins to rise in late childhood and increases to adult levels during puberty.^{73,88} Studies in the rat suggest that hypothalamic GnRH increases through puberty.⁸⁹

Knobil subsequently showed that puberty can be induced in the immature female rhesus monkey by administering GnRH in hourly pulses that yield blood levels of about 2000 pg/mL.⁹⁰

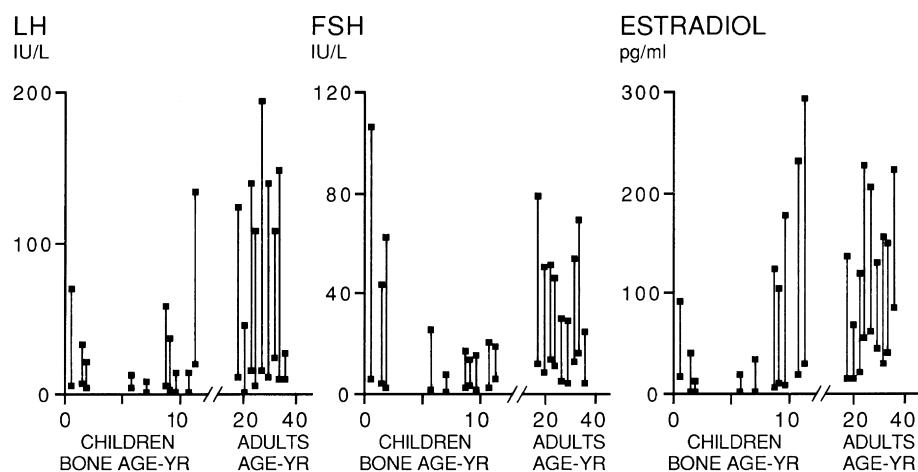


Fig. 16.6 Basal and peak responses to the gonadotropin-releasing hormone agonist nafarelin (1 mcg/kg subcutaneously) during development. Lines connect the basal and peak responses in control children. The responses are related to bone age in children and chronological age in adults. Note the biphasic pattern of the responses. They are high in infancy, lower in midchildhood, and rise again during puberty. The peak gonadotropin responses occur at approximately 4 hours, and peak estradiol responses occur at 20 hours. *FSH*, Follicle-stimulating hormone; *LH*, luteinizing hormone. (From Rosenfield, R.L., Burstein, S., Cuttler, L., et al. (1989). Use of nafarelin for testing pituitary-ovarian function. *J Reprod Med*, 34, 1044.)

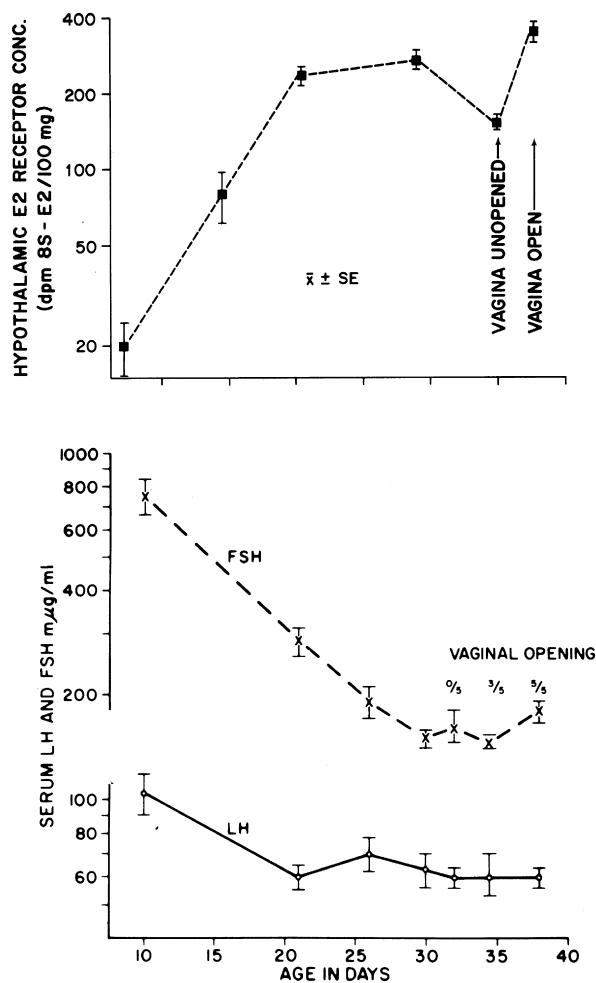


Fig. 16.7 Relationship of maturation of hypothalamic estrogen receptors (top) to serum gonadotropin levels (bottom) in the developing female rat. *FSH*, Follicle-stimulating hormone; *LH*, luteinizing hormone. (From Rosenfield, R.L. (1977). Hormonal events and disorders of puberty. In: Givens, J.R. (ed.), *Gynecologic Endocrinology*. Chicago, Year Book Medical. By permission of Mosby-Year Book.)

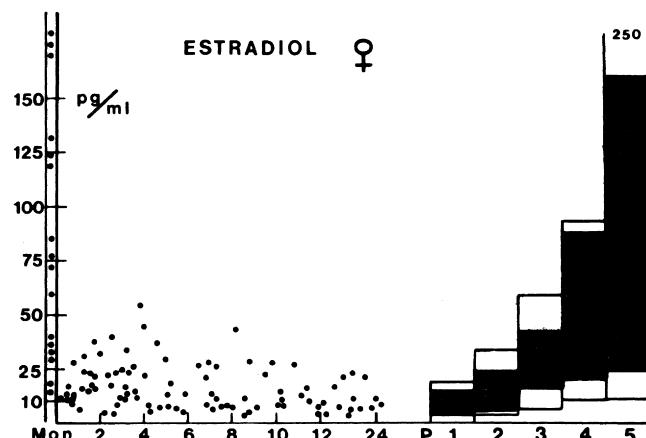


Fig. 16.8 The distribution of plasma estradiol levels in infant females compared with pubertal and adult female levels. The columns represent the normal ranges for the various stages of puberty. The area between 10th and 90th percentiles is dark. Stage P1 includes all prepubertal girls older than 2 years. The values between the ordinates were found between 2 and 5 days of age. (From Bidlingmaier, F., Knorr, D. (1978). Oestrogens: physiological and clinical aspects. *Pediatr Adolesc Endocrinol*, 4, 41-84.)

Prolonged administration of GnRH according to this regimen first gradually brings about transient increases in LH and FSH. This then induces cyclic follicular development. The resultant moderate estradiol surge is of such magnitude as to result in menarche because of withdrawal menstrual bleeding in an anovulatory cycle (Fig. 16.10). Continuation of the same GnRH regimen leads to development of normal monthly ovulatory menstrual periods. Physiological pulses of GnRH in man probably attain lower concentrations (200 pg/mL) and occur at slightly wider intervals than in monkeys.⁹² Consequently, LH pulses in mature women occur at intervals of approximately 1.5 hour during the follicular phases, slowing during the luteal phase.

Puberty begins in response to increased GnRH secretion. Serum LH first begins to rise disproportionately to FSH; this LH-FSH disparity is particularly evident during sleep, which is

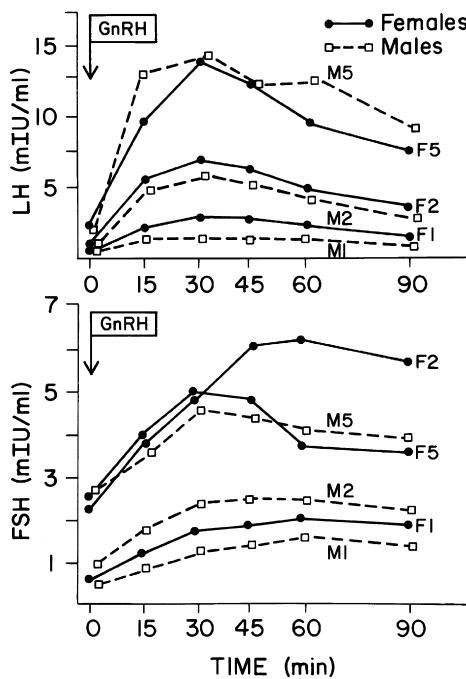


Fig. 16.9 The luteinizing hormone (LH) and follicle-stimulating hormone (FSH) responses to gonadotropin-releasing hormone (GnRH) bolus (50 mcg/kg/day) in males (M) and females (F) in prepuberty (age 5–6 years: F1, M1), early puberty (F2, M2), and later puberty (F5, M5). The responses to GnRH tend to progress with advancing puberty. However, early pubertal girls have a readily releasable FSH pool that is greater than that of more advanced adolescents. The peak responses of girls tend to be somewhat greater than those of boys at comparable stages. (Modified with permission from Ehrmann et al, Polycysticovary syndrome as a form of functional ovarian hyperandrogenism due to dysregulation of androgen secretion. *Endocr Rev*. 1995;16:322–353.)

reflected in responses to GnRH or GnRH agonist (Table 16.1). Puberty becomes clinically apparent as thelarche when estradiol levels are sustained >10 pg/mL.⁹³ It seems likely that a rise in inhibin-B as increasing ovarian follicles develop plays a key negative-feedback role in limiting further increase in FSH levels during puberty.⁷⁷ The mechanisms for differential regulation of FSH and LH are discussed later in this chapter.

Pubertal gonadotropin cycles seem to develop well before menarche^{79,84} and are capable of inducing cyclic estrogen production.^{94,95} Our working model of the nature of pituitary-ovarian dynamics in early puberty is illustrated in Fig. 16.11.

Puberty progresses as LH rises. Whereas serum FSH levels rise about 2.5-fold over the course of puberty, LH levels rise 25-fold or more.⁷⁷ The initial change in LH secretion at the beginning of puberty is a nightly increase in LH secretion that begins within 20 minutes of the onset of sleep. Subsequently, LH increases more with the onset of sleep, stays up longer, and falls less during waking hours. As the child approaches menarche, the daytime LH levels continue to increase until the diurnal rhythm is typically lost. FSH levels follow a similar pattern, although the FSH changes are less striking. The gonadotropin diurnal rhythm during puberty seems entirely related to sleep, unlike the cortisol circadian rhythm.⁹⁶ There is a delay of about 12 h between the peak LH level during sleep and the estradiol zenith, such that estradiol levels are maximal between late morning and early afternoon.^{97,98} The gonadotropin and estradiol rhythms in an early pubertal girl are shown in Fig. 16.12.⁹⁸

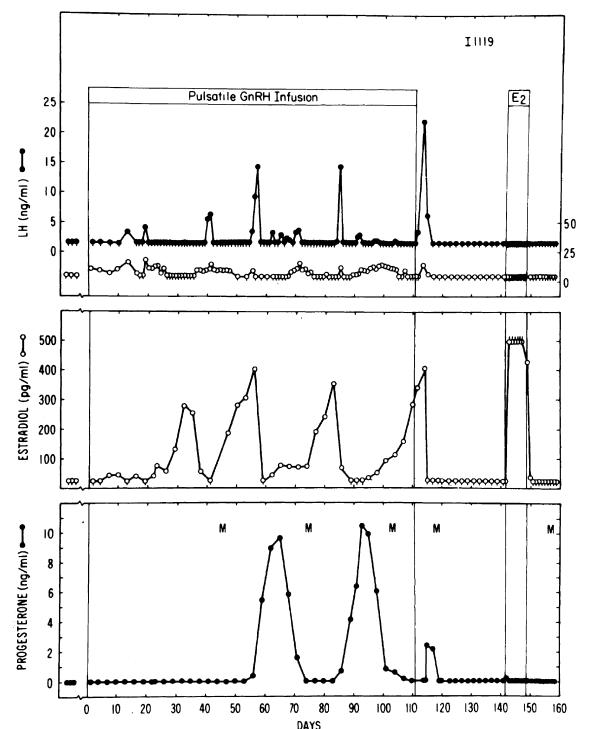


Fig. 16.10 Induction of puberty in a 13-month-old prepubertal rhesus monkey by an unvarying pulsatile gonadotropin-releasing hormone (GnRH) regimen (1 mcg/min \times 6 min hourly). Luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol (E2), and progesterone were undetectable before the GnRH infusion. On GnRH infusion, a rise in FSH was the first change detectable by midmorning sampling midway between GnRH pulses. A substantial E2 surge occurred approximately 1 month later. The subsequent LH surge was too modest to elicit ovulation, but menses (M) occurred a few days after subsidence of the week-long E2 surge—menarche resulting from an anovulatory cycle. Continuation of the GnRH led to the sustained occurrence of ovulatory menstrual cycles at 28-day intervals. An identical outcome results if an arcuate-lesioned adult animal undergoes this GnRH regimen. The third of the LH surges occurred 2 days after GnRH was discontinued. Progesterone secretion from the corpus luteum was blunted and transient in the absence of sustained LH secretion. A subsequent increase in plasma E2 produced by E2 implantation subcutaneously failed to elicit a gonadotropin surge, indicating that the animal had reverted to an immature state. Menarche eventually spontaneously recurred in such animals at the usual age (approximately 27 months). Small vertical lines beneath data points indicate values below the sensitivity of the assay. Note that gonadotropins and E2 were often undetectable (prepubertal range) during the induced puberty. (From Knobil, E. (1980). The neuroendocrine control of the menstrual cycle. *Recent Prog Horm Res*, 36, 53.)

Augmentation of the bioactivity of serum LH occurs during pubertal progression. Plasma LH bioactivity rises nearly fivefold more during the course of puberty than does LH as measured by polyclonal RIA.^{99,100} The change in bioactive LH is mirrored well by the “third-generation” monoclonal antibody-based immunometric (“pediatric”) assays that have very high specificity for bioactive LH epitopes. However, disparities in the ratio of bioactive to immunoreactive LH (B/I) persist with these assays, for reasons related to the molecular microheterogeneity of gonadotropins, which is discussed later. Serum FSH rises during puberty according to immunoassay more so than by bioassay.¹⁰¹

TABLE 16.1 Typical Female Normal Ranges for Luteinizing Hormone, Follicle-Stimulating Hormone, and Ovarian Steroids at Baseline and in Response to Adrenocorticotrophic Hormone and Gonadotropin-Releasing Hormone Agonist Tests^a

	LH (U/L)	FSH (U/L)	Estradiol (pg/mL)	Estrone (pg/mL)	Testosterone (ng/dL)	Androstene-dione (ng/dL)	DHEA (ng/dL)	17PROG (ng/dL)	17PREG (ng/dL)	DHEAS (mcg/dL)
BASELINE (8:00 AM)										
Preterm infants, 26–28 wk, day 4	0.1–175	2–200	—	—	<45	60–940	80–1485	100–2000	375–3550	125–880
Term infants, day 1	—	—	300–500	300–500	16–75	100–410	300–2600	150–850	110–3000	20–410
Term infants, day 3–7	—	—	<15	<20	<20	280	40–1300	<80	35–800	90–360
Term infants, maximum 1–6 mo	≤1.1	1.2–19	<7–55	≤20	<10–45	≤40	≤950	≤110	40–765	≤115
Children, 1–5 y	<0.15	<0.16–3.5	0.5	0.5	0.5	10–50	20–130	5–115	10–105	5–35
Children, 6–10 y	≤0.3	≤2.9	0.9	0.9	0.9	10–75	20–345	5–115	10–200	10–115
Premenarcheal pubertal, 9–13 y	≤7.2	1.1–9.0	≤55	10–35	10–35	40–175	40–600	16–220	35–350	35–130
Postmenarcheal, early follicular phase	1.5–5.6	3.6–7.9	20–85	20–50	20–60	50–200	100–850	≤130 ^b	55–360	75–255
PEAK AFTER ACTH_{1–24} (30–60 MINUTES AFTER ≥10 mcg/m² IV)										
Children, 1–5 y	—	—	—	<20	16–70	25–100	50–270	45–350	5–35	
Children, 6–10 y old	—	—	—	<20	25–100	70–320	85–300	60–650	10–115	
Premenarcheal pubertal, 9–13 y	—	—	—	10–35	55–230	70–725	90–400	150–750	35–130	
Postmenarcheal, early follicular phase	—	—	—	20–60	60–250	250–1470	35–160 ^b	150–1070	75–255	
PEAK AFTER GnRH AGONIST (LEUPROLIDE ACETATE 10 mcg/kg SC)										
Prepubertal, 6–9 y	1.2–8.9	9.3–37	≤55	—	<20 ^c	25–50 ^c	25–70 ^c	<25 ^c	—	—
Premenarcheal pubertal, 9–13 y	2.8–99	14–40	30–350	—	10–45 ^c	25–165 ^c	60–185 ^c	<155 ^c	—	—
Postmenarcheal, early follicular phase	30–135	16–60	65–260	—	10–60 ^c	50–180 ^c	60–450 ^c	30–135 ^c	—	—
Conversion multipliers to SI units				3.67 (pmol/L)	3.70 (pmol/L)	0.0347 (nmol/L)	0.0349 (nmol/L)	0.0347 (nmol/L)	0.0303 (nmol/L)	0.0316 (nmol/L)
									0.0271 (nmol/L)	

17PREG, 17-Hydroxypregnенолон; 17PROG, 17-hydroxyprogesterone; ACTH, adrenocorticotropin hormone; DHEA, dehydroepiandrosterone; GnRH, gonadotropin releasing hormone; FSH, follicle-stimulating hormone; IV, intravenous; LH, luteinizing hormone; SC, subcutaneous.

^a5th to 95th percentile for third-generation gonadotropin immunoassays and high-specificity steroid assays after preparatory chromatography, except for DHEAS. Values differ slightly among laboratories.

^b17-Hydroxyprogesterone early follicular phase baseline levels >130 ng/dL are found in women who are heterozygous for 21-hydroxylase deficiency, and they often have responses to ACTH greater than those shown. 17PROG begins rising during the late follicular phase and peaks as high as 400 ng/dL in the luteal phase of the cycle.

^cAt 1600hr after dexamethasone administration (0.5 mg po at 1200hr) to blunt coincidental adrenocortical secretion.

(Data from Rosenfield, R.L. (2007). Identifying children at risk of polycystic ovary syndrome. *J Clin Endocrinol Metab*, 92, 787–791; Rosenfield, R.L., Bordini, B., Yu, C. (2013). Comparison of detection of normal puberty in girls by a hormonal sleep test and a gonadotropin-releasing hormone agonist test. *J Clin Endocrinol Metab*, 98, 1591–1601; Mortensen, M., Ehrmann, D.A., Littlejohn, E., Rosenfield, R.L. (2009). Asymptomatic volunteers with a polycystic ovary are a functionally distinct but heterogeneous population. *J Clin Endocrinol Metab*, 94, 1579–1586; Forest, M.G. (1979). Function of the ovary in the neonate and infant. *Eur J Obstet Gynecol Reprod Biol*, 9, 145–160; de Peretti, E., Forest, M.G. (1982). Pitfalls in the etiological diagnosis of congenital adrenal hyperplasia in the early neonatal period. *Horm Res*, 16, 10–22; Bidlingmaier, F., Knorr, D. (1978). Oestrogens: physiologic and clinical aspects. *Pediatr Adolesc Endocrinol*, 4, 41–84; Chellakooty, M., Schmidt, I.M., Haavisto, A.M., Boisen, K.A., Damgaard, I.N., Mau, C., et al. (2003). Inhibin A, inhibin B, follicle-stimulating hormone, luteinizing hormone, estradiol, and sex hormone-binding globulin levels in 473 healthy infant girls. *J Clin Endocrinol Metab*, 88, 3516–3520; Greaves, R.F., Pitkin, J., Ho, C.S., Baglin, J., Hunt, R.W., Zacharin, M.R. (2015). Hormone modeling in preterm neonates: establishment of pituitary and steroid hormone reference intervals. *J Clin Endocrinol Metab*, 100, 1097–1103; Johannsen, T.H., Main, K.M., Ljubicic, M.L., Jensen, T.K., Andersen, H.R., Andersen, M.S., et al. (2018). Sex differences in reproductive hormones during mini-puberty in infants with normal and disordered sex development. *J Clin Endocrinol Metab*, 103, 3028–3037; Endocrine Sciences/LabCorp. 2018 Expected Values and S.I. Unit Conversion Tables. <https://www.endocrinesciences.com/sites/default/files/Endocrine%20Sciences%20Expected%20Values.pdf>.)

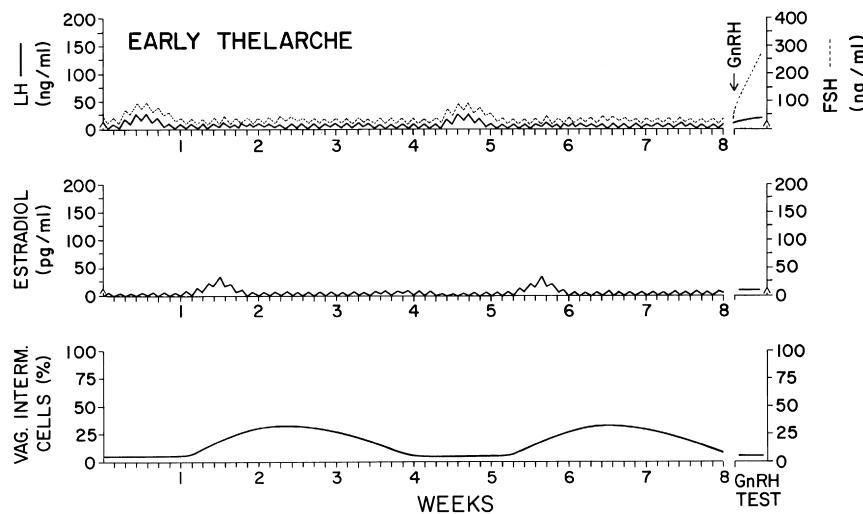


Fig. 16.11 Diagram depicting our working hypothesis of the hormonal patterns in girls during very early puberty. We conceptualize this pattern as occurring both cyclically in the earliest stage of normal puberty and occasionally in unsustained sexual precocity (i.e., most US cases of idiopathic premature thelarche). Daytime and nighttime serum concentrations of hormones (gonadotropins relative to the LER-907 standard) and the percentage of intermediate cells on vaginal smear are shown. The typical response to a gonadotropin-releasing hormone (*GnRH*) test is illustrated. Subclinical hormonal cycles lasting approximately 1 month result from a few days of increased follicle-stimulating hormone (*FSH*) and luteinizing hormone (*LH*) secretion. Because the drive to gonadotropin release is relatively weak, *FSH* and *LH* production are suppressed promptly and for long periods of time by the resultant modest amounts of estradiol (*E2*) secretion. Estradiol is detectable in plasma for only a few days a month. Maturation of the vaginal mucosa, however, is detectable for approximately 2 weeks after *E2* production has waned.

Estradiol output increases rapidly in the year approaching menarche.¹⁰² This seems to be the result of a variety of autoamplification phenomena that facilitate puberty, maturation of the dominant follicle, and ovulation. These are summarized in **Box 16.1**.^{103–116} These phenomena occur at all levels of the axis. The CNS is stimulated by preovulatory levels of

estradiol to increase *GnRH* pulse amplitude. At the pituitary level, there is the self-priming effect of *GnRH*, whereby a pulse of *GnRH* sensitizes the pituitary to have a greater *LH* response to a subsequent identical *GnRH* pulse. Critical patterns of estradiol and progesterone secretion enhance the pituitary *LH* and *FSH* responsiveness to *GnRH*. At the gonadal level, the cascade of events is augmented by the *FSH* induction of aromatase activity and progestin production in granulosa cells, phenomena in which androgens play a synergistic role. Furthermore, *FSH* stimulates granulosa cell mitosis and induces *LH* receptors, phenomena in which estradiol may play a synergistic role. Subsequently, *LH* is able to further enhance the aromatase and

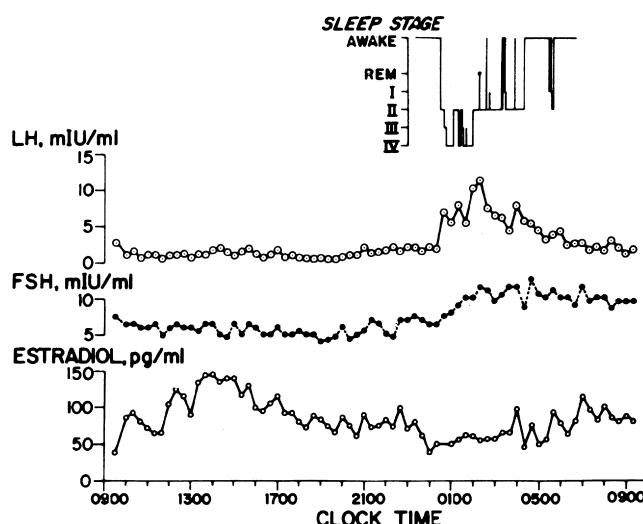


Fig. 16.12 The patterns of serum luteinizing hormone (*LH*), follicle-stimulating hormone (*FSH*), and estradiol (*E2*) typical of early female puberty. Note that daytime gonadotropin levels are in the prepubertal range. Note also the episodic nature of *LH* release at intervals of 1 to 3 hours. Estradiol levels are seen to fluctuate considerably in the course of the daytime, rising to peak levels about 12 hours after the maximum nocturnal gonadotropin surges. (From Boyar, R.M., Wu, R.H.K., Roffwarg, H., et al. (1976). Human puberty: 24-hour estradiol patterns in pubertal girls. *J Clin Endocrinol Metab*, 43, 1418.)

BOX 16.1 Autoamplification Processes Involved in Pubertal Progression, Follicle Maturation, and Ovulation^{38,99, 103–116}

Central nervous system KISS1 and *GnRH* secretion increases^{103–104} via:

- *E2*-inducing progesterone receptors¹⁰⁵
- Progesterone synergization with *E2*¹⁰⁶

Pituitary *LH* and *FSH* responsiveness to *GnRH* increases via:

- *GnRH* self-priming¹⁰⁷
- Critical patterns of *E2* secretion-stimulating *LH/FSH* responsiveness^{108,109}
- Progesterone synergization with *E2*^{109–111}
- *LH* bioactivity increases⁹⁹

Gonadal responsiveness to *FSH* and *LH* increases via:

- *FSH*-inducing aromatase and progesterone in granulosa cells: androgens and progesterone synergization with this effect^{112–114}
- *FSH*-stimulated granulosa meiosis³⁸ and *FSH*-inducing granulosa *LH* receptors; *IGF-1* synergization^{115,116}

E2, Estradiol; *FSH*, follicle-stimulating hormone; *GnRH*, gonadotropin-releasing hormone; *IGF-1*, insulin-like growth factor-1; *KISS1*, kisspeptin; *LH*, luteinizing hormone.

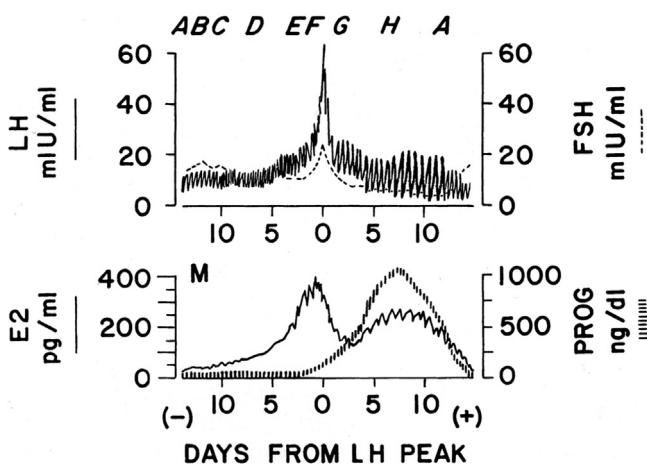


Fig. 16.13 Diagram of gonadotropin and female hormone levels during the normal menstrual cycle. The levels are centered in reference to the day of the midcycle luteinizing hormone (LH) peak (day 0). Letters A through F above the top panel correspond to stages of follicular development in **Fig. 16.14**. G and H are discussed in the text. M (bottom panel) shows time of menses. E2, Estradiol; FSH, follicle-stimulating hormone; PROG, progesterone. (Data from Abraham, G.E. (1974). Ovarian and adrenal contributions to peripheral androgens during the menstrual cycle. *J Clin Endocrinol Metab*, 39, 340; Ross, G.T., Cargille, C.M., Lipsett, M.B., Rayford, P.L., Marshall, J.R., Strott, C.A., Rodbard, D. (1970). Pituitary and gonadal hormones in women during spontaneous and induced ovulatory cycles. *Recent Prog Horm Res*, 26, 1; and Soules, M., Steiner, R., Clifton, D., Cohen, N., Aksel, S., Bremner, W. (1984). Progesterone modulation of pulsatile luteinizing hormone secretion in normal women. *J Clin Endocrinol Metab*, 58, 378.)

progesterone effects. Progesterone itself plays a synergistic role in stimulating granulosa cell progesterone and prostaglandin synthesis in concert with both FSH and LH. In the rat, ovarian GnRH receptor sites also diminish just before ovulation,¹¹⁷ and at about this time the ovary changes its pattern of metabolism so that the secretion of androstanediol- 3β -monosulfate decreases to levels that are no longer inhibitory to LH secretion.¹¹⁸

The preovulatory gonadotropin surge occurs when all these cascading processes culminate in activation of the positive feedback mechanism, a unique feature of the female neuroendocrine system.^{119,120} Positive feedback refers to the neuroendocrine system acquiring the ability to secrete a midcycle surge of LH in response to the increasing estrogen secretion by a preovulatory follicle, that is, when the ovary signals via increasing estrogen secretion that it is prepared for ovulation.

Menarche does not necessarily indicate full maturation of the neuroendocrine-ovarian axis. As the studies of Knobil illustrate (see **Fig. 16.10**), menarche can be caused by estrogen-withdrawal bleeding—and it is about half of the time—but ovulatory cycles may follow in short order. General characteristics of the mature ovary are shown in **Fig. 16.4**.

The morphology of the normal adolescent ovary has long been considered polycystic, and histological examination typically has shown thecal luteinization.^{80,121} In the perimenarcheal period, the combination of a high number of follicles and mature gonadotropin stimulation leads to a greater number of 2 to 9 mm antral follicles within a year after menarche than at any other stage (see **Fig. 16.3**).³⁰ This often leads to a "multi-follicular" ultrasonographic appearance^{82,122,123} that overlaps adult criteria for polycystic ovary morphology in one-third to one-half of normal adolescents (see section on polycystic ovary syndrome).¹²⁴

Adult

The follicular phase of each menstrual cycle recapitulates puberty in many respects. Gonadotropin and sex hormone levels are low during the premenstrual phase of the mature cycle (**Fig. 16.13A**).^{125,126} Gonadotropin concentrations then increase at the time of menstruation, FSH predominating in the early follicular phase, whereas nocturnal LH pulsation is slow¹²⁷ (**Fig. 16.13B**). LH pulsation increases to a circannual pattern around a stable baseline, and estradiol production slowly begins as antral follicles develop (**Fig. 16.13C**). Estradiol levels gradually increase and serum FSH levels fall reciprocally (**Fig. 16.13D**). Upon formation of a dominant follicle, serum estradiol concentrations increase geometrically. This selectively begins to amplify the pituitary's LH response to GnRH as estradiol reaches about 90 pg/mL for over 3 days^{109,110,128} (**Fig. 16.13E**).

When the serum estradiol rises to over 200 to 300 pg/mL for 36 hours, the positive feedback mechanism is activated and the midcycle gonadotropin surge commences (**Fig. 16.13F**). Estradiol then appears to induce PR expression in the hypothalamus and pituitary.¹²⁹ An increase in progesterone to 100 ng/dL facilitates the LH surge, shortens the duration of time over which estradiol is required for the surge to 24 hours, and brings about an FSH surge. The mechanism of progesterone action involves inhibition of GnRH cleavage.¹¹¹ Androgens may also play a role in facilitating FSH and GnRH release.^{130,131} The LH surge is then primarily responsible for luteinizing the preovulatory ovarian follicle (see **Fig. 16.13F**). At this time, LH pulses become larger in amplitude but slower in frequency and their apparent bioactivity increases. Ovulation then results.

As the follicle is disrupted by ovulation, estrogen levels fall (**Fig. 16.13G**). Meanwhile, serum progesterone increases steadily as the corpus luteum begins to form, and comes to be sustained at very high levels for several days, along with a lesser increase in 17-hydroxyprogesterone (17-OHP) and a return of estradiol to late follicular phase levels (**Fig. 16.13H**).^{125,126,132} In response to the high progesterone level, LH pulses become slow and large.^{127,132} In the absence of increasing human chorionic gonadotropin (hCG) from a conceptus, the corpus luteum's life span is exhausted and its production of progesterone and estradiol wanes. Subsequently, FSH begins to rise out of proportion to LH. Shortly after the sex steroids withdraw from the scene, the endometrium sloughs, giving rise to menstrual flow. Meanwhile, the follicular growth induced earlier by FSH begins to gain momentum and the next cycle begins.

Follicular (Proliferative) Phase Ovary. The hormonal functions of the follicle have dual purposes that must be closely coordinated: to change the milieu of the ovum to prepare for ovulation and to signal the pituitary to send the signal to ovulate, that is, the LH surge. Thus the ovary is the zeitgeber for the cycle; the normal cyclic pattern of ovarian hormone secretion induces the midcycle surge of pituitary gonadotropins, even in the presence of unchanging circannual pulses of GnRH.⁹⁰ Ovarian hormones also augment the amplitude of the GnRH response,^{103–106} which is a "fail-safe" mechanism that "guarantees" a preovulatory gonadotropin surge.

Ovarian follicular development and steroid secretion in relationship to changing gonadotropin levels are illustrated in **Fig. 16.14**.^{38,133–135} FSH and LH play major roles in granulosa and thecal cell differentiation, respectively, whereas a host of local factors modulate gonadotropin action. For example, follicular maturation in response to gonadotropins is enhanced by insulin-like growth factors (IGFs), TGF- β , and fibroblast growth factor, whereas it is inhibited by TGF- α .

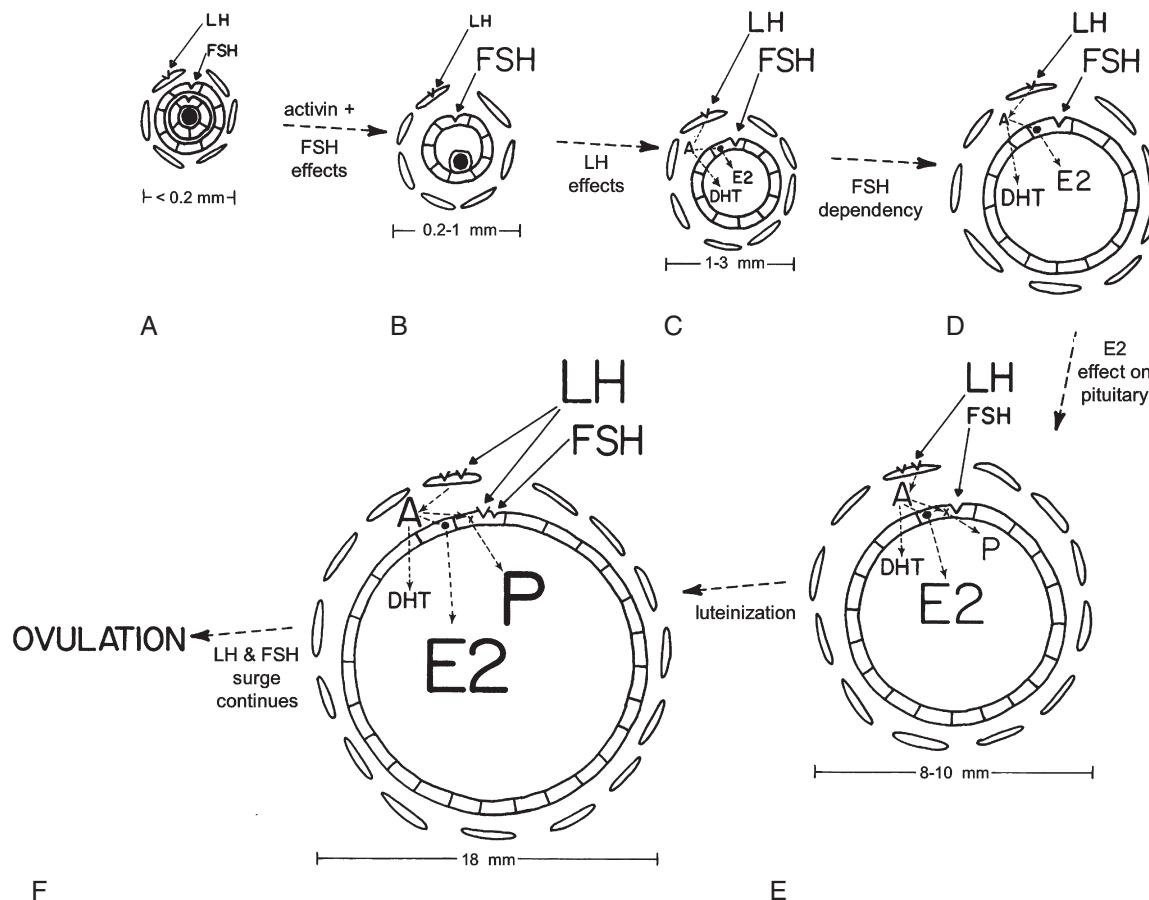


Fig. 16.14 Relationships among gonadotropins, the ovarian follicle, and ovarian steroids according to the two-cell two-gonadotropin model of ovarian steroidogenesis. A through F: Stages of ovarian follicular development found during the times of the menstrual cycle designated by the corresponding letters on Fig. 16.13. The size of the letters designating hormones relates to the magnitude of their serum and/or follicular concentrations. (A) Preantral follicle with luteinizing hormone (*LH*) and follicle-stimulating hormone (*FSH*) receptors in theca and granulosa cells, respectively. There is no antrum surrounding the ovum (*stippled in center*). (B) Small antral follicle. Activin upregulates *FSH* receptors, and *FSH* receptor activation is required to initiate antrum formation. (C) Larger antral follicle (≥ 1 mm). Aromatase activity (A) has been induced in granulosa cells. Interactions between theca and granulosa cells, the former producing androgens (androstenedione [A]), result in increasing estradiol [E2] and dihydrotestosterone [DHT] synthesis. (D) *FSH*-dependent granulosa cell multiplication (not shown) is responsible for more follicular growth and more E2 synthesis. (E) Estradiol enhances pituitary *LH* secretion in response to GnRH, while at the same time inhibiting pituitary *FSH* secretion. The increased *LH* induces more theca *LH* receptors and stimulates androgen production. Androgens serve as a substrate for E2 formation and synergize with *FSH* to stimulate progesterone (P) secretion. (F) In the preovulatory follicle, *FSH* induces *LH* receptors on the granulosa cell, which completes luteinization. Steroid secretion is augmented further. Then, increasing progesterone amplifies the positive feedback effect of E2 to initiate the preovulatory gonadotropin surge.

Primordial follicle growth and development is gonadotropin independent. Subsequently, granulosa cells of preantral follicles develop *FSH* receptors, and theca cells, which encircle granulosa cells, develop *LH* receptors (see Fig. 16.14A). Activin causes *FSH*-independent upregulation of *FSH* receptors in preantral follicles,¹¹⁶ although it opposes *FSH* stimulation of antral follicle development.³⁸ Primordial follicle growth is constitutively repressed by nuclear forkhead transcription factor Foxo3; when Foxo3 is released in response to stimulation of the PTEN-PI3K-Akt pathway, follicular growth progresses to the point where follicles become responsive to *FSH*.¹³⁶

Antrum formation requires a trace (prepubertal) amount of *FSH* receptor activation (see Fig. 16.14B).^{38,137–140} *FSH* stimulates androgen receptor expression in primary follicles, and androgens in turn stimulate further expression of *FSH* receptors and the early stages of follicular growth.¹⁴¹ Androgen action is also necessary for the development of a full complement of follicles, and androgen excess stimulates excessive follicle number.^{142,143} *LH* stimulates the appearance in thecal cells of the enzymes necessary for androgen biosynthesis.¹⁴⁴ Evidence that theca cells of small antral follicles form estradiol is meager.¹⁴⁵

As antral follicles grow over 2.5 mm in diameter, their granulosa cells begin to form estradiol from androgen supplied by theca cells (see Fig. 16.14C).^{146–150} Androgen production at low levels may synergize with *FSH* to stimulate aromatase activity within the granulosa cells.^{112,151,152}

At this stage, follicles are increasingly *FSH* dependent and consequently uniformly *FSH* responsive.^{38,137} IGF-1 is required for follicular growth beyond the early antral stage in response to *FSH*.¹⁵³ Antral follicles do not grow over 5 mm in diameter without a pubertal degree of *FSH* stimulation.¹³⁹ By the midfollicular phase, the proliferation of *FSH*-responsive granulosa cells results in an accelerating rate of estradiol production and preferential conversion of androstenedione to estradiol rather than dihydrotestosterone (DHT) by these cells (see Fig. 16.14D).^{146–148,150,154,155} Estradiol itself clearly stimulates proliferation of granulosa cells and oocyte survival in rodents.¹⁵⁶ In humans, estradiol appears to promote antral growth independently of *LH*,¹⁵⁷ and is synergistic with *FSH* in bringing about the development of the dominant follicle.^{158,159}

A dominant follicle is selected at the beginning of the menstrual cycle from a crop of follicles that were recruited

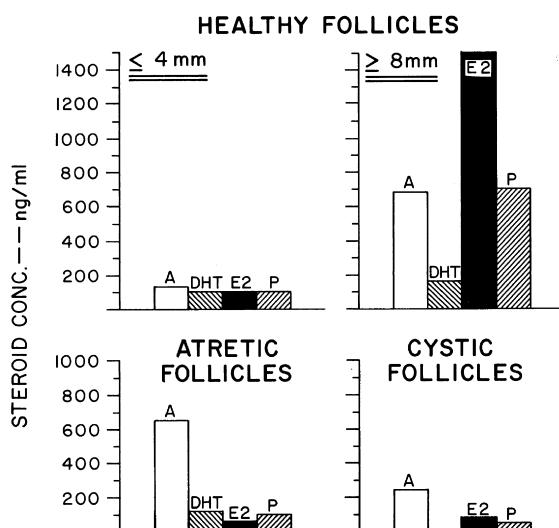


Fig. 16.15 Normal human antral fluid steroid concentrations. Healthy follicles are well populated by granulosa cells (50% of maximal complement). Healthy follicles seem capable of further development because many of them (75%) contain healthy-appearing oocytes (histologically intact germinal vesicles), 96% of which are viable in culture. Moderately large follicles (8 mm or larger in diameter) make their appearance only in the midfollicular phase of the cycle and contain follicle-stimulating hormone. Data are shown only for those large follicles well populated by granulosa cells, only one of which usually arises in the follicular phase of each menstrual cycle. Atretic follicles are small follicles beginning to show degenerative changes in the number of granulosa cells and appearance of the oocyte. Cystic follicles tend to be larger follicles with only a sparse granulosa cell lining. The testosterone content of antral fluid is about a third that of dihydrotestosterone (DHT) owing to the pattern of granulosa cell metabolism of androstenedione (A). E2, Estradiol; P, progesterone. (Data from McNatty, K.P., Makris, A., Reinhold, V.N., De Grazia, C., Osathanondh, R., Ryan, K.J. (1979). Metabolism of androstenedione by human ovarian tissues in vitro with particular reference to reductase and aromatase activity. *Steroids*, 34, 429–443.)

2.5 months prior.³⁸ Recruitment of a group of follicles is normally promoted by the midcycle FSH surge and regresses with increasing corpus luteum progesterone secretion. Another wave of follicle growth in the late luteal phase is promoted by the rise of FSH as luteal progesterone and estradiol secretion wanes. The selected follicle is the one that is the most sensitive to FSH (lowest "FSH threshold"). FSH is critically important during the follicular phase for optimal development of this dominant follicle. By the midfollicular phase of the cycle this follicle becomes virtually the sole source of estradiol (see Fig. 16.14E). Typically, there is only one such follicle. Only this follicle continues to grow so as to reach a diameter of 10 mm or more. All other gonadotropin-dependent follicles undergo atresia.

At this stage, the rising estradiol level is suppressing FSH secretion and augmenting pituitary LH responsiveness to GnRH. FSH is more bioactive in the dominant follicle because it is more efficiently concentrated¹⁵⁴ and because local factors increase ovarian responsiveness to FSH. The increased LH causes further proliferation of thecal cells and an increase in their LH receptor content.¹¹³ Androgen production is consequently increased. This synergizes with FSH to both augment aromatase activity and bring about increasing progesterone secretion by the well-estrogenized granulosa cells of these follicles. Progesterone then enhances the synthesis of both itself and estradiol.^{113,115} The increased thecal androstenedione production is diverted much more to estradiol than to DHT biosynthesis. Antral fluid steroid concentrations reflect these changes (see Fig. 16.15).^{146,147,154}

Activin acts to prevent premature luteinization of granulosa cells, and activin tone seems to wane as the preovulatory phase approaches.^{38,116}

FSH next induces LH receptors in the granulosa cells, luteinizing them (see Fig. 16.14F).⁴⁰ Androgen and insulin synergize with FSH in this induction of LH receptors. LH then joins FSH in acting on luteinized granulosa cells to augment estradiol and progestin production.

The LH and FSH surge then occurs in response to the positive feedback action of estradiol at both the CNS and pituitary levels, an effect amplified by the rising levels of progesterone. The final steps in follicle maturation ensue rapidly: the LH surge induces granulosa cell PR and prostaglandin synthase while inhibiting cyclin gene transcription,^{26,160} and the FSH surge upregulates vascular endothelial growth factor.¹⁶¹ In the absence of these critical steps, ovulation and follicular rupture do not occur. Then the follicle promptly becomes desensitized to LH and FSH and ceases to grow.¹⁶² This is followed by an inflammatory-type response. Protease activity, prostaglandin production, and vascular permeability increase; cell junctions loosen; and cumulus cells form a mucopolysaccharide envelope around the oocyte (cumulus expansion).

Oocyte meiotic maturation resumes in response to a specific phosphodiesterase,¹⁶³ forming the haploid gamete (secondary oocyte) and the first polar body in response to the LH surge.¹⁶⁴ Ovulation of the cumulus-oocyte complex then occurs. The presence of a favorable follicular steroid milieu is necessary both for ovulation (a premature LH surge in a subject with an unripe follicle will not result in ovulation) and subsequent developmental competence of the oocyte.^{165,166} Meiosis will go to completion and the second polar body will be extruded only in response to contact with a sperm.

The processes stimulating dominant follicle emergence are delicately balanced by those preventing it. It seems critical that the intraovarian concentration of androgens not become excessive. Androgen excess interferes with follicle viability beyond about the 8-mm stage¹⁴⁸ and synergizes with FSH to cause premature luteinization.⁴⁰ These interfere with the emergence of dominant follicles. Follicles arrested in their growth become atretic, and atretic follicles contain relatively high concentrations of androgens (see Fig. 16.15). Progesterone also suppresses further differentiation of nondominant follicles¹⁶⁷ by some of the same mechanisms.¹⁶⁸ High concentrations of estrogen play a critical role in inhibiting selection of the dominant follicles in primates.¹⁶⁹ If there is interference with estrogenization, multiple large cystic follicles develop that are impaired in their ability to ovulate and undergo androgen-dependent atresia.^{170–172}

Anti-Müllerian hormone (AMH) and inhibins have emerged as other granulosa cell factors important in the regulation of follicular development. AMH is the major hormonal paracrine inhibitor of primordial follicle progression.⁴⁰ It is produced by the granulosa cells of small growing follicles. As follicles grow, intrafollicular AMH levels rise sufficiently to inhibit recruitment of primordial follicles to the primary follicle stage; it also inhibits P450c17 activity, GnRH release, and FSH stimulation of aromatase activity.^{40,173} Because estradiol inhibits AMH production,^{174,175} there exists an intrafollicular short negative feedback loop confining AMH expression to follicles up to about 8 mm in diameter. Thus AMH appears to act as a follicular gatekeeper, ensuring that each small antral follicle produces little estradiol before selection of the dominant follicle, which allows a direct ovarian-pituitary dialogue regulating the development of the follicle selected to undergo ovulation.¹⁷⁶

Inhibin-B is the predominant form of inhibin.^{177,178} It arises from granulosa cells in small follicles before aromatase is expressed¹⁷⁹ and is regulated by FSH in a sluggish negative feedback loop. It upregulates thecal steroidogenesis, as discussed later. Inhibin-A is a product of the preovulatory follicle (and corpus luteum) that responds to both LH and FSH.

Atresia is the fate of all except the few hundred follicles chosen for ovulation during an individual's life span. Most follicles beyond the primordial stage become atretic. Atresia occurs by the process of programmed cell death.³⁸ This apoptotic process has diverse determinants, including cell death inducer and repressor genes.^{135,180} FSH support becomes increasingly necessary for survival as the follicle matures, and it is normally only the follicle that has the lowest FSH threshold that escapes atresia.

Luteal (Secretory) Phase Ovary. Ovulatory rupture of the dominant follicle (see Fig. 16.13G) is followed by invasive proliferation of capillaries and fibroblasts from the theca that breaks down the separating basement membrane. The luteinized granulosa and theca cells then intermingle and complete the luteinization process by forming the corpus luteum.¹⁸¹

Histologically, luteinization is a process of lipid droplet accumulation that begins as the dominant follicle forms. The biochemical hallmark of the luteinized granulosa cell is the acquisition of LH receptors, with the subsequent capacity to form progesterone, 17-OHP, and estrogen in response to LH/hCG.^{182–184}

During its functional life span, the corpus luteum is normally the major source of the sex hormones secreted by the ovary. Corpus luteum function reaches its peak about 4 days after ovulation and begins to wane about 4 days before menstruation (see Fig. 16.13H). Loss of sensitivity to LH and estradiol heralds luteal senescence. Regression of the corpus luteum—luteolysis—occurs if pregnancy does not provide hCG. Luteolysis is probably mediated by prostaglandins. Transformation of the corpus luteum into an avascular scar, the corpus albicans, then occurs.

Early luteal phase increases in secretion of both estradiol and progesterone cause secretory transformation and hyperplasia of the endometrium, which is necessary for implantation of the fertilized egg. Later falloff in secretion of female hormones to a level insufficient to maintain the endometrium results in menstruation (see Fig. 16.13A). Withdrawal of progesterone is specifically responsible for constriction of spiral arteries, local prostaglandin accumulation, and subsequent ischemic necrosis of the endometrium. Normal menstrual flow then results from a complete slough of the secretory endometrium.

Documentation of ovulation can be accomplished by demonstrating collapse of the dominant follicle by ultrasonographic monitoring¹⁸⁵ or by assessing the luteal transition in the estradiol/progesterone ratio,¹⁸⁶ detecting the LH surge,¹⁸⁷ or demonstrating a normal midluteal phase rise of serum progesterone either directly¹⁸⁵ or indirectly by a rise in basal body temperature.¹⁸⁸ A significant rise in basal body temperature, averaging 0.55° C, usually occurs when serum progesterone reaches 400 ng/dL or more and continues as long as that level is maintained. While the results of these methods are correlated, LH surges are sometimes inadequate to stimulate a follicle sufficiently mature to develop into a normally functioning corpus luteum, particularly during adolescence^{124,189} (see Luteal Phase Defects).

Regulation of the Neuroendocrine-Ovarian Axis

Factors Controlling the Onset of Puberty

Pubertal onset is under the control of a complex regulatory network that is able to dynamically respond to numerous endogenous and environmental signals. GnRH neurons play a critical hierarchical role in the direct and indirect integration of these central and peripheral signals. Reproductive development is coupled with metabolic cues that may disrupt the maturation process. The mechanisms by which neuroendocrine and genetic factors control pubertal development remain unknown. Epidemiological studies indicate that nutrition, ethnicity, and genetic factors, are normally important in the pubertal process.¹⁹⁰ Environmental chemicals and chronic inflammatory disease can disrupt the process.^{190–193}

Evidence that there are genetic factors involved in the timing of puberty comes from multiple studies.^{194–204} It has been estimated that between 50% and 80% of the variation in the timing of puberty is genetically determined. Several large genome-wide association studies (GWAS) of age at menarche, examined pubertal timing in healthy females^{205–207} to identify the genes responsible. These studies demonstrated that there is significant genetic heterogeneity in pubertal timing in the general population that is likely to involve hundreds of common variants. The gene Lin-28 homolog B (*LIN28B*)²⁰⁸ was the first locus associated with age of menarche. *LIN28B* is the human ortholog of the *Caenorhabditis elegans* gene that controls developmental timing through micro ribonucleic acid (microRNA). Mutations in *LIN28B* have not been identified in humans with disorders of puberty.^{209,210} The 1000 Genomes Project studied genotype data in about 370,000 women and identified 389 independent signals ($P < 5 \times 10^{-8}$) for age at menarche,²¹¹ with effect sizes per allele ranging from 1 week to 5 months. These signals explain only about 7.4% of the population variance in age at menarche. Genes implicated in GnRH signaling, pituitary development, hormonal regulation, fatty acid biosynthesis, and energy homeostasis have been implicated.^{207,212–215} Although mutations in these genes have been shown to cause physiological interruptions in development, their role in the initiation of puberty remains unknown. Specifically, single nucleotide polymorphisms (SNPs) in the GnRH and GnRH receptor genes have not been associated with variations in the timing of puberty in the general population.²¹⁶

The key in the initiation of puberty is the activation of the hypothalamic GnRH pulse generator. The molecular events that control the pulse generator include a complex interplay between both inhibitory and stimulatory factors. The mechanism of central activation of puberty first appears to be a consequence of a removal of a restraint mechanism, with a rise in gonadotropin secretion (initially during sleep).²¹⁷ This restraint in the GnRH pulse generator is independent of the presence of gonads⁷⁰ and more intense in males.²¹⁸ A targeted gene approach in mice has confirmed that ER α (also termed ESR1) in Kiss1 neurons mediates feedback suppression of both Kiss1 expression and gonadotropin secretions during the prepubertal period.²¹⁹

However, the high levels of testosterone to which the male fetus was exposed during the period of sexual differentiation may be responsible for the more prolonged suppression of GnRH release in males than females. A role for decreased estrogen feedback sensitivity by the hypothalamic pulse generator near the time of puberty has also been shown.²²⁰

Recent evidence points to an important role for the kisspeptin 1 receptor (*KISS1R*), a G-protein-coupled receptor (previously known as GPR54), and its ligand, kisspeptin, an excitatory neuropeptide, as a signal for pubertal GnRH release. Expression of both proteins has been found to increase before pubertal onset in association with the increase in GnRH pulse generator activity in the hypothalamus.²²¹ Kisspeptin binding to its receptor on GnRH neurons stimulates GnRH secretion. Mice with knockout of *Kiss1r* were found to be infertile despite having normal GnRH neurons.^{222,223} Leptin and androgen synergistically upregulate this system, and estrogen antagonizes it.²²⁴ Mutations in *KISS1R* result in hypogonadotropic hypogonadism.^{213,222,225} However, mutations in *KISS1R* have not been found in boys with pubertal delay, nor have polymorphic sequences been associated with delay of pubertal development.²²⁶ Elegant studies in primates have demonstrated an increase in kisspeptin during pubertal development with a corresponding increase in *KISS1R* associated with an increase in LH. The maximum level of expression of kisspeptin and *KISS1R* in the hypothalamus in both males and females occurs at puberty.^{227,228} For each Tanner stage, girls tend to have higher kisspeptin levels than boys, potentially explaining their earlier onset of puberty.²²⁹

Chronic administration of kisspeptin to immature female rats induces precocious activation of the central axis.²²⁷ In addition, chronic treatment with kisspeptin restores pubertal development in a rat model of undernutrition.²³⁰ Kisspeptin may thus not only influence the priming of puberty, but also the integration of nutritional and energy status.²³¹ Although it is clear that kisspeptin activation of GnRH neurons occurs at puberty and that GnRH is increasingly sensitive to kisspeptin activation during development,^{232,233} other pathways contribute to GnRH activation since the hypogonadism associated with deficiency of *KISS1* or *KISS1R* is not complete.²³⁴

Neurokinin B (NKB) signaling seems to be critical for the initiation of puberty.²³⁵ Some kisspeptin neurons, KNDy neurons, coexpress NKB, dynorphin A, and their receptors (TAC3R and KOR), the primary function of which seems to be synchronizing kisspeptin neuron pulsatility.²³⁶ Receptors for NKB are also located on GnRH neurons, where they seem to modulate GnRH release or transport.²³⁷ Loss-of-function mutations in TAC3 and its receptor *TACR3* in patients with normosmic GnRH deficiency and pubertal failure²³⁸ have identified a role for this neuropeptide in the control of GnRH secretion. Although kisspeptin directly regulates GnRH expression and secretion, NKB agonists failed to stimulate GnRH release in rodents. It appears most likely that a collaborative mechanism that includes both kisspeptin and NKB signaling to GnRH neurons is necessary for reproductive function in females.^{239,240} To investigate the interactions of kisspeptin and NKB in humans,

the effects of the coadministration of kisspeptin-54, NKB, and an opioid receptor antagonist, naltrexone, on LH pulsatility were studied. Subjects receiving kisspeptin and naltrexone increased LH and LH pulsatility, whereas NKB alone did not affect gonadotropins. NKB and kisspeptin given together had significantly lower increases in gonadotropins compared with kisspeptin alone. These results suggest significant interactions between the KNDy neuropeptides on GnRH pulse generation in humans.²⁴¹ Further, *Tacr3* knockout mice are infertile,^{242,243} although they appear to have reversible central hypogonadism.²⁴⁴ Interestingly, a mutation in *TAC3R* was found in one patient with constitutional delay of growth and pubertal development (CDGP) in a study of 50 patients,²⁴⁵ whereas none have been reported in *TAC3*.

Disrupting mutations in makorin ring finger protein 3 (MKRN3), a paternally expressed, imprinted gene located in the Prader-Willi syndrome locus, are associated with central precocious puberty.²⁴⁶ This indicates the presence of a GnRH release-inhibiting pathway centered in the arcuate nucleus.

Initiation of puberty involves coordinated changes in transsynaptic and glial-neuronal communication.²⁴⁷ Mediating pubertal restraint are the major inhibitory systems: gamma-aminobutyric acid (GABA)ergic,²⁴⁸ some opioidergic contribution,²⁴⁹ and gonadotrophin-inhibiting hormone (GnIH), an RFamide-related peptide (RFRP).²⁵⁰ The major excitatory systems involve glutamate and kisspeptin signaling, with glial cells facilitating GnRH secretion in diverse ways (Fig. 16.16).^{236,247}

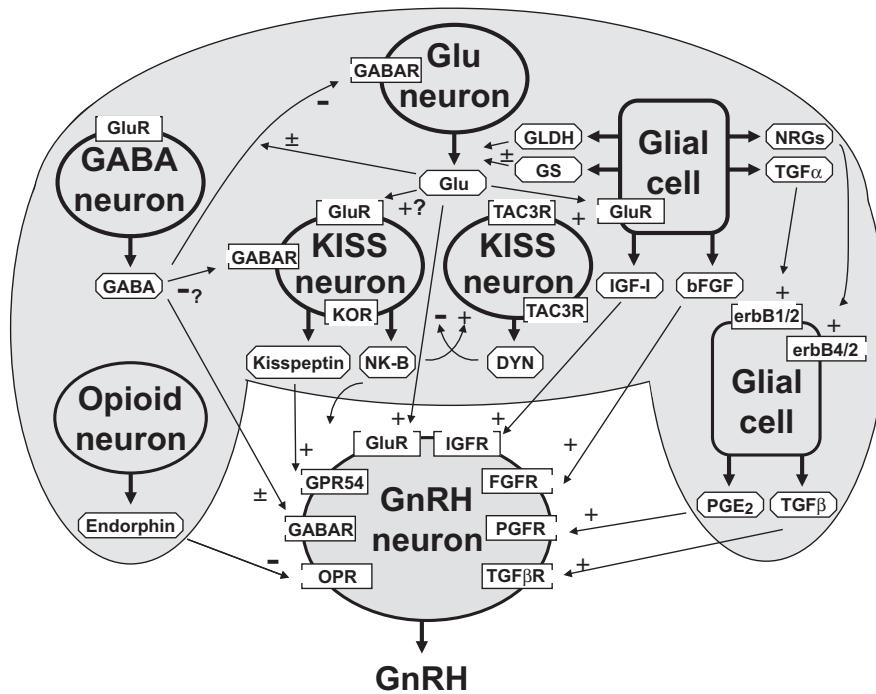


Fig. 16.16 The molecular biological basis for the major known proximate hypothalamic pathways regulating gonadotropin-releasing hormone (GnRH) secretion. The left-hand column depicts the major inhibitory pathways, which involve gamma-aminobutyric acid (GABA) signaling through the GABA receptor and opioidergic signaling through the endorphin receptor (OPR). The central column depicts the major excitatory pathways, which involve glutamate (Glu) signaling through the family of glutamate receptors and kisspeptin (KISS) signaling through GPR54. The right column shows the major glial factors that facilitate GnRH release. These include the elaboration of the enzymes glutamic dehydrogenase (GLDH) and glutamine synthase (GS), which regulate the concentration of glutamate and the elaboration of a variety of growth factors. Most kisspeptin neurons coexpress neurokinin B (NK-B), dynorphin A (DYN), and their receptors (TAC3R and KOR), the primary function of which seems to be synchronizing kisspeptin neuron pulsatility; receptors for NKB are also located on GnRH neurons. +, Positive stimulation; -, inhibition; ±, either; ?, unknown; bFGF, basic fibroblast growth factor; erbB 1-4, subunits for the TGF- β and NRG receptors; IGF-1, insulin-like growth factor 1; NRG, neuregulins; PGE, prostaglandin E; R, receptor; TGF β , tumor growth factor β . (Modified from Ojeda, S.R., Lomniczi, A., Mastronardi, C., et al. Minireview: the neuroendocrine regulation of puberty: is the time ripe for a systems biology approach? *Endocrinology*, 147, 1166–1174).

It appears that GABA receptor signaling develops in advance of glutamate signaling.²⁵¹ Increased signaling via glutamate receptors of several types (ionotropic and metabotropic) appears to be the major proximate change in neurotransmission involved in puberty onset.^{217,218,247} At puberty, however, seemingly as a consequence of glutamate receptor signaling, GABA-A receptor signaling on GnRH neurons increases GnRH secretion.^{217,221,252,253} Glial cells facilitate the process through elaboration of TGFs (especially TGF- β 1), IGF-1, neuregulins, prostaglandin E₂, and the elaboration of enzymes that control the concentration of glutamate (glutamic dehydrogenase, which catalyzes the synthesis of glutamate, and glutamine synthase, which converts glutamate to glutamine).²⁵⁴

The basis of the change in neurotransmitter balance is becoming clearer. A second tier of control seems to be modulation of these processes by increased hypothalamic expression at puberty of tumor-suppressor genes that act to integrate glial-neuronal interactions. A yet higher echelon of candidate hypothalamic genes have been identified that are transcriptional regulators of the second-tier genes. These genes include *Oct-2*, a regulator of the POU-domain homeobox genes, enhanced at puberty 1 (*EAP1*), knock-out of which delays puberty and decreases fertility of mice, thyroid transcription factor I (*TTF1*), yin yang 1 (*YY1*), and *CUX1*.²⁵⁵ Genes contiguous to elastin appear to be involved in the pace of puberty: deletion of chromosome 7q11.23 in Williams syndrome typically leads to an early normal onset but rapid pace of puberty with an abbreviated pubertal growth spurt.²⁵⁶ Substantial redundancy of these networks and the signaling neurochemicals exists since the onset of puberty is dependent on the expression of many genes, likely arranged in a coordinated network. The gene products may function as activators or repressors of targets important for pubertal onset and progression. Sex steroids have been implicated as important modulators in pubertal onset.²⁵¹

MicroRNAs, specifically the miR-200/429 family and miR-155, have been shown to be important in the epigenetic regulation of puberty by regulating GnRH gene transcription.²⁵⁷ miR-7a2 is critical for normal pituitary development and deficiency results in gonadotropin deficiency.²⁵⁸

Thus the onset of puberty is controlled by an opposing increase in excitatory and a corresponding decrease in inhibitory signaling from neural networks targeting the GnRH neuron. Lesioning studies indicate that inhibitory tracts mainly seem to be routed through the posterior hypothalamus and stimulatory ones through the anterior hypothalamic preoptic area.^{1,259} These studies have been complemented by studies in genetically engineered mouse models. In one such model, the anteroventral periventricular nucleus (AVPV) population of neurons was shown to be the site of estrogen positive feedback in the control of pubertal progression, and kisspeptin cells in the arcuate nucleus of the hypothalamus were shown to be critical for estradiol negative feedback.²⁶⁰ Indeed, it appears that KNDy neurons integrate negative feedback of sex steroids to regulate GnRH secretion.^{261,262} Postmortem hypothalamic tissues were collected by The Netherlands Brain Bank, and sections were stained for kisspeptin by immunohistochemistry to determine the number of kisspeptin-immunoreactive neurons within the infundibular nucleus. This study showed that the number of kisspeptin neurons is greater in the infant/prepubertal and elderly periods compared with the adult period. In MTF transsexuals, but not homosexual men, female-typical kisspeptin expression was observed. The authors suggest that infundibular kisspeptin neurons are sensitive to circulating sex steroid hormones and that the sex reversal observed in MTF transsexuals might in part reflect an atypical brain sexual

differentiation.²⁶³ Neonatal androgenization, which ablates the ability to generate a midcycle LH surge, was shown to selectively inhibit development of the AVPV population of kisspeptin neurons.²³⁶

An overview of the systems involved in regulating the initiation of puberty is shown in Fig. 16.17. Pubertal maturation and skeletal maturation seem to have common determinants.

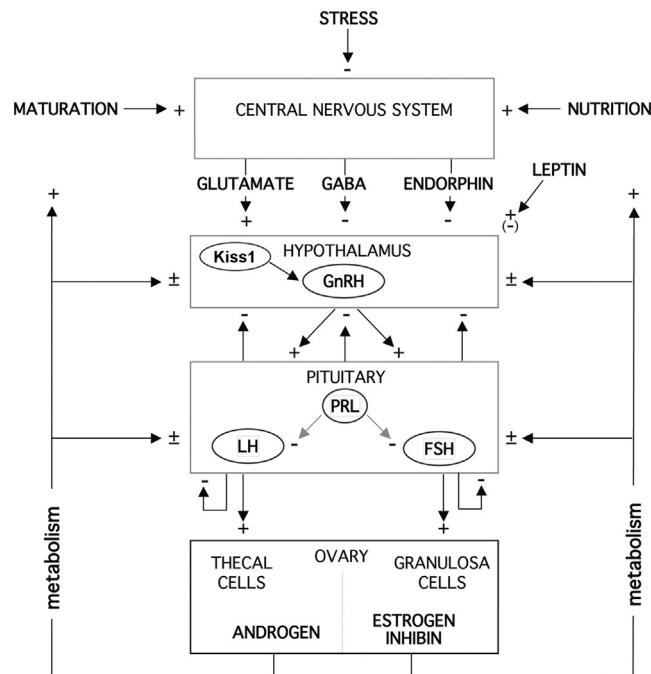


Fig. 16.17 Diagram of the major mechanisms controlling the development and function of sex hormone secretion by the unripe antral follicle. Regulation may be either stimulatory (+) or inhibitory (-). The central nervous system (CNS) influences kisspeptin (KISS1) and gonadotropin-releasing hormone (GnRH) secretion both negatively and positively. For the CNS to relinquish its inhibitory control over GnRH secretion, it must achieve a high level of maturity. Even after this is achieved, psychological or physical stress may negatively influence the system. Nutrition must be optimal. Leptin is a critical mediator of the nutrition effect. Sex steroids have a maturing effect. Whether efferent tracts from the hypothalamus to the cerebrum play a role in reproductive function is unknown. Pineal secretion of melatonin and other substances are known to exert inhibitory influences on GnRH in lower animals (not shown). Kisspeptin stimulates GnRH, which in turn stimulates luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Paracrine and autocrine feedback of the gonadotropins on GnRH release and on their own release, respectively, are shown. Prolactin (PRL) has multiple effects on gonadotropin secretion. In unripe antral follicles, LH acts on thecal and interstitial cells and FSH acts on granulosa cells. Androstenedione and testosterone secreted by the theca cells are aromatized by the granulosa cell, under the influence of FSH, to estradiol. The granulosa cell is also the site of production of the FSH inhibitor inhibin B. Estradiol has a biphasic effect on the mature pituitary and on hypothalamic GnRH release as well. Androgens seem normally to be of minor importance in regulating gonadotropin release in females. Intraovarian mechanisms seem to modulate LH action so as to coordinate thecal formation of androgens with granulosa cell formation of estrogens. Paracrine and autocrine factors, including insulin-like growth factors, are involved. GABA, Gamma-aminobutyric acid.

Abundant clinical evidence indicates that sex steroid hormones are among these determinants.^{264,265} Thus genes involved in sex steroid hormone metabolism and action are candidate regulators of the onset of puberty. There is limited and inconsistent data on the role of endocrine-disrupting chemicals on the timing of puberty, although some animal and epidemiological evidence supports the potential for some compounds to accelerate the time of pubertal onset and for others to delay the timing.^{190–192,266} Experience with diethylstilbestrol indicates that fetal exposures can have epigenetic effects.²⁶⁷ The growth hormone (GH)-IGF system is another determinant. GH facilitates the onset and tempo of puberty.²⁶⁸ Experimental studies suggest that this occurs through GH or IGF actions at all levels of the neuroendocrine-ovarian axis.^{269,270} Girls generally enter puberty when they achieve a pubertal bone age. Pubertal stage normally correlates better with the bone age ($r = 0.82$) than with the chronological age ($r = 0.72$, RLR unpublished data), particularly as menarche approaches.²⁷¹ Skeletal age correlates better with menarche than chronological age, height, or weight, and its variance at menarche is half that of chronological age.²⁷² The bone age at the onset of breast development averages about 10.75 years, and that at menarche averages about 13.0 years. Disorders that accelerate bone maturation, such as congenital adrenal hyperplasia (CAH) or hyperthyroidism, tend to advance the age of onset of true puberty.²⁷³ Disorders that retard skeletal maturation, such as GH deficiency, hypothyroidism, or anemia, tend to delay the onset of puberty.²⁷⁴ On the other hand, some data suggest that factors linked to intrauterine growth retardation, although not necessarily the growth retardation itself, predispose to sexual precocity.¹⁹⁰

Optimal nutrition is clearly necessary for initiation and maintenance of normal menstrual cycles. The hypothesis that body fat is the weight-related trigger for pubertal development originated with the discovery by Frisch and coworkers that weight correlated with initiation of the pubertal growth spurt, peak growth velocity, and menarche better than chronological age or height.²⁷⁵ Midchildhood may be a critical period for weight to influence the onset of puberty.¹⁹⁰ Suboptimal nutrition related to socioeconomic factors is an important factor in the later onset of puberty in underdeveloped than in developed countries.¹⁹⁰ Conversely, obesity appears to be an important factor in advancing the onset of puberty in the United States.²⁷⁶ Some of the obesity effect may be mediated by IGF-1 and adrenal androgen.²⁷⁷

Leptin appears to be an important link between nutrition and the attainment and maintenance of reproductive competence.^{218,278,279} Leptin deficiency causes obesity and gonadotropin deficiency. Paradoxically, prolonged leptin excess can downregulate the leptin receptor and GnRH release.²⁸⁰ Leptin is secreted by white adipose cells, acting on the hypothalamus to reduce appetite and stimulate gonadotropin secretion.²⁸¹ A critical threshold level appears to signal that nutritional stores are sufficient for mature function of the GnRH pulse-generator and, thus, to be permissive for puberty. Blood leptin levels rise throughout childhood and puberty to reach higher levels in girls than boys²⁸² and are positively related to adiposity and negatively related to testosterone levels.²⁸³ Leptin binding protein, a truncated form of the leptin receptor, falls as puberty begins, which suggests that circulating leptin becomes more bioavailable.²⁸² Whether leptin has a direct role in the pubertal activation of the GnRH pulse generator is unknown. In models of leptin insufficiency, the administration of kisspeptin induced LH secretion.²³⁷ Conversely, leptin's effect on puberty did not require signaling in kisspeptin neurons in other mouse models.²⁸⁴

Other factors also link nutrition and gonadotrophic function. Part of the leptin effect is mediated by inhibition of

hypothalamic neuropeptide Y (NPY) formation.²⁸⁵ NPY is a potent appetite-stimulating member of the pancreatic polypeptide family that directly inhibits GnRH release during food deprivation.²⁸⁵ However, in the preovulatory state, it stimulates GnRH release,²⁸⁶ an effect mediated by a different neural network acting on a different NPY receptor subtype on the GnRH neuron.²⁸⁷ NPY is also inhibited by the anorexogenic peptide YY (PYY), a gut hormone secreted in response to food and inhibited by GH; the pubertal fall in PYY has been postulated to permit the coordinated pubertal rise in appetite and gonadotropins.²⁸⁸ Insulin may also signal nutritional status to KNDy neurons, since deletion of the insulin receptor in KNDy neurons in genetically modified mice resulted in pubertal delay and reduced serum LH levels in both sexes. Interestingly, adult fertility was not affected.²⁸⁹

GWAS studies of pubertal timing implicated several genes associated with body weight other than leptin and leptin receptor and include fat mass and obesity-associated protein (*FTO*), SEC16 homolog B (*SEC16B*), transmembrane protein 18 (*TMEM18*), and neuronal growth regulator 1 (*NEGR1*).²¹¹ Rare heterozygous variants in *FTO* have been identified in pedigrees with CDGP associated with low body mass index (BMI) and growth and pubertal delay.²⁹⁰ Mice made heterozygous for the *FTO* gene knockout displayed delayed puberty, but did not manifest low body mass. Other mediators linking nutrition and puberty include melanocortin (MC)3/4 receptors, signaling from alpha-melanocyte-stimulating hormone (MSH) to increase *Kiss1* expression and mediate the permissive effects of leptin on puberty,²⁹¹ and ghrelin and mutations in the ghrelin receptor growth hormone secretagogue receptor (GHSR).^{292,293} A small cohort of 31 CDGP patients was analyzed for mutations in *GHSR*, and 5 patients were found to have point mutations in this gene.²⁹⁴

Other cues that provide information on nutritional status to the central reproductive axis may include glucose,²⁹⁵ ghrelin,²⁹⁶ and insulin.²⁹⁷ The effect of these factors on LH pulsatility may be mediated directly at the level of the gonadotroph or indirectly by changes in GnRH secretion. There is little evidence for the role of pineal secretions in human reproduction that is found in lower animals.^{298,299}

The essential element for the onset of puberty is an increase in pulsatile hypothalamic GnRH secretion that is regulated by a complex interplay of excitatory and inhibitory signals that have yet to be fully understood or elucidated.²⁴⁷ During childhood the activity of the GnRH pulse generating system is restrained, an awakening of the pulse generator occurs gradually during late childhood, and the tempo of GnRH neuronal activation increases during puberty. The underlying mechanisms for all these changes are unclear. The pubertal diminution in tone of the CNS centers that inhibit hypothalamic GnRH secretion during childhood has traditionally been considered to result from decreasing sensitivity of a "gonadostat" to negative feedback by sex steroids.^{6,300} However, this now seems an overly simplistic concept for a mechanism that seems to involve a change in the balance of neural inhibitory and stimulatory signals that impinge upon the GnRH neuron.

Many studies have been performed to help understand the initiating developmental events or the "trigger" for pubertal onset. In fact, it is becoming increasingly clear that there is no single "trigger" for puberty, but a gradual increase in GnRH pulsatility associated with a complex interplay of factors and hypothalamic developmental programs. Thus the apparent "sensitivity of the gonadostat" seems increasingly likely to reflect the degree of activity of the GnRH neuron. That is, when GnRH secretory activity is attenuated, the pulse generator is easily inhibited; when the GnRH neuron is active, the pulse generator is relatively insensitive to negative feedback.

The integration of hypothalamic signaling systems along with the developmental changes in the control of GnRH neuronal function seem to converge to trigger the onset of puberty. In the rat, structural remodeling of the GnRH neuron was demonstrated during pubertal progression by an increase in the density of dendritic and somal spines; the percentage of total neurons with spines being lowest at birth and increased gradually postnatally until puberty.³⁰¹ The spiny processes of neurons are the location of excitatory synapses important in neuronal plasticity. The greatest percentage of complex neurons is in the peripubertal period, with the percentage decreasing after completion of puberty.³⁰² These developmental changes are correlated with an increase in excitatory synaptic input to the GnRH neuron triggering the onset of puberty in mice.^{302,303} Which excitatory synaptic input (e.g., glutamatergic, kisspeptinergic, or yet unknown neurochemical signals) plays a role in the pubertal increase in GnRH secretion is unknown. Whether primate or human GnRH neurons undergo synaptic excitatory remodeling during development is also unknown.³⁰⁴

Since its discovery, numerous studies have demonstrated the expression of the kisspeptin-signaling system in several peripheral sites implicating it in biological processes, such as the regulation of ovarian function, embryo implantation, placentation, angiogenesis, and insulin secretion. However, whether kisspeptin is secreted from sites of peripheral expression and the impact on the reproductive axis are currently unclear.^{305,306}

Regulation of Gonadotropin Secretion

An essential feature of the mature HPG axis is the long-loop, negative-feedback control of gonadotropin secretion by gonadal secretory products, as depicted in Fig. 16.17. The generally tonic nature of gonadotropin secretion is punctuated by two prominent types of periodicity: two- to threefold pulsations of LH above trough levels at 1.5-hour intervals and, in the sexually mature female, by a transient, midcycle, preovulatory gonadotropin surge. The latter is characterized by a greater than 10-fold, rapid rise of LH and a lesser rise of FSH. This surge

is brought about by positive feedback when a critical level of estradiol, facilitated by a modest rise in progesterone, is achieved for a critical period of time, as discussed in relation to Fig. 16.13.

Estradiol, in concert with inhibin, reciprocally regulates FSH in a sensitive, log-dose, negative-feedback loop.³⁰⁷ Progesterone in high (luteal phase) concentrations is a major negative regulator of GnRH-LH pulse frequency.¹²⁹ Androgens have a biphasic long-loop feedback relationship with gonadotropins: at modest elevations they stimulate gonadotropin release and at very high levels they inhibit it.³⁰⁸

Estradiol exerts triphasic, and progesterone biphasic, effects on gonadotropin secretion. As estradiol rises after the midpoint of the follicular phase it selectively reduces the FSH response to GnRH, and when it reaches preovulatory levels it transiently exerts positive feedback effects on LH and, to a lesser extent, FSH.³⁰⁹ At sustained high levels estradiol suppresses both gonadotropins. As progesterone reaches a preovulatory level, it enhances the estradiol positive feedback effect, but at the higher levels that ensue during the luteal phase, it suppresses LH pulse frequency while enhancing LH pulse amplitude.¹²⁹

The GnRH neurons primarily responsible for maintenance of the reproductive cycle are those of the arcuate (infundibular) nucleus (Fig. 16.18).⁹⁰ GnRH neurons are inherently pulsatile.³¹⁰ Synchrony is promoted by fluxes of ionic calcium into these cells and autocrine GnRH inhibitory feedback. GnRH secretion is modulated by the variety of neurotransmitters and growth factors involved in initiating puberty.²⁴⁷ Synchrony of the network of GnRH neurons that accounts for pulsatility is conferred when the hypothalamic concentration of GABA periodically falls from levels inhibitory to GABA receptors in the presence of an excitatory neurotransmitter.^{311,312} EAP1, a hypothalamic protein previously shown to be important for pubertal onset, has also been implicated in the control of menstrual cyclicity in primates.³¹³

Sex steroid signals are in part conveyed to GnRH neurons indirectly. Regulation of GnRH secretion by estrogen involves

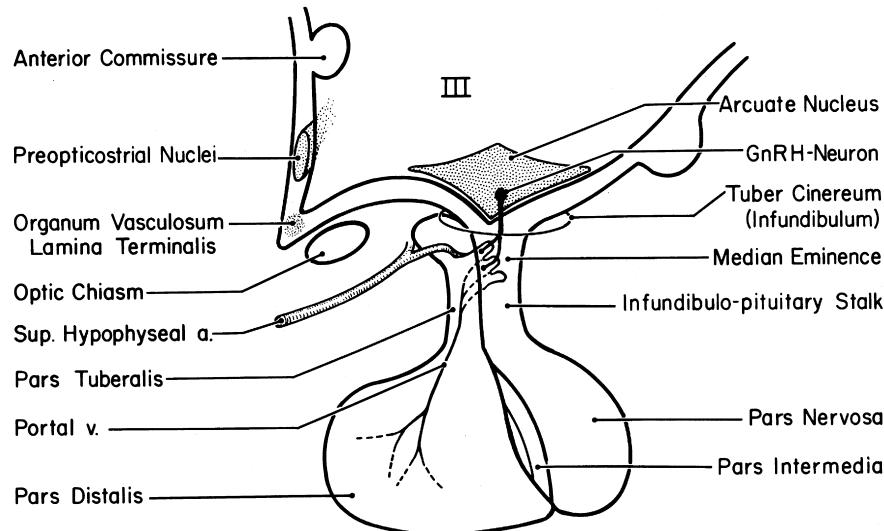


Fig. 16.18 The location of major gonadotropin-releasing hormone (GnRH)-containing neurons (shaded) in relation to the hypothalamus and pituitary gland. The neurons are of greatest density in the arcuate nuclei and in the periventricular wall of the medial basal hypothalamus. These neurons project to the adjacent median eminence, the second most dense population of GnRH neurons lies in the preopticostriatal area. The development of some is altered by early androgenization. Some are connected by the stria terminalis to the amygdala. Other projections from this area appear to connect indirectly with the median eminence, perhaps via the organum vasculosum lamina terminalis—a midline structure that resembles the median eminence. The pituitary portal veins transport blood rich in releasing factors to sinusoids engulfing anterior pituitary cells.

in part induction of PRs in the hypothalamus.^{129,314} GnRH neuronal cell lines have been studied in which estradiol directly stimulates and inhibits GnRH gene expression under different experimental conditions.^{315,316} Although progesterone exerts its main inhibitory effect on GnRH secretion, it has effects at higher CNS levels and at the pituitary level.^{317–319} Prolactin suppresses both hypothalamic and gonadotropin GnRH receptor expression.^{320,321}

Other clinically relevant factors affecting GnRH release are sleep, endorphins (endogenous opioids), and interleukins. In sexually mature women, sleep inhibits GnRH pulse frequency and this effect seems to be amplified by female hormones.³²² Endorphins are important physiological regulators of GnRH release after puberty has begun. Hypothalamic β-endorphin suppresses oophorectomy-initiated GnRH secretion, and opiate antagonists reverse this effect, as well as the sleep effect. The inhibitory effect of stress on gonadotropin release appears to be mediated by β-endorphin released from proopiomelanocortin in response to corticotropin releasing hormone (CRH).³²³ Interleukins also inhibit gonadotropin release.³²⁴ Serotonin seems to modulate LH pulsatility and facilitate the LH surge.³²⁵

GnRH receptors on the gonadotroph are maintained in an optimally active state only when GnRH is delivered in pulses approximately 1 to 2 hours apart in man.^{90,326} Pulses substantially less frequent result in a hypogonadotropic state. Paradoxically, continuous administration of an initially stimulatory dose of GnRH results in downregulation of gonadotropin production, after an initial burst of gonadotropin release.³²⁷ This is the physiological basis for the success of long-acting gonadotropin agonists in suppressing puberty in children with true central precocious puberty. However, while gonadotropins are downregulated, free alpha-subunit production is elevated and responsive to GnRH.

Hypothalamic GnRH receptor function is modulated by autocrine and paracrine factors, including GnRH itself and kisspeptin.⁵ Pituitary GnRH receptors appear to be directly and indirectly downregulated by GnRH, gonadotropins, and inhibins, as well as sex steroids.³²⁸ LH and FSH themselves inhibit GnRH release (short-loop feedback) and inhibit their own release (autocrine feedback).^{328,329}

How is differential regulation of gonadotrope LH and FSH release accomplished in response to a single GnRH pulse? The frequency of the GnRH pulse is one determinant. An increased frequency of this signal stimulates LHβ-subunit gene expression, whereas slowing this signal stimulates FSH β-subunit and suppresses follistatin gene expression, altering the FSH/LH ratio.³³⁰ Pituitary adenylate cyclase activating polypeptide amplifies LH responses to GnRH while blocking its effect on FSH.³³¹

The sex hormone milieu is also clearly a major differential modulator of gonadotrope LH and FSH release.^{90,319,332–334} FSH is more sensitive than LH to inhibition by estrogen; this effect of modest levels of estradiol is of rapid onset and sustained. LH is the more sensitive to the stimulatory effects of higher estradiol levels; this effect is of later onset and short-lived. Similar relationships pertain in aromatase null mice. ER null mice have revealed ER-alpha as the predominant receptor isoform that conveys negative feedback regulation to the gonadotroph.³³⁵ Progesterone exerts both negative and positive feedback effects at the pituitary level, and these effects are antagonized by androgen. The progesterone metabolite 3α-hydroxyprogesterone suppresses FSH release.³³⁶

Androgens have complex effects on gonadotropin dynamics. Normal androgen action facilitates the midcycle gonadotropin surge in response to positive feedback.^{337,338} Elevated testosterone increases baseline LH pulse amplitude and frequency^{64,339,340} while inhibiting the capacity to mount the

gonadotropin surge.³³⁷ These actions appear to result from antagonizing progesterone action.^{16,339,340}

Inhibins of gonadal origin seem to be the major nonsteroidal-specific negative feedback regulator of pituitary FSH synthesis and secretion.^{341,342} Inhibins inhibit FSH release at the pituitary level, but they may act at a higher level as well.³⁴³ Serum levels of both inhibins rise upon FSH stimulation.^{177,178} Inhibin-B, produced by small antral follicles in response to FSH, is virtually the only inhibin moiety in blood during puberty. Its blood levels rise during the early follicular phase and then fall thereafter except for a small postovulatory peak, generally paralleling the changes in serum FSH; the latter peak may function to attenuate the FSH surge. Serum inhibin-A, a marker of the preovulatory follicle and corpus luteum, begins to rise in the late follicular phase and thereafter parallels levels of progesterone; its fall late in the luteal phase appears to contribute to the early follicular phase rise in the FSH level.

The structurally related activins seem to be important as regulators of both pituitary and ovary function.³⁴⁴ Activin is formed by gonadotropes themselves and its primary role is to stimulate FSH release. It also upregulates the activin binding protein follistatin, which arises within folliculostellate cells of the anterior pituitary.²⁸⁵ Follistatin, by competitively inhibiting binding of activin to its receptor, specifically inhibits activin stimulation of FSH secretion.³⁰⁷

Infant and Child

Neuroendocrine Unit. The hypothalamic-pituitary-gonadal (HPG) axis is transiently active during the neonatal period. This is sometimes termed the *minipuberty of the newborn*; unlike true puberty, the clinical manifestations are only nascent and do not progress. The regulation of neonatal gonadotropin secretion, like that during puberty, is incompletely known.

Serum FSH and LH are low in cord blood and remain low until estrogen concentrations fall from inhibitory levels upon disruption of the fetoplacental unit at birth. Then the LH and FSH levels of neonates promptly begin to rise in pulsatile fashion to early pubertal levels in the first week of life (see Fig. 16.5).^{7,11,58–61}

Serum LH and FSH levels rise higher in female than in male premature infants, reaching into the postmenopausal range.^{17,62} This sexual dimorphism seems to be related to lack of negative feedback because of lagging ovarian follicular development: antral follicle development begins near term gestational age.¹⁸ There is parallel hyperprolactinemia without sexual dimorphism.⁶³

At their peak between term and 4 months of age, serum gonadotropins and LH/FSH ratios are lower in girls than in boys,¹⁷ apparently because girls lack androgen-programmed accentuation of GnRH pulsatility.^{16,64,65} Responses to GnRH and GnRH agonist are similar to those of early puberty (see Fig. 16.6).^{55,66–69} In congenital agenesis, gonadotropins reach postmenopausal levels during the neonatal period.⁷⁰

After about 4 months postterm, gonadotropin and prolactin levels begin to gradually fall into the prepubertal range (see Fig. 16.5). FSH is higher in girls than in boys, a tendency that tends to persist into early childhood.^{58,71} This appears in part related to negative feedback by the higher activin-A and lower inhibin-B serum levels of girls than boys.⁷² GnRH secretion also appears to be greater in girls than in boys at this time.⁷³

The decline in gonadotropins may in part be related to the maturation of neural tracts that conduct inhibitory signals from the CNS and/or to an increase in hypothalamic sex steroid receptors. Hypothalamic ERs increase in a pattern reciprocal to the fall in serum gonadotropins in the rat (see Fig. 16.7),⁷⁴ as do hypothalamic DHT receptors.⁷⁵ Increasing sensitivity of the hypothalamus to sex steroid hormone negative feedback

could account for the inhibitory effect of the small amounts of circulating estradiol and testosterone.

A nadir in both serum gonadotropins occurs by about 6 years of age (see Figs. 16.2 and 16.5). At this age, the LH and FSH response to GnRH is minimal, apparently from lack of GnRH stimulation. Furthermore, at this stage, agonadism is seldom reflected in a rise in serum gonadotropins or gonadotropin reserve.⁷⁰

However, gonadotropin production is not completely suppressed in midchildhood. Gonadotropins have been detected in the urine of young prepubertal children, at the limits of sensitivity of classic bioassays: LH excretion averaged 3% and FSH 15% of the adult amounts.⁷⁶ Specific monoclonal antibody-based assays have revealed that LH falls to less than 0.2 U/L during the day whereas FSH remains detectable and that the gonadotropins produced at this stage are secreted in micro-pulses that approximately double in association with sleep.⁷⁷ The gonadotropins also appear to be bioactive judging from their sensitivity to estradiol negative feedback in the primate⁷⁸ and the active formation of antral follicles during childhood, which indicates gonadotropin stimulation, as discussed in the following section on the adult.

Between 7 and 10 years of age, even prepubertal girls experience subtle but significant increases in gonadotropin levels.⁷⁹ This change corresponds with rising secretion of GnRH.⁷³ These data indicate that the hormonal secretory pattern of the prepubertal 10-year-old child is different from that of the 7-year-old and indicate that the hormonal changes signaling the development of puberty are found late in the first decade of life, antedating by some time the development of secondary sex characteristics.

Ovary. The ovary of the infant and child is not quiescent. Initiation of growth and development of resting follicles occurs throughout childhood. The neonatal ovary typically contains an antral follicle with thecal luteinization,^{80,81} and the number of antral follicles approximately doubles over that in infancy by 7 years and quadruples by 9 years (see Fig. 16.3).³¹ All these antral follicles normally undergo atresia in childhood, and this augments the amount of stroma.³¹ As a result, by midchildhood, the ovaries of normal girls have up to five antral follicles 4 to 9 mm in diameter, and ovarian volume increases up to approximately 3.5 cc. Ovarian follicular development begins to accelerate just before the onset of clinical signs of puberty.^{31,82–86}

During the first few months of life, early pubertal blood levels of ovarian hormones are found as part of the transient activation of the HPG axis that occurs in the newborn. Serum estradiol and inhibin-B levels parallel those of FSH. In the neonatal period they begin rising to early pubertal levels, remain there for the first few months of life, and fall to low levels during childhood (see Fig. 16.8).^{66,69} Specifics about the hormonal changes are discussed later (see Normal Hormonal and Sexual Developmental Stages).

Regulation of Ovarian Secretion

Ovarian secretion results from the combined actions of LH and FSH, as discussed earlier with regards to Figs. 16.13 and 16.14. The early follicular phase follicle functions according to the two-cell, two-gonadotropin model illustrated in Fig. 16.19.^{144,345,346} In response to LH, androstenedione, the most abundant steroid formed in the ovary, is secreted by the theca-interstitial-stromal (thecal) cell compartment. In response to FSH regulation, aromatase then forms estrogen from precursor androstenedione in granulosa cells. FSH also stimulates granulosa cells to secrete inhibins. As by-products of the secretion of both ovarian estradiol and adrenal cortisol, androgens do not normally contribute to negative feedback regulation of gonadotropins. However,

they have a biphasic effect on gonadotropin secretion: modest elevations increase GnRH pulse frequency by interfering with progesterone negative feedback and very high levels directly inhibit gonadotropin secretion.³⁰⁸

The regulation of the intraovarian androgen concentration is critical to ovarian function.^{40,144} Androgens are important for ovarian function. Androgens are obligate substrates for estradiol biosynthesis. Androgens also increase recruitment of primordial follicles into the growing follicle pool¹⁴² and then act in conjunction with gonadotropins on granulosa cells to stimulate preantral follicle development into small antral follicles,³⁴⁷ which enhances FSH upregulation of aromatase activity.¹⁴¹ Androgens also synergize with FSH to luteinize follicles by inducing LH receptors. However, in excess androgens impair selection of the dominant follicle of women; this appears likely to result from premature luteinization of follicles, thus committing the follicle to atresia. Therefore androgen synthesis must be kept to the minimum necessary to optimize follicular development. This means that the synthesis of ovarian androgens must be coordinated with the needs of the follicle. This is achieved by intraovarian intracrine, autocrine, and paracrine modulation of LH action (see Fig. 16.19).

LH stimulates theca cell development and steroidogenesis and is necessary for the expression of gonadal steroidogenic enzymes and sex hormone secretion. However, once adult LH levels are achieved, further LH increase normally has little further effect on androgen levels because excess LH causes homologous desensitization of theca cells.^{144,348} Desensitization involves downregulation of LH receptor expression and steroidogenesis. Because steroidogenic downregulation is primarily exerted on 17,20-lyase activity, which converts 17-hydroxycorticoids to 17-KS, 17-OHP levels rise in response to increased LH levels, but the rise in androgens is limited.³⁰⁸

A model of the intraovarian interaction among the major regulators of steroidogenesis is shown in Fig. 16.19.¹⁴⁴ Stimulation of androgen secretion by LH appears to be augmented by specific intraovarian FSH-dependent factors, such as inhibins and IGFs. These processes seem to normally be counterbalanced by other FSH-dependent processes that downregulate androgen formation as LH stimulation increases. Androgens and estrogens themselves seem to mediate at least a portion of this desensitization to LH, with estrogens being critical through an ER α -dependent mechanism.^{335,349}

Insulin and IGFs are important coregulators of ovarian function. Insulin upregulates theca cell LH receptor sites and action⁴⁰ and to a lesser extent estrogen biosynthesis in response to FSH.³⁵⁰ The entire IGF system is represented in the ovary: IGF-1 augments FSH receptor expression and action,^{153,351} and appears to mediate GH promotion of granulosa cell steroidogenesis.^{352,353} Insulin is equipotent with IGF-1 in stimulating thecal androgen biosynthesis,⁴⁰ and, although insulin can act through hybrids of the insulin and IGF-1 receptors³⁵⁴ and at very high levels interacts with the IGF-1 receptor, it appears to primarily act through its own receptor.^{40,355}

Androgen-expressing steroidogenic cells express a previously unrecognized protein variant, DENND (differentially expressed in normal and neoplastic development) 1A.V2, that facilitates steroidogenesis: it upregulates basal and cAMP-stimulated cytochrome P450c17 and side chain cleavage activities.^{356,357} The mechanism by which DENND1A.V2 regulates steroidogenesis is currently unknown. DENND1A is a member of the connedenn family of proteins, which are involved in protein trafficking, endocytotic processes, and receptor recycling. Thus it is tempting to speculate that it acts by upregulating LH receptor signaling.

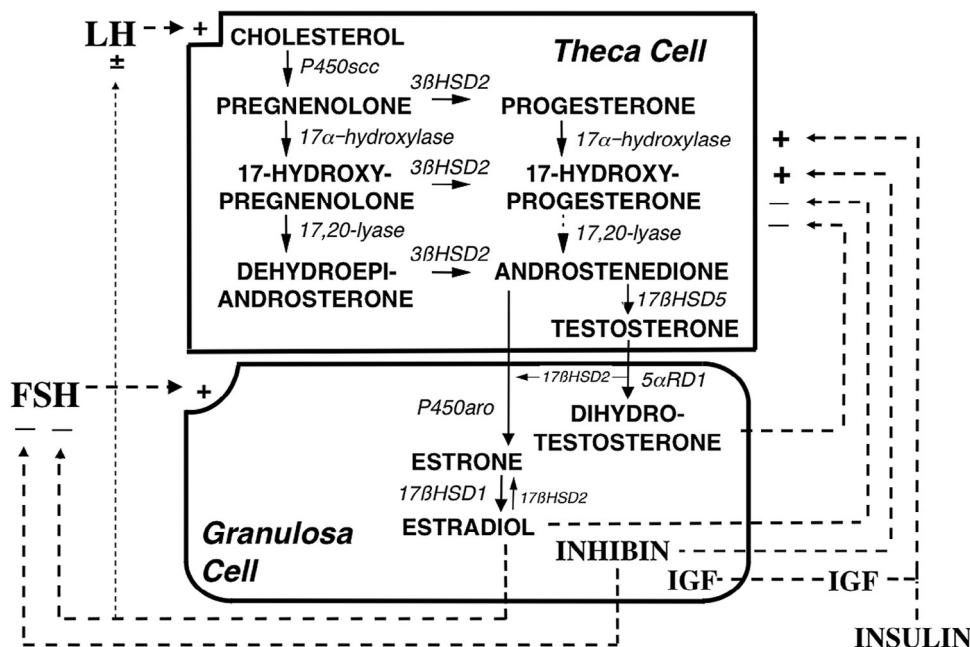


Fig. 16.19 Depiction of the organization and regulation of the major steroid biosynthetic pathways in the small antral follicle of the ovary according to the two-gonadotropin, two-cell model of ovarian steroidogenesis. Luteinizing hormone (LH) stimulates androgen formation within theca cells via the steroidogenic pathway common to the gonads and adrenal glands. Follicle-stimulating hormone (FSH) regulates estradiol biosynthesis from androgen by granulosa cells. Long-loop negative feedback of estradiol on gonadotropin secretion does not readily suppress LH at physiological levels of estradiol and stimulates LH under positive feedback circumstances. Androgen formation in response to LH appears to be modulated by cytochrome P450c17 that is expressed only in theca cells. The relative quantity of androstenedione formation via 17-OHP (dotted arrow) in the intact follicle is probably small, as is the amount of progesterone formed from granulosa cell P450scc activity in response to FSH (not shown). 17βHSD2 activity is minor in the ovary, and estradiol is primarily formed from androstenedione. Androgens and estradiol inhibit (-) and inhibin, insulin, and insulin-like growth factor-I (IGF) stimulate (+) 17-hydroxylase and 17, 20-lyase activities. The sites of aromatase and IGF gene expression appear to vary with the stage of follicular development. Other peptides also modulate the steroidogenic response to LH. Pertinent enzyme activities are italicized: the 17-hydroxylase and 17,20-lyase activities of P450c17 are shown, otherwise enzyme abbreviations are as in the text. 5α-R, 5α-Reductase; 17β-HSD5, type 5 17β-HSD; HSD, hydroxysteroid dehydrogenase. (Modified with permission from Ehrmann et al, Polycysticovary syndrome as a form of functional ovarian hyperandrogenism due to dysregulation of androgen secretion. *Endocr Rev*. 1995;16:322–353. Reproduced with permission from Rosenfield, R.L., Ehrmann, D.A. (2016). The pathogenesis of polycystic ovary syndrome (PCOS): the hypothesis of PCOS as functional ovarian hyperandrogenism revisited. *Endocr Rev*, 37, 467–520.)

Many other peptides modulate ovarian cell growth or function in response to gonadotropins.^{144,348} Inhibin stimulates ovarian androgen production, whereas androgens reciprocally stimulate ovarian inhibin production. Activin opposes the inhibin effect. A variety of other ovarian peptides are also capable of modulating thecal androgen synthesis.¹⁴⁴ Stimulators include catecholamines, for which an intraovarian system exists,³⁵⁸ prostaglandin, and angiotensin. Inhibitors include leptin, CRH, epidermal growth factor (EGF), tumor necrosis factor, TGF-β, and GDF9.³⁵⁹ Leptin antagonizes IGF-1 effects.³⁶⁰ TGF-β is particularly interesting because it suppresses androgen biosynthesis and stimulates aromatase activity; it also stimulates meiotic maturation of the oocyte.³⁶¹ Other peptides acting on granulosa cells include cytokines, which have diverse effects, and AMH, which inhibits aromatase.³⁶² GnRH is also capable of modulating thecal steroidogenesis. A GnRH-like protein has been described in the ovary that may act through ovarian GnRH receptors to suppress steroidogenesis in the human ovary.^{363,364} It inhibits FSH induction of progesterone secretion, aromatase activity, and LH receptors in granulosa cells, downregulates LH receptors, and inhibits the hCG stimulation of progesterone secretion by luteal cells.^{115,134}

Prolactin has complex effects on steroidogenesis. In low concentrations, it enhances ovarian estradiol and progesterone secretion by increasing LH receptors.³⁶⁵ On the other hand, high levels of prolactin inhibit ovarian estradiol and progesterone biosynthesis.³⁶⁶ Prolactin also stimulates adrenal androgen production.³⁶⁷

Adrenarche and the Regulation of Adrenal Androgen Secretion

Adrenarche denotes the onset of the increase in adrenal androgen production that gradually begins in midchildhood well before the pubertal maturation of the neuroendocrine-gonadal axis.^{40,368} Adrenarchal androgens contribute to the appearance of pubic hair (pubarche) and sebaceous gland and apocrine gland development.

Adrenarche results from a change in the pattern of adrenal secretory response to ACTH (Fig. 16.20). It is characterized by disproportionate rises in the responses to ACTH of the Δ^5 -3 β -hydroxysteroids 17-hydroxypregnolone and dehydroepiandrosterone (DHEA), whereas cortisol secretion does not change. Dehydroepiandrosterone sulfate (DHEAS) is the predominant marker for adrenarche. A DHEAS level over 40 mcg/dL is usually considered adrenarchal. Other serum androgens and precursors are ordinarily at the upper end of the prepubertal range at the onset of adrenarche.

Adrenarche reflects the development of the adrenocortical zona reticularis.⁴⁰ Humans and some higher primates are unique in having an adrenal zone with similar structure-function-developmental stage relationships.^{369,370} Although the zona reticularis resembles the fetal zone of the adrenal cortex in its location and function, it appears to originate from stem cells located in the outer definitive zone of the fetal adrenal gland.^{371,372} This zone becomes continuous at about 5 years of age and enlarges steadily over the subsequent decade. Its increasing development correlates with DHEAS levels.

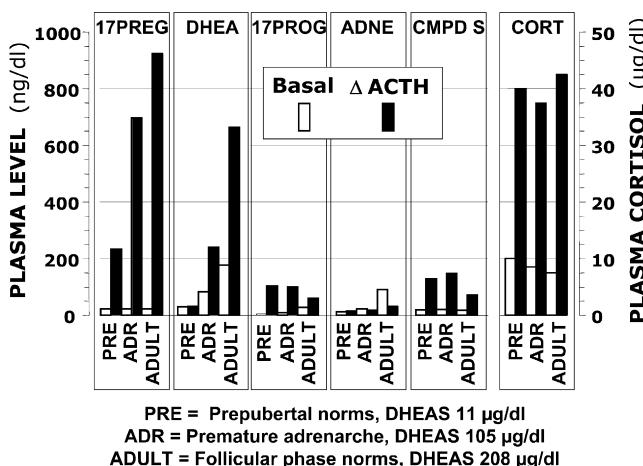


Fig. 16.20 Changing pattern of adrenal steroidogenic response to adrenocorticotrophic hormone with maturation. Shown are plasma steroid levels before (basal, 8:00 a.m. after dexamethasone 1 mg/m²) and the rise (Δ) 30 minutes after cosyntropin (ACTH) administration (10 $\mu\text{g}/\text{m}^2$) in healthy prepubertal children, children with premature adrenarche as an isolated phenomenon, and follicular phase adult women. Note that 17-hydroxypregnенolone (17PREG) and dehydroepiandrosterone (DHEA) responses of children with premature adrenarche are intermediate between prepubertal and adult responses. 17PROG, 17-hydroxyprogesterone; ADNE, androstenedione; CMPD S, 11-deoxycortisol; CORT, cortisol; DHEAS, dehydroepiandrosterone sulfate. (Data from Rich, B.H., Rosenfield, R.L., Lucky, A.W., Helke, J.C., Otto, P. (1981). Adrenarche: Changing adrenal response to adrenocorticotropin. *J Clin Endocrinol Metab*, 52, 1129.)

This zone's secretion pattern results from a unique enzyme expression profile: it expresses low 3 β -hydroxysteroid dehydrogenase type 2 (HSD3B2), but high cytochrome b5 (an enhancer of the 17,20-lyase activity of cytochrome P450c17) and steroid sulfotransferase (SULT2A1) activities.⁴⁰ The high secretion of DHEA and DHEAS is primarily attributable to these activities.⁴⁰ Enhanced expression of 17 β -hydroxysteroid dehydrogenase type 5 (HSD17B5) by this zone accounts for the small but significant adrenal contribution to testosterone secretion. Both testosterone and androstenedione are further metabolized by zona reticularis 11 β -hydroxylase type 1 (CYP11B1), which underlies adrenal 11 β -hydroxyandrostenedione and 11 β -hydroxytestosterone secretion.^{373,374} These are further metabolized, primarily in the periphery, by 11 β -hydroxysteroid dehydrogenases to their 11-keto cogeners. These 11-oxy metabolites of testosterone are one-fifth as potent as testosterone at half-maximal dosage (ED₅₀).³⁷³

The factors causing and regulating zona reticularis development are unclear. Body growth is related to adrenarche.^{375–378} Insulin, IGF-1, and leptin have been suggested as determinants of this relationship. Insulin and IGF-1 stimulate expression of adrenal P450c17 and 3 β HSD2 activities^{379,380} and may be involved in progenitor cell proliferation.³⁸¹ Leptin, an adipocyte hormone, stimulates the 17,20-lyase activity of adrenocortical cells, which shunts adrenal steroidogenesis toward DHEAS production.³⁸²

Nutritional status, in particular, seems to play a role in the development of adrenarche, particularly in girls.^{376,383} Infants born small for gestational age have higher DHEAS levels at 5 to 8 years of age, and children born large for gestational age have lower levels than those with normal birth weight.³⁸⁴ Obesity is related to DHEAS levels, and rapid weight gain during early childhood is associated with adrenal androgen levels independently of birth weight.³⁸³

A pituitary hormone ("adrenarche factor") may well be required to bring about their adrenarchal development.⁴ It has been postulated to be an ACTH-related hormone distinct from ACTH because adrenal androgen production is more sensitive to glucocorticoid suppression than is cortisol production,³⁸⁵ falls more slowly than cortisol after dexamethasone administration,¹²⁵ and rises more sluggishly after its withdrawal.³⁸⁶ Candidates for a dexamethasone-suppressible adrenarchal factor include pro-ACTH-related peptides and CRH, but the data have not been convincing.³⁸⁷ Prolactin seems to be required.^{40,388} Currently the only established adrenal androgen-stimulating hormone in postnatal life is ACTH. Because the adrenarchal secretion pattern represents a change in the pattern of steroidogenic response to ACTH, an adrenarche factor need only control the growth and differentiation of zona reticularis cells or regulate their unique pattern of steroidogenic enzyme expression.

ACTH effects on adrenal androgen production are modulated by diverse signaling networks.^{40,389} Modulators of the androgenic response to ACTH include a stimulatory isoform of DENND1A (DENN/MADD domain-containing protein 1A; DENND1A.V2) that is known to be overexpressed in polycystic ovary syndrome (PCOS) theca cells, and BMP4, which is inhibitory. Intraadrenal cortisol may participate in the regulation of adrenal DHEA secretion through inhibition of 3 β -hydroxysteroid dehydrogenase (3 β -HSD) activity.³⁷⁸ In addition, interleukin-6 is strongly expressed in the zona reticularis, where it directly stimulates production of all classes of adrenal steroids independently of ACTH.^{390,391} Although gonadal dysgenesis is associated with earlier adrenarche,³⁹² paradoxically, ovariectomy precipitates an early decline in DHEAS levels that is not reversed by estrogen replacement.³⁹³

Adrenarchal levels of androgens suffice to successively initiate sebaceous gland development, apocrine gland development, and the growth of pubic hair. Sulfation of DHEA within the adrenal cortex prevents adrenal hyperandrogenism,³⁹⁴ and circulating DHEAS is a precursor for ovarian testosterone formation.³⁹⁵ It has been proposed that DHEAS elevation in response to obesity exerts a protective effect on plasma lipids.³⁹⁶ Whether adrenarche plays a more fundamental role in normal puberty is not established.³⁶⁸

There has been considerable interest in the possibility that adrenarchal steroids play a role in human neurobiological development. DHEA and testosterone serum levels differentially correlate with specific structural developmental changes in the cerebral corticolimbic system.³⁹⁷ DHEAS and its precursor, pregnenolone sulfate, as well as the progesterone metabolite allopregnanolone, have direct nongenomic neuroactive effects, which include modulation of neurotransmitter signaling and neuroplasticity.^{398–400} These steroid sulfates are actively transported across the blood-brain barrier.⁴⁰¹ The association of adrenarchal changes with the emergence of sexually dimorphic sexual attraction, stress-adaptive, and social maturational behavior during middle childhood, before true puberty, has led to the suggestion that adrenarchal steroids play a role in activating these behaviors.^{402–404} Many functions attributed to DHEAS have been inconsistent, and whether they differ from those of low-dose testosterone remains to be established.³⁶⁸

Hormonal Secretion, Transport, Metabolism, and Action

Peptide Hormones

Peptide hormones act after binding to specific receptors located in the plasma membranes of target cells. GnRH receptors and

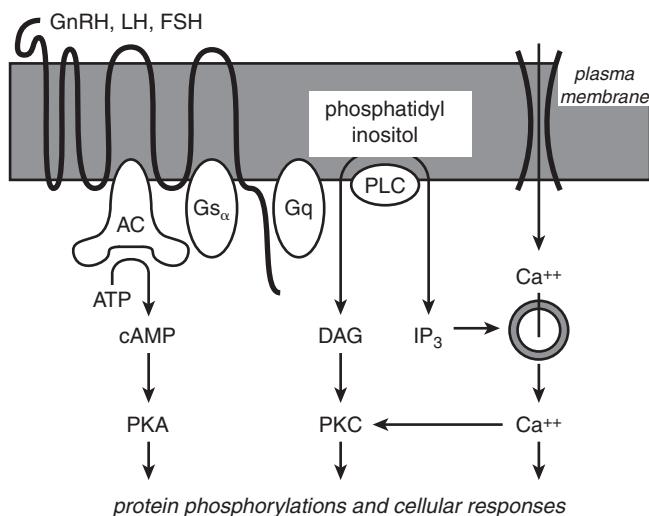


Fig. 16.21 Overview of the pathways established to mediate gonadotropin-releasing hormone (GnRH) and gonadotropin action. The receptors for these hormones are members of the seven-transmembrane family of receptors. Hormone-receptor binding alters receptor configuration. One consequence is to couple the receptor to adenylate cyclase (AC) via the stimulatory alpha-subunit of G-protein ($G_{\alpha s}$). This permits the efficient generation of cyclic adenosine monophosphate (cAMP) from adenosine triphosphate (ATP). Another consequence is to couple phospholipase C (PLC) to the receptor through $G_{\alpha q}$. PLC is a phosphodiesterase that hydrolyzes phosphatidyl inositol to diacylglycerol (DAG) and inositol-1,4,5-triphosphate (IP₃). DAG stimulates the calcium-sensitive protein kinase C (PKC). IP₃ mobilizes ionic calcium (Ca^{2+}) from intracellular organelles and stimulates Ca^{2+} influx through calcium ion channels. Protein kinase A (PKA), PKC, and Ca^{2+} then bring about cellular responses through protein phosphorylations. *FSH*, Follicle-stimulating hormone; *LH*, luteinizing hormone.

gonadotropin receptors are members of the 7-transmembrane receptor family. These receptors are necessary for the actions of their cognate hormones. Receptors expressed in nonclassical sites are not necessarily functionally mature.⁴⁰⁵ Mature receptors signal after coupling to a guanine nucleotide (G-protein) subunit (Fig. 16.21).^{5,326,330,406–408} G_s signaling activates adenylate cyclase and acts via phosphodiesterase-regulated cAMP to activate protein kinase A. G_q signaling activates phospholipase C, which acts via protein kinase C and Ca^{2+} ; Ca^{2+} may also be mobilized by other factors that influence ion channels. Phosphorylation of various cytoplasmic and nuclear proteins ultimately mediates the action of the peptide hormones, and secondarily involves the RAS and EGF signaling cascades in the case of gonadotropins.²⁶ The diversity among target cells in their responses to the action of protein kinases in part relates to diversity and type of kinase, intracellular compartmentalization, substrate availability, and other differences in gene expression that are specific to each type of target cell.

GnRH is a decapeptide [pyro]Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH₂.³²⁶ One gene encodes the single precursor protein for both GnRH and prolactin release-inhibiting factor.⁴⁰⁹ GnRH not only effects prompt release of preformed gonadotropins (the “readily releasable pool”), but also stimulates the synthesis of gonadotropins (the “reserve pool”).⁴¹⁰ Repeated administration of GnRH augments the pituitary responsiveness to subsequent GnRH pulses (“self-priming”).¹⁰⁷ This has been ascribed partly to upregulation of GnRH receptors. GnRH has an important paradoxical effect. As discussed earlier, it acutely stimulates gonadotropin secretion, but, upon protracted, continuous administration it downregulates pituitary gonadotropin secretion. The significance of the

expression of GnRH and its receptor in nonhypothalamic reproductive tissues⁴¹¹ is unclear.

An evolutionarily conserved form of GnRH (GnRH-II) acts primarily through the type 2 GnRH receptor.⁴¹² GnRH-II and the type 2 GnRH receptor are products of unique genes, rather than being modified products of the GnRH or type 1 GnRH receptor genes.⁴¹³ The cell bodies of the GnRH-II neurons lie predominantly in the midbrain and only a minority project to the hypothalamic-pituitary area. GnRH-II function in humans is unknown; there is speculation that it is a neurotransmitter involved in sexual behavior. A recent study using functional neuroimaging demonstrated that kisspeptin administration enhanced limbic brain activity in response to sexual and couple-bonding stimuli and attenuated negative mood. Whether kisspeptin may become a therapeutic agent for patients with reproductive function dysfunction is currently unclear.⁴¹⁴

LH and FSH are synthesized in a single type of cell, and both are sometimes identified within the same cell.⁴¹⁵ A vestigial population of hCG-secreting pituitary cells has been described.⁴¹⁶ LH, FSH, and hCG are glycoprotein hormones that consist of two chains.⁴¹⁷ After synthesis of these hormones on the ribosomes, the carbohydrate moieties, which constitute about 16% of the weight, are added in the rough endoplasmic reticulum and Golgi apparatus. The α chains of LH, FSH, hCG, and thyroid-stimulating hormone (TSH) are identical in amino acid sequence (92 amino acids). Although the β chain of each hormone is different in both primary amino acid sequence and length, these β chains nevertheless share 30% to 80% amino acid homology. Biological activity is conferred when an α and β chain are glycosylated and assemble within the cell. The α/β dimer is stabilized by a β -subunit derived “seat belt” that wraps around the α -subunit. Neither the isolated α nor the β glycosylated protein subunit exhibits biological activity unless noncovalently bound to one another.

The gonadotropins exhibit considerable molecular heterogeneity.^{100,418–420} The major basis for this is variation in the relative degree of glycosyl sialylation or sulfonation, steps which occur reciprocally in the pituitary gland.⁴²¹ These differences affect in vitro and in vivo bioactivity. Polymorphisms in amino acid sequence of the LH- β and hCG- β gene also may affect the expression or bioactivity of LH or hCG.⁴²² Reproductive status affects isoform distribution, with sialylated forms predominating in the hypogonadal state.⁴²³ Androgens increase and estrogens decrease in vitro LH biopotency by altering LH sialylation.^{424,425} Thus the pituitary gland contains multiple isoforms of LH and FSH that vary in bioactivity. Consequently, different pituitary LH and FSH standards, as well as serum, contain variable proportions of immunoreactive material of varying bioactivity.

One corollary of the molecular heterogeneity is that the antibodies generated from these gonadotropin moieties detect heterogeneous epitopes that are not necessarily bioactive; indeed some may even act as gonadotropin antagonists.⁴²⁶ These factors combine to cause the gonadotropin B/I to vary in a wide variety of circumstances.^{100,418} The purest of standards, even recombinant ones, interact very differently in the diverse immunoassay systems. Likewise, the putative level of LH or FSH in a serum sample differs substantially among immunoassays. Furthermore, bioactivity assessments vary with the bioassay model system.^{100,427} Monoclonal antibody-based immunometric assays yield results that correlate with, but are not necessarily equivalent to, those by bioassay.^{100,428} The “third-generation” immunometric assays have the advantage of being more sensitive and specific for low levels of gonadotropins in serum than polyclonal antiserum-based RIA, but B/I discrepancies remain.

The major determinant of in vivo gonadotropin bioactivity is the serum half-life. Terminal sialic acid residues retard clearance by the liver, the primary site of metabolism, whereas

TABLE 16.2 Average Hormone Blood Production Rates in Midfollicular Phase Women.^a

Hormone	Production Rate
Luteinizing hormone	615 IU/day ^b
Follicle-stimulating hormone	215 IU/day ^b
Androstenedione	3.4 mg/day
Dehydroepiandrosterone	7.0 mg/day
Dehydroepiandrosterone sulfate	7.0 mg/day ^c
Dihydrotestosterone	0.06 mg/day
Estradiol	0.1 mg/day
Estrone	0.1 mg/day
Progesterone	1.1 mg/day
17-Hydroxyprogesterone	1.2 mg/day
Testosterone	0.2 mg/day

^aThese production rates are roughly equivalent to those in midpuberty. The average daily production of those hormones that fluctuate cyclically is substantially greater. For example, estradiol production transiently peaks to about 0.5 mg/day, and thus the average production over the monthly cycle is about 0.2 mg/day or 6 mg/mo.^{434–440}

^bIn terms of second International Reference Preparation, human menopausal gonadotropin.

^cApproximate urinary production rate, expressed as unconjugated steroid.

sulfonated ones facilitate clearance.⁴²¹ About 10% to 15% of gonadotropins are excreted in urine according to RIA⁴²⁹; only about one-third of this is in a biologically active form.⁴³⁰

LH is cleared more rapidly from the blood than FSH or hCG.^{431,432} LH disappears from blood in an exponential pattern: RIA indicates that the half-life of the first component is about 20 minutes and that of the second component is about 4 hours. The bioactive LH half-life is about 25% to 50% shorter.⁴³³ These respective components for immunoreactive FSH are 4 and 70 hours; those for hCG are 11 and 23 hours. Hormone production rates in follicular phase women, which approximate midpubertal values, are given in Table 16.2.^{434–440}

Prolactin has structural and functional similarities to GH and placental lactogen. Prolactin has a considerable degree of structural heterogeneity; this results from genetic and posttranslational events within pituitary cells, as well as modifications, such as glycosylation in the periphery.⁴⁴¹ Lactotrope growth and prolactin secretion are stimulated by estrogens. Prolactin release from the anterior pituitary is primarily under the control of hypothalamic inhibition, probably primarily mediated by dopamine.⁴⁴² A prolactin release-inhibiting factor has been described within the same precursor protein as GnRH,⁴⁰⁹ thus providing a potential mechanism for reciprocal control of these two peptides. Prolactin secretion also is inhibited by thyroxine and is directly responsive to thyrotropin-releasing hormone (TRH). Estrogen and suckling are stimulatory. These signals may be positively mediated by α-MSH.

Inhibins and activins are members of the TGF-β superfamily and signal accordingly.^{343,344} Inhibin was discovered as the result of the search for the nonsteroidal gonadal hormone capable of specifically suppressing FSH. Activin was serendipitously discovered as the FSH-stimulating activity in the side-fractions in these studies. These hormones are formed by the differential disulfide-linked dimerization of two of three subunits (α, β_A, and β_B), each encoded by a distinct gene. The combination of an α- and β-subunit yields the inhibins, inhibin-A (αβ_A) and inhibin-B (αβ_B). Activins are dimers of β-subunits, β_Aβ_A, β_Bβ_B, and β_Aβ_B (activin-A, B, and AB). Inhibin antagonizes all known actions of activin. The genes for all three subunits are differentially expressed in a wide variety of tissues. Furthermore, these factors, particularly activin, have proven to exert effects not only on gonadotropes, but within other pituitary cells, the gonads, and in nonsexual target tissues.

Steroid Hormones

The ovary and adrenocortical zona reticularis share the core of the steroid biosynthesis pathway (Fig. 16.22).^{40,444,445} Gonadal cholesterol seems to be derived mostly from the cholesterol esters of low-density lipoprotein in man.⁴⁴⁶ Most steroidogenic steps are mediated by cytochrome P450 family members. These are the terminal enzymes in electron transfer chains, which include P450 oxidoreductase (POR) as the clinically relevant electron donor for all in the endoplasmic reticulum. The initial step in the biosynthesis of all steroid hormones is the conversion of cholesterol to pregnenolone. This is a two-stage process. The rapidity of the process depends upon the transport of cholesterol from the outer to the inner mitochondrial membrane by the steroidogenic acute regulatory protein (StAR). The conversion itself is carried out by the cholesterol side chain cleavage activity (scC) of cytochrome P450scC. The next steps are either the 3β-HSD step or 17α-hydroxylation. 3β-HSD converts Δ⁵-3β-hydroxysteroids to steroids with the Δ⁴-3-keto configuration (e.g., pregnenolone to progesterone, 17-hydroxypregnenolone to 17-OHP, and DHEA to androstenedione). This step is obligatory for the synthesis of all potent steroid hormones. The type 2 3β-HSD isozyme accounts for the vast majority of the 3β activity in the human ovary and adrenal; the type 1 isozyme accounts for 3β-HSD activity in liver and skin. Pregnenolone alternatively undergoes a two-step conversion to the 17-KS DHEA along the Δ⁵-steroid pathway: this conversion is accomplished via cytochrome P450c17. P450c17 is a single enzyme with 17α-hydroxylase and 17,20-lyase activities, the latter being less efficient and critically dependent on electron transfer from cytochrome b. Progesterone undergoes a parallel transformation to androstenedione in the Δ⁴-steroid path: 17α-hydroxylation of progesterone by P450c17 forms 17-hydroxyprogesterone, but in humans P450c17 does not efficiently utilize 17-hydroxyprogesterone as a substrate for 17,20-lyase activity, so P450c17 seems to form little if any androstenedione. There is some evidence for the existence of a P450c17-independent Δ⁴-pathway to androstenedione, but most seems to be formed from DHEA by the action of 3β-HSD.⁴⁴⁷ Sulfotransferase 2A1 is uniquely expressed in the adrenal zona reticularis and requires the cofactor 3'-phosphoadenosine-5'-phosphosulfate synthase type 2.³⁹⁴ Other sulfotransferases (e.g., for formation of estrone sulfate) and steroid sulfatase (for the reverse reaction) are widely expressed.⁴⁴⁸

17β-Hydroxysteroid dehydrogenase (17β-HSD) and aromatase activities are required for the formation of potent sex steroids. In the ovary, androstenedione is the major precursor for sex steroids. The conversion of 17-KS to 17β-hydroxysteroids by 17β-HSDs is essential for the formation of both androgen and estrogen: testosterone is formed in the ovary by 17β-HSD type 5 (also termed *aldoketoreductase*, AKR1C3), whereas estradiol formation requires 17β-HSD type 1.⁴⁴⁹ Aromatase activity, effected by P450arom, is essential for estradiol formation. Alternate promoters are used by the P450arom gene in the gonads, placenta, and adipose tissue, which yields alternatively spliced forms of aromatase. The organization and regulation of steroidogenesis in the developing follicle is depicted in Fig. 16.19.

The ovary normally accounts for about 25% of testosterone secretion in the mature female (0.06 mg daily), but it secretes about 30 times as much androstenedione (1.6 mg daily).⁴³⁶ These amounts are similar to those secreted by the adrenal. However, the ovary secretes less than 1/10 as much DHEA as the adrenal.

The "production rate" of a hormone equals its secretion rate plus (in the case of hormones formed outside of endocrine glands) the rate of formation of the hormone by peripheral conversion of secreted precursors. The "blood production rate" is calculated as metabolic clearance rate × serum concentration;

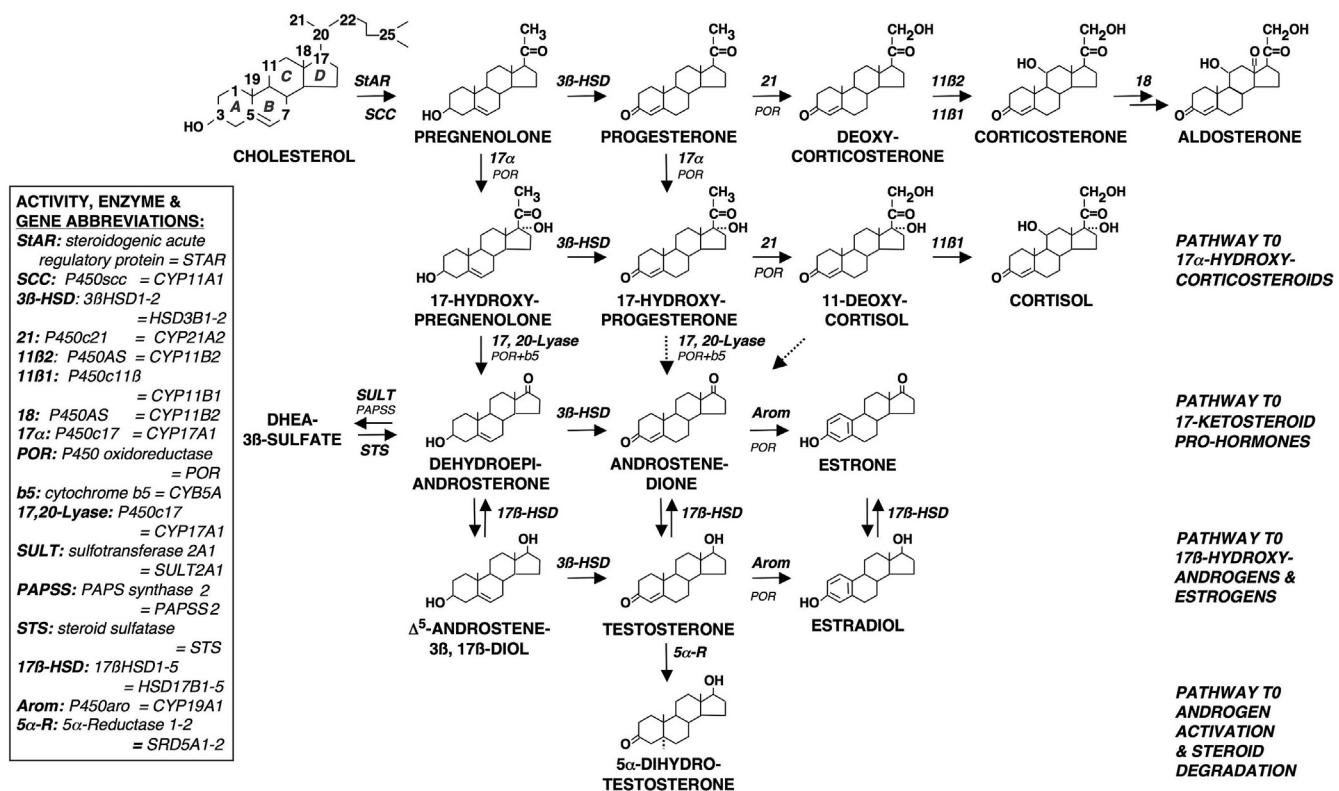


Fig. 16.22 Major pathways of steroid hormone biosynthesis from cholesterol. Carbon atoms of cholesterol are designated by conventional numbers and rings by conventional letters. The flow of hormonogenesis is generally downward and to the right. The top row shows the pathway to progesterone and mineralocorticoids, the second row the pathway to glucocorticoids, the third row the 17-ketosteroid prohormones, and the fourth row the potent 17 β -hydroxysteroids, and the bottom row the activation of androgen. The steroidogenic enzymes are italicized. Abbreviations for enzymes include the following cytochrome P450 enzyme activities: cholesterol side chain cleavage (scc); 17 α -hydroxylase (17 α); 21-hydroxylase (21); 11 β -hydroxylase (11B1); aldosterone synthase (11B2, 18-hydroxylase/oxidase); aromatase (Arom). Non-P450 enzyme activity abbreviations include Δ^5 -isomerase-3 β -hydroxysteroid dehydrogenase (3 β) and 17 β -hydroxysteroid dehydrogenase (17B-HSD). Clinically relevant electron transfer enzymes include P450 oxidoreductase (POR), cytochrome b5 (b5), and 3'-phosphoadenosine-5'-phosphosulfate synthase type 2 (PAPSS). (Modified from Rosenfield, R.L., Lucky, A.W., Allen, T.D. (1980). The diagnosis and management of intersex. *Curr Prob in Pediatr*, 10, 1.)

in the steady state the amount of hormone irreversibly leaving the plasma compartment equals the amount entering it. Because of extensive steroid interconversions, the quantity of these hormones excreted in urine is not necessarily indicative of the amount reaching target tissues.⁴³⁶ For example, so large a fraction of urinary testosterone glucuronide is formed directly from androstenedione by compartmentalized metabolism within the liver that the range of urinary excretion of testosterone in women overlaps that in men (Fig. 16.23).⁴⁵⁰ Estrone sulfate, like DHEAS in the androgen pathway, forms a circulating reservoir of inactive estrogen that can be returned to the active pool by hepatic sulfatase activity.⁴⁵¹ The blood production rates of representative steroid hormones are given in Table 16.2 and are shown for estrogens in Fig. 16.24. During the luteal phase of the menstrual cycle, estradiol production doubles⁴³⁹ and progesterone production rises 16-fold or more.⁴⁵²

Sex hormones also have environmental origins. Structurally distinct biological estrogens include equine estrogens and plant-derived phytoestrogens.⁴⁵¹ Synthetic estrogens include pharmacological compounds, such as ethinyl estradiol, diethylstilbestrol, selective ER modulators (SERMs), and some industrial chemicals, such as organochlorines (p,p"-dichlorodiphenyltrichloroethane [DDT] and others) and plasticizers (such as bisphenol A and phthalates). Endocrine-disrupting chemicals (EDCs) interfere with any aspect of hormone action, with mechanisms including mimicking or blocking hormone signaling through its receptor, and modulating

the synthesis, release, transport, metabolism, binding or elimination of natural hormones. These compounds therefore may impact development of the reproductive tract and function of the reproductive axis.^{192,266} Animal studies have indicated that EDCs can impair ovarian development, inhibit ovarian follicle growth, increase follicular atresia, and disrupt steroid hormone levels.²⁶⁶

Peripheral conversion of secreted prehormones by non-endocrine organs accounts for a major portion of sex hormone production. The ovary and the adrenal cortex are sources of prehormones, as well as secreted hormones. About 50% of serum testosterone (0.1 mg daily) normally is formed indirectly by peripheral conversion. Although 85% of normal estrogen production in women arises by secretion in midcycle, 50% of estrogen production can arise from extraglandular sources during the low-estrogen phases of the menstrual cycle.⁴⁵³ Peripheral formation of active steroids occurs in a wide number of sites, including liver, fat, and target organs.^{40,454} For example, the liver has high levels of 3 α -, 3 β -, and 17 β -hydroxysteroid dehydrogenase and 5 α -reductase activities (see Fig. 16.22).

Peripheral androgen metabolism is not tightly regulated by the neuroendocrine system. It seems determined to some extent by the perinatal androgenic milieu,⁴⁵⁵ the effect of which is possibly mediated by GH.⁴⁵⁶ Postnatally, it is influenced by the sex hormone binding globulin (SHBG) level and the state of nutrition. Adipose tissue becomes a major site of conversion of androstanedione to both estrone and testosterone in the obese.^{308,457}

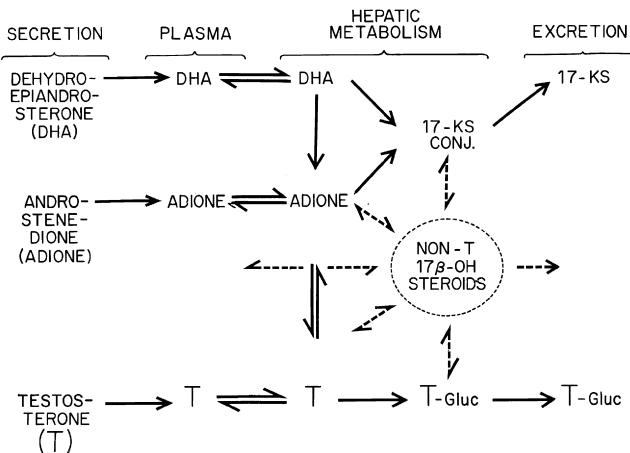


Fig. 16.23 Diagram illustrating the relationship among secreted, plasma, and urinary steroids. 17-Ketosteroid (17-KS) excretion does not reflect accurately the excretion of the most important plasma androgens. Only 25% or less of testosterone is excreted as 17-KS metabolites. Therefore important changes in testosterone production may not appreciably affect urinary 17-KS excretion. Furthermore, even the major 17-KS (DHA-sulfate) is excreted poorly until its production rate becomes quite high. On the other hand, about half of 17-KSs are not identified by the standard colorimetric test and 2 mg daily of 17-KS in adults results from hydrocortisone metabolism. In addition, testosterone glucuronide excretion does not accurately reflect the plasma testosterone level: less than 2% of testosterone appears in the urine as such. Furthermore, the plasma 17-KS androstenedione may be converted to testosterone glucuronide without ever circulating as unconjugated testosterone. (From Rosenfield, R.L. (1973). Relationship of androgens to female hirsutism and infertility. *J Reprod Med*, 11, 87.)

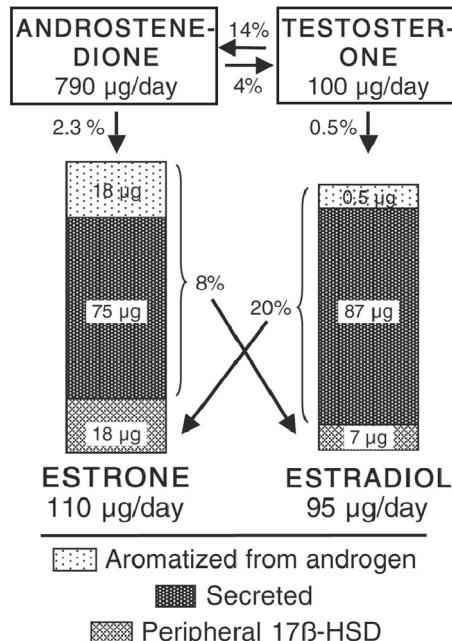


Fig. 16.24 Sources of estrone and estradiol in blood of follicular phase premenopausal women. Estrogen is derived from direct secretion by the gonad, aromatization of androgen, or conversion of an estrogen precursor by 17 β -hydroxysteroid dehydrogenase (17 β -HSD) activities. The percentage of substrate converted per day and total approximate production in micrograms per day are noted for each source. (Modified from Alonso, L.C., and Rosenfield, R.L. (2002). Oestrogens and puberty. *Best Pract Res Clin Endocrinol Metab*, 16, 13.)

Cytochrome P450 mixed function oxidases, the most important of which is CYP3A4, affect steroid efficacy by forming hydroxylated steroid metabolites of varying potency.^{458,459} They are subject to induction or inhibition by numerous drugs. Phytoestrogens increase estradiol bioavailability by inhibiting hepatic sulfotransferase.⁴⁶⁰

Plasma steroids appear to reach their sites of action and metabolism by simple diffusion from the vascular compartment.⁴⁶¹ The bioactive portion of serum testosterone seems to be the free testosterone and a portion of the albumin-bound testosterone that differs among tissues according to the diffusion characteristics of the vascular bed.⁴⁶² About 98% of serum testosterone and estradiol are bound to albumin and SHBG. The SHBG concentration determines the fraction of serum testosterone and other ligands (e.g., estradiol, DHT) that are free or bound to albumin. It is also a major determinant of ligand egress from plasma (Fig. 16.25).⁴⁶³ Some sex steroid effects may be mediated by SHBG binding to membrane receptors and activation of adenylate cyclase.^{464,465} A number of physiological and pathological states affect the SHBG level. It is increased by estrogen and thyroid hormone excess; it is decreased by androgen, insulin-resistant obesity, glucocorticoid, GH, and inflammatory cytokines.^{466–468}

Target cell metabolism influences the cell's response to the steroid hormones that reach it (Fig. 16.26).⁴⁶⁹ The intracellular conversion of testosterone to DHT by one of the two isozymes of 5 α -reductase is important for many but not all effects of testosterone,⁴⁷⁰ dependent upon the tissue-specific pattern of

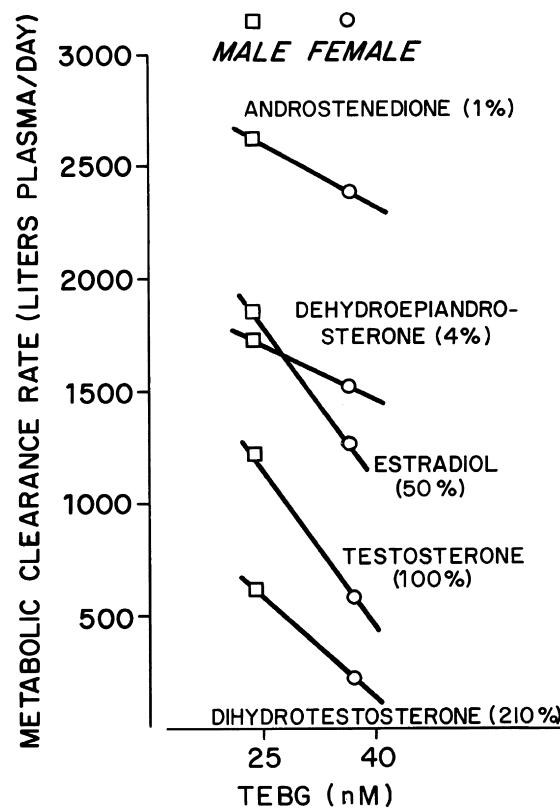


Fig. 16.25 The relationship between the metabolic clearance rate (MCR) and binding of sex hormones to sex hormone binding globulin (SHBG = testosterone-estradiol binding globulin [TEBG]). The MCR of each steroid has been related to the mean SHBG levels of men and women. The approximate affinity of each steroid for SHBG relative to testosterone is indicated in parentheses. (From Rosenfield, R.L. (1975). Studies of the relation of plasma androgen levels to androgen action in women. *J Steroid Biochem*, 6, 695.)

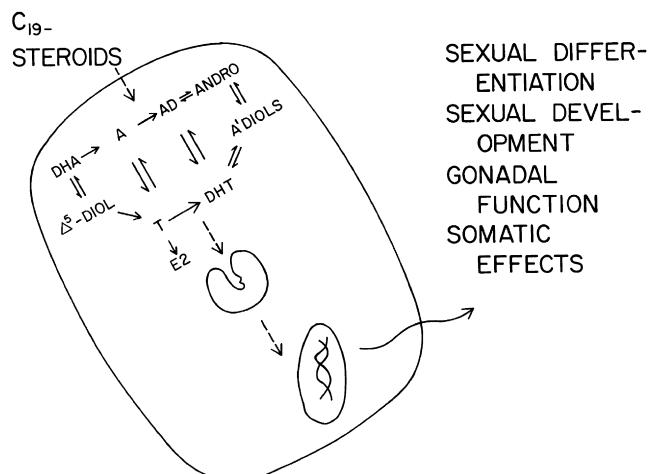


Fig. 16.26 Model of the mechanism of androgen action emphasizing the effect of steroid metabolism within a target cell on the mode of action. Solid arrows indicate pathways of steroid metabolism from 17-ketosteroid precursors as laid out in Fig. 14.23. Broken arrow indicates transport. The cell-specific intracellular pattern of C₁₉-steroid metabolism determines the relative availability of testosterone or dihydrotestosterone (DHT) to the cytosol receptor for translocation to the nucleus. In cells, such as the rat granulosa cell in which $\Delta^5,3\beta$ -hydroxysteroid dehydrogenase activity is high, androstenediol (Δ^5 -diol) is as potent as testosterone. The human sebaceous gland has a similar pattern of steroid metabolism. A, androstenedione; AD, androstanedione; A'DIOLS, androstane diols; ANDRO, androsterone; DHA, dehydroepiandrosterone. E2, estradiol; T, testosterone. (From Nimrod, A., Rosenfield, R.L., Otto, P. (1980). Relationship of androgen action to androgen metabolism in isolated rat granulosa cells. *J Steroid Biochem*, 13, 1015. With permission from Elsevier Science.)

steroid metabolism. An important mode of testosterone action is via estradiol, notably within the brain. Although transformation is not fundamental to the mode of action of estradiol, estradiol effectiveness is influenced by target cell metabolism: the induction of 17β -hydroxysteroid oxidation in target tissues by progesterone, resulting in conversion of estradiol to the less potent estrogen estrone, counterbalances estrogenization.⁴⁷¹ There is also evidence that novel steroid metabolites exert tissue-specific effects.^{472,473}

Within target cells, all steroid hormones regulate the genome similarly, starting with binding to high-affinity intracellular receptors (Fig. 16.27).^{474–476} The steroid hormone receptors belong to the superfamily of nuclear hormone receptors. The estrogen, progesterone, and androgen receptors are, thus, homologous. Classic sex hormone effects are exerted by the interaction of steroid with receptor, not by either alone. Steroid binding triggers the dissociation of inhibitory chaperone heat shock proteins from the receptor.⁴⁷⁷ The active receptor-ligand complex then undergoes noncovalent dimerization and binding to its specific hormone response element on the gene. The deoxyribonucleic acid (DNA) bound steroid-receptor complex acts as a transcriptional regulator of the target gene promoter. Sensitivity to steroids is also modulated by molecular chaperone proteins that influence receptor configuration, intracellular trafficking, and receptor turnover, all which are determinants of steroid action.^{478,479}

The binding properties of steroids to their cognate receptors are the initial determinants of classical steroid action.^{474,475} Ligand-based selectivity is one element of this interaction. Estradiol is a more potent estrogen than estrone and estriol partly because it binds best to the steroid binding domain of the ER.⁴⁸⁰ DHT is an inherently more potent androgen than

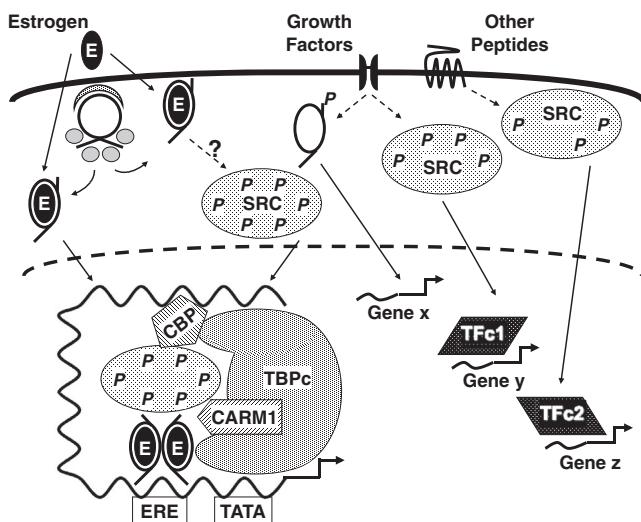


Fig. 16.27 A model for the mechanism of estrogen (E) action that emphasizes the role of interactions of the estrogen receptor with steroid receptor coregulator (SRC) and phosphorylation signaling. Estrogen causes the 4S subunit and heat-shock proteins to dissociate from the unliganded estrogen receptor. Then estrogen entry into the binding pocket causes a conformational change in the receptor. Estrogen also stimulates phosphorylation of SRC in a specific pattern (Ps), possibly via liganded membrane-bound estrogen receptor (ER) as it does some transcription factors, and recruits it to the nuclear deoxyribonucleic acid (DNA) steroid-receptor complex with the estrogen response element (ERE). SRC in turn recruits other coactivators, such as the cyclic adenosine monophosphate (cAMP) response element binding protein-binding protein (CBP) and coactivator-associated methyltransferase (CARM1) to the hormone binding complex. This aggregate then interacts with the TATA binding protein initiation complex (TBPC) to initiate estrogen-specific gene transcription. The genomic estrogen effect is modulated by the effects of environmental signals on other cell-specific transcription factors (TFs), some of which involve differentially phosphorylated SRC complexes (TFc) in gene activation, others of which involve ligand-independent ER. Dotted lines indicate diverse kinase pathways. ER recycling is not shown. (From Katzenellenbogen, B.S., Montano, M.M., Ediger, T.R., et al. (2000). Estrogen receptors: selective ligands, partners, and distinctive pharmacology. *Recent Prog Horm Res*, 55, 163–193; O'Malley, B.W. (2005). A life-long search for the molecular pathways of steroid hormone action. *Mol Endocrinol*, 19, 1402–1411; McDevitt, M.A., Glidewell-Kenney, C., Jimenez, M.A., et al. (2008). New insights into the classical and nonclassical actions of estrogen: evidence from estrogen receptor knock-out and knock-in mice. *Mol Cell Endocrinol*, 290, 24–30.)

testosterone mainly because of its higher association rate constant and its lower dissociation rate constant.⁴⁸¹

The antiestrogens tamoxifen and clomiphene and the anti-androgens cyproterone acetate and spironolactone competitively inhibit the active ligands from binding to their specific receptor sites by weakly and transiently occupying receptor sites. These differences result from potent agonists snugly fitting into the binding pocket, which induces a receptor conformation different than that of antagonist-bound receptor. One such change is the C-terminal tail of the receptor flipping over to close the “door” when a potent agonist enters; this simultaneously provides a different outer surface for interaction with coregulator proteins.

Thus ligand-based selectivity arises not only because of tighter ligand binding, but because alternative ligands produce both intermediate and unique conformational changes in the receptor, which in turn induce altered receptor interactions

with coregulator proteins that result in a spectrum of activities.⁴⁸² Thus steroids do not simply switch receptors on; they induce selective functions that depend on the nature of the coregulators that are recruited to the complex.^{483–486} In part, this selectivity arises because different domains of these receptors mediate these different functions. For example, the activation function (AF)-1 domain of the ER mediates interactions with mitogen-activated protein (MAP) kinase and TGF- β 3, whereas the AF-2 domain mediates interactions with coregulator proteins.⁴⁸⁷ Coactivators, in turn, regulate alternative splicing, gene activation and repression (in some cases via their dual enzymatic functions), ubiquitin-proteosome-mediated turnover of the receptor-coregulator complex,⁴⁸⁸ and also determine cell-specific, site-based actions,⁴⁷⁵ as discussed later.

Receptor-based selectivity is a second element in steroid action. There are now known to be two isoforms of each of the sex steroid receptors. The α and β forms of the ER, although homologous, are coded by separate genes.¹⁷¹ A and B forms of the progesterone and androgen receptors exist.^{489,490} These forms of the PR arise by transcription from alternate promoters within the same gene, whereas those of the androgen receptor arise from posttranslational modification of a single messenger (m)RNA. These isoforms have variously been shown to manifest a differential tissue expression pattern and respond differentially to antagonists. Interactions of a steroid with different forms of receptors can regulate some target genes differentially. One role of ER β is apparently to modulate ER α activity: ER α and ER β can have opposite actions at AP-1 and SP-1 sites, and studies of transcriptional activity in bone and breast tissue of mice indicate a restraining effect of ER β on responses to estradiol.^{171,491} Thus different target tissues exposed to the same hormone may respond selectively because of a distinct repertoire of receptor isoform expression. Some examples are notable. Although both forms of ER are expressed in most target tissues, the classic form of the ER, ER α , plays the key role in regulation of LH and estrogen actions on the uterus, breasts, sex-specific behavior, and bone.^{171,492,493} In the mouse ovary, knockout experiments show that ER α is expressed in thecal cells where it prevents androgen excess in response to LH. In contrast, ER β is expressed only in granulosa cells, where its inhibition of androgen receptor expression is critical to prevent premature follicular atresia.¹⁷² Both are necessary for oocyte survival and the ability of preovulatory follicles to rupture. Furthermore, loss of both causes transdifferentiation of granulosa cells to Sertoli-like cells and massive oocyte death.¹⁵⁶ Liganded PR A is essential for ovulation⁴⁹⁴ and is the more effective antagonist of ER action.⁴⁷⁴ In addition, sequence variation in hormone response elements contributes to differential gene regulation.⁴⁹⁵

Effector site-based selectivity is a third variable in classical sex hormone action. In other words, the potency and character of a response to a ligand-receptor complex are not simply inherent properties of the complex. Rather, they depend on the array of effector molecules present in the site of action. Thus the array of genes expressed locally and the relative expression level of coregulators (coactivators and corepressors) are extremely important in the determination of appropriate and graded responses to a ligand by a target cell.^{474,496} Heterodimerization of the ER with other nuclear receptors can modulate its action.⁴⁹⁷ Androgens appear to exert some of their genomic effects by directly complexing with transcription factors other than the androgen receptor.⁴⁹⁸ Both estrogen and androgen appear to exert antiapoptotic effects in osteoblasts and osteocytes by activating a ligand-dependent, but nongenomic, kinase-mediated signaling pathway.⁴⁹⁹

Nuclear receptor coactivators are critical in sensing cell-specific environmental signals and to coordinate signals emanating from membrane receptors with nuclear receptor

action.⁴⁷⁵ Surface receptors send signals through kinase pathways that result in specific serine/threonine phosphorylation patterns of coactivators. These phosphorylation patterns serve as a code for the coactivator to preferentially bind and activate distinct sets of downstream transcription factors (see Fig. 16.27). Overexpression of steroid receptor coactivator-3 (SRC-3) is as important in the pathogenesis of some breast cancers as is ER positivity.

The effects of a given ligand-ER complex often differ from those of the estradiol (E2)-ER complex among cell types. This is the basis for the development of SERMs: these compounds exert effects in a tissue-specific manner depending on the cell context.^{474,482} The chemical structure of a SERM—or any ER ligand, for that matter—determines the configuration of the ER, resulting in a spectrum of activities from agonist to antagonist, depending on which coregulators are available for recruitment in the target cell. Raloxifene is an estradiol agonist in bone and epiphyseal cartilage but is antiestrogenic in uterus and breast; tamoxifen is estrogenic in uterus but antiestrogenic in breast and bone. Both appear to retain neural and endothelial estrogenic activity.^{500,501}

Nonclassical mechanisms play a role in sex steroid action.⁴⁷⁵ The nonclassical mechanisms are of two general types: (1) genotropic estrogen response element (ERE)-independent signaling, in which liganded ER acts as a coregulator of other transcription factors that act through their specific DNA response elements and (2) nongenotropic signaling, in which E2 binding to membrane-associated receptors, including ER α , rapidly stimulates phosphorylation pathways.^{502,503}

The nongenomic effects via membrane signaling occur rapidly (within minutes) and can mediate cell proliferation, apoptosis, and migration in cell-specific ways.^{499,504} Nongenomic E2 actions account for most of the LH-inhibitory and energy balance effects of E2.^{503,505} These effects can be mediated by binding to nuclear ER in plasma membrane domains provided by scaffolding proteins, such as caveolin. On such platforms, the E2-ER complex acts like a membrane receptor, coupling with G-proteins and activating cytoplasmic pathways involving SRC and MAP kinase. Androgens appear to act similarly. Nongenomic actions of nuclear PR have also been reported. Some nongenomic effects seem to involve the activation of novel G-protein-coupled transmembrane receptors for E2 and progesterone that interact either with steroids or their metabolites.

Genomic ER signaling may also be ligand independent. For example, cell membrane signaling by growth factors or other peptides stimulate ER phosphorylation. EGF activates phosphorylation of the ER and simulates diverse estrogen effects.⁵⁰⁶ Activation of unliganded ER α seems to be involved in repressing expression of the androgenic 17 β -HSD testicular isoform in the ovary.³³⁵

Steroids that act by binding to membrane-bound receptors in the brain are termed *neuroactive*.³⁹⁷ Neuroactive steroids synthesized in the brain are termed *neurosteroids*.^{507–509} The best documented of these effects are on neurotransmitters, which control ion channels. Allopregnanolone (3 α -hydroxy-5 α -tetrahydroprogesterone) and 3 α -androstaneol are GABA A receptor agonists and so have sedative and antiepileptic properties.⁵¹⁰ Pregnenolone sulfate and DHEAS have the opposite effect, the former also stimulating the glutamate receptors. Receptors for 5-hydroxytryptamine have been implicated in mediating some of the effects of sex steroids and certain of their metabolites.^{511,512} Some estrogen effects in brain are membrane-mediated.⁵¹³

The tissue-specific posttranscriptional events involved in sex steroid signaling are poorly understood. Estradiol and progesterone modulate the actions of each other through effects on their specific receptors: increased estrogens in the preovulatory phase of the cycle upregulate target organ receptors for both estradiol and progesterone; luteal phase levels of progesterone

then suppress the production of both receptors.^{514,515} Estrogen prevents bone loss by blocking the production of proinflammatory cytokines.⁵¹⁶ Androgen action has been reported to be mediated by prostaglandins in genitalia,⁵¹⁷ and testosterone stimulates the IGF-1 system in epiphyseal cartilage.⁵¹⁸

Maturation of Sex Hormone Target Organs

Genital Tract

The Müllerian system of the embryo gives rise to the uterus, cervix, upper vagina, and fallopian tubes in the absence of AMH secretion by fetal testes during the first trimester of gestation.⁴⁴⁴ Genital swelling develops to engulf the base of the penis-like clitoris between 11 and 20 weeks' gestation in parallel with the development of the ovarian follicular system.⁵¹⁹ ERs are expressed in the labia minora, prepuce, and glans in females, but not in the homologous structures of males.⁵²⁰ An association between antiestrogen and genital ambiguity has been reported.⁵²¹ Diethylstilbestrol induces dysplasia of the genital tracts.⁵²² These data suggest that estrogen may play a direct role in female genital tract differentiation. However, knock-out of ERs has no obvious effect on genital tract differentiation.¹⁵⁶

The infantile uterus and cervix enlarge under the influence of estrogen during puberty. The endometrium and cervical glands then undergo cyclical changes in concert with cyclic ovarian function. In response to rising estrogen during the follicular phase of the cycle, the endometrial epithelium and stroma proliferate. The uterine glands increase in number and lengthen. Endometrial hyperplasia is prevented by progestin⁵²³ and androgen excess.⁵²⁴ In response to progesterone secretion after ovulation, the endometrium increases in thickness: stromal edema occurs, and the uterine glands enlarge, become sacculated, and secrete a glycogen-rich mucoid fluid. The coiled arteries lengthen further during this time and become increasingly spiral. These changes are critical to permit implantation. High-dose progestin is an effective postcoital contraceptive because it prevents implantation when taken within 3 days of unprotected intercourse.⁵²⁵

Endocervical gland secretions lubricate the vaginal vault. The endocervical mucus is scanty and relatively thin during the low-estrogen phase of the cycle. The increase in mucus flow with advancing follicular development seems to require tissue-specific stimulation of the cystic fibrosis transmembrane regulator by estrogen.⁵²⁶ Cervical mucus becomes more viscous and elastic as estrogens rise in the later follicular phase of the cycle—the extent to which it can be stretched into a long spindle, *spinnbarkeit*, is a function of the estrogen level.

The mucosa of the vagina and the urogenital tract is comprised of hormone-responsive stratified squamous epithelium (Fig. 16.28).² The basal layer is the regenerative area. In the absence of estrogen, there is only a parabasal layer of cells over this, and the vagina is thin, with a tendency to alkalinity, which predisposes it to local infection (nonspecific vaginitis).⁵²⁷ In response to estrogen, epithelial proliferation occurs, with formation of successive intermediate and superficial layers. With this maturation, the cytoplasm of each cell first expands, leading to formation of small intermediate cells. With further estrogenization, the nuclei become pyknotic and large intermediate cells form. Greater estrogenization brings about their transformation to cornified squamous superficial cells: the cytoplasm changes from basophilic to acidophilic with the accumulation of glycogen. Resistance to infection of the fully developed vaginal mucosa results from its thickness and from its acid pH, which occurs from the fermentation of the glycogen of the superficial cells. In response to luteal phase progesterone, degenerative changes appear in vaginal mucosal cells:

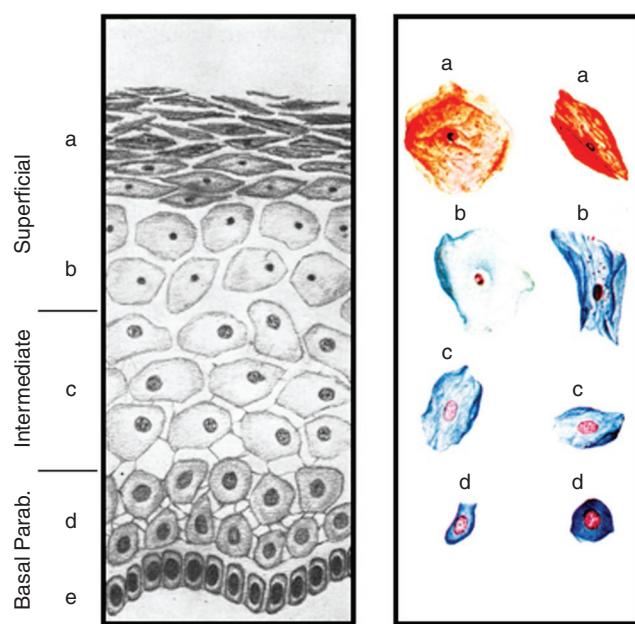


Fig. 16.28 The layers of vaginal epithelium of the well-estrogenized adult. The superficial layer contains surface cells that are cornified (squamous) with eosinophilic cytoplasm and pyknotic nuclei (a) as well as large intraepithelial cells that are also karyopyknotic but basophilic (b). The intermediate zone contains basophilic cells that have less cytoplasm and intermediate-size nuclei (c). The basal and parabasal cells have a relatively small amount of basophilic cytoplasm and relatively vesicular nuclei (d, e). (Modified from Wilkins, L. (1968). The Diagnosis and Treatment of Endocrine Disorders in Childhood and Adolescence. Springfield, IL, Charles C Thomas.)

superficial cells decrease, the cytoplasm assumes a "crinkled" appearance, cells degenerate, and bacterial proliferation increases.

Vaginal smears show the characteristic cyclic changes in the cell types comprising the vaginal epithelium (see Fig. 16.28).⁵²⁸ In the prepubertal years, parabasal cells predominate, and characteristically 10% or less are small intermediate cells. A pattern consisting entirely of intermediate cells is typical of early puberty. The early follicular phase of the menstrual cycle is characterized by the predominance of large intermediate cells with few, if any, superficial cells. Peak maturation is reached at midcycle, at which time 35% to 85% of the cells seen on vaginal smear are superficial; the remainder are large intermediate cells. This cornification develops over a 1-week period in response to estradiol levels of about 70 pg/mL and persists 1 to 2 weeks after estrogen withdrawal (see Fig. 16.11).⁵²⁹

Progesterone antagonizes estrogen effects on the vaginal epithelium and cervix. Inhibition of cervical ripening by progestins is used to prevent recurrent spontaneous preterm delivery.^{530,531}

Many normal variations have been recognized in the appearance of the hymen. The transverse diameter increases with age.^{532,533}

Mammary Glands

Multiple rudimentary branching mammary ducts are found beneath the nipple in infancy; they grow and branch very slowly during the prepubertal years.⁵³⁴ Estrogen stimulates the nipples to grow, mammary terminal duct branching to progress to the stage at which ductules are formed, and fatty stromal growth to increase until it constitutes about 85% of the

mass of the breast. GH (via IGF-1) and glucocorticoids play a permissive role.^{535,536} These hormones interact with breast stroma and local growth factors to stimulate the development of breast epithelium. Lobulation appears around menarche, when multiple blind saccular buds form by branching of the terminal ducts. These effects are caused by the presence of progesterone. The breast stroma swells cyclically during each luteal phase. Full alveolar development normally only occurs during pregnancy under the influence of additional progesterone and prolactin. Prolactin does not play a role in breast growth without priming by female hormones.⁵³⁷

Estrogen and progesterone also play a role in breast cancer susceptibility.⁵³⁸ Earlier than average age at menarche is a modest risk factor for breast cancer, regardless of BRCA status;^{539,540} notably, however, breast cancer risk has not been shown to be increased in precocious puberty. The *BRCA1* gene normally restrains mammary growth, at least in part, by inhibiting expression of ER α and PRs, and cancer-related mutations reverse these processes.⁵⁴¹

Pilosebaceous Unit

The pilosebaceous unit (PSU), with but few exceptions, consists of both a piliary and a sebaceous component.⁵⁴² Androgens are a prerequisite for the growth and development of PSUs in their characteristic pattern. Androgens exert their effects both on the dermal papilla, which regulates the hair growth cycle, and on PSU epithelium. Before puberty, the androgen-dependent PSU consists of a prepubertal vellus follicle in which the hair and sebaceous gland components are virtually invisible to the naked eye (Fig. 16.29). Under the influence of androgens, in the sexual hair areas, the PSU switches to producing a medullated terminal hair follicle that expresses a unique type of keratin that is androgen responsive.⁵⁴³ The difference in the apparent density of sexual hair between men and women is caused by differences in the density of terminal hairs that develop in response to androgen. In the balding-prone area of scalp in individuals genetically predisposed to male-pattern alopecia, androgens weakly attenuate the hair growth cycle, so that the PSU gradually generates only vellus follicles.⁵⁴⁴ In acne-prone areas, androgen causes the prepubertal vellus

follicle to develop into a sebaceous follicle, in which the sebaceous epithelium develops and the hair remains vellus. Adrenarchal levels of androgens suffice to successively initiate sebaceous gland development and the growth of pubic hair. Progressively, greater amounts of androgen are in general required to stimulate terminal hair development along a pubic to cranial gradient. All these effects of androgen are to some extent reversible by antiandrogens.

Estrogens modestly stimulate hair growth, probably by inhibiting the catagen (resting) phase of the hair cycle;⁵⁴⁵ this may well be caused by induction of androgen receptors by estrogen. Estrogens also directly inhibit sebum secretion. GH synergizes with androgen action on the PSU, in part via IGF-1 signaling. Retinoic acid receptor agonists antagonize the effects of androgen on the sebaceous gland by inhibiting sebocyte proliferation and differentiation. Insulin, prolactin, glucocorticoids, thyroxine, and catecholamines also play roles in PSU growth, development, and function.

Bone

Increased secretion of sex hormones clearly initiates the pubertal growth spurt. About half of this effect of sex hormones is caused by their stimulation of the GH-IGF axis.⁵⁴⁶ The remainder of the effects of sex steroids on skeletal growth is direct.^{547,548}

Differences between the actions of sex hormones contribute to women's bones being shorter and narrower than men's.^{549,550} The basis for these differences are diverse and involve interactions with IGF-1 and effects on cortical, cancellous, and periosteal bone formation.

Estrogen and androgen both stimulate epiphyseal growth. Estradiol is the critical hormone that brings about epiphyseal closure.⁵⁴⁸ Estrogen also is particularly effective in reducing bone turnover. To some extent these effects may be prenatally programmed.⁵⁵¹ Bone accrual during puberty is a major determinant of adult fracture risk. Menarche after 15 years carries a 1.5-fold increase in fracture risk, and the risk rises with age of menarche.⁵⁵²

Adipose Tissue

Women have a greater percentage of body fat than men.⁵⁵³ During puberty, they develop both more and larger fat cells than men in the lower body, which favors a lower body (gluteofemoral) fat distribution, in contrast to men's upper-body (visceral) fat accumulation. The critical periods for establishment of the adipocyte population are fetal life and adolescence, after which lipid accumulation occurs primarily by cell hypertrophy.⁴⁰ Serum levels of leptin rise throughout puberty to reach higher levels in females than males,²⁸² whereas levels of the antilipolytic adipocytokine adiponectin remain stable in females but fall in males⁵⁵⁴ in response to androgen.⁵⁵⁵

Insulin signaling is of major importance to the size and function of adipose tissue—stimulating adipogenesis (development of preadipocytes into adipocytes) and lipogenesis, while inhibiting lipolysis.⁴⁰ Beta-adrenergic catecholamines stimulate lipolysis, countering inhibition by insulin.^{556,557} Visceral white adipose tissue (VWAT) lipolysis is less sensitive to insulin and more sensitive to catecholamines than subcutaneous SCWAT.⁵⁵⁷

Androgens cause a masculine physique at puberty primarily by inhibiting adipogenesis reciprocally to stimulating myogenesis.⁵⁵⁸ They act by inhibiting adipogenic differentiation of human mesenchymal pluripotent stem cells reciprocally to their stimulation of the myogenic lineage, in a dose-dependent fashion.⁵⁵⁹ Local androgen generation by adipose tissue as it differentiates in response to insulin⁵⁶⁰ likely serves to limit insulin-generated adipogenesis.⁵⁶¹ In adipocytes, androgen has been reported to inhibit lipogenesis.^{562,563} Androgen also

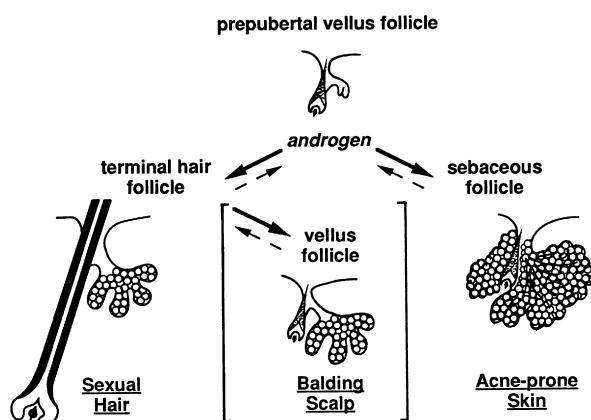


Fig. 16.29 Role of androgen in the development of the pilosebaceous unit. Androgens (solid lines) are responsible for the patterned differentiation of the pilosebaceous unit at puberty. Dotted lines indicate effects of antiandrogens. Hairs are depicted only in the anagen (growing) phase of the growth cycle. In balding scalp (bracketed area), terminal hairs not previously dependent on androgen regress to vellus hairs under the influence of androgen. (From Rosenfield, R.L., Deplewski, D. (1994). Role of androgens in the developmental biology of the pilosebaceous unit. *Am J Med*, 97(5A), 80.)

inhibits catechol-stimulated lipolysis in female SCWAT, opposing the effect of insulin, but not in omental WAT.^{564,565}

Estrogen has been reported to suppress lipogenesis through inhibition of adipocyte lipoprotein lipase activity, according to most *in vitro* studies.⁵⁶⁶ Estradiol attenuates the lipolytic response to catecholamines, specifically in SCWAT adipocytes,⁵⁶⁷ but promotes lipolysis by stimulating hormone-sensitive lipase.⁵⁶⁶ The development of obesity in postmenopausal women and oophorectomized animal models has led to the concept that estrogen deficiency causes obesity,⁵⁶⁸ but the role of FSH elevation in mediating this obesity by inhibiting the formation of brown adipose tissue⁵⁶⁹ confounds the interpretation of all such studies. Progesterone generally counters estrogen and androgen effects on white and brown adipose tissue in experimental models.^{568,570}

Female sex steroid effects on serum lipids are modest.^{571,572} Physiological (transdermal) estradiol replacement slightly raises high-density lipoprotein cholesterol (HDL-C)⁵⁷² and lowers very low-density lipoprotein (VLDL) triglycerides.⁵⁷³ The lower LDL-C during the normal luteal phase seems primarily because of its consumption by the corpus luteum as steroidogenic substrate.⁵⁷² Oral estrogen replacement therapy raises HDL-C more than does transdermal replacement, but differs from it in raising VLDL-triglycerides and decreasing LDL-C, whereas androgenic progestins lower HDL-C.⁵⁷⁴

Progesterone deficiency is responsible for the increased postprandial chylomicron triglyceride concentrations that occur as the result of low peripheral lipoprotein lipase activity when the pituitary-gonadal axis is acutely suppressed.⁵⁷⁵ In humans, use of the progesterone analogue megestrol acetate is approved to stimulate appetite and weight gain; use of progestins is associated with insulin resistance.⁵⁷⁶

Muscle

Testosterone administration increases muscle mass and decreases fat mass reciprocally. Androgen does so by promoting the commitment of mesenchymal pluripotent stem cells to the myogenic lineage while inhibiting adipogenesis.^{559,577} Testosterone effects are exerted via androgen-receptor-mediated and nonclassical pathways. Human skeletal muscle formation of DHT is mediated primarily by type 3 5-alpha-reductase.⁵⁷⁸ Androgens then exert dose-related stimulation of muscle cell hypertrophy, as well as hyperplasia along with associated tissues, such as motor neurons. Hyperandrogenic women have increased muscle mass and strength, which seems to give them an advantage in athletic competition.⁵⁷⁹ Consequently, there is active debate about regulation of women's androgen levels in elite athletic competition.

Central Nervous System

Concordance of gender identity (self-identification as male or female) and gender orientation (sexual preference) with gender assignment on the basis of genital anatomy is the norm, which is consistent with an important role of androgen in programming these aspects of neuropsychosocial development. However, nonhormonal genetic and epigenetic factors influence sexually dimorphic aspects of human development.⁵⁸⁰⁻⁵⁸²

Before sex hormone differences are detectable, several genes are differentially expressed in the brains of male and female mice.⁵⁸³ Sex chromosomes directly program sexually dimorphic neuronal differentiation⁵⁸⁴ and behaviors, such as aggression, parenting, and social interaction.⁵⁸⁵ The maternally inherited X chromosome is preferentially expressed in glutamatergic neurons of the cerebral cortex, and sex-specific imprinting of autosomal genes of the hypothalamus is common and appears to be the default state in females.^{586,587}

Testosterone exposure during both the period of transient activation of the HPG axis in the fetal-perinatal period and again during puberty plays a role in organizing neural gene expression and development in a sexually dimorphic manner according to extensive studies in animal models, which are consistent with observations in humans.⁵⁸⁸⁻⁵⁹⁰ The critical period for this hormonal programming on behavioral patterns closes after puberty. This has consequences both for sex-typical neuroendocrine function and sex-typical and nonsexual behavior that is activated by the pubertal hormonal milieu.^{404,588,591,592}

The critical period for hormonal sensitivity of sexually dimorphic areas of the brain occurs during the early newborn period of rodents, which is thought to be comparable to the early second trimester of humans.⁵⁹³ In rats, the preoptic nucleus of the hypothalamus is larger in males, and treatment of newborn females with testosterone (or estradiol) permanently increases neuronal development to duplicate this effect and causes subsequent masculinized sexual behavior and anovulation.^{13,594} The anovulation results from masculinization of the LH secretory pattern (that is, both increased LH secretion and suppressed capacity to mount LH surges in response to estrogen priming) that appear to be the consequence of permanent suppression of hypothalamic PR expression.¹⁶

Testosterone administration to experimental animals stimulates the growth of sexually dimorphic brain areas to adult male size.⁵⁹⁵ Maintenance of differences in adult nuclear size and androgen receptor expression of sexually dimorphic areas of the brain is dependent upon the ambient androgen level.^{596,597} Peripubertal testosterone and female hormone administration have different effects on behavior.^{595,598}

Several human brain structures are sexually dimorphic, some becoming so at puberty.⁵⁹⁹⁻⁶⁰¹ Characteristic cortical and subcortical sex differences are discernable at puberty by structural magnetic resonance imaging (MRI),^{589,602,603} although most human brains have a mosaic of "male" and "female" features.⁶⁰⁴ Fetal amniotic testosterone reportedly correlates positively with many of the regional male sexual dimorphisms in the gray matter, including the amygdala, as with diverse differences in gender behavior.^{603,605} Functional MRI has shown endogenous testosterone levels to correlate with, and exogenous testosterone administration to females to activate, amygdala and parahippocampal regions and other brain areas in response to social-affective stimuli.⁶⁰³ Studies in transsexuals have shown that virilizing doses of testosterone affects the size of specific cortical and subcortical areas of the brain, and antiandrogen/estrogen treatment robustly inhibited hippocampal size.⁶⁰⁶

Some testosterone effects are androgen specific.^{607,608} However, many testosterone behavioral effects appear to be mediated by intraneuronal aromatization of testosterone to estradiol in a manner that is regulated in site-specific fashion by androgen and estrogen.⁶⁰⁹⁻⁶¹¹ Thus it has been postulated that low levels of estradiol promote the development of the brain and greater amounts masculinize it. These higher levels of estradiol are generated in the male brain by neuronal aromatization of circulating testosterone. ER α knock-out in female mice reduces sexual behavior and parenting behavior, while increasing aggressiveness.⁶¹²

The mechanism by which estrogens mediate androgenic masculinization of rodent sexual behavior involves prostaglandin E₂ mediation.^{593,613} Estradiol acts through ER α to induce microglia, the resident immune cells of the brain, to secrete prostaglandin E₂, which reduces DNA methyl transferase activity so as to release epigenetic repression of the default female behavioral state;⁵⁸⁷ this initiates enhanced neurite dendritic spine formation and masculinized behavior. Release of epigenetic repression appears to be an important postreceptor mechanism of testosterone action in the brain, also affecting

embryonic neural stem cells.⁶¹⁴ The involvement of prostaglandin E₂ in mediating the androgen effect on neural synapsing lends credence to a role for androgen excess contributing to autism spectrum disorders.^{593,615}

This hormonal organization of the brain involves hormone-specific effects on cell proliferation versus survival and synapse formation versus pruning.^{588,589} Androgens have a trophic effect on the dendritic spine cells of sexually dimorphic nuclei of rodents that promotes increased synaptic density.⁵⁹³ Estrogen alters the pattern of synaptic connections in spatially-specific and precise patterns that appear to fine-tune the sensitivity of certain regions of the brain to excitatory and inhibitory amino acids.^{616,617} Hypothalamic changes in synaptic remodeling have been correlated with the preovulatory surge of GnRH. There is also sexual dimorphism in cerebral progestin receptors, and progesterone attenuates testosterone effects on the brain.^{618,619} Progestin-estrogen administration is neuroprotective in animal studies.⁶²⁰ These hormones may counteract brain and spinal cord injury in adult women,^{620,621} but not in the sexually immature state.⁶²²

On average, women tend to perform better than men on tasks that involve object memory, verbal skills, processing speed and accuracy, and fine-motor skills, whereas men tend to excel in visual-spatial memory, while the sexes do not differ in vocabulary or math skills.^{590,592,623–625} These differences are quantitatively modest, of the order of 0.4 to 1.0 standard deviation (SD), leading, therefore, to large overlaps in these skills among the sexes. The male advantage in visual-spatial skills is established by 4.5 years of age. Because both boys and girls who are congenitally sex hormone deficient are relatively poor in visual-spatial abilities, and sex hormone treatment at puberty does not ameliorate these deficits, this difference seems to be the result of estrogen-mediated patterning in both sexes. The extent to which this difference is innate or because of socio-cultural factors is a subject of considerable debate.

A wide variety of gender dimorphic behaviors are found in young children, but normally they have a different character than in adults.⁶²⁶ Gender identity is established in midchildhood,⁵⁸¹ probably by 3 years of age.⁶⁰⁰ Sexual orientation is established by 10 years of age; it has been postulated that this is dependent upon adrenarche rather than true puberty.⁴⁰⁴ Early pubertal amounts of androgen or estrogen have little effect on sexual behaviors, but increase some aspects of aggressive behavior.^{627,628} Only in later puberty is there activation of the sex drive, which has been programmed in earlier development.

Discordant gender identity (transsexualism/transgenderism) and sexual orientation (homosexuality, bisexuality) occur in a small proportion of the population.^{580,581} Their prevalence seems to be increased in disorders of sex development (DSD). Studies of DSD indicate that a male level of androgen acting through the androgen receptor pre- or perinatally is an important determinant of male gender behavior (role) and mildly disruptive to female gender identity.^{600,629} However, DSD is uncommon among homosexuals and transsexuals, whereas heritability estimates approximate 20% to 60%.^{580,581} Neuroimaging studies suggest that these disorders have a biological basis. Homosexuality is associated with loss of sex differences in brain structures^{630,631} and transsexuality with less differentiation of brain areas dealing with body and self-perception.⁶³⁰ Neuroimaging indicates that male homosexuals have a pattern of nuclear activation in response to pheromone-like chemosignals resembling heterosexual women rather than that of heterosexual men, and homosexual women have an intermediate type of activation.⁶³²

Androgen and estrogen metabolites in sweat and urine, which contain unusual steroids, such as androst-4,14-diene-3-one,⁶³³ have been found to exert sexually dimorphic activation of the anterior hypothalamus that is independent of their

odor.⁶³² Therefore they appear to act as pheromone-equivalent chemosignals. Human pheromones appear to modulate the timing of ovulation⁶³⁴ and mood.⁶³⁵ It is likely that a dedicated population of olfactory receptors that project to GnRH neurons act as pheromone receptors.⁶³⁶

Other Targets of Sex Hormone Action

Sex steroid hormones affect a wide variety of tissues in ways that are often unrecognized. An estrogen effect on stabilizing muscle integrity has been noted in muscular dystrophy.⁶³⁷

Autoimmune disorders are in general more common in females, particularly after puberty.⁶³⁸ Estrogen downregulates blood levels of the inflammatory cytokine interleukin-6⁶³⁹ and thymic autoimmune regulator (AIRE) gene expression.⁶⁴⁰ Progesterone has a similar effect and androgen the opposite effect on AIRE. Sex dimorphism in predisposition to autoimmune disorders is partly explicable by sex hormone action on AIRE network genes during the neonatal minipuberty.⁶⁴¹ However, sex genotype influences the autoimmune system independently of sex steroids.^{585,638}

The cardiovascular effects of estrogen include upregulation of estrogen and PRs in vascular tissue and nongenomic effects on endothelial nitric oxide synthase.⁶⁴² Estrogen improves the disturbed endothelial dysfunction of young hypogonadal women and is necessary for the cardioprotective effect of exercise.⁶⁴³ Estradiol replacement therapy, oral or transdermal, lowers blood pressure, although estradiol causes salt and water retention.⁵⁷¹ This contrasts with contraceptives containing the more potent estrogen ethynodiol dienoate, which raise blood pressure significantly, unless containing an antiminerocorticoid progestin.

Estrogens and progestins also exert hemostatic effects that are associated with increased resistance to the anticoagulant action of activated protein C.⁶⁴⁴ Combined oral contraceptives containing estrogen carry about a fourfold increased risk of venous thromboembolism in first-time users.^{645,646} The risk falls with decreasing dose of estrogen and duration of use and rises about 50% in those containing third-generation (e.g., desogestrel) and antiandrogenic progestins.⁶⁴⁷ Nevertheless, the risk is less than that of pregnancy. Progestin-only contraceptives are not associated with any increased risk of venous or arterial thrombosis.^{645,648}

The differences between the sexes in lipid levels are not explained by physiological differences in estrogen levels.⁶⁴⁹ Although oral estrogens raise triglycerides, this is caused by a first-pass hepatic effect. Differences in androgen (lowers HDL-cholesterol) and progesterone (lowers triglycerides and HDL-cholesterol) levels only explain part of the difference.

NORMAL HORMONAL AND SEXUAL DEVELOPMENTAL STAGES

The Fetus and Neonate

The fetus grows in a richer steroidial milieu than the pubertal female owing to the function of the fetoplacental unit. Concentrations of estrogens in fetal serum are extremely high. Umbilical cord plasma free testosterone levels are modestly greater than those of normal adult females.⁴³⁶ Dehydroepiandrosterone sulfate is at an adrenarchal level. The newborn shows some signs of the pubertal degree of hormonal stimulation from the intrauterine environment. Hypertrophic labia minora and superficial cell transformation of the urogenital epithelium are consistently observed estrogen effects, and a palpable breast bud is present at term in one-third of babies.⁶⁵⁰ The mean (SD) uterine length at birth is 4.15 ± 0.56 cm.⁶⁵¹ Sebaceous gland hypertrophy results from the androgenic state,⁶⁵² and the

clitoral shaft sometimes is prominent, particularly in small premature babies.⁵¹⁹

Steroid hormone levels from birth through puberty are shown in Table 16.1.^{15,66,69,93,650,653–656} Upon birth, withdrawal from the intrauterine environment occurs. Pituitary-gonadal axis hormone levels fall to a prepubertal-like nadir within days of withdrawal from the intrauterine environment.¹⁷ Menstrual bleeding and colostrum production sometimes occur as the newborn is withdrawn from the estrogenic environment. The mini-puberty of the newborn then begins.

This neonatal mini-puberty evolves according to a developmental program determined by gestational age. At term gestational age, it commences with a gradual but transient rise to pubertal hormone levels. In girls these reach maximal values in the early pubertal range at 3 to 4 months of age, about 2 months later than in boys, before they regress as seen in Figs. 16.5 and 16.8. The activation of the HPG axis of the newborn stimulates breast and genital tract development that commonly persists for several months.^{657,658}

Premature neonates, in contrast to term newborns, develop high gonadotropin levels, of the magnitude seen in ovarian insufficiency, that persist until antral follicle development begins near 40 weeks, gestational age.^{17,18,659} As antral follicles develop, ovarian estrogen and AMH secretion commence, and the compensatorily high gonadotropins gradually fall to the low levels normal for term infants.¹⁷ Coincidentally, adrenal contributions to steroid intermediate levels are higher in premature infants because of the persistence of the fetal adrenocortical zone and immaturity in size and apparent 11 β -hydroxylase activity of the definitive adrenocortical zones.^{660–664}

Transient ovarian hyperstimulation has been reported in preterm babies as a consequence of high gonadotropin levels persisting until or beyond late-term corrected gestational age.^{665–667} It manifests at several months of age as ovarian cysts with hyperestrogenism, causing genital swelling, persistent breast development, and/or delayed menstrual bleeding.

These phenomena then regress progressively through later infancy as the inhibitory tone of the neuroendocrine-gonadal axis undergoes juvenile maturation. Nevertheless, according to an ultrasensitive recombinant cell bioassay, girls' estrogen levels in late infancy are several fold greater than those of boys, averaging 1 pg/mL and ranging up to 3 pg/mL.⁶⁶⁸ On occasion, there may be subclinical but detectable estrogen effects on urogenital cytology.⁹⁵ Whether the transient "minipuberty" activity of the neuroendocrine-gonadal axis in the newborn has a programming influence on subsequent behaviors remains unclear.⁶⁰⁰

The developmental pattern of serum AMH differs from that of other reproductive hormones because it reflects follicular growth and development rather than neuroendocrine activity. AMH rises from undetectable to low in cord blood to 0.6 to 4.1 ng/mL (4.3–29 pM) at 3 months; it then continues to slowly rise about 1.5-fold more to reach an adult level by the postmenarcheal period.⁶⁶⁹

Childhood

As the neuroendocrine-gonadal axis becomes quiescent and the fetal zone of the adrenal cortex regresses, steroid hormone levels fall through infancy to reach a nadir in midchildhood (see Table 16.1). The earliest hormonal change during childhood is the adrenarchal rise in serum DHEAS that is discernable at about 6 years (see Table 16.1). Although childhood gonadotropin levels are low and there is seldom obvious sexual development as a consequence of prepubertal gonadotropin production, there is a low level of bioactive gonadotropin production and ovarian follicular development^{31,86,670} and occasional evidence of transient estrogen secretion.⁶⁷¹ In

midchildhood, GnRH agonist stimulation of gonadotropin secretion elicits a prompt small rise in estradiol secretion.^{93,672} AMH levels of girls rise minimally in midchildhood, to levels about 3% those of boys.⁶⁶⁹

In late prepuberty, girls begin to experience increasing diurnal production of gonadotropins, and estradiol levels rise in diurnal fashion to approximate 10 pg/mL in midmorning.^{79,97}

Adolescence

Hormonal

The earliest hormonal changes of true puberty occur gradually during late preadolescence. Clinically prepubertal 10-year-olds develop greater average gonadotropin and sex hormone levels than do prepubertal 7-year-olds.⁷⁹

In the average girl, serum gonadotropins achieve pubertal levels after 8 years of age. However, the chronological age at which puberty begins varies considerably among children. Therefore the pubertal rise in gonadotropins is best appreciated by relating gonadotropin levels to pubertal stage. Daytime serum LH rises 25-fold from prepuberty to late puberty according to bioassay, but this rise is underestimated by polyclonal RIA.⁹⁹ "Third-generation" immunometric assays, using monoclonal antibodies and a more purified standard than earlier RIAs, show a rise similar to that found by bioassay.^{77,79}

The hallmark of early puberty is an increase in the sleep-related rise in LH (see Fig. 16.12).^{79,98} Daytime sampling underestimates the rise in gonadotropins in early puberty because it does not detect most of this sleep-related increase. In early puberty, current assays show that LH rises during sleep to reach peaks in the lower adult range, generally above 1.0 U/L and then typically falls during the day to 0.6 U/L or less.^{79,93,673,674} A single daytime sample also does not necessarily truly represent a child's pubertal status because it does not account for episodic and cyclic changes in gonadotropin secretion.^{94,675} The serum LH response to GnRH is slightly more indicative of the pubertal status than a morning basal sample (see Fig. 16.9) (Table 16.1).^{87,673,674} An LH level 1-h post-GnRH agonist of 3.2 U/L or more is 90% sensitive and 5.5 U/L or more is 95% specific for the onset of puberty in girls.⁹³ The response of LH to GnRH or GnRH agonist administration increases more than that of FSH during puberty, with a resultant increase in the LH:FSH ratio.^{77,676} GnRH agonists add a dimension to GnRH testing: they provide a sufficiently potent and prolonged stimulus to LH and FSH release to bring about an increase in ovarian estradiol secretion in pubertal girls.⁶⁷⁷ These responses likewise increase characteristically with sexual maturation (see Fig. 16.6).⁶⁸

Sex hormone levels rise further as the consequence of ovarian and adrenal maturation. Pubertal levels are intermediate between those of prepubertal and sexually mature individuals. Table 16.1 shows typical normal ranges for serum levels of the major steroid hormones. Once pubertal levels of estrogens and androgens are achieved, their effects ordinarily become obvious within 6 months.

Serum AMH levels stabilize at an adult level of 0.5 to 6.2 ng/mL (3.5–45 pM) in postmenarcheal females with normal ovarian morphology.^{40,669} They do not fluctuate during the normal menstrual cycle,⁶⁷⁸ reflecting a balance between recruitment of growing follicles and growth of antral follicles.⁶⁶⁹ The AMH serum level is an indicator of the number of growing follicles, and thus indexes the size of the oocyte pool ("ovarian reserve"); AMH begins to fall with the oocyte pool in the premenopausal adult⁶⁶⁹ and becomes undetectable after menopause.⁶⁷⁹ AMH levels also reflect the intrafollicular androgenic status of fertile females, probably because androgens stimulate the early phases of follicular growth.⁴⁰ Serum prolactin rises moderately

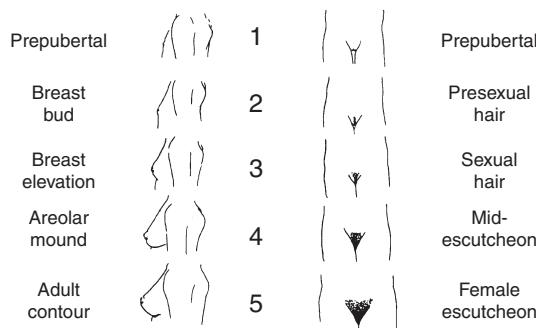


Fig. 16.30 The stages of breast and pubic hair development. (Modified from Ross, G.T., Vande Wiele, R. (1974). The ovary. In: Williams, R.H. (ed.), *Textbook of Endocrinology*, 5th ed. Philadelphia, WB Saunders.)

in females at about 14 years of age.⁶⁸⁰ This may be a response to estrogen secretion because it does not occur in boys.

Clinical

The first physical sign of puberty is breast development (thelarche). In a minority of girls, pubic hair development (pubarche) occurs before thelarche. Thelarche represents a response to estrogen, and pubarche a response to androgen. When pubarche precedes thelarche, it is usually a reflection of adrenal androgen production (adrenarche) rather than a sign of true puberty. The stages of breast and pubic hair development are shown in Fig. 16.30.^{681,682} Tanner stage 1 is prepubertal. The initial stage of breast development (stage 2, B2) is appreciated as a palpable subareolar bud before it can be seen as an elevation. Stage B3 is obvious enlargement and elevation of the whole breast. Stage B4, the phase of areolar mounding, is very transient and may not necessarily appear. Stage B5 is the stage of attainment of mature breast contour. Pubic hair first starts as presexual pubic hair development (PH2)—hairs which are shorter, lighter, and straighter than sexual pubic hairs, but longer than vellus body hair. Hypertrichosis is sometimes mistaken for stage 2 pubic hair; these can be differentiated by comparing genital hair to that on the forearm. Sexual pubic hair development (PH3)—curly terminal (long, dark) hair—usually commences on the labia majora before spreading to the pubis. Pubic hair then gradually progresses to the mature female escutcheon (inverted triangle pattern, stage 5). Axillary hair usually appears about a year later than pubic hair and passes through similar stages.

The age at which pubertal milestones are normally attained is not known with certainty. There has been considerable debate about the normalcy of pubertal changes between 6.0 and 8.0 years of age.^{190,203,683} The debate stems from office practice observations that breast and pubic hair development were found more frequently than expected in black girls in this age range. The prevalence of pubertal milestones has subsequently been estimated for the general US population by modeling cross-sectional data collected 1988 to 1994 on children 8.0 years of age and older by the National Health and Nutrition Examination Survey III (NHANES III).^{198,199,276} NHANES data had the advantage of nationally representative sampling, but the breast stage data was based on observation and so data quality was questionable, and modeling assumptions that permit extrapolation to younger ages may not be valid.^{276,684,685} The median ages at which the major pubertal milestones were attained in normal-weight US girls according to this database are given in Table 16.3.²⁷⁶ These data indicated that puberty begins before 8.0 years in less than 5% of the

TABLE 16.3 Pubertal Milestone Attainment in Normal Body Mass Index Girls in the General US Population, NHANES III, 1988-1994.

Stage	5%	50%	95%
Breast Stage 2	8.25	10.2	12.1
Pubic Hair Stage 3	9.25	11.6	13.9
Menarche	11.0	12.6	14.1

Before age 8.0 years breasts appeared in 12% to 19% and sexual pubic hair (stage 3) in ≤3% of normal-body mass index in non-Hispanic black and Mexican American girls. Menarcheal milestones are attained at similar ages in these ethnic groups except for the 5th percentile being significantly earlier in blacks (10.5 y) than non-Hispanic whites (11.3 y). (Modified from Rosenfield, R.L., Lipton, R.B., Drum, M.L. (2009).

Thelarche, pubarche, and menarche attainment in children with normal and elevated body mass index. *Pediatrics*, 123, 84–88.)

normal general female population, although breasts may normally appear during the seventh year in blacks and Mexican Americans.

A more contemporary study of female puberty was conducted from 2004 to 2011. The design was improved: data collection was longitudinal and breast development was ascertained by palpation. However, although the design included broad racial/ethnic and socioeconomic representation, it was not nationally representative, the study population enriched for Asians, for example.^{686,687} The overall distribution of pubertal timing was shifted to earlier ages by approximately 0.75 year for breast development and 0.35 year for menarche compared with NHANES III. The data confirmed that normal-weight non-Hispanic black girls achieved pubertal milestones earlier than white girls, by about 0.75 to 1.0 year for thelarche and 0.5 to 0.75 year for menarche; thus thelarche normally may occur in the seventh year of life in blacks. Pubertal milestones in Asian girls were similar to those in whites, and in Hispanics to blacks.

Obesity and ethnic factors now appear to be independent factors that advance the onset of puberty and menarche, with obesity having the greater influence.^{276,686} Longitudinal study indicates that obesity is associated with advancement of the age of thelarche (0.7 year) more closely and twice as much as advancement of the age of menarche (0.3 year).

Pubertal tempo, the span of time between the onset of breast development (B2) and menarche, is normally 2.3 ± 1.0 years. However, obesity is paradoxically associated with significant advancement of the age of thelarche while significantly slowing pubertal tempo and preserving height potential.^{687,688} It is possible that the advancement of the age of thelarche by obesity is not entirely because of an advancement of neuroendocrine puberty but rather arises in part from extragonadal formation of estrogen from adrenal precursors in excess adipose tissue; this may explain, in part,⁶⁸⁹ the apparent blunting of early neuroendocrine puberty in obese girls with early thelarche.^{93,687,690} On the other hand, a subgroup of early maturers with a history of intrauterine growth retardation seem to have an unusually rapid tempo of puberty and lose height potential.⁶⁹¹

The onset of puberty is more closely related to an individual's bone age than to chronological age. This is particularly important in the case of subjects who are later than average in entering puberty, as discussed previously. The great majority of girls can be expected to begin puberty by the time their skeletal age reaches 12.5 years and to experience menarche by the time skeletal age reaches 14 years.

The pubertal growth spurt in girls occurs during early adolescence. The peak of linear growth velocity corresponds most closely with stage B2⁶⁹² and the increase in serum alkaline phosphatase levels with B3.⁶⁹³ Fat accumulation increases

and fat distribution changes as well.⁶⁹⁴ As a consequence of these pubertal changes occurring out of phase with chronological age, girls begin to differ considerably in size and habitus during the ninth year of life.

Adult Menstrual Cycle

The menstrual cycle of young adults averages 28 days in length (normal adult 24–38 days).⁶⁹⁵ The variation in cycle length is almost entirely because of differences in the duration of the follicular phase. The luteal phase, the time between ovulation and the onset of menses, invariably lasts 14 ± 1 (SD) days.¹²⁶

The cyclic changes of LH, FSH, estradiol, and progesterone serum levels during the menstrual cycle are shown in Fig. 16.13. Diurnal and episodic fluctuations are superimposed upon these cyclic changes. Since testosterone and androstenedione have both adrenal and ovarian origins, their levels fluctuate to some extent in cyclic, diurnal, and episodic patterns. For example, testosterone levels tend to be 20% greater in the morning than in the evening and to double in midcycle.^{125,696} The normal range for most of the important ovarian sex hormone levels of women during the early follicular phase of the menstrual cycle is given in Table 16.1. Progesterone levels are below 100 ng/dL until the periovulatory phase of the cycle and then peak to over 500 ng/dL in the midluteal phase. Hormonal production rates for the midfollicular phase in women are given in Table 16.2. Serum prolactin increases transiently in midcycle with maximum ovarian estradiol secretion.⁶⁹⁷ Prolactin levels also transiently rise in response to mammary stimulation and psychological factors.⁶⁹⁸

Normal Variations in Pubertal Development

Although the onset of breast development (stage B2) characteristically precedes the appearance of sexual pubic hair (PH3) and the onset of menses substantially (see Table 16.3), there is considerable variation in the sequence of these events. Pubic hair may appear before breasts begin to develop, a situation arising from lack of direct linkage between adrenarche and gonadarche. Menarche may occur within months after the appearance of breasts; however, this is so unusual that its occurrence demands exclusion of an abnormal hyperestrogenic state.

A common normal variant is the unilateral onset of breast development. Unilateral breast development may exist up to 2 years before the other breast becomes palpable. This phenomenon seems related to an asymmetry that normally persists into adulthood. Excisional biopsy of a normal unilateral breast papilla in search of a nonexistent tumor should be avoided, because such a procedure excises the entire breast anlage.

Two extreme variations of normal are the most common causes of premature sexual development.⁶⁹⁹ These are the isolated appearance of breast development (premature thelarche) and the isolated appearance of sexual hair (premature pubarche).

Premature Thelarche

Breast development before 8.0 years is traditionally considered premature. Premature thelarche is a very mild, non- or slowly progressive incomplete form of premature puberty that is a variant of normal. In the 6- to 8-year age group, it is usually because of obesity, which may either accelerate the onset of puberty, account for increased peripheral estrogen production, or cause artefactual adipomastia.^{93,686,687,690,700} Otherwise, it usually seems to be caused by idiopathic subtle overfunction of the pituitary-ovarian axis, occurring in those girls whose FSH levels tend to be sustained about the upper end of the

prepubertal normal range.⁶⁷¹ Average serum levels of FSH at baseline and in response to GnRH are significantly increased, whereas those of LH are not. Estradiol levels are generally below the level of detection in most standard assays, but are significantly elevated according to ultrasensitive assay,⁷⁰¹ and intermittent low-grade estrogenization of the urogenital mucosa is sometimes found (see Fig. 16.11). Ovarian ultrasound examination shows an increased prevalence of antral follicles ("microcysts") and uterine enlargement.⁷⁰² Nevertheless, a growth spurt does not occur, the bone age advancement rate is not abnormal, and menses do not appear until the usual age.

In infants, the syndrome seems to be caused by a lag in inhibition of the transient activation of the HPG axis of the newborn and is usually unsustained. In older children, the breast development is more likely to persist. A subgroup with "exaggerated thelarche" has an increased growth rate with relatively proportionate bone age advancement. Their unsustained or intermittent neuroendocrine activation seems to lie on a spectrum between ordinary premature thelarche and true sexual precocity (Fig. 16.31).⁷⁰³ However, the McCune-Albright syndrome mutation is found in the peripheral blood of about 25% of such patients.⁷⁰⁴ Premature thelarche may be the first sign of feminizing disorders (see Precocious Puberty). Therefore follow-up of these patients is indicated.

Premature Pubarche

The isolated appearance of sexual pubic hair before 8.0 years of age in girls (premature pubarche) is usually caused by prepubertal adrenarche, as discussed next. However, it may occur at androgen levels that are normal for preschool children (idiopathic premature pubarche). This likely reflects increased sensitivity of the PSU to low preadrenarchal androgen concentrations, analogous to idiopathic hirsutism in adults, in which increased sexual hair growth occurs in the absence of other evidence of hyperandrogenism. The mechanism may be caused by increased androgen receptor gene activity.⁷⁰⁵

Premature adrenarche is a very mild, slowly progressive incomplete form of premature puberty that is usually a variant

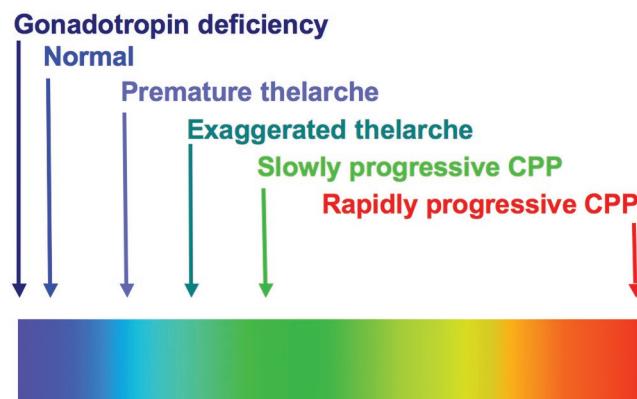


Fig. 16.31 The spectrum of gonadotropin secretion in girls with normal and abnormal puberty. Normal girls are conceptualized as having a small amount of pituitary-ovarian axis activation, which is more than that of congenitally hypogonadotropic girls. Premature thelarche, exaggerated thelarche, slowly progressive precocity, and rapidly progressive precocity fall along a spectrum of increasing activation of the axis—with deterioration of height potential occurring only in those near the most activated end. CPP, central precocious puberty. (Modified from Kreiter, M.L., Cara, J.F., Rosenfield, R.L. (1993). Modifying the outcome of complete precocious puberty: To treat or not to treat. In: Grave, G.D., Cutler, G.B. (eds.), Sexual Precocity: Etiology, Diagnosis, and Management. New York, Raven Press, p. 109–120.)

of normal. It usually appears to be caused by advanced development of the zona reticularis as indicated by increase of adrenal androgen levels above those of preschool children.^{15,706} The condition is ordinarily detected when a child presents with premature pubarche or other clinical manifestation of androgen action, such as adult-type body odor or acne.⁷⁰⁷ The androgen excess is ordinarily so subtle that there is no obvious growth spurt, the bone age typically does not advance abnormally, and there are no other signs of sexual maturation. The diagnosis requires biochemical demonstration of a serum steroid pattern indicative of adrenarche before 8 years of age in girls, for example, DHEAS 40 to 130 mcg/dL and other androgen levels only slightly elevated for age (see Table 16.1). The term is sometimes used to describe biochemical evidence of adrenarche irrespective of clinical manifestations.

Exaggerated adrenarche is a clinically extreme type of premature adrenarche.^{15,706} These girls have clinical features that suggest subtle androgen excess (e.g., significant bone age advancement, but not clitoromegaly) or insulin resistance (e.g., central adiposity or acanthosis nigricans). Such children generally have a slightly advanced onset of true puberty, but height potential is not compromised. The term exaggerated adrenarche has been variously applied but is here when the atypical clinical picture is associated with serum androgen levels above normal for early puberty (see Table 16.1), for example, DHEAS above 130 mcg/dL or testosterone over 35 ng/dL, or bone age above height age by 20%. Virilizing disorders should be excluded in girls with exaggerated adrenarche so defined.

The cause of premature adrenarche usually seems simply to be caused by advanced onset of normal zona reticularis development, but it sometimes seems to be an early manifestation of the steroidogenic dysregulation of polycystic ovary syndrome.^{15,706} Premature adrenarche appears to carry about a 10% to 20% risk of developing polycystic ovary syndrome; it is unclear whether those with exaggerated adrenarche are primarily at risk. Premature adrenarche with insulin resistance is independently associated with obesity, rapid weight gain during early childhood, and low birth weight.^{15,383,708} The IGF-1 excess of GH treatment may be a risk factor.⁷⁰⁹ Associations also exist between premature adrenarche and temporal lobe lesions⁷¹⁰ and poliomyelitic scoliosis.⁷¹¹ Heritable factors are also important. The heritability of DHEAS sulfate blood levels is estimated at 26% to 66%.⁷¹² Carriers for CAH may be overrepresented in this group.^{713,714} Epigenetic programming seems to be a factor: prenatal virilization of nonhuman primates causes functional adrenal hyperandrogenism as part of the PCOS spectrum,⁷¹⁵ and fetal undernutrition has been postulated to program for insulin resistance by activating the limbic-hypothalamic-pituitary-adrenal system to cause fetal corticoid excess.⁷¹⁶

The differential diagnosis of premature pubarche and adrenarche includes virilizing disorders, of which nonclassic CAH is the most common. Girls with premature adrenarche should be followed through puberty for the possible development of hyperandrogenism. A suggested approach to rule out serious hyperandrogenic disorders in childhood is suggested in the accompanying algorithm (Fig. 16.32).⁷¹⁷

Constitutional Delay of Growth and Pubertal Development

By statistical definition, delayed puberty occurs in 3% of girls. Most of these girls are otherwise normal, in which case this is termed *constitutional delay of growth and pubertal development* (CDGP). It is familial;^{200,201} 80% in a large series had a parent with objectively delayed puberty.⁷¹⁸ A family history of delayed puberty is found in 50% to 75% of patients with CDGP.²⁰¹ Although its inheritance is likely complex, some predisposing

genetic factors seem to have a dominant effect. It has long been recognized that delayed puberty was overrepresented in families with idiopathic hypogonadotropic hypogonadism or hypothalamic amenorrhea cases, and rare variants in genes underlying these conditions appear to contribute to the etiology.^{719,720} Recent molecular investigation revealed that CDGP indeed segregates within families with complex patterns of inheritance that include X-linked, autosomal dominant and recessive and bilineal,²⁰⁰ although sporadic cases also occur. Autosomal dominant is the most prevalent pattern of inheritance (with or without complete penetrance).^{200,721} Initially, candidate genes associated with CDGP have been identified using GWAS, linkage analysis, and targeted sequencing strategies.^{718,722} However, recently whole exome and genome sequencing are increasingly being used to identify novel candidate genes. Despite these advances, the genetic basis and neuroendocrine pathophysiology remains unknown in the majority of patients with CDGP.

Mutations in several genes have been associated with CDGP. Six unrelated families from a Finnish cohort with delayed puberty were found to have two mutations in immunoglobulin superfamily member 10 (IGSF10)⁷²³ and four other families were found to have two rare variants with unknown significance. Mutations in IGSF10 appear to cause decreased levels of IGSF10 expression during embryogenesis resulting in delayed migration of GnRH neurons from the olfactory bulbs to the hypothalamus. The dysregulation of GnRH neuronal migration results in an abnormal configuration of the GnRH neuronal network and a functional defect in GnRH secretion that becomes crucial when a threshold level of GnRH secretion must be achieved for pubertal onset. IGSF10 mutations were also found in women with a hypothalamic amenorrhea and in patients with congenital hypogonadotropic hypogonadism (CHH), although these mutations did not alone appear sufficient to cause the phenotype. Loss-of-function mutations in IGSF1 have been identified in patients with X-linked central hypothyroidism,⁷²⁴ delayed pubertal growth, and delayed increase in testosterone levels.⁷²⁵

Mutations in genes found in patients with CHH are also found in CDGP. Mutations in heparan sulfate 6-O-sulfotransferase 1 (HS6ST1), fibroblast growth factor receptor 1 (FGFR1), and Klotho β (KLB) have been found in several kindreds with CHH and their relatives with CDGP.^{726–728} Variants in the genes encoding gonadotropin-releasing hormone receptor (GNRHR), tachykinin 3 (TAC3) and its receptor (TACR3), interleukin-17 receptor D (IL17RD), and semaphorin 3A (SEMA3A) known to cause CHH have been found in patients with CDGP.⁷²⁹ Mutations in 24 genes associated with GnRH deficiency were found in probands with CHH with greater frequency than in CDGP, leading to the conclusion that CHH and CDGP are likely to have different genetic backgrounds.⁷³⁰ Mutations in Kallmann syndrome genes such as anosmin 1 (ANOS1) and N-methyl-D-aspartic acid receptor synaptosomal signaling and neuronal migration factor (NSMF) have not to date been identified in pedigrees with CDGP. Loss-of-function mutations within the GnRH receptor are the most frequent cause of autosomal recessive CHH, accounting for 16% to 40% of patients. Mutations have been found within the extracellular, transmembrane, and intracellular domains of the receptor leading to impaired GnRH action.⁷³¹ A homozygous partial loss-of-function mutation in GNRHR was found in two brothers, one with CDGP and one with idiopathic HH,⁷³² and a further heterozygous mutation was found in one male with self-limited CDGP.⁷³³ Thus the genetic background of CHH and CDGP may be different, or shared by as yet undiscovered genes.⁷³³

Girls with constitutional delay are generally more slight in habitus and have lower bone mineral density upon entering

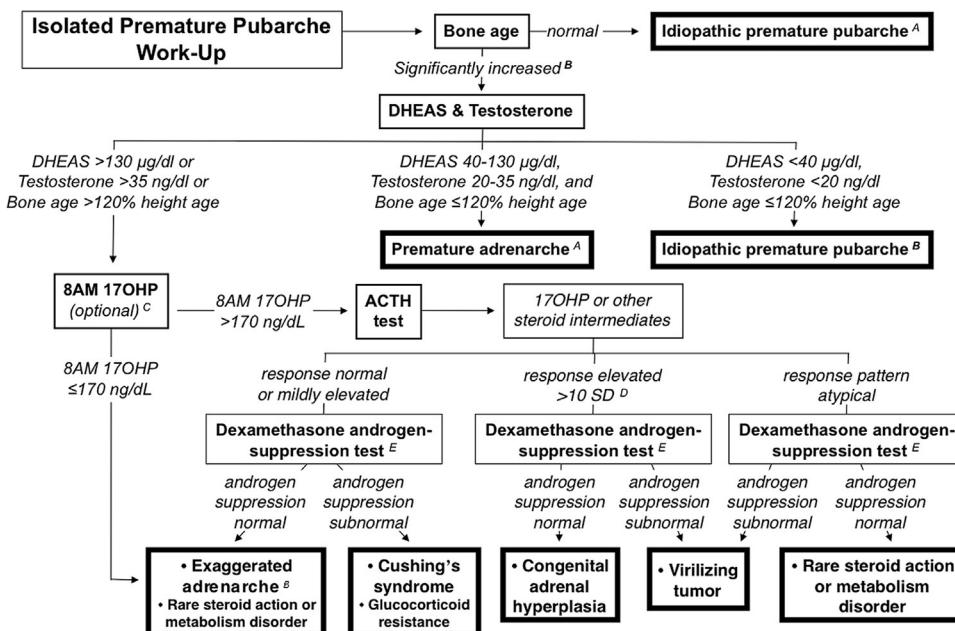


Fig. 16.32 A workup to screen for hyperandrogenic-virilizing disorders as a cause of premature pubarche. Premature or exaggerated adrenarche accounts for the great majority of premature pubarche; nonclassical congenital adrenal hyperplasia caused by 21-hydroxylase deficiency, the most common hyperandrogenic cause, accounts for less than 10% in most populations/ethnic groups. SD, Standard deviations. (Modified with permission from Rosenfield, R.L. (2018). Premature adrenarche. UpToDate.)

Footnotes:

- A. Diagnoses of idiopathic premature pubarche, premature adrenarche, or exaggerated adrenarche, or based on bone age and/or hormonal measurements are provisional diagnoses. Even if these tests are normal or only mildly elevated, clinical follow-up is indicated to rule out inordinate progression of pubarche or evidence of virilization. This is because bone age interpretation and the precision of testosterone assays at low levels are often problematic, and because this algorithm will not detect some very mild hyperandrogenism cases.
- B. A bone age that is significantly advanced for chronological age is an indication for a screening laboratory workup for childhood hyperandrogenism, consisting of dehydroepiandrosterone sulfate (DHEAS) and testosterone levels. A bone age that exceeds height age indicates less than average height potential. A compromised height potential (indicated by a predicted adult height less than expected for the family or a bone age >120% of height age in a young child) or DHEAS or testosterone level above the normal adrenarchal range (130 mcg/dL and 35 ng/dL, respectively) suggests that the premature pubarche may be caused by a virilizing disorder rather than ordinary premature adrenarche.
- C. An 8:00 AM baseline measurement of 17-hydroxyprogesterone (17-OHP) is an option to an adrenocorticotropin hormone (ACTH) test in a family, population, or ethnic group in which the risk is relatively low for nonclassical congenital adrenal hyperplasia caused by 21-hydroxylase deficiency. A level over 170 ng/mL (5.1 nmol/L) has a 95% or greater sensitivity and specificity for this disorder among premature pubarche patients when obtained at 8:00 AM, before the level wanes with diurnal ACTH secretion. A normal baseline level does not necessarily exclude this or more rare forms of nonclassical congenital adrenal hyperplasia.
- D. For ACTH testing, the typical response in congenital adrenal hyperplasia (CAH) is that the steroid immediately before the enzyme block is extremely elevated, and steroids earlier in the biosynthetic pathway are successively less elevated the further removed they are from the block. For example, in 21-hydroxylase deficiency, 17-OHP is extremely elevated and androstenedione and testosterone are successively less elevated; in 3beta-hydroxysteroid dehydrogenase type 2 deficiency, 17-hydroxypregnolone and DHEA responses are extremely elevated and 17-hydroxyprogesterone, androstenedione, and testosterone are mild to moderately elevated. An elevated baseline level of 17-OHP may preclude a clear response to the ACTH test. For ACTH testing, an atypical response pattern is one that is not typical for any type of CAH. An example of an atypical response would be baseline or post-ACTH androstenedione or testosterone elevations greater than those of 17-OHP.
- E. The dexamethasone androgen-suppression test consists of administering dexamethasone, 1 mg/m²/day in three to four divided doses daily for 4 days, and then measuring the serum cortisol, DHEAS, and androgens on the morning of the fifth day after a final dexamethasone dose. Normally, serum cortisol falls below 1 mcg/dL (28 nmol/L), testosterone to less than 10 ng/dL, and DHEAS to less than 40 mcg/dL. Serum androgen intermediates also fall to prepubertal concentrations.

puberty than earlier maturing girls.⁷³⁴ Girls do not usually become concerned about this until they enter high school at 14 years of age and realize that not only has pubertal development not begun, but also most of their friends are menstruating. When puberty does ensue in such subjects it is perfectly normal in tempo. Endocrinological status is normal for the stage of puberty. The differential diagnosis includes chronic endocrine, metabolic, and systemic disease of almost any kind, as well as gonadotropin deficiency, which it closely resembles and from which it is distinguished with difficulty (see Gonadotropin Deficiency and Functional Hypothalamic Anovulation).

Physiological Adolescent Anovulation

Immaturity of the hypothalamic-pituitary-ovarian axis causes menstrual cycles to be longer and more irregular during the early postmenarcheal years (Fig. 16.33).^{124,695,735} About half of menstrual cycles during the first 2 years after menarche are anovulatory by standard criteria; half of these actually have evidence of the attenuated ovulation that results in luteal insufficiency (see Luteal Phase Defects).^{124,736} Although about half of these cycles with ovulatory abnormalities are irregular, half are of normal length by adult standard, so normal adolescent

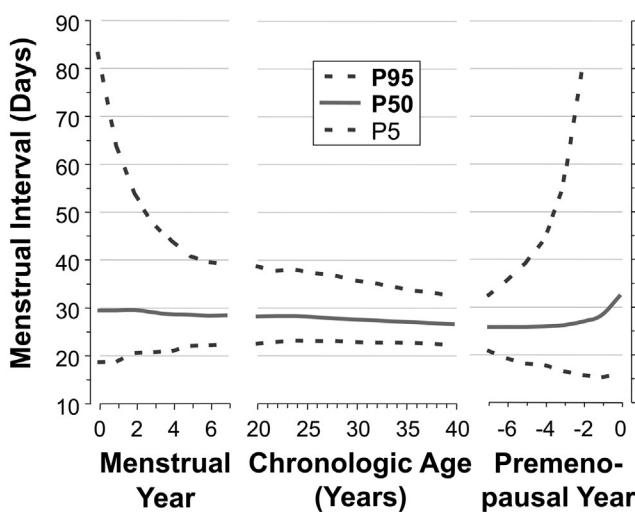


Fig. 16.33 Normal range for interval between menstrual cycles. Note that cycle intervals averaging more than 90 days or fewer than 21 (19 days in first year after menarche) days are abnormal at any age. P5 and P95 = 5th and 95th percentiles, respectively. (Modified from Treloar, A., Boynton, R., Benn, B., Brown, B. (1967). Variation of human menstrual cycle through reproductive life. *Int J Fertil*, 12, 77–84.)

menstrual cyclicity differs only slightly from that of reproductive-age adults. Thus most adolescent ovulatory abnormalities are asymptomatic, with cyclic menstrual bleeding occurring at 21- to 45-day intervals even in the first postmenarcheal year (Fig. 16.34): this paradox arises because immature cyclic ovarian function is usually occurring during these intervals.⁷³⁷ Serum hormonal changes during normal adolescent menstrual cycles confirm that substantial but immature cyclic follicular development occurs in such girls and some anovulatory adolescents (Fig. 16.35).^{735,736,738}

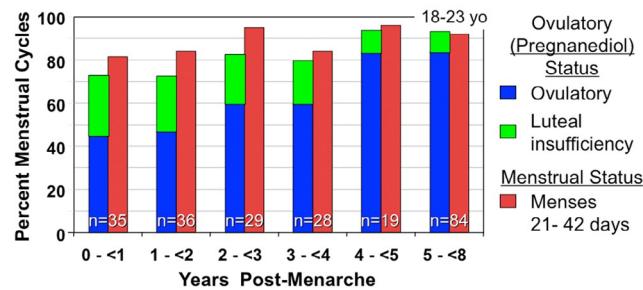


Fig. 16.34 Comparison of the percent of menstrual cycles that are 21 to 42 days duration (red) and percentage of menstrual cycles that are ovulatory (blue) by postmenarcheal age through young adulthood. Many more menstrual cycles are regular (21–42 days) than are normal, mature ovulatory cycles. Ovulation normalcy was determined with reference to adult standards of the major urinary progesterone metabolite, pregnanediol glucuronide, in weekly samples collected during the last 12 days of each menstrual cycle. Clearly detectable but subnormal pregnanediol is here designated as having luteal insufficiency (green). It can be seen that most of the cycles that are not mature ovulatory had sufficient cyclic follicular activity to generate an immature corpus luteum, which indicates antecedent ovulation, rather than being truly anovulatory as the investigators had labeled them. (Data from Metcalf, M.G., Skidmore, D.S., Lowry, G.F., Mackenzie, J.A. (1983). Incidence of ovulation in the years after the menarche. *J Endocrinol*, 97, 213–219; Rosenfield, R.L. (2015). The diagnosis of polycystic ovary syndrome in adolescents. *Pediatrics*, 136, 1154–1165. With permission.)

Although there is considerable variation in the time it takes for menstrual cycles to mature, menstrual regularity approximates adult standards in most girls within a year of menarche: three-quarters have a cycle length between 21 and 45 days, and 5% more fall within these bounds each of the next 3 years.^{739,740} By 5 gynecological years, 90% of menstrual cycles last 22 to 40 days, and about 80% of cycles are normal ovulatory ones. Menstrual cycle length narrows to 24 to 38 days in midadulthood⁶⁹⁵ as ovulatory rates approach 90%.¹⁸⁷

Menstrual abnormalities in adolescence can be defined similarly to abnormal uterine bleeding in adults.^{736,741} Primary amenorrhea is failure to begin menses at a normal age (by 15 years of age or within 3 years of thelarche when it has been delayed). Secondary amenorrhea is the absence of menstrual periods for 90 days or more after initially menstruating. Oligomenorrhea is defined as subnormal menstrual frequency, the normal limits for which gradually change during the 6 years after menarche (Box 16.2). Anovulatory cycles may also cause excessive uterine bleeding, as discussed in the later section Ovulatory Dysfunction: Dysfunctional Uterine Bleeding.

Symptomatic menstrual abnormalities in adolescents are increasingly unlikely to represent “physiological” adolescent anovulation with time. By 1 year postmenarche, failure to establish and sustain a normal adult menstrual pattern carries approximately a 50% risk of persistent oligoovulation, and failure to do so by 2 years after menarche carries approximately a two-thirds risk (Fig. 16.36).⁵⁷⁵ Thus persistence of menstrual irregularity for 1 to 2 years or more is a strong indication for investigation (see section Abnormal Puberty).

Serum LH, testosterone, and androstenedione levels are significantly higher in adolescents with anovulatory than those with ovulatory cycles.^{742,743} It is unclear whether this is the cause or the result of the anovulation; however, if hyperandrogenemia is found, it seldom regresses.¹²³ A polycystic ovary is common in adolescents and is usually a variant of normal, unless associated with menstrual abnormality or hyperandrogenism.^{83,123,656,744–746}

Other Normal Adolescent Variations

Three-quarters of adolescent girls experience mild to severe comedonal acne, and one-quarter mild inflammatory acne.⁷⁴⁷ Mild hirsutism also arises commonly among perimenarcheal girls. However, inflammatory acne that is moderate or severe (i.e., >10 lesions of face or other region) is uncommon during the perimenarcheal years, and hyperandrogenism should be considered in such girls as it should in those with mild hirsutism and menstrual irregularity (see Hyperandrogenism in Adolescence). The initiation of acne is more closely related to blood levels of DHEAS than of other androgens, as is cystic acne.⁵⁴²

Profound psychological changes occur during adolescence. Sexually immature girls tend to be socially immature, and the onset of puberty is associated with increased independence and profound changes in outlook on life and intellectual capacities. The extent to which these developments occur in reaction to the physical changes of puberty and the extent to which they are direct effects of sex hormones are unknown. Masculine tomboyish traits usually have no clear hormonal basis, although there is some evidence that they may have prenatal hormonal determinants. Social interactions have effects on these aspects of development. They affect even the synchrony of the menstrual cycle.⁷⁴⁸

Despite the popular notion that adolescence is inherently a period of turmoil, the majority of teenagers do not develop significant social, emotional, or behavioral difficulties.⁷⁴⁹ Occasional experimentation and risk-taking are normal, as are withdrawal from and conflict with parents. Adolescent behavior must be understood in the context of individual

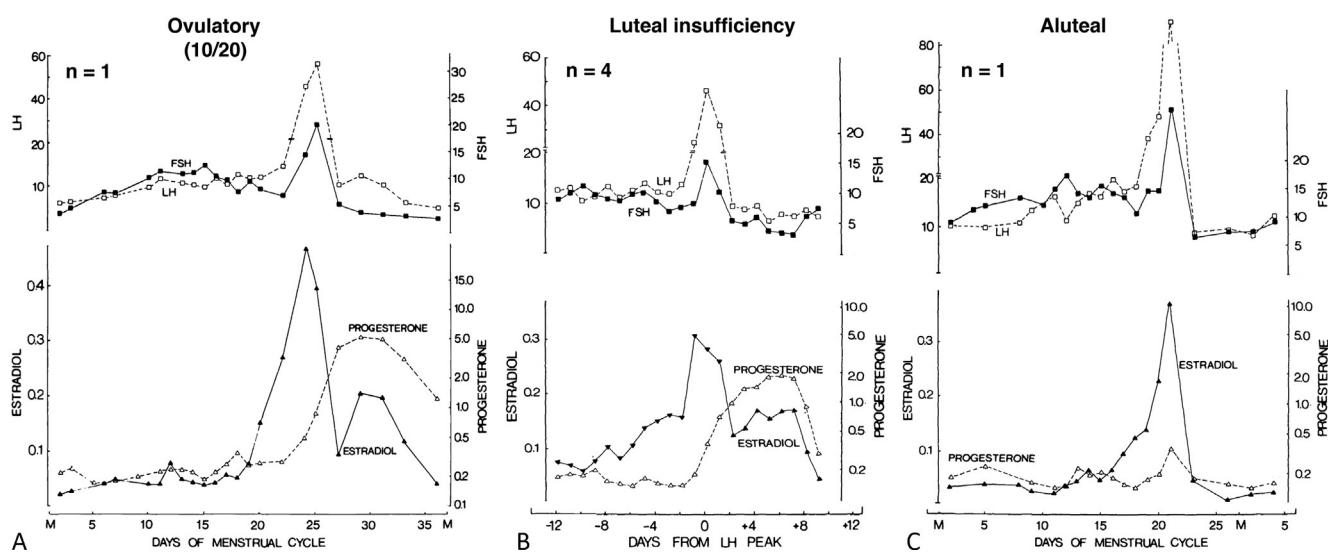


Fig. 16.35 Serum hormonal patterns of normal adolescent menstrual cycles among 20 healthy schoolgirls. **Panel A.** A normal ovulatory cycle in a 16-year-old female, representative of 10/20 of the study group who were ovulatory. Note normal preovulatory estradiol and progesterone rises, the subsequent midcycle luteinizing hormone (LH) and follicle-stimulating hormone (FSH) surges, followed by progesterone levels that reach 5.0 ng/mL or higher during the luteal phase of the menstrual cycle. Although this is a standard criterion for an ovulatory cycle, the illustrated cycle would be considered luteally insufficient in an adult. **Panel B.** Mean hormone levels of the group defined as having luteal insufficiency: their short luteal phases lasted 4 to 8 days, but cycle lengths were normal (23–30 days) caused by prolonged follicular phase. Note the slightly low preovulatory estradiol and absent progesterone rises, followed by blunted midcycle LH and FSH surges that presage the low progesterone levels of the short luteal phases. **Panel C.** An aluteal anovulatory cycle in a 14-year-old female. Note normal preovulatory estradiol and LH surges, but absent preovulatory progesterone rise that presaged an absent luteal phase progesterone increase. The remainder of the five anovulatory study subjects did not attain clear preovulatory development and progesterone levels were consistently under 1.0 ng/mL, yet the menstrual cycles were abnormally long in only 2/5. M, menses. Scales differ slightly, and progesterone scales are logarithmic. Steroid levels in ng/mL LH and FSH levels in mIU/mL. (Data from Apter, D., Viinikka, L., Vihko, R. (1978). Hormonal pattern of adolescent menstrual cycles. *J Clin Endocrinol Metab*, 47, 944–954; Modified from Rosenfield, R.L. (2013). Clinical review: adolescent anovulation: maturational mechanisms and implications. *J Clin Endocrinol Metab*, 98, 3572–3583. With permission.)

susceptibility, family upbringing and interactions, peer group interactions, changes in brain maturation, and adolescents' reaction to their perception of the bodily changes and to the sexual urges that are the direct consequences of puberty. Simply because a problem is displayed during adolescence does not mean that it is a direct consequence of puberty.

Many behavioral problems that emerge during adolescence have earlier roots. Although the prevalence of depression increases during puberty, many children who develop depression during adolescence have had preexisting symptoms of psychological distress. Likewise, most delinquent teenagers have had antecedent problems at home and school.

Early-maturing girls in Western cultures are more popular, but they have more emotional problems; lower self-image; and higher rates of depression, anxiety, and disordered eating than their peers. Early maturation appears particularly to be a risk factor for problem behavior among girls who have had a history of difficulties before adolescence, when they have more opposite sex friendships and relationships, and when they attend coeducational schools.

Short-term administration of testosterone or estrogen has minimal effects on behavior or mood in adolescents.^{627,750} Thus variation in hormone levels accounts for only a small fraction of adolescents' affective issues, and social influences account for considerably more. Although there is little evidence that psychological difficulties stem directly from hormonal changes during normal puberty, it is likely that the bodily changes of adolescence play a role in the development of a negative body image when they occur out of synchrony with sociocultural norms.

Problems with initiating and maintaining sleep are common in adolescents and contribute a small amount to poor

school performance.⁷⁵¹ Although insufficient sleep might be caused by environmental factors (e.g., social and academic pressures), intrinsic factors clearly play an important role. A 50% decline in the intensity of deep (slow wave, delta) sleep occurs during adolescence, and one-half of this change occurs between 12 and 14 years of age.^{752,753} Recent evidence indicates that this change is related to age and sex, beginning earlier in girls, but not to pubertal stage. It has been proposed that this shift is a manifestation of the widespread synaptic pruning that is related to the emergence of adult cognitive capacity.

The causal direction of the link between pubertal development and the quality of family relationships has come into question. Several studies have indicated that family dynamics may affect the timing and course of puberty, with earlier and faster maturation observed among adolescents raised in homes characterized by more conflict and among girls from homes in which the biological father is not present.⁷⁴⁹

ABNORMAL PUBERTY

Abnormal Development

Disorders of Sex Development

Patients with DSD (formerly termed *intersex*)—those whose genitalia are ambiguous or inappropriate for their gonadal sex as a result of endocrinopathy—may come to a physician's attention for the first time at puberty. These syndromes have been categorized as 46, XX DSD, which encompasses cases formerly termed *female pseudohermaphroditism* and including 46, XX testicular DSD (formerly termed *XX sex reversal*); 46, XY DSD, which encompasses cases

BOX 16.2 Definition of Types Of Abnormal Uterine Bleeding in Adolescents

Descriptor	Definition
Primary amenorrhea	Lack of menarche by 15 years of age or by 3 years after the onset of breast development ^a
Secondary amenorrhea	Over 90 days without a menstrual period after initially menstruating (in consecutive periods during first year post-menarche)
Oligomenorrhea (infrequent abnormal uterine bleeding)	Postmenarcheal year 1: average cycle length >90 day (fewer than four periods in the year) Postmenarcheal year 2: average cycle length >60 days (fewer than six periods in the year) Postmenarcheal years 3: average cycle length >45 days (fewer than eight periods per year) Postmenarcheal years ≥4: cycle length >38 days (<9 periods per year)
Excessive uterine bleeding ^b	Menstrual bleeding that occurs more frequently than every 21 days (19 days in year 1) or is prolonged (lasts >7 days) or heavy (soaks more than one pad or tampon every 1–2 h large clots, or gushing)

(Rosenfield, R.L. (2015). The diagnosis of polycystic ovary syndrome in adolescents. *Pediatrics*, 136, 1154–1165. With permission; Modified according to Teede, H.J., Misso, M.L., Costello, M.F., et al. (2018). Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. *Fertil Steril*, 110, 364–79).

^aBone age of 15 years may be substituted for chronological age in girls with earlier than average age at puberty onset.

^bEncompasses frequent, intermenstrual, heavy, and/or prolonged abnormal uterine bleeding. Alternatively termed *dysfunctional uterine bleeding* or *excessive abnormal uterine bleeding caused by ovulatory dysfunction*.

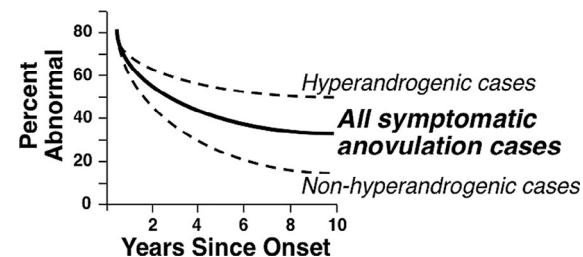


Fig. 16.36 Probability that an adolescent with a symptomatic menstrual abnormality severe enough to result in gynecological consultation will have continued menstrual abnormality. The lines show the cumulative rates at which subjects with menstrual abnormalities converted to normal patterns. The *heavy curve* shows the average incidence of continued menstrual abnormality in adolescents when considering all symptomatic cases of abnormal uterine bleeding presenting to a reproductive endocrine clinic regardless of time of symptom onset. (Data from Southam, A.L., Richart, E.M. (1966). The prognosis for adolescents with menstrual abnormalities. *Am J Obstet Gynecol*, 94, 637.) Dysfunctional uterine bleeding of onset within 1 year of menarche carries the furthest from average poor prognosis for continuing menstrual abnormality, and oligomenorrhea of relatively short duration occurring after a normal menstrual pattern has been established carries the furthest from average best prognosis. Note that if the menstrual abnormality persists for 1 year there is about a 50% probability, and if for 2 years over a 60% probability, that the patient will not spontaneously evolve to normal cycles. Similarly, if the problem persists for 5 years there is an 80% likelihood of persistence of the abnormality. “Hyperandrogenic” and “Nonhyperandrogenic” curves are hypothetical, based on data reviewed in Rosenfield, R.L. (2015). The diagnosis of polycystic ovary syndrome in adolescents. *Pediatrics*, 136, 1154–1165. Hyperandrogenic cases are predominantly a mix of physiological anovulation and polycystic ovary syndrome (PCOS), with PCOS persisting. Nonhyperandrogenic cases are a mix of physiological anovulation and hypogonadal cases, ranging from primary hypogonadism through hypothalamic amenorrhea to hypogonadotropic hypogonadism, with hypogonadal cases persisting. (Modified from Rosenfield, R.L. (2015). The diagnosis of polycystic ovary syndrome in adolescents. *Pediatrics*, 136, 1154–1165.)

in response to estradiol. Experimental animals exposed to androgen excess early in gestation develop classic PCOS features: these animals have elevated LH levels, ovarian and adrenal hyperandrogenism, oligomenorrhea, and polyfollicular ovaries. They also have abdominal obesity, insulin resistance, impaired glucose tolerance, and dyslipidemia, which likewise appear to result from developmental programming.

Genetic males with complete androgen insensitivity have low LH levels and poor LH responsiveness to GnRH in the neonatal period.⁶⁴ However, gonadotropin levels are normal-to-high at puberty, and, paradoxically, androgen signaling via the androgen receptor enhances the capacity of females to mount an LH surge in response to estrogen positive feedback.^{337,338,755}

Other Dysgenetic Disorders

Failure of the onset of menses can result from structural abnormalities of the genital tract that do not have an endocrinological basis. The hymen may be imperforate, which results in hydrocolpos if the vagina is intact. The vagina may be aplastic, which will result in hydrometrocolpos if the uterus is intact.⁷⁵⁶ The uterus may be congenitally aplastic. Uterine synechiae develop as the consequence of endometritis, which may result from infection or irradiation (Asherman syndrome). Congenital absence of the vagina may be associated with varying degrees of uterine aplasia; this is the Mayer-Rokitansky-Kuster-Hauser syndrome.⁷⁵⁷ This syndrome seems to occur as a

formerly termed *male pseudohermaphroditism* and including 46, XY complete gonadal dysgenesis (XY sex reversal, Swyer syndrome); and sex chromosome DSD, which includes Turner syndrome, Klinefelter syndrome, mixed gonadal dysgenesis, and chimeric ovotesticular DSD.⁶⁰⁰ In the absence of chromosomal mosaicism, ovotesticular DSD, formerly termed *true hermaphroditism*, is categorized as either 46, XX DSD or 46, XY DSD. Patients with any of these disorders may undergo inappropriate puberty. They may present with clitoromegaly and be found upon examination to have a degree of genital ambiguity which was previously overlooked. Virilization beginning at puberty is sometimes the presenting complaint. Ovotesticular DSD or 46, XX DSD because of CAH are compatible with fertility.⁴⁴⁴ Androgen insensitivity syndrome in a genetic male may present as primary amenorrhea in an otherwise phenotypically normal adolescent girl. The disorders of sexual differentiation are reviewed in detail in Chapter 6.

Congenital virilization of the female developmentally programs the emergence of PCOS at puberty. These observations are consistent with studies of fetal androgenization of the female in several species, including primates.^{15,16,754} There is a persistent increase in LH pulse frequency and impairment of the negative feedback effect of progesterone on LH release that appear to be related to suppression of hypothalamic PR

single gene defect or as an acquired teratogenic event involving mesodermal development and the mesonephric kidney, the latter resulting in abnormalities of the genital tract and sometimes the urinary tract. A subtype caused by *Wnt4* gene defects is associated with hyperandrogenism.⁷⁵⁸

Precocious Puberty

Causes

When breast or sexual pubic hair development begins before the age of 8.0 years or menses begin before the age of 9.5 years, puberty is traditionally considered precocious, or premature. It should be kept in mind that breast development during the seventh year is within normal limits in ethnic minority girls. In addition, presexual pubic hair (stage 2) may be normal in 6- and 7-year-old ethnic minority girls.

Puberty can occur prematurely as an extreme variation of normal, because of a disturbance in the HPG axis normally involved in sexual maturation, or because of a disturbance outside the HPG axis. Depending on which part of this hormonal axis is involved, different forms of precocious puberty are distinguished. A classification of the causes of premature puberty together with typical findings is given in Table 16.4.

It is important to distinguish between true precocious puberty and pseudoprecocious puberty. True precocious puberty is gonadotropin dependent; thus *central* is another term applied to this type of precocity. Maturation is complete: both breasts and pubic hair develop as the result of CNS activating pituitary secretion of the respective gonadotropins FSH and LH (although breast development may be the sole manifestation of early complete precocity for as long as 6–12 months). Patients with true precocious puberty have “*isosexual*” precocity because the secondary sexual characteristics are appropriate for the sex of the child. Pseudoprecocious puberty is gonadotropin independent; it is not mediated by pubertal pituitary gonadotropin secretion and is sometimes

termed *peripheral*. Maturation is incomplete, with only one type of secondary sexual characteristic developing early. Peripheral precocity has diverse causes. In some patients with pseudoprecocity, pubertal development is isosexual, in others it is “*contrasexual*,” meaning that characteristics of the opposite sex are manifested.

Complete Precocious Puberty. True isosexual precocity results from pubertal function of the HPG axis. About 95% of true precocity in girls is idiopathic. Idiopathic true sexual precocity appears to be caused by premature triggering of the normal pubertal mechanism. Pubertal development usually is qualitatively and quantitatively normal except for its early occurrence. The predominance of the idiopathic cases in females and its benign nature are compatible with the likelihood that this disorder is an extreme exaggeration of the normal tendency of girls to have higher gonadotropin levels than boys. Most cases are sporadic, a few familial. The majority of these patients seem to go on to have normal menstrual cycles and fertility.⁷⁵⁹ Indeed, pregnancy has been documented to occur as early as 4 years of age.

Rapidly progressive puberty with a growth spurt ensues when activation of the pituitary-ovarian axis is sustained. However, precocious puberty is not necessarily sufficiently intense or sustained to cause inexorable progression or bring about deterioration of height potential.⁷⁶⁰ Precocity in the 6- to 8-year age range usually is not rapidly progressive and most commonly seems to be caused by excessive adiposity.²⁷⁶

Any type of intracranial disturbance can cause true isosexual precocity. These neurogenic disturbances are presumed to cause true sexual precocity by increasing the prevalence of excitatory inputs or by interfering with CNS inhibition of hypothalamic GnRH secretion.⁷⁶¹ These include congenital brain dysfunction, such as cerebral palsy or hydrocephalus, or acquired disorders, such as irradiation,⁷⁶² trauma, chronic inflammatory disorders, or masses in the region of the hypothalamus. The activation of GnRH release by hypothalamic injury may be

TABLE 16.4 Typical Findings in Female Sexual Precocity

Locus	Type	HA ^a	BA ^a	Estrogens ^Ü	Androgens ^Ü	LH/FSH ^Ü	Pathology	Characteristics
COMPLETE PRECOCITY (CENTRAL, GONADOTROPIN-DEPENDENT)								
Hypothalamic	Isosexual	+	++	+	+	+	Idiopathic Neurogenic Advanced somatic maturation	95% of female cases
INCOMPLETE PRECOCITY (PERIPHERAL, GONADOTROPIN-INDEPENDENT)								
Normal variant	Isosexual	-	-	±	-	±	None	Thelarche
	Isosexual	-	-	-	±	-	None	Pubarche/adrenarche
Neuroendocrine	Contrasexual	+	++	-	+	+/-++	LH/hCG excess	Familial or tumor
	Isosexual	Low	Low	-	-	±	Hypothyroid	Growth arrest
Ovary	Isosexual	+	++	+/-++	-	-	McCune-Albright	Bone lesions ± nevi ± ovarian cysts
	Isosexual/ contrasexual	+	++	+/-++	+/-++	-	Tumor	
Adrenal	Contrasexual	+	++	±	+++	-	Congenital adrenal hyperplasia	Dexamethasone suppressible
	Contrasexual/ isosexual	+	++	+/-++	+/-++	-	Tumor	
Ectopic	Isosexual	+	++	+/-++	-	-	Aromatase excess	
Exogenous	Contrasexual/ isosexual	±	±	-	-	-	Sex steroid exposure	
End organ	Isosexual/ contrasexual	-	-	-	-	=	Vaginal foreign body, abuse, tumor	

Hormone levels: – normal prepubertal; + pubertal level; ++ adult level; +++ abnormally high.

FSH, Follicle-stimulating hormone; hCG, human chorionic gonadotropin; LH, luteinizing hormone.

^aHA (height for age) and BA (bone age); – normal; + advanced; ++ markedly advanced.

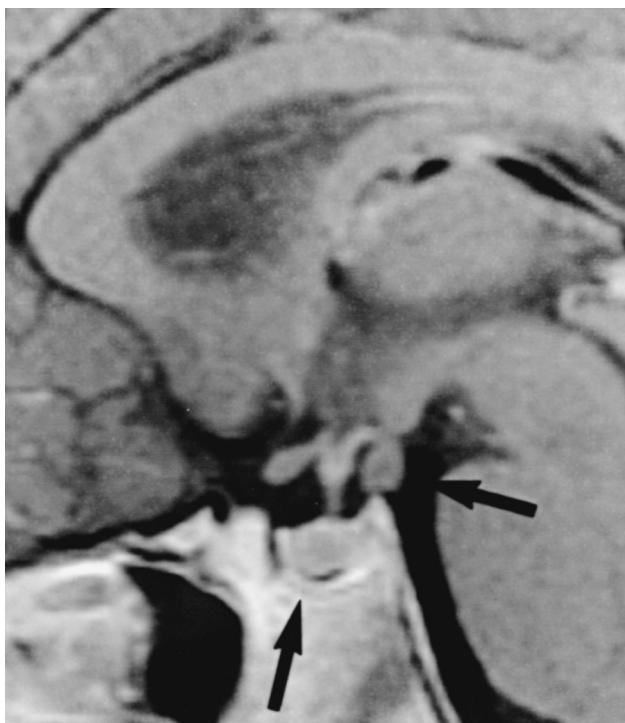


Fig. 16.37 Magnetic resonance image showing a hypothalamic hamartoma (right-hand arrow) as the cause of true sexual precocity in a 2.5-year-old girl. The hamartoma is hanging from the floor of the hypothalamus just posterior to the pituitary infundibulum. The sella turcica (bottom arrow) contains a normal pituitary gland with pituitary stalk hanging from the infundibulum.

mediated by TGF- β , trauma, chronic inflammatory disorders, or masses in the region of the hypothalamus. An empty sella is occasionally found.⁷⁶³ The precocity of neurofibromatosis type 1 (von Recklinghausen disease) usually results from an optic glioma, which is often of low-grade malignancy, or from a hamartoma,⁷⁶⁴ although occasionally with neither.⁷⁶⁵ Hamartoma of the hypothalamus may cause central sexual precocity; this effect is most likely caused by an anatomic effect on hypothalamic structures rather than by acting as an "accessory hypothalamus" that releases pulses of GnRH into the pituitary portal circulation.^{766,767} Fig. 16.37 shows a hypothalamic hamartoma.

A small proportion of pineal tumors cause true sexual precocity.^{768,769} The incidence of sexual precocity is about 3.5 times as great in nonparenchymatous neoplasms (such as gliomas and teratomas) as in parenchymatous pineal tumors. This suggests that these tumors cause sexual precocity via absence of a normal pineal inhibitory factor rather than by destructive effects on inhibitory tracts. Although pineal masses may cause paralysis of upward gaze by pressure on the corpora quadrigemina, this sign is present in only a minority of cases.

Pineal or hypothalamic hCG-secreting germ cell tumors occasionally cause true sexual precocity.^{770,771} Because hCG is an LH receptor agonist, possible explanations for this unusual situation are disinhibition of hypothalamic GnRH release attributed to a mass effect of the neoplasm, the weak FSH effect of massive elevation of hCG, or the capacity of some dysgerminomas to secrete estradiol, as well as hCG.⁷⁷²

Advancement of somatic maturation caused by peripheral endocrine disorders that advance the bone age to a pubertal level sometimes cause true sexual precocity. Thus true puberty may begin after correction of virilizing or feminizing disorders that have advanced the bone age to 10 to 12 years.^{264,265}

The hypergonadotropinism of premature ovarian insufficiency (POI) has been reported to cause sexual precocity or rapid progression of puberty before premature ovarian failure.^{773,774} Although PCOS has occasionally been reported to follow true sexual precocity,⁷⁷⁵ there is currently no clear evidence of a significant association.^{759,776}

A family history of complete precocious puberty (CPP) is common in patients with early puberty; however, a clear genetic etiology is usually unknown. Monogenic causes of CPP have recently been identified in four genes. Gain-of-function mutations have been found in kisspeptin and its receptor, KISS1R.^{777,778} The patients were heterozygous for the mutations, with an autosomal dominant inheritance.⁷⁷⁹ The first mutation to be described was in an adopted girl who had progressive thelarche from birth, with accelerated growth velocity and skeletal maturation noted at age 7 years. A heterozygous activating mutation of KISS1R (p. Arg386Pro) was identified and in vitro studies showed that the mutation led to prolonged activation of intracellular signaling pathways resulting in higher sustained inositol phosphate accumulation.⁷⁷⁷ A mutation in kisspeptin, p. Pro74Ser, was identified in a boy with CPP at age 1 year, with high concentrations of serum LH and testosterone. Interestingly, his mother and maternal grandmother had normal pubertal development, carried the p. Pro74Ser mutation in the heterozygous state, suggesting incomplete penetrance. In vitro, the mutated protein was able to stimulate signal transduction to a greater extent than the wild type, suggesting it may be more resistant to degradation, resulting in greater kisspeptin bioavailability.⁷⁷⁸ However, large-scale studies in children with CPP have not been able to identify additional patients and families with CPP with these mutations, hence kisspeptin and KISS1R activating mutations are relatively rare causes of CPP.⁷⁷⁹

Another genetic abnormality linked to CPP involves loss-of-function mutations in the Makorin RING-finger protein 3 (MKRN3) gene, a maternally imprinted gene located on the long arm of chromosome 15 (Prader-Willi region) encoding a protein that is involved in gene transcription and ubiquitination. Expression of MKRN3 was found to be high in the arcuate nucleus in prepubertal mice, decreases before puberty, and is low after puberty, thus MKRN3 appears to be acting as an inhibitor of puberty. Interestingly, MKRN3 polyubiquinylates Nptx1, another protein of unclear function that is also expressed at low levels prepubertally, and highly during puberty.⁷⁸⁰ Fifteen families with a history of CPP were studied, and mutations in MKRN3 that resulted in truncated proteins or missense mutations predicted to disrupt protein function were found in one-third of them. At present, 10 different mutations have been found, and include frameshift, missense, or nonsense mutations predicted to result in loss-of-function of the protein.^{246,781-787} MKRN3 is maternally methylated, explaining the autosomal dominant paternal inheritance in all cases, with no de novo mutations described.⁷⁸¹ All patients described to date exhibit a typical pattern of early pubertal development. A few patients with MKRN3 mutations were described as having syndromic features that included esotropia, a high-arched palate, dental abnormalities, clinodactyly, and hyperlordosis.⁷⁸⁵ In a genetic study of 20 boys with idiopathic CPP, eight were found to have MKRN3 mutations and one had a KISS1-activating mutation,⁷⁸⁸ perhaps indicating that MKRN3 mutations are a relatively frequent cause of CPP.

Delta-like homolog 1 (DLK1), a paternally expressed imprinted gene, encodes a protein expressed in kisspeptin-expressing neurons.⁷⁸⁹ DLK1 is a part of the delta-notch pathway, which is an evolutionarily conserved signaling pathway with roles in proliferation and differentiation during development.⁷⁹⁰ In the pituitary, DLK1 and notch signaling appear to be important in pituitary cell type differentiation.⁷⁹⁰

A mutation in DLK1 was found in a family with five girls with CPP. The mutation was a 14-kb deletion along with a 269-bp duplication. Serum levels of DLK1 were undetectable in these girls.⁷⁸⁹ In another study, the *DLK1* gene was sequenced in 60 girls with idiopathic CPP, and no mutations were found.⁷⁹¹

Distinct chromosomal abnormalities associated with specific syndromes may include CPP. These include: 1p36 deletion, 7q11.23 microdeletion (Williams-Beuren syndrome),⁷⁹² 9p deletion, maternal uniparental disomy of chromosomes 7 (Silver-Russell syndrome) and 14 (Temple syndrome),⁷⁹³ inversion duplication of chromosome 15,⁷⁹⁴ de novo interstitial deletion and maternal uniparental disomy of chromosome 15 (Prader-Willi syndrome),⁷⁹⁵ and a de novo deletion in the cyclin-dependent kinase-like 5 gene (*CDKL5*; located in the Xp22 region)⁷⁹⁶ (phenotype similar to Rett syndrome).

Incomplete Precocity. The most common causes of incomplete sexual precocity in girls are the extreme variants of normal mentioned previously, premature thelarche and premature pubarche. These are incomplete forms of sexual precocity in which either breast development (thelarche) or sexual hair development (pubarche) is of a degree appropriate for an early stage of puberty and isosexual. Isolated prepubertal menses is a rare disorder that has been attributed to transient ovarian activity.⁷⁹⁷

LH- or hCG-producing tumors have not been reported to virilize girls, perhaps because of their limited capacity for thecal androgen production over short periods of time. However, familial isolated elevation of LH has been reported to cause mild virilization of siblings:⁷⁹⁸ one was a girl who developed premature pubarche and clitoral hypertrophy at 4 years of age, with slight to moderate advances in height and bone age in association with an adrenarchal level of DHEAS and a moderately elevated testosterone level (91 ng/dL).

The van Wyk-Grumbach syndrome is one of the most puzzling pediatric complexes.⁷⁹⁹ This is an unusual syndrome of sexual precocity associated with juvenile hypothyroidism. A case is illustrated in Fig. 16.38. This syndrome is often characterized by galactorrhea, which is often not spontaneous; a few drops of milky fluid may become apparent only upon "milking" the subareolar ductal tissue. Multicystic ovaries are often demonstrable by ultrasonography.⁸⁰⁰ Modern assays show that levels of LH at baseline and post-GnRH are suppressed and those of FSH are early pubertal.⁸⁰¹ There is little, if any, sexual hair development. There is another clinically unique feature about the sexual precocity of hypothyroidism: it is the only form of sexual precocity in which growth is arrested rather than stimulated and is an exception to the general rule, indeed followed by most chronically hypothyroid children, that a delayed growth pattern is associated with delayed puberty.

Van Wyk and Grumbach postulated that this syndrome resulted from hormonal "overlap" in the negative feedback regulation of pituitary hormone secretion, with overproduction of gonadotropins, as well as TSH in response to the thyroid deficiency. The specific nature of hormonal overlap has been considerably clarified in recent years, but the pathogenesis of the precocity remains unclear. The increases in serum TSH and prolactin that characterize the syndrome could well be accounted for by common neurohumoral control systems, TRH stimulating and dopamine inhibiting both hormones. It has been suggested instead that the ovarian FSH receptor is activated by the weak intrinsic FSH activity of extreme TSH elevation,⁸⁰² analogous to the rare ovarian hyperstimulation syndrome in which pregnancy levels of hCG activate the FSH receptor.⁸⁰³

Prolactin excess has been postulated to underlie the FSH-predominant gonadotropin pattern by slowing GnRH pulsations, and it is this FSH stimulation of the TSH-sensitized ovary⁸⁰⁴ that seems to be the proximate cause of the sexual precocity.



Fig. 16.38 Sexual precocity caused by hypothyroidism in a 9.1-year-old with breast development since 7 years and menarche at 9.0 years. Growth failure had occurred, and her height age was 6 years. In addition to breast enlargement and galactorrhea, the labia minora were noted to be enlarged and pigmented. There was no sexual hair or clitoromegaly. Rectal examination revealed an enlarged and palpable cervix without adnexal masses. There were typical physical findings of hypothyroidism. Bone age was 6.2 years. Thyroxine was less than 1 mcg/dL. Thyrotropin-stimulating hormone was 438 µU/mL. Prolactin was 66 ng/mL. Serum estrogens were 72 to 182 pg/mL. Vaginal smear showed 45% superficial cells and 55% large intermediate cells. Immunoreactive luteinizing hormone (LH) and follicle-stimulating hormone were 300 and 174 ng LER-907/mL, respectively (see Fig. 16.5 for reference ranges). However, bioactive LH was undetectable. Immunoreactive gonadotropins failed to suppress upon estrogen administration. Their response to a 100-mcg gonadotropin-stimulating hormone bolus was minimal, and they seemed responsive to thyrotropin-releasing hormone. All of these hormonal findings were not obviously different from those of hypothyroid girls without sexual precocity except for the higher estrogens. She had withdrawal bleeding and evidence of regression of breast development within the first 3 months of thyroid hormone replacement treatment. After 6 months' treatment, normal puberty began. Menarche occurred at 12.5 years of age.

Hyperprolactinemia alone does not correspond with pubertal development in normal or hypothyroid children. However, hyperprolactinemia may itself also sensitize the ovaries to gonadotropins. Induced hyperprolactinemia causes sexual precocity in female rats.³⁶⁵ Ovarian estrogen and progesterone responsiveness to hCG is increased by prolactin, possibly by its induction of ovarian LH receptors. Conversely, suppression of hyperprolactinemia in experimental hypothyroidism blocks the ovarian cyst formation characteristic of hypothyroidism.⁸⁰⁵

McCune-Albright syndrome is another intriguing disorder causing incomplete isosexual feminization.^{806,807} This is a syndrome of precocious puberty, cafe-au-lait pigmentation

occurring in nevi that have an irregular ("coast of Maine") border, and polyostotic fibrous dysplasia. The disorder is caused by a somatic activating mutation of the G_s-alpha subunit protein that couples transmembrane receptors to adenylate cyclase. The syndrome has been recognized predominantly in females and occurs in incomplete, as well as expanded forms. Precocious puberty or monoostotic bone lesions may occur in the absence of cutaneous pigmentation; not all patients have sexual precocity. The sexual precocity is of the gonadotropin-independent type. Luteinized follicular cysts within the ovaries function autonomously. Pituitary adenomas capable of secreting excess LH, FSH, GH, and/or prolactin have been reported. Patients may have Cushing syndrome and hyperthyroidism because of autonomous multinodular hyperplasia. These girls may be at increased risk of breast carcinoma.⁸⁰⁸ Nonendocrine abnormalities include cardiopulmonary disease, hypertension, and hepatobiliary disease (including severe neonatal cholestasis).⁸⁰⁹ Molecular studies have shown an R201H mutation in over 90% of cases where an affected tissue could be studied, but in only 50% of blood samples.⁸¹⁰ Because of the variation in the number and degree of tissue involvement in individual patients, caused in large part by the extent of mosaicism present, precocious puberty may be the only feature present in an individual who is mosaic for the activating mutation of G_s-alpha. Thus these mutations have been found in blood samples from 25% to 33% of subjects with isolated gonadotropin-independent precocity or exaggerated thelarche.^{704,810}

CAH is a well-known cause of premature pubarche. Either nonclassic CAH, a form of the disorder which is so mild that there is no genital defect in girls, or poor control of classic CAH may be responsible. Each form on occasion has been reported to mimic true sex precocity.^{811,812}

Tumor may cause isosexual or contrasexual development. The most common tumor is the feminizing benign ovarian follicular cyst.^{813,814} Most are isolated and large (>1.0 cm in diameter). The cells lining these cysts are often luteinized. Estrogen levels may be markedly elevated. Testosterone levels tend to be in the adult female range (about 40 ng/dL). Many function intermittently. They may be gonadotropin dependent and respond to GnRH agonist or progestin therapy. A case is illustrated in Fig. 16.39. The second most common hormonally active ovarian neoplasm in girls is the juvenile granulosa cell tumor.^{815,816} These have variable degrees of ovarian sex cord-stromal elements and are usually localized and benign in spite of having a malignant histological appearance. They are more commonly feminizing than masculinizing in young children. They may produce hCG, AMH, and inhibin. Elevation of hCG is found in many ovarian dysgerminomas (a primitive germ cell tumor), with hypercalcemia in some, although less frequently than in small cell carcinoma.^{815,817} Granulosa-theca cell tumor is occasionally associated with mesodermal dysplasia syndromes. It has been reported in the adrenal gland, presumably arising in an ovarian rest.⁸¹⁸ FOXL2 mutations are typical of adult-type granulosa cell tumors, but are found in only 10% of the juvenile type. A related feminizing ovarian sex cord-stromal tumor may be caused by loss of a tumor-suppressor gene, as in Peutz-Jeghers syndrome.⁸¹⁹ Adult-type ovarian carcinoma of epithelial cell origin is rare. Ovarian masculinizing tumors are discussed in the section Hyperandrogenism in Adolescence.

Adrenocortical tumors, as discussed in Chapter 14, typically cause rapid virilization characterized by very high DHEAS production; however, androstenedione is the predominant androgen in many cases. A case discussion is given with Fig. 16.40. Many are accompanied by cushingoid changes. On occasion, they cause feminization. When adrenocortical tumors secrete both androgen and estrogen, the clinical picture may resemble complete isosexual precocity.⁸²⁰ Structural abnormalities of

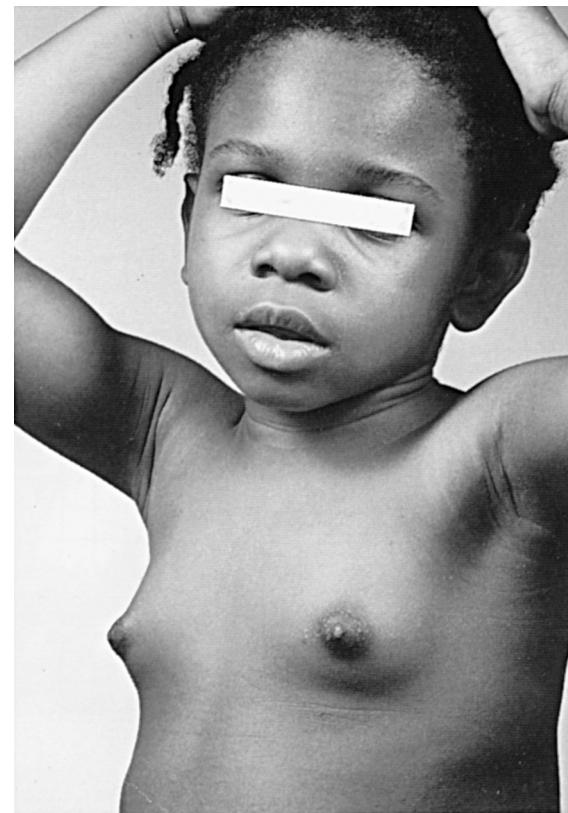


Fig. 16.39 The appearance of a 5.2-year-old child with complete isosexual precocity caused by a luteinized follicular cyst. Her breast development is no different from that of girls with idiopathic premature thelarche. She presented at 4.7 years with a 2-week history of breast development. Height and bone ages were 5 years. Over a 5-month follow-up period, breast development progressed, she developed sexual pubic hair and presexual axillary hair, and menstrual flow commenced at 3- to 5-week intervals. Four weekly determinations of plasma unconjugated estrogens (estradiol and estrone) showed them to consistently range between 158 and 215 pg/mL. Luteinizing hormone was pubertal and follicle-stimulating hormone was suppressed (averaging 50 and 13 ng LER-907/mL, respectively; see Fig. 16.5 for reference ranges). Testosterone was 37 ng/dL, and dehydroepiandrosterone sulfate (DHEAS) was 82 mcg/dL. Exploratory laparotomy was performed when she was 5.2-years-old. Her height age was 5.8 years, and her bone age was 7.5. The laparotomy revealed a right ovarian cyst about 5 cm in diameter, which was removed. Subsequently, there was a rapid fall in plasma estrogens and testosterone to prepubertal levels. However, DHEAS was unchanged. Menses ceased, but intermittent vaginal cornification (maturation index 90/10/0 to 0/90/10) was repeatedly found. Breast enlargement and sexual hair development resumed at 8.5 years, with normal pubertal sex hormone levels. Menarche occurred at 9.5 years.

chromosome 11p15 and mutations of the tumor suppressor p53 are fairly common in pediatric adrenocortical tumors.⁸²¹

Aromatase excess syndrome is a rare cause of ectopic feminization.⁸²² This is an autosomal dominant disorder with variable penetrance that results from constitutive aromatase gene overexpression.

Exogenous steroids can cause sexual precocity. Estrogen-containing contraceptive pills and creams are widely available. Some cases of precocious thelarche may be caused by ingesting food contaminated with artificial estrogens.⁸²³ Soy formulas are potential sources of phytoestrogens, as are many commonly consumed foods, herbs, and topicals (including products containing lavender oils and tea tree oils).^{824–827} It has been



Fig. 16.40 Abdominal ultrasound (decubitus view) showing a pedunculated encapsulated 5-cm adrenal adenoma (large arrow) near upper pole of kidney (small arrow). This 1.3-year-old girl was virilized. Pubic hair had appeared 2 months previously, height had changed from the 10th to the 30th percentile, and clitoromegaly had occurred. Bone age was 2.3 years. Dehydroepiandrosterone sulfate was 3000 to 4271 mcg/dL. Testosterone was 121 ng/dL. A, Anterior; P, posterior.

proposed that childhood exposure to estrogenic chemical contaminants in underdeveloped countries predisposes to sexual precocity when children emigrate to developed countries.¹⁹⁰ Premature pubarche or acne can result from anabolic steroid use. Topical nonprescription androgen use by a parent, such as for sexual dysfunction or anabolic effects, can cause premature pubarche without necessarily being detectable by standard tests.⁸²⁸

Vaginal bleeding in the absence of breast development suggests foreign body, sexual abuse, or tumors of the genital tract. A rare cause is "premature menarche," which may result from an extreme variation in the normal intermittent ovarian activation of young girls.⁹³ Malodorous discharge is highly suggestive of foreign body. A hymenal opening of greater than 5 mm or posterior notches is compatible with sexual abuse.^{532,533} Neurofibromas have been reported to simulate breast development and clitoral hypertrophy.

Differential Diagnosis

A physician need not be experienced in endocrinology to diagnose and manage most girls presenting with early breast or pubic hair development. For the most part, the isolated appearance of one of these signs is caused by the benign processes of either premature thelarche or premature pubarche, respectively. An otherwise normal history and physical examination, together with a normal bone age, constitutes sufficient workup.

In the history, the physician should inquire about the possibility of exposure or access to exogenous steroids in the form of pills, diet, or topical substances (such as estrogen creams or personal care products containing lavender oil or tea tree oil). The possibility of sexual abuse, vaginal infection, or foreign body must be kept in mind when evaluating the child with

isolated vaginal bleeding. In the examination, the physician should search for nevi, acanthosis nigricans, signs that might suggest intracranial or abdominal-pelvic disease, and inspect the external genitalia. The child's height and weight should be carefully recorded, the growth curve examined, and the BMI percentile plotted.

If the history and examination are unremarkable, only a bone age determination is indicated to screen for whether the symptom is indeed an isolated phenomenon or whether appreciable hormone excess exists. If the skeletal age is not abnormally advanced relative to height age, it is likely that the presenting symptom is an isolated and benign extreme variant of normal, which requires no treatment.^{671,829} To confirm the diagnosis of premature thelarche or premature adrenarche, the child must be similarly reevaluated after 3 to 6 months. If the results are still negative, the family can be reassured with a high degree of confidence that true puberty, including menses, will not occur until the usual age.

If more than one sign of precocious puberty is present or develops or if the growth is accelerated, a more extensive workup is indicated. For example, if a young girl with early breast development begins to grow pubic hair, or vice versa, then something more than premature thelarche or premature adrenarche is involved. The same is true if she develops a growth spurt or if she begins menstruating. These additional signs indicate the need for more extensive studies. Isolated vaginal bleeding, that is, bleeding in the absence of secondary sex characteristics, suggests sexual abuse, foreign body, or genital tract tumor, rarely isolated menarche. Cytology, anaerobic culture, pelvic ultrasound, and serum estradiol examinations are indicated.

Bone age advancement that is or becomes disproportionate to height (as indicated by compromised height potential or bone age $\geq 20\%$ greater than height age) suggests a sustained excess of sex hormone, and is an indication for a more extensive investigation to determine the cause of the precocity. An algorithmic approach to the differential diagnosis of premature pubarche is presented in Fig. 16.32. The importance of the recheck is illustrated by the case presented in Fig. 16.39.

One must be particularly aware that girls with neurogenic precocity, especially those who have had cranial irradiation, are at risk of paradoxically having concomitant GH deficiency.⁷⁰³ Coexistent GH deficiency masks the seriousness of the precocity: the growth rate is normal (not accelerated) and breast development is attenuated. However, the disparity between bone age and height age is extreme.

If the clinical picture and bone age are suggestive that premature breast development may be due to sex precocity, the laboratory investigation of premature pubertal development requires determinations of sex steroids, LH, and FSH by assays of high sensitivity: at least 10 pg/mL for estradiol, 10 ng/dL for testosterone, 5 mcg/dL for DHEAS, and 0.2 U/L for LH and FSH.⁷⁵⁹ The modern multichannel platform assays that are widely available generally meet these specifications for the assays of DHEAS, LH, and FSH, but they are totally inadequate for testosterone and estradiol. These require assays of high sensitivity and specificity, such as are provided by postchromatographic RIA or tandem mass spectrometry, in laboratories with well-established normal ranges for children.⁸³⁰ Prepubertal estradiol levels are normally less than 10 pg/mL, and prepubertal testosterone is less than 20 ng/dL. Measurement of serum thyroxine and prolactin is indicated if the sexual precocity is accompanied by growth arrest and/or galactorrhea.

In central precocity, daytime serum estradiol concentration is usually pubertal, 10 pg/mL (37 pmol/L) or more.⁸³¹ An estradiol level in the upper premenarcheal normal range (≥ 75 pg/mL) is atypical and necessitates a prompt workup to distinguish ovarian or adrenal tumor from true isosexual precocity.⁸³² If the estradiol level is atypically high, weekly determinations of estradiol may be helpful to determine

whether the level is fluctuating in the normal cyclic fashion of true precocious puberty. Because of the episodic and cyclic nature of sex hormone secretion, examination of the vaginal mucosa (see Fig. 16.28) for estrogen effect is a more sensitive indicator of the presence of early puberty than is an estradiol blood level, because it represents the integrated effect of estrogen over the preceding 2 to 3 weeks. Uterine size (e.g., uterine length >3.8 cm and endometrial thickness of ≥2 mm) has been used as an objective indicator of overall estrogenization.^{85,86} Androgen levels are appropriate for the stage of pubarche in true precocity.

Third-generation, monoclonal-antibody-based, "pediatric" immunoassays for gonadotropins are necessary for early detection and monitoring of therapy.⁷⁵⁹ Early morning basal LH greater than 0.3 to 0.6 U/L has been reported to be 62% to 95% sensitive and 92% to 100% specific for the diagnosis of central precocious puberty in girls.^{673,833,834} A post-GnRH peak LH greater than 6.9 U/L has been reported to be 92% sensitive and 100% specific,⁶⁷³ whereas a post-GnRH agonist peak LH over 4.0 to 5.0 U/L has been reported to be 90% or more accurate for the diagnosis of central precocious puberty.^{676,835,836}

A study of GnRH agonist-stimulated LH levels in healthy prepubertal girls found mean levels of 5.2 ± 4.0 and 2.9 ± 2.5 U/L in girls 0.8 to 3 years and 3 to 6 years, suggesting an LH cutoff level of 9 U/L and LH/FSH ratio of 0.4 in girls under 3 years.⁸³⁷ The diagnostic specificity of GnRH agonist testing is complicated by the overlap between the gonadotropin responses of prepubertal and pubertal girls. A study of healthy 6- to 13-year-old girls showed the 95th percentile for prepubertal girls to be 8.9 U/L and the 5th percentile for pubertal girls to be 2.8 U/L (Table 16.1).⁹³ In a subgroup of peripubertal 8- to 11-year-old girls, the most discriminating LH values were 3.2 U/L (85% specific) and 5.5 U/L (72% sensitive) 1 hour post-GnRH agonist. The GnRH agonist test also permits assessment of the ovarian gonadotropin-responsiveness: an estradiol peak of 34 pg/mL or more is approximately 90% sensitive and 60 pg/mL or more 95% specific for puberty (see Table 16.1).⁹³

FSH levels are not as helpful diagnostically because prepubertal values overlap considerably with pubertal ones and they may be elevated in premature thelarche. In response to GnRH or GnRH agonist testing, children with unsustained pseudopuberty as variants of normal will have a minimal gonadotropin response, whereas children with gonadotropin-independent precocity will have suppressed responses.^{838,839} Demonstration of a sleep-related rise of serum LH is an alternate diagnostic procedure. Box 16.3 summarizes laboratory criteria for the diagnosis of complete sexual precocity.⁸⁴⁰

Premature pubarche must be distinguished from hypertrichosis, the generalized excessive growth of vellus body hair that is prominent in nonsexual areas. The most common cause, by far, of premature pubarche is premature adrenarche. Premature adrenarche must be distinguished from virilizing disorders, the most common of which is virilizing nonclassic CAH and the most serious of which, although rare, is virilizing tumor.

Determination of the early morning baseline serum androgen pattern is useful in discriminating among the causes of premature pubarche and virilization. Premature adrenarche is characterized by a pubertal level of DHEAS, whereas serum testosterone and androstenedione are, at most, marginally elevated above the prepubertal range. A greatly elevated level of DHEAS suggests either adrenal tumor or the 3 β -HSD deficiency form of CAH. Androstenedione and 17-OHP levels are disproportionately elevated compared with testosterone or DHEAS levels in other forms of virilizing CAH and many ovarian tumors.

An ACTH stimulation test is the definitive test to exclude CAH. We advise performing an ACTH test in children with premature pubarche who have unusually high serum

BOX 16.3 Laboratory Criteria for Diagnosis of Complete Precocious Puberty in Girls

BONE AGE ADVANCEMENT

Bone age >height age >chronological age

Compromised predicted adult height

LH LEVEL PUBERTAL^a

Sleep-associated LH peak >1.0 U/L

LH (early morning) ≥ 0.6 U/L^b

Post-GnRH (1 h) agonist LH $\geq 3.2\text{--}5.5$ U/L^b

Suppressible by chronic GnRH agonist administration

SEX HORMONE LEVEL PUBERTAL

Estradiol (early morning, cyclically): >9 pg/mL^a

DHEAS normal for age or early puberty

DHEA, Dehydroepiandrosterone; GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone.

^aEssential criterion.

^bTypical values for sensitive assays. Exact values vary among laboratories.

androgens and bone age advancement and an early morning serum 17-OHP concentration above 170 ng/dL (5.1 nmol/L) (Fig. 16.32),⁸⁴¹ although levels above 1500 ng/dL do not require a confirmatory ACTH stimulation test. The diagnosis of CAH is discussed in detail in Chapter 14. The differential diagnosis of hyperandrogenic disorders is discussed further in the section Hyperandrogenism in Adolescence.

Ultrasonography is indicated to screen for abdominal or pelvic masses when feminizing or virilizing disorders are suspected.^{842,843} The ovaries of girls with true sexual precocity resemble those of normal pubertal girls.^{82,84,844} A "cyst" of 10 mm or more in diameter is usually caused by a transient preovulatory follicle. However, the differential diagnosis of a persistent cyst or multicystic ovaries includes McCune-Albright syndrome,⁸⁰⁷ tumor,⁸⁴⁵ and premature ovarian failure.⁸⁴⁶

The ability of ultrasonography to detect small adrenal neoplasms is highly dependent on the expertise of the ultrasonographer. On rare occasions, ultrasonography has been insensitive in detecting an ovarian tumor in adults.⁸⁴⁷ Computed tomography and MRI permit better visualization and more detailed assessment of tumors.

A systematic review that included 1853 subjects found that brain MRI identified a pathological finding in 9% of girls with central precocious puberty, with a much higher prevalence in those under 6 years of age (25%), compared with those that were 6 to 8 years of age (3%).⁸⁴⁸ The most common finding was a hypothalamic hamartoma in approximately 3.7%, whereas 1.3% of the subjects had another type of CNS tumor. Thus MRI of the hypothalamic-pituitary area is indicated in progressive central precocious puberty in those less than 6 years old or those at risk of organic causes by virtue of their underlying condition or neurological symptoms and signs,^{831,849} while the necessity of such imaging in girls presenting at 6 to 8 years of age without neurological indications is less certain. An argument for genetic testing of patients with CPP has been made, citing cost-effectiveness and safety in cases where MRIs with sedation are required.⁸⁵⁰

Management

The goals in management are to rule out an organic disorder that requires treatment in-and-of itself and to ascertain whether sexual precocity is either compromising height potential or resulting in important secondary emotional disturbances in the child.

The situation of a girl presenting with the onset of breast development or pubic hair between 6.0 and 8.0 years of age warrants special consideration. Breast development between 7.0 and 8.0 years of age is normal in blacks and Hispanics. Although presexual pubic hair (stage 2) may be seen in 6- to 8-year-old black and Hispanic girls, sexual pubic hair (stage 3) is abnormal if present before 8 years of age (see Table 16.3). However, pubertal development in girls in the 6.0- to 8.0-year age range may be associated with pathology, with excessive adiposity being the major consideration in most.²⁷⁶

Many 6.0- to 8.0-year-old girls with central precocious puberty, including whites, have slowly progressive precocity, with a normal timing of menarche, and are at low risk of short adult stature. Most such girls do not require GnRH agonist therapy to preserve adult stature.^{759,760,851,852} Thus a less comprehensive investigation may be warranted in selected girls presenting with thelarche or stage 2 pubic hair between 6 and 8 years of age. For most such girls, a complete history and physical examination, including obesity evaluation and a bone age determination, may be all that is needed, along with careful longitudinal follow-up to rule-out a disorder that requires therapy.^{760,852} However, 6- to 8-year-old girls with a suggestion of rapidly progressive or excessive androgenization or feminization, neurological symptoms, linear growth acceleration, or significant bone age advancement should be more completely evaluated, as outlined earlier.

Intracranial lesions must be treated by appropriate measures, such as neurosurgery or irradiation. Shunting for hydrocephalus may stop the precocity. Granulosa cell tumors confined to the ovary have a good prognosis for cure by unilateral oophorectomy. Recurrence of tumor may occur up to 20 years after the initial operation, however. Biopsy of the opposite ovary is indicated in unilateral ovarian neoplasms. Compensatory ovarian hypertrophy can be expected at any age after removal of a single ovary.⁸⁵³

The only permanent physical complication of true isosexual precocity, all else being normal, is short adult height. There is no increase in the risk of obesity, metabolic derangements (diabetes, hypertension, dyslipidemia), or cancer in adults with a history of central precocious puberty, whether they were or were not treated with GnRH agonist.⁸⁵⁴ Women with a history of central precocious puberty, whether treated or not, do have a higher rate of hirsutism and irregular menses, and women who were not treated for the sexual precocity may have decreased fertility and a higher need for assisted fertilization.⁷⁷⁶ However, fertility is normal in women who received treatment with GnRH agonist or with cyproterone acetate for central precocious puberty, with a spontaneous pregnancy rate that is no different than in controls.⁷⁷⁶ Pregnancy outcome is normal in both treated and untreated women with a history of central precocious puberty.⁷⁷⁶ With regard to height, excessive sex hormone production in the first decade of life causes early maturation of the epiphyses, resulting in their premature closure. About half the girls with this disorder reach an adult height of 53 to 59 inches and the remainder are over 60 inches tall.^{760,852} The mismatch between physical, hormonal, and psychological development may cause behavior changes ranging from social withdrawal to aggression or sexuality. However, frank behavioral problems are unusual in girls and so are by themselves seldom indications for treatment.

When central precocity is accompanied by documented progression of pubertal development that accelerates growth and compromises normal height potential, GnRH agonist treatment is indicated.⁷⁵⁹ Documentation typically requires 3 to 6 months' observation, although this may be unnecessary if puberty is substantially advanced clinically and hormonally on presentation. The downregulating effect of GnRH agonists

BOX 16.4 Indications for Gonadotropin-Releasing Hormone Agonist Therapy of Precocious Puberty

1. Documentation of central precocious puberty
2. Documentation of pubertal progression
3. Plus presence of one of the following:
 - Progressive compromise of predicted adult height or
 - Emotional or behavioral disturbance or
 - Menses in the emotionally immature or disabled

(Modified from Rosenfield, R.L. (1994). Selection of children with precocious puberty for treatment with gonadotropin releasing hormone analogs. *J Pediatr*, 124, 989–991.)

on pituitary gonadotropin release inhibits gonadotropin secretion within 1 month. Suggested criteria for the use of these drugs are presented in Box 16.4.^{759,840} The commonly used agents in the United States are leuprolide acetate (ordinarily given as Lupron Depot-Ped® 7.5–15 mg/mo or 11.25–30 mg/3 mo intramuscular [IM]),^{855,856} nafarelin acetate (Synarel® 800 mcg bid intranasal),⁸⁵⁷ a histrelin implant (Suprelor® LA 50 mg implant yearly),⁸⁵⁷ and triptorelin (Triptodur® 22.5 mg/24wk IM).⁸⁵⁸ Dosage can be adjusted later as necessary.

Treatment is adequate if the estradiol and baseline LH levels become prepubertal⁸⁵⁹ or LH is below 4.0 (1 hour) to 6.6 U/L (2 hours) after GnRH agonist^{856,860} 1 month after institution of therapy. Withdrawal menses may occur at that time, but none should be expected thereafter. Arrest of breast development and the pubertal growth spurt become apparent by 3 to 6 months. Concomitantly, epiphyseal closure is delayed and adult height potential is improved, because a type of catch-up growth occurs in which height age catches up to bone age. Adult height is greatest when treatment is started soon after onset at an early age, yielding an average height gain above pretreatment height prediction of about 1.4 cm for each year of therapy.⁷⁵⁹ Adult height prediction at the end of treatment tends to be overestimated from bone age. Therefore prolonging treatment beyond a chronological or bone age of 12.0 to 12.5 years of age generally leads to little further increase in adult height potential, regardless of the prediction of residual height potential. Menses occurs an average of 12 to 20 months after discontinuation of GnRH agonist treatment.^{861,862}

Coincident GH deficiency must be treated for optimal growth.⁸⁶³ GH-sufficient patients with central precocity who are started on treatment relatively late and whose height velocity falls below 4 cm/y after 2 to 3 years, appear to gain an average of 2 cm/y when GH therapy is added.^{864,865}

Use of the depot form of GnRH agonist is complicated by sterile abscesses at injection sites in about 5% of cases. The histrelin implant breaks on its removal over 20% of the time,⁸⁶⁶ necessitating care that the complete implant is removed. Anaphylaxis is a rare complication.⁸⁶⁷ No other serious side effects have come to light. During treatment, girls with central precocious puberty do not differ in their cognitive or psychosocial functioning compared with controls.⁸⁶⁸ Long-term safety data remain incomplete, but current studies following subjects into young adulthood are reassuring, including no difference in menstrual cycle characteristics or pregnancy outcomes of treated compared with untreated subjects⁸⁶⁹ and some evidence of improved fertility with GnRH agonist treatment compared with that in women with a history of untreated central precocious puberty.⁷⁷⁶ GnRH agonist treatment does not seem to cause

or aggravate obesity, as judged from BMI.^{759,854} Bone mineral density dips with the onset of treatment, but becomes normal after discontinuation of GnRH agonist therapy of precocious puberty.⁷⁵⁹

Girls with idiopathic slowly progressive puberty of onset between 6 and 8 years of age or with early fast puberty between 8 and 9 years of age tend to be tall at the onset of puberty, follow an advanced growth pattern, and reach their target height without GnRH agonist therapy.^{760,852,870–872} Therefore this treatment is only indicated if height potential is compromised or there are other compelling reasons to slow the pace of puberty.

Medroxyprogesterone acetate (Depo-Provera) is useful for stopping menses and as a contraceptive in girls with intellectual disability in whom preservation of height potential is not important. It is begun in a dosage commencing at 50 mg/month IM. Doses as high as 400 mg/month have been used, although cushingoid side effects may be observed at this level.⁸⁷³ Although this treatment reverses some of the physical changes of premature puberty, it does not reverse the inordinately rapid maturation of the skeleton, possibly because of its inherently weak androgenicity. In addition, use of medroxyprogesterone acetate is associated with a loss of bone mineral density, which must be considered if long-term use is being considered.⁸⁷⁴

A variety of drugs have been used off-label to treat gonadotropin-independent precocity. Both antiestrogen and aromatase inhibitor treatments have demonstrated partial efficacy for McCune-Albright syndrome.^{856,875–877} However, there should be a period of observation before initiating treatment for patients with McCune-Albright syndrome, as there can be marked variability in the clinical course, with some patients having extended periods of disease inactivity in whom treatment may not be indicated.⁸⁷⁷ There have been reports of surgical intervention in children with McCune-Albright syndrome, including resection of an ovarian cyst or ovariectomy. In many of these cases, however, there has been recurrence of precocious puberty symptoms.⁸⁷⁸ Therefore such treatment should only be considered in very select situations, such as a girl with a large ovarian cyst at risk of ovarian torsion. Ketoconazole, an anti-fungal agent that inhibits 17,20-lyase activity and other steroidogenic enzymes, may be useful.⁸⁷⁹ GnRH agonist treatment may be necessary for those in whom true puberty becomes superimposed because the bone age has reached a pubertal level.^{264,265,880} Bisphosphonates are often effective at relieving the bone pain of fibrous dysplasia in McCune-Albright syndrome, although they do not appear to have an effect on the course of the lesions and are not a suitable long-term treatment.⁸⁸¹

Patients with premature thelarche or pubarche as variations of normal are counseled as follows. The child's early development seems to be a matter of a normal stage of puberty occurring early. It is caused either by an incomplete, slow kind of puberty or by increased sensitivity to the trace levels of hormones that are normally present in childhood. Feminization with breast development and eventual menstruation can be expected to occur at an appropriate age. No treatment is indicated. To exclude subtle sex hormone excess or eventual anovulatory syndromes, long-term follow-up is advisable.

Besides dealing with the physical consequences of true iso-sexual precocity, the physician must be ready to help the family and child cope with the psychological problems that come with early physical maturation. The doctor can help the family by explaining that even though their child looks older and more mature than other children of the same age, the child will not behave more maturely. The libido of young children with precocity is not increased. The family should be advised to take some precautions to downplay their child's development, for example, in the choice of clothing and swimsuits. Early on,

children with these disorders tend to be withdrawn because they feel that they are different from their peers. Later on, they tend to enter into romantic relationships early. It is important to remind the family and child that in a few years, the child will not be unique from the standpoint of sexual development.⁷⁰³ The following books may be helpful in explaining precocious puberty: for children, *What's Happening to Me?*, by Peter Mayle (Lyle Stuart, Inc., Secaucus, NJ, 1973); for parents, *Sex Errors of the Body*, by John Money (Paul H Brookes Publishing Co., Inc, Baltimore, MD, ed. 2, 1994).

Hypogonadism

Causes

If hypogonadism is complete and present prepubertally, it causes sexual infantilism. In genetic males, congenital primary hypogonadism may also cause a completely female or ambiguous phenotype (see Chapter 6). If hypogonadism in girls is partial or of onset in the early teenage years, feminization will be of too limited a degree to permit the onset of menses at a normal age (primary amenorrhea). Milder, partial, or incomplete forms of hypogonadism may present in adolescence with abnormal uterine bleeding (Box 16.2). Consequently, disorders causing hypogonadism appear in the differential diagnosis of disorders of sexual differentiation, sexual infantilism, failure of pubertal progression, and menstrual irregularity. The causes of hypogonadism are listed in the differential diagnosis of amenorrhea in Box 16.5.

Primary Ovarian Failure. Primary ovarian failure is characterized by high levels of gonadotropins, particularly FSH. Two exceptions exist to this rule. First, the gonadotropins may not be elevated until CNS maturation has reached a pubertal stage, as indicated by a bone age of approximately 10 to 11 years (Table 16.5).⁸⁸² Secondly, patients with early or partial ovarian failure (primary ovarian insufficiency), as normally occurs during the menopausal transition, do not have high baseline gonadotropin levels.^{883,884} FSH may hyperrespond to GnRH and estrogen hyporespond to GnRH agonist challenge. It seems as if relatively few ovarian follicles—too few to permit the cyclic emergence of preovulatory follicles—suffice to prevent the characteristic rise in basal FSH levels. Serum AMH levels are a less sensitive indicator of ovarian failure than FSH but may be useful in prognosticating the potential for fertility.^{885,886}

Primary ovarian failure may occur before or during puberty, causing primary amenorrhea, or after puberty has occurred, causing secondary amenorrhea. The latter is termed *premature ovarian insufficiency* (POI; in its complete form termed *premature ovarian failure*) and clinically resembles premature menopause, except that about 25% of the cases sometimes resume ovarian function and there is a 4.4% spontaneous pregnancy rate.⁸⁸⁷

Gonadal dysgenesis caused by deficiency of genes on the X-chromosome is the most common cause of primary ovarian failure and POI. It is usually caused by a relatively large-scale deletion of X-chromosomal material, which is associated with a characteristic, but variable, phenotype and is termed *Turner syndrome* (see Chapter 17). Fetuses with a 45,X karyotype have a normal number of oocytes in the ovary at midgestation, but a drastic reduction in the number of follicles,⁴¹ which appears to cause gonadal streaks via an accelerated rate of apoptosis. However, the gonadal dysgenesis, like other features of the syndrome, is often incompletely expressed.^{888,889} Thus Turner syndrome should be considered in all girls with primary hypogonadism or secondary amenorrhea whether or not they have the typical stigmata of Turner syndrome.

BOX 16.5 Differential Diagnosis of Amenorrhea: Structural and Anovulatory Disorders**ABNORMAL GENITAL STRUCTURE**

- Ambiguous genitalia
 - Disorders of sex development
 - Pseudointersex
- Aplasia^a
 - Hymenal
 - Müllerian
 - Disorders of sex development
- Endometrial adhesions

ANOVULATORY DISORDERS**Hypoestrogenism, FSH Elevated**

- Primary ovarian failure
 - Congenital
 - Gonadal dysgenesis
 - Chromosomal
 - Genetic
 - Other genetic disorders
 - Acquired
 - Oophorectomy
 - Radiotherapy or chemotherapy
 - Ophoritis
 - Idiopathic

Hypoestrogenism, FSH not Elevated

- Primary ovarian insufficiency
 - Complete if bone age <11 years^a
 - Incomplete if bone age >11 years

FSH, Follicle-stimulating hormone.

^aCause only primary amenorrhea.

- Delayed puberty
 - Constitutional delay of growth and puberty^a
 - Growth-retarding disease
- Gonadotropin deficiency
 - Congenital
 - Acquired
 - Organic
 - Functional
 - Virilization

Estrogenized

- Hypothalamic anovulation
 - Functional hypothalamic amenorrhea
 - Athletic amenorrhea
 - Psychogenic amenorrhea
 - Epilepsy
- Nonhypothalamic nonovarian disorders
 - Pregnancy
 - Obesity or undernutrition
 - Chronic disease
 - Cushing syndrome
 - Hypothyroidism
 - Drug abuse
 - Hyperprolactinemia
 - Postpill amenorrhea
- Hyperandrogenism
 - Polycystic ovary syndrome
 - Other hyperandrogenic disorders

TABLE 16.5 Bone Age in Workup of Sexually Infantile Girls With Normal Follicle-Stimulating Hormone Level

	Bone Age (Years)		
	<11	11–13	>13
Primary hypogonadism	Yes		
Delayed puberty	Yes	Yes	
Gonadotropin deficiency	Yes	Yes	Yes

(From Rosenfield, R.L., Barnes, R.B. (1993). Menstrual disorders in adolescence. *Endocrinol Metab Clin North Am*, 22, 491.)

Specific loci on the X-chromosome are associated with primary ovarian failure. Xp11.2 harbors BMP15, a specific ovarian differentiation factor, heterozygous mutation of which is a rare cause of gonadal dysgenesis. Xq harbors two independent loci, in addition to the fragile X premutation, that are associated with about 5% of sporadic and 14% of familial POI.⁸⁹⁰ The premutation is an expansion of CGG repeats in the fragile X mental retardation 1 (*FMR1*) gene that is insufficiently long to cause fragile X syndrome. Women with the premutation allele have a substantially increased risk of POI, possibly because raised intracellular mRNA concentrations might sequester CGG binding proteins that are important for RNA processing.

Gonadal dysgenesis also results from 46, XY complete gonadal dysgenesis and certain forms of autosomal aneuploidy.^{600,890,891} A rare cause is ER β (ESR2) receptor inactivating mutation. A variable degree of ovarian dysgenesis occurs in trisomy 21; delayed menarche, anovulatory cycles, and primary gonadal failure are occasionally seen.⁸⁹² However, pregnancy

has been reported; trisomic offspring are common.⁸⁹³ Oocytes are virtually absent in trisomies 13 and 18. Ovarian dysgenesis also occasionally occurs as part of the Denys-Drash syndrome caused by a *WT-1* mutation.⁸⁹⁴ Gonadal dysgenesis also occurs in DNA-repair disorders that impair meiosis, such as ataxiatelangiectasia and Fanconi syndrome.^{895,896} Other autosomal genetic disorders causing premature ovarian failure include inactivating mutations of *FOXL2*, which are found in sporadic cases, as well as in the autosomal dominant type 1 blepharophimosis syndrome.⁸⁹⁷ POI also occurs in galactosemia,⁸⁹⁸ leukodystrophies, and myotonia dystrophica.^{899,900} Mutation of the inhibin alpha-subunit predisposes to POI, an effect that appears to vary depending on ethnicity; this is postulated to be caused by deficient paracrine interactions with TGF β -family receptors.⁹⁰¹ Mutations in other autosomal genes that have been associated with POI include: *NOBOX*, *FSHR* (encoding the FSH receptor), and *TRIM37* (mutations of which cause Mulibrey nanism disorder).⁹⁰² Mutations in *NR5A1*, encoding the transcription factor SF-1, can result in 46, XY gonadal dysgenesis or POI, as well as being a cause of adrenal insufficiency because of impaired adrenal gland development.⁹⁰³ Variants in additional genes that have been identified in patients with POI include *PGRMC1* at Xq22-q24 (coding for a putative progesterone-binding membrane receptor) and the autosomal genes *SPIDR*, *GDF9*, *FIGLA*, *NANOS3*, *SYCE1*, *MCM8/9*, and *HFMI*.⁹⁰⁴ There are a number of other candidate genes with less clear evidence of causality,⁹⁰⁴ and mutations incriminated in the ovarian failure of mouse models⁹⁰⁵ or in genes involved in follicle development²⁶ may also be identified in the future as causes of human primary ovarian failure.

Injury to the ovary is a common cause of primary ovarian failure. Mumps oophoritis is a classic but rare cause of ovarian

failure. Very high-dose estrogen treatment in adolescence increases the risk of hypergonadotropic subfertility.⁸⁸⁶

Irradiation and chemotherapy for childhood neoplasia are frequent causes of primary ovarian failure now that life is effectively prolonged.^{890,906–909} Such treatments can cause either acute ovarian failure or a decreased follicular reserve. Acute ovarian failure results in a lack of pubertal development when it occurs prepubertally, or in arrested pubertal development or secondary amenorrhea when it occurs in pubertal or postpubertal girls. Decreased follicular reserve results in premature ovarian failure.⁹¹⁰ Ionizing radiation and alkylating agents damage DNA whether or not a cell is replicating.⁹¹¹ Therefore nonreplicating primordial follicles are not spared from these agents, although prepubertal ovaries may be less sensitive to damage than pubertal or postpubertal ovaries.^{910,912} The damage is dose related and the damage is worse with combined irradiation and chemotherapy compared with treatments with just one of these modalities. A radiation dose of 15 Gy or more causes acute ovarian failure in over 80% of prepubertal girls, whereas a dose more than 10 Gy will cause equivalent damage in postpubertal girls.⁹¹³ However, doses as low as 2 Gy will also impact long-term ovarian function by depleting the follicular pool by as much as 50%.⁹¹⁴ A cumulative cyclophosphamide dose⁹¹⁵ over 7.5 g/m² will cause acute ovarian failure in over 80% of females younger than 20 years.⁹¹³ Green et al. developed a method to quantitate a “cyclophosphamide equivalent dose (CED)” for other alkylating agents.⁹¹⁵ After prepubertal cancer treatment, 94% of girls can be anticipated to enter puberty and menstruate regularly, but 8% of these will develop nonsurgical premature menopause because of the reduced number of oocytes.⁹⁰⁸ The risk for premature menopause is 30% in those who have received both radiation and chemotherapy, 5% to 13% for those receiving either alone. Some with early hypergonadotropinism will experience ovarian recovery with normal pituitary-ovarian function after several years,⁹¹⁶ but infertility occurs. Several nonalkylating chemotherapies are also gonadotoxic (including platinum agents and anthracyclines), while others (such as methotrexate, fluorouracil, and vincristine) have very low or no gonadotoxic risk.^{917–920} However, data are scarce, and interactions among the various classes of chemotherapeutic agents is poorly understood. Radiation treatment for malignancy can also lead to infertility by causing hypogonadotropic hypogonadism, or through injury to the uterus. As a consequence of gonadotropin elevation when gonadal failure begins, puberty may progress rapidly.⁷⁷⁴

Sterilization by irradiation can be obviated by transposing the ovaries out of the irradiation field if possible. The evidence that the prepubertal ovary appears less sensitive to injury from irradiation and chemotherapy has suggested that GnRH agonist therapy might protect ovarian function in pubertal/postpubertal girls receiving such treatment. While there are some conflicting data, there is no clear benefit to such treatment.⁹¹² Imitinab (Gleevec[®]) is a potential oocyte-protective treatment, as it blocks an apoptotic pathway activated by radiation and cisplatin in mouse oocytes.⁹²¹

Gonadotropin resistance (Savage syndrome) can arise from autosomal recessive loss of function mutations of the LH or FSH receptor.^{917–919} The reported cases have had some degree of pubertal development followed by primary or secondary amenorrhea or oligomenorrhea. The ovaries of LH receptor mutants contain follicles in all stages of development, while those of FSH receptor mutants vary from hypoplastic to normal size, with antral follicle development varying from nil to 5 mm. Partial gonadotropin resistance is common in the Albright osteodystrophy form of pseudohypoparathyroidism, as part of the generalized defect in G-protein signal transduction.⁹²⁰

Autoimmune oophoritis is the basis of approximately half of spontaneous premature ovarian failure, although estimates vary from 5% to 85% in various series.⁹²² It is diagnosed by

its association with any of a variety of autoimmune endocrine or nonendocrine disorders, manifest or subclinical, that have in common defects in T cell suppressor function. Autoimmunity may be directed against the granulosa cell, oocyte, or theca cell. The clinical picture may resemble relatively selective resistance to FSH or, less frequently, to LH. The latter results from lymphocytic destruction of theca cells with sparing of granulosa cells from small antral follicles, which lack substrate to form estradiol and can only respond to the compensatory increase of FSH by producing inhibin-B.⁹²³ These patients have autoantibodies to steroidogenic cells and are at risk for adrenal failure. Usually these antibodies are directed against 21-hydroxylase, less frequently to side chain cleavage enzyme or 17-hydroxylase, seldom to 3 β -HSD. Replacement glucocorticoid therapy may temporarily ameliorate the immune oophoritis in such cases.⁹²⁴ A case with autoantibodies to testosterone has been reported.⁹²⁵ AIRE gene mutations have been identified as causative of type 1 polyendocrine failure. Ultrasonographic and histological findings are variable in premature ovarian failure and include large or small ovaries, inactive or polyfollicular ovaries, loss or preservation of primordial follicles, and infiltration by lymphocytes or plasma cells.⁸⁴⁶

Functional ovarian failure can also result from specific autosomal recessive defects in the biosynthesis of sex steroids. Both androgen and estrogen deficiency occur in lipoid CAH (Star and side chain cleavage mutations, see Fig. 16.22), 17 α -hydroxylase deficiency, P450 oxidoreductase deficiency, and 17,20-lyase deficiency.^{926,927} Thus affected genetic males may present with a female phenotype. Hypoestrogenism is associated with virilization in aromatase and 3 β -HSD deficiency. Aromatase is unique among the ovarian steroidogenic defects in not being associated with CAH. Congenital lipoid adrenal hyperplasia is unique in that underlying Star deficiency has too little direct impact on ovarian function to interfere with the early phases of puberty, but the gradual buildup of intraovarian lipid deposits resulting from enzyme deficiency—a “second hit”—causes ovarian damage with anovulation and late ovarian failure. SF-1 (NR5A1) deficiency can cause primary ovarian failure in the absence of adrenal insufficiency.⁹⁰³

Estrogen resistance caused by inactivating mutation of ER α has been reported.^{928,929} Hypoestrogenism and hyperestrogenemia were profound, the ovaries were enlarged and multicystic, and gonadotropin and testosterone levels were marginally to clearly elevated.

Gonadotropin Deficiency (Hypogonadotropic Hypogonadism). Congenital gonadotropin deficiency can occur in association with cerebral, hypothalamic, or pituitary dysfunction as an isolated defect.⁹³⁰ Congenital defects in hypothalamic-hypophyseal formation may be associated with midline facial defects. Congenital hypothalamic dysfunction may be associated with other neurological or endocrine dysfunction, such as in the Prader-Willi syndrome (congenital hypotonia, and neonatal failure to thrive followed by hypothalamic obesity, sometimes with hypopituitarism)⁹³¹ or the Laurence-Moon-Biedl syndrome (retinitis pigmentosa, obesity, mental deficiency).

Congenital hypogonadotropic hypogonadism often results from mutations (Table 16.6).^{212–214,220,222,226,235,719,932–942} The autosomal recessive forms of congenital combined pituitary hormone deficiency caused by PROP1, HESX1, LHX3, and OTX-2 mutations are associated with gonadotropin deficiency. Leptin and leptin receptor inactivating mutations cause gonadotropin deficiency in combination with moderate or extreme obesity.^{941,943} The report of a natural pregnancy in a woman with a homozygous mutation in the leptin receptor lends controversy to the current concept that leptin function is essential for reproduction.⁹⁴⁴

Gonadotropin deficiency may be associated with anosmia (olfactory-genital dysplasia or Kallmann syndrome).²¹⁴

TABLE 16.6 Gene mutations causing congenital hypogonadotropic hypopituitarism

Gene Mutation	Disorder
<i>CCDC141, CHD1, DUSP6, FGF8 and 17, FEZF1, FGFR1, FLRT3, IGF510, IL17RD, KAL1, HS6ST1, KLB, PROK2, PROKR2, SEMA3A and 3E, SMCHD1, SPRY4, WDR11</i>	Disrupted migration of gonadotropin-releasing hormone (GnRH) neurons (Kallmann syndrome)
<i>DAX1 (NR0B1), HESX-1, LHX3, NR5A1, PROP-1, SOX2</i>	Abnormal development of the hypothalamus and pituitary
<i>LEP, LEPR, PC1</i>	Associated with obesity
<i>GNRH1, KISS1, KISS1R, TAC3, TACR3</i>	Abnormal GnRH pulsatility
<i>GNRHR, FSHB, LHB</i>	Abnormal gonadotropes
<i>DMXL2</i>	Associated with type 1 diabetes mellitus, central hypothyroidism, developmental delay, peripheral neuropathy
<i>OTUD4, PNPLA6, RNF216, STUB1</i>	Gordon Holmes syndrome (cerebellar ataxia, retinal dystrophy)
<i>POLR3A and 3B</i>	4H syndrome (hypomyelination, hypodontia, hypogonadotropic hypogonadism)
<i>RAB18, 3GAP1 and 3GAP2</i>	Warburg Micro syndrome (microcephaly, developmental delay, microcornea, optic atrophy)

This syndrome is 1/10th as frequent in females as in males. Mutations in the *KAL-1* gene in the pseudoautosomal region of the X-chromosome, which encodes anosmin, a key protein for GnRH neuronal migration, cause the highly penetrant X-linked form and rarely affect females. Inactivating mutations of other genes in the anosmin signaling pathway (*FGF8/FGFR1, PROK2/R2, NELF*, and *CHD7*) account for the vast majority of female cases; these are inherited as autosomal dominant or recessive (heterozygous, compound heterozygous, or digenic) traits with variable penetrance.^{214,719,933,934,945} Neurological and somatic abnormalities, such as synkinesia, cerebellar ataxia, sensorineural deafness, mental retardation, unilateral renal agenesis, and cleft palate, are variably associated genetic features of Kallmann syndrome. Rare affected individuals have only delayed puberty. These same mutations sometimes are responsible for normosmic idiopathic GnRH deficiency; *CHD7* mutations typically have features of CHARGE syndrome. Research is increasingly revealing new elements in the GnRH developmental and signaling pathway. For example, a single-nucleotide polymorphism in the *EAP1* gene has been associated with amenorrhea/oligomenorrhea in primates.⁹⁴⁶

GnRH receptor mutations account for about half of autosomal recessively inherited cases of isolated, normosmic gonadotropin deficiency.⁹³⁵ The degree of hypogonadism is variable, even within a family, with delayed puberty and delayed menarche as presentations.^{936,937,947}

Loss-of-function mutations of GnRH⁹⁴⁸ and in signaling systems that modulate GnRH release (*KISS/KISS1R, NKB/TAC3R*) are rare causes.^{222,235} The hypogonadism of most subjects with neurokinin B/TAC3R mutations is reversed by sex steroid therapy, which suggests that this signaling system is important for the start-up of puberty, but not its maintenance. Isolated hypogonadotropic hypogonadism also has been reported in a woman homozygous for a nonsense mutation of the X-linked *orphan nuclear receptor DAX1* gene, which was associated with X-linked adrenal hypoplasia congenita in her brothers.⁹⁴⁹

Isolated FSH deficiency caused by mutation in the β-subunit has been reported to cause primary amenorrhea in association with a unique test panel—low FSH, elevated LH, and low

testosterone levels.^{139,919} Carbohydrate-deficient glycoprotein syndrome (phosphomannomutase deficiency) is characterized by high levels of immunoreactive, but bioinactive gonadotropins, mimicking primary ovarian insufficiency.⁹⁵⁰ Isolated LH deficiency because of *LHβ* gene inactivating point mutations has been reported to lead to secondary amenorrhea following normal pubertal development, but undetectable LH, high FSH, low estradiol, and macrocystic ovaries.⁹⁵¹ However, some inactivating LH mutations in men have high or low immunoreactive LH levels.⁹¹⁹ Mutations in genes responsible for anterior pituitary development may cause gonadotropin deficiency resulting in pubertal delay or CHH. LIM Homeobox 3 (*LHX3*), SRY-Box 2 (*SOX2*), and HESX homeobox 1 (*HESX1*) are responsible for early forebrain patterning and pituitary cell development.⁹⁵² Paired like homeodomain factor 1 (*PROP1*) is important for the development of somatotrophs, lactotrophs, thyrotrophs, and gonadotrophs⁹⁵³ and patients with *PROP1* mutations have variable phenotypes ranging from CDGP to CHH.⁹⁵² Gonadotropin deficiency may also be associated with other conditions, particularly with neurological phenotypes. Mutations in RNA polymerase III subunit A and B (*POLR3A/B*) result in the 4H syndrome (hypomyelination, hypodontia, and hypogonadotropic hypogonadism).⁹⁵⁴ Ring finger protein 216 (*RNF216*), OTU deubiquitinase 4 (*OTUD4*) and Patatin-like phospholipase domain containing 6 (*PNPLA6*) CHH,^{955,956} and ataxia (also known as *Gordon-Holmes syndrome*).^{955,956} DMX like 2 (*DMXL2*) mutations are associated with CHH, other endocrine deficiencies, and polyneuropathies.⁹⁵⁷ Mutations in RAB3 GTPase activating protein catalytic subunit 1 (*RAB3GAP1*) result in dysregulation of the RAB3 cycle, leading to Warburg Micro syndrome with ocular, neurodevelopmental, and central reproductive defects.^{958,959}

Acquired gonadotropin deficiency can be a consequence of tumors, trauma, autoimmune hypophysitis,^{960,961} degenerative disorders involving the hypothalamus and pituitary,⁹⁶² irradiation,⁹⁶³ chemotherapy,⁹⁶⁴ or chronic illness.⁹⁶⁵ Hypogonadotropic hypogonadism will develop in about one-third of those receiving 20 to 30 Gy cranial irradiation, whereas it is typical in those receiving >50 Gy.^{762,966} Pituitary adenoma, craniopharyngioma, and dyserminoma are the most common neuroendocrine neoplasms responsible for hypopituitarism in children. Most "nonfunctioning" pituitary adenomas are gonadotrope adenomas, which secrete gonadotropin subunits in response to TRH.⁹⁶⁷ A case of hypothalamic tumor is presented with Fig. 16.41. Pinealomas most commonly cause sexual infantilism. They may act by secreting an inhibitory substance,⁷⁶⁹ rather than by compressing key areas of the hypothalamus.

Anorexia nervosa is the prototypic form of eating disorders, and is a common cause of hypogonadotropic hypopituitarism in teenagers. It is a syndrome of undernutrition because of voluntary starvation with a particular psychological dysfunction that results in amenorrhea.^{968–970} These patients uniformly consider themselves too fat in the face of objective evidence that they are underweight. The psychiatric criteria that distinguish this disorder from food faddism and fear of obesity consist of: (1) restriction of energy intake relative to requirements, leading to a significantly low body weight; (2) intense fear of gaining weight or of becoming fat, or persistent behavior that interferes with weight gain, even though at a significantly low weight; and (3) disturbance in the way in which one's body weight or shape is experienced, undue influence of body weight or shape on self-evaluation, or persistent lack of recognition of the seriousness of the current low body weight. A 10th percentile BMI should be the initial maintenance goal.⁹⁷¹

Bulimia nervosa, the binge-eating/purging variant eating disorder, is similar in the overvaluation of body shape and weight and the use of extreme weight control behaviors. Physical activity tends to be high. These disorders may be manifest at an early stage as atypical eating disorders, before weight or

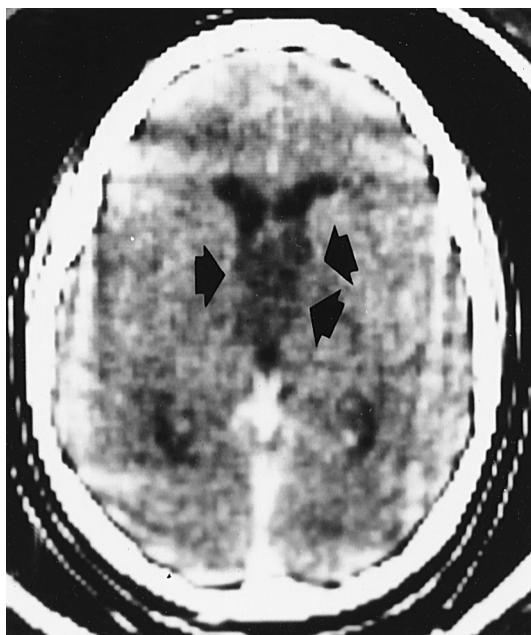


Fig. 16.41 Computed tomography of the brain of a 16-year-old girl with hypothalamic astrocytoma. The low-density tumor mass (arrows) extends superiorly from the hypothalamus, obliterates the third ventricle, and partially compresses the frontal horns of the lateral ventricles (particularly the right). This patient presented with secondary amenorrhea. Menarche had occurred at age 13 years, and menses were normal until 15.3 years. The patient then became amenorrheic in association with lethargy, episodic headaches, polyuria, and weight gain—despite little change in appetite. Physical examination was negative. The skull radiograph, electroencephalogram, visual fields, and serum prolactin and thyroxine levels were normal—and urine-specific gravity was 1.016. After biopsy of the cyst wall, studies revealed her to have gonadotropin, growth hormone, and partial antidiuretic hormone deficiencies.

amenorrhea criteria are met or when the binge is subjective. The cognitive defect that weight can serve as the predominant value in judging self-worth is central to anorexia nervosa. In contrast to other depressive individuals, these patients are generally content with themselves in the areas of intellectual and vocational achievement.

The cause is multifactorial. It involves a genetic predisposition. Concordance rates for the anorexic type are about 50% for monozygous twins, compared with about 5% for dizygotic twins. Many other risk factors have been implicated. Familial factors also include eating disorders of any type, depression, substance abuse, and adverse family interactions. Premorbid experiences, such as sexual abuse or social pressures, or premorbid characteristics, such as low self-esteem, compulsiveness, and perfectionism, are also important. Dieting meets a need for approval in our culture with its emphasis on dietary restriction and thinness as goals for women. Anorexia is often precipitated in vulnerable children by a new experience, such as puberty, leaving home or beginning college, or by adverse life events. The disorder is perpetuated by the complications of starvation, such as depression and reduced gastric emptying.

The onset tends to be at 12 years of age or later. Earlier onset is associated with growth arrest, delay of puberty, and primary amenorrhea.⁹⁷² Dysfunction of the hypothalamic-pituitary axis in anorexia nervosa includes not only hypogonadotropic hypogonadism, but GH deficiency^{973,974} and hypercortisolism;

GH resistance and nonthyroidal illness also occur as consequences of malnutrition.⁹⁷⁵

The medical complications of anorexia nervosa are serious. The risk of death is approximately 10-fold increased: electrolyte imbalance, hypoglycemia, cardiovascular instability, bone marrow hypocellularity predisposing to silent infection, and renal failure account for about half of the mortality, suicide for the rest.

The weight changes leading to cessation or restoration of menstrual cycles are in the range of 10% to 15% of body weight. Recovery is associated with achieving a critical level of body fat stores above the 10th percentile (over approximately 20% body fat) (see Fig. 16.42), at a BMI approximately 20, that is, midnormal.^{976,977} There is an inverse relationship between body weight and the maturity of gonadotropin release in these patients. The 24-hour pattern of gonadotropin release tends to be immature (prepubertal or pubertal), and the diurnal LH pattern becomes mature upon recovery from undernutrition.⁹⁷⁸ LH pulsatility is low and may be restored by opiate antagonists.⁹⁷⁹ The gonadotropin response to GnRH and ovulatory response to clomiphene citrate are blunted in the malnourished state and become normal with weight gain to about 80% of ideal.^{979,980} Leptin levels are significantly decreased and are a major contributor to both the gonadotropin deficiency and to changes in the thyroid and GH axes.⁹⁸¹

Mild hypercortisolism is frequent and may contribute to the anovulation by mechanisms discussed further under Hypothalamic Anovulation.⁹⁸² Afternoon ACTH and cortisol levels are significantly higher and the response to CRH is significantly lower than normal. In contrast to Cushing syndrome, DHEAS levels tend to be blunted as a consequence of undernutrition.⁹⁸³

A fundamental neuropsychological flaw or hypothalamic disturbance⁹⁸⁴ has been suspected because some patients become amenorrheic before losing weight, and about half of the cases remain amenorrheic after treatment. The serotonergic systems implicated in the regulation of feeding and mood seem to remain altered even after weight restoration. Evidence exists for marked individual differences in reactivity of the neuroendocrine system to stress.⁹⁸⁵ The authors favor the concept that psychological problems lead to amenorrhea only in women who are predisposed by a unique preexisting hypothalamic dysfunction.

A number of features attributed to hypothalamic dysfunction, such as cold intolerance, may be caused by the subtle hypothyroid state that is secondary to the malnutrition.⁹⁸² Serum triiodothyronine levels are consistently low, serum thyroxine levels tend to be lower than average (although usually within normal limits), the pattern of TSH release indicates TRH deficiency, and the state of deep tendon reflexes and metabolism is consistent with hypothyroidism. Hypothyroidism may in part complicate the GH resistance of malnutrition that occurs as a consequence of the interference with IGF-1 generation: low IGF-1 initiates GH excess, compensatory somatostatin release, and subsequent inhibition of the thyrotropin response to TRH. Undernutrition also diverts the generation of thyroxine metabolites away from triiodothyronine toward reverse triiodothyronine.

Hyperprolactinemia is a potentially reversible cause of gonadotropin deficiency.⁹⁸⁶ Galactorrhea is present in about half of the patients, particularly those with residual estrogen production. The causes of hyperprolactinemia are diverse, including hypothalamic or pituitary disorders, drugs, hypothyroidism, renal or liver failure, peripheral neuropathy, stress, autoimmune, macroprolactinemia, genetic, and idiopathic.⁹⁸⁷⁻⁹⁹⁰ Elevated serum prolactin levels occur with a variety of tumors that cause functional or anatomic pituitary stalk section, thereby preventing dopaminergic inhibitory pituitary control. About

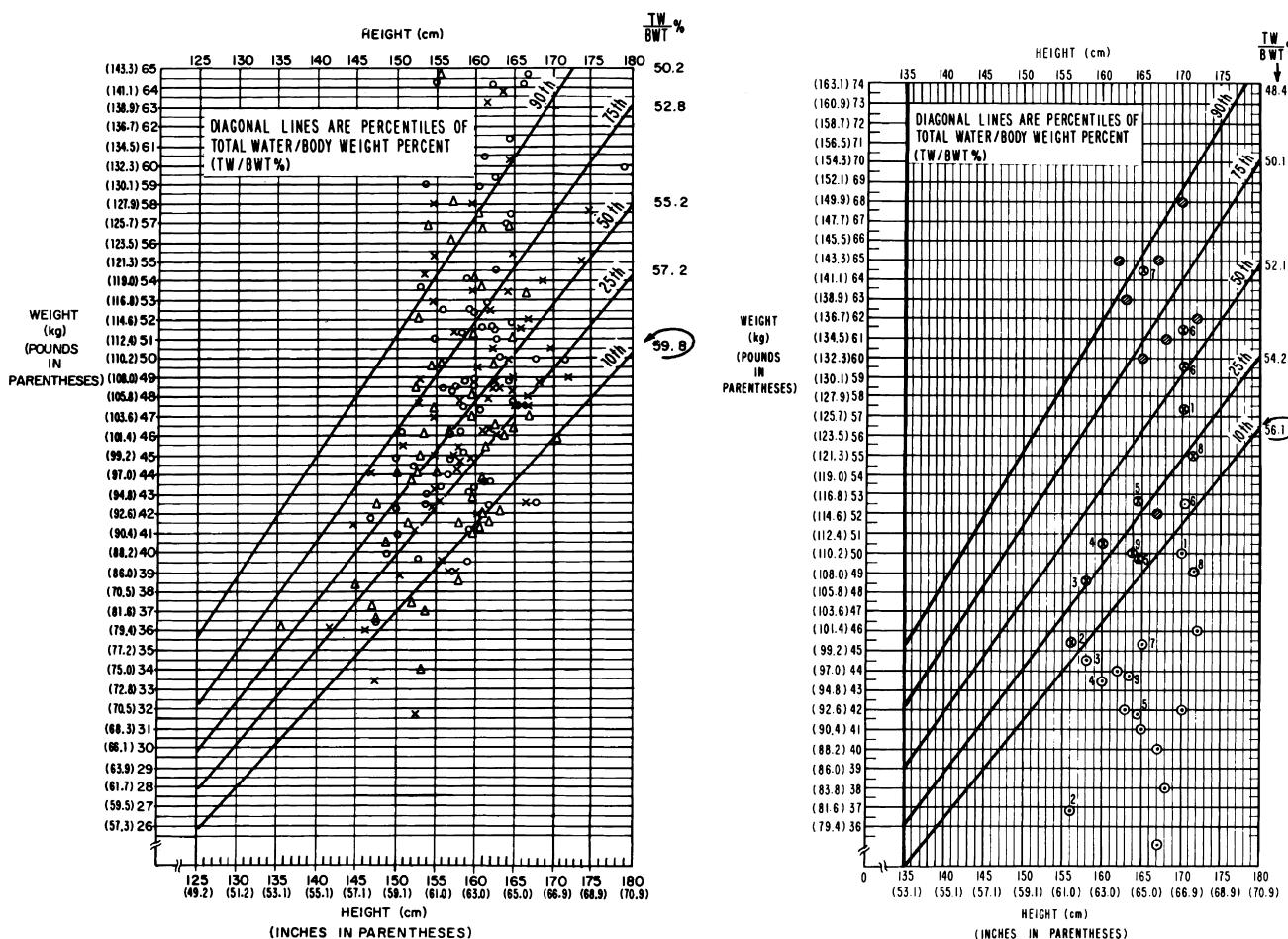


Fig. 16.42 Percentiles of fatness (diagonal lines) for white girls at menarche (left) and after menarche (right) equated with computed percentiles of total water as a percentage of total body weight. The minimal weight necessary at a particular height for the onset or maintenance of menses is very close to the 10th percentile of fatness on these respective charts. Data for anorexia nervosa cases are shown on the right-hand chart: • at presentation; x at resumption of menses. (From Frisch, R.E., McArthur, J.W. (1974). Menstrual cycles: Fatness as a determinant of minimum weight for height necessary for their maintenance or onset. *Science*, 185, 949. Copyright © by the American Association for the Advancement of Science.)

one-third of hyperprolactinemic women have an identifiable pituitary adenoma. Prolactinomas less than 1 cm in diameter (microadenomas) cause no problems by local extension. Prolactinoma may be associated with multiple endocrine neoplasia type 1.⁹⁹¹ In about a quarter of adult hyperprolactinemia, the malfunction is caused by the ingestion of drugs, such as phenothiazines, estrogen, or cocaine.⁹⁹² Considerable hyperprolactinemia is idiopathic: decreased sensitivity to dopaminergic inhibition may underlie such cases.⁹⁹³

Macroprolactinemia is caused by a variant molecule or auto-antibody formation comprised of prolactin and plasma proteins, most often caused by immunoglobulin (Ig)G prolactin-autoantibodies.⁹⁹⁴ In this situation, direct immunoassay indicates elevated levels of prolactin. However, the biologically available or active prolactin level is normal; thus there is no physiological consequence to the macroprolactinemia.

Hyperprolactinemia results in LH pulses that tend to be infrequent and LH secretion that is variable in response to GnRH.⁹⁹⁵ Selective prolactin excess causes variable degrees of gonadotropin deficiency, ranging from severe to partial (hypothalamic amenorrhea). Adrenal hyperandrogenism, hirsutism, and seborrhea are common.³⁶⁷

Frank virilization as a result of very high androgen levels suppresses gonadotropin levels and so causes defeminization.

However, the moderately hyperandrogenic disorders discussed later, which are more common, are associated with normal estrogenization.

Differential Diagnosis

The differential diagnosis of hypogonadism is included in Box 16.5. Investigation should begin for hypogonadism when puberty is delayed or does not progress normally. Delayed puberty is indicated by lack of thelarche by the chronological or bone age of 13 years. Abnormal progression of puberty is suggested by failure of menses to occur within 3.0 years of the onset of thelarche or if secondary amenorrhea or oligomenorrhea has persisted for 1 year. As discussed earlier, normal menstrual frequency varies with the time after menarche, but an interval persistently greater than normal (Box 16.2) should prompt an evaluation.⁹⁹⁶ A family history of delayed puberty is compatible with the delay being constitutional rather than having an organic basis. The history should include a thorough past medical history and systems review, including intracranial, visual, olfactory, emotional, abdominal, or pelvic symptoms and systemic symptoms that might indicate chronic endocrine, metabolic, or systemic disorders that delay puberty. Upon examining the patient, the height and weight should be

carefully measured and growth rate and appropriateness of weight for height determined (see Fig. 16.42). Careful categorization of the stage of breast and sexual hair development are essential. Inspection of the external genitalia is indicated, but an internal pelvic examination seldom is necessary for diagnosis.⁹⁹⁷ Examination of the mature breast should include an attempt to express milk from the ducts to the nipple. The finding of a structural genital abnormality may indicate that amenorrhea is caused by abnormal genital tract development, while clitoromegaly⁹⁹⁸ is a clue to a virilizing disorder. Neurological examination should include evaluation of eye movements, visual fields, and optic fundi, as well as a search for anosmia and midline defects.

If a disorder or syndrome associated with hypogonadism is recognized in the newborn period, advantage may be taken of the mini-puberty of the newborn to attempt to make a diagnosis during the first few months of life. For example, the hypergonadotropic hypogonadism of Turner syndrome and the hypogonadotropic hypogonadism of hypopituitarism may be documented during this critical period before the physiological restraint of puberty during late infancy and childhood makes it difficult to do so before the normal age of puberty.^{999–1002}

An algorithmic approach to the workup of patients with menstrual disorders is shown in Figs. 16.43 to 16.45.¹⁰⁰³ The laboratory workup depends on the degree of estrogenization, as initially assessed from the stage of breast development: it includes a bone age radiograph in adolescents who are not sexually mature and generally begins with a chronic disease panel, and determination of gonadotropins, estradiol, and testosterone level. A pregnancy test is indicated in a sexually mature adolescent. The diagnostic considerations differ in the anovulatory girl without FSH elevation, depending upon whether she is hypoestrogenic or estrogenized (see Box 16.5 and see Figs. 16.43 and 16.44).

FSH elevation indicates primary ovarian failure (see Figs. 16.43–16.45). Chromosome abnormalities are ordinarily the first consideration because the most common cause is Turner syndrome and its variants. Those individuals with primary ovarian failure that is not caused by Turner syndrome and its variants should be investigated for the fragile X premutation, autoimmune oophoritis, and steroidogenic defects.

Lack of FSH elevation in a prepubertal patient does not rule out primary ovarian failure if bone age is below 11 years because neuroendocrine puberty may not have occurred; in this situation, primary ovarian failure is not hypergonadotropic (see Table 16.5).⁸⁸² If FSH is not elevated and bone age has reached 11 years, in a prepubertal girl without a growth-attenuating or retarding disorder, one is dealing either with constitutional delay of puberty or isolated gonadotropin deficiency (see Fig. 16.43). "Constitutional" delay of puberty is the most likely diagnosis until the bone age reaches 11 to 13 years (see Table 16.5).⁸⁸² Its distinction from isolated gonadotropin deficiency may be difficult. The features that help to distinguish it from isolated gonadotropin deficiency are listed in Box 16.6 and discussed in Fig. 16.43 footnotes. The single most useful test is the LH level in response to GnRH testing because random LH levels in hypogonadotropic patients often overlap those of pre- and midpubertal normal children.¹⁰⁰ GnRH agonist testing may discriminate between these disorders better because the LH response at 3 to 4 hours is the best indicator of gonadotropin secretory reserve and because this test permits assessing the gonadal secretory response to the secreted gonadotropins at 24 hours.⁸³⁸ Kisspeptin was given in an experimental protocol to children with CDGP to determine whether it may be a diagnostic test to predict entry into puberty. Children showed a wide range of responses, ranging from a robust response to little to no response. Hence its utility as a predictor of future reproductive development remains questionable.¹⁰⁰⁴

BOX 16.6 Features That Distinguish Gonadotropin Deficiency From Constitutional Delay of Puberty

In a healthy delayed prepubertal girl with BA >11 y and prepubertal FSH, gonadotropin deficiency is:

- Possible if:
 - Weight loss greater than 5% to 8% (BMI <10th–15th percentile for height age)
 - Midline facial defect
 - CNS dysfunction
 - CT or MRI brain scan abnormal
- Probable if:
 - BA > 13 years and LH <0.15 U/L in early daytime
 - Anosmia or panhypopituitarism
- Diagnostic if:
 - Sleep-associated increase in LH lacking
 - GnRH agonist test subnormal response
 - Chronological age >16 y

BA, Bone age; BMI, body mass index; CNS, central nervous system; CT, computed tomography; FSH, follicle-stimulating hormone; LH, luteinizing hormone; MRI, magnetic resonance imaging.

(Modified from Rosenfield, R.L., Barnes, R.B. (1993). Menstrual disorders in adolescence. *Endocrinol Metab Clin North Am*, 22, 491–505. With permission.)

The assessment of an adolescents' degree of estrogenization is often difficult. Breast development indicates that there has been estrogen exposure, but does not mean that it is current. Determination of serum estradiol is the simplest test, but diurnal and cyclic variations must be considered. Determination of hormonal effects on vaginal cytology is the most indicative of overall estrogen exposure (see Fig. 16.28), but less well accepted by patients. A progestin challenge test is often helpful. A female who does not experience progestin withdrawal bleeding (see Fig. 16.44) probably has an ambient estradiol level of less than about 40 pg/mL.¹⁰⁰⁵ If bleeding does not occur in response to this maneuver, the integrity of the uterus can be demonstrated by eliciting withdrawal bleeding after a 3-week course of estrogen-progestin, most conveniently administered in the form of birth control pills.

A prolactin level is indicated in the initial workup of normogonadotropic patients, regardless of their estrogen status (Fig. 16.45). The prolactin level correlates with the size of prolactinomas, and a level over 200 ng/mL is typical of a macroadenoma. A prolactin level that does not correlate with the size of a large pituitary tumor suggests either that the tumor is not a prolactinoma and is causing a functional pituitary stalk section or it is a macroadenoma elaborating such high levels of prolactin as to artefactually lower the immunoassayable prolactin level by a "hook effect."¹⁰⁰⁶ Very high blood or cerebrospinal fluid prolactin levels suggest invasiveness. The workup for this should include formal testing of visual fields (Goldman perimetry or evoked response). Pituitary microadenomas may be "incidentalomas" of no clinical significance, judging from an approximate 10% incidence in autopsy material.¹⁰⁰⁷ However, they require careful assessment of pituitary function and follow-up.¹⁰⁰⁸ Macroadeninemia should be considered in the absence of clearly related symptoms and when MRI is negative or in the setting of autoimmune disease.^{988,989} Macroadeninemia is confirmed when the prolactin level measured after precipitation of serum using polyethylene glycol is normal (or substantially reduced compared with the level measured in untreated serum).

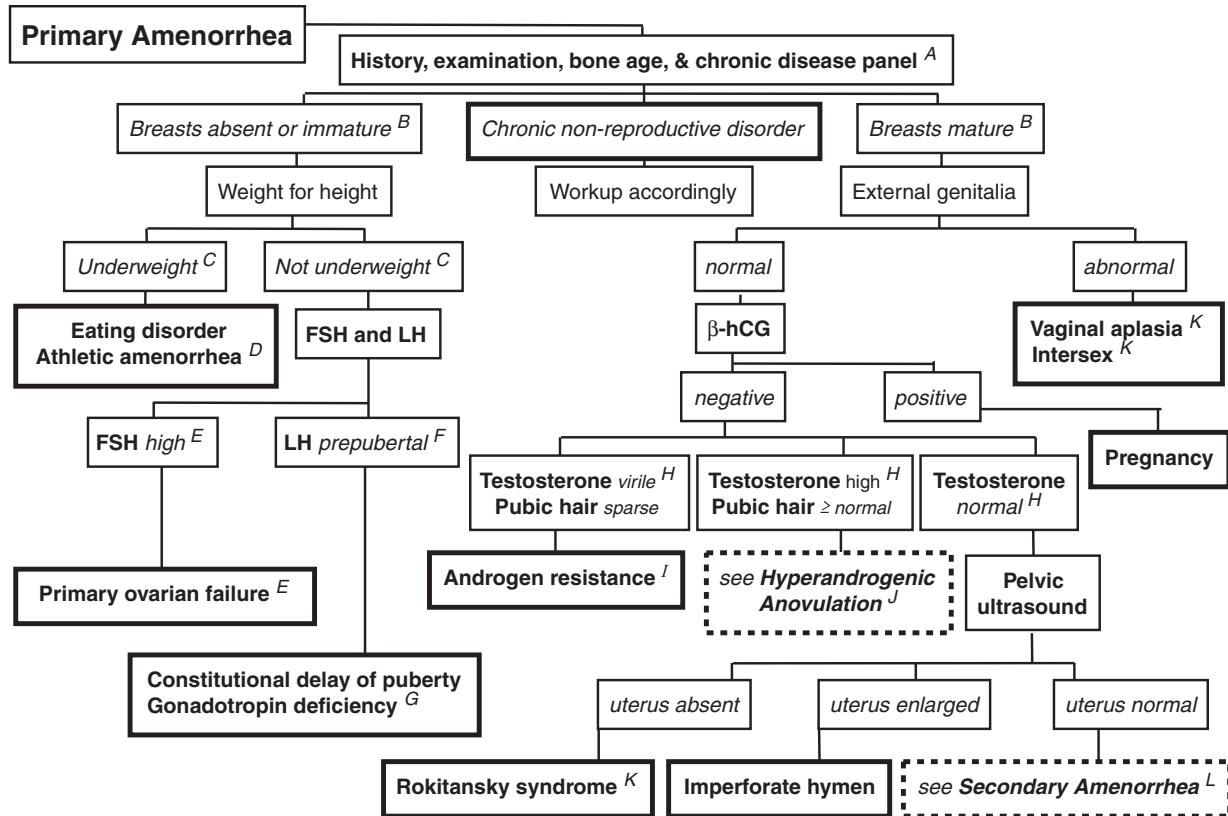


Fig. 16.43 Differential diagnosis of primary amenorrhea. (Modified from Rosenfield, R.L. (2003). Menstrual disorders and hyperandrogenism in adolescence. In: Radovick, S., MacGillivray, M.H. eds. Pediatric Endocrinology a Practical Clinical Guide. Totowa, N. J., Humana Press, Inc., p. 451–478. With permission.)

Footnotes:

- A. Prime among the causes of primary amenorrhea are growth-retarding or growth-attenuating disorders. In the absence of specific symptoms or signs to direct the workup, laboratory assessment for chronic disease typically includes bone age radiograph if the adolescent is not sexually mature and a chronic disease panel (complete blood count and differential, sedimentation rate, comprehensive metabolic panel, celiac panel, thyroid panel, cortisol and insulin-like growth factor-I levels, and urinalysis).
- B. Breast development ordinarily signifies the onset of pubertal feminization. However, mature breast development does not ensure ongoing pubertal estrogen secretion.
- C. Body mass index (BMI) under the 10th percentile generally corresponds to body composition <20% body fat, which is the critical factor.
- D. BMI may not accurately reflect body fat in serious athletes (who have a disproportionately greater muscle mass) or bulimia nervosa.
- E. Follicle-stimulating hormone (FSH) is preferentially elevated over luteinizing hormone (LH) in primary ovarian failure. The most common cause of primary amenorrhea caused by primary ovarian failure is gonadal dysgenesis caused by Turner syndrome, but acquired causes must be considered (such as cytotoxic therapy). The workup of primary ovarian failure is considered in detail in the next algorithm (secondary amenorrhea and oligomenorrhea). Lack of FSH elevation virtually rules out primary ovarian failure only when the bone age is appropriate for puberty (≥ 11 years).
- F. "Pediatric" gonadotropin assays sensitive to 0.15 U/L or lower are critical to the accurate diagnosis of gonadotropin deficiency and delayed puberty. A low LH level is more characteristic of these disorders than a low FSH level. Congenital gonadotropin deficiency is closely mimicked by the more common extreme variation of normal, constitutional delay of puberty.
- G. History and examination may yield clues to the cause of hypogonadotropic hypogonadism, such as evidence of hypopituitarism (midline facial defect, extreme short stature, visual field defect) or anosmia (Kallmann syndrome) or functional hypothalamic disturbance (bulimia, excessive exercise). Random LH levels in hypogonadotropic patients are usually below 0.15 IU/L, but often overlap those of normal pre- and midpubertal children. The gonadotropin-releasing hormone (GnRH) test, measuring the gonadotropin response to a 50- to 100-mcg bolus, in the premenarcheal teenager strongly suggests gonadotropin deficiency if the LH peak is less than 4.2 IU/L by monoclonal assay. Inhibin-B under 20 pg/mL or responses to GnRH agonist testing (e.g., leuprolide acetate injection 10 mcg/kg SC) are reported to discriminate well between gonadotropin deficiency and constitutional delay (see text). It may not be possible to definitively establish the diagnosis of gonadotropin deficiency until puberty fails to begin by 16 years of age or progress at a normal tempo.
- H. Plasma total testosterone is normally 20 to 60 ng/dL (0.7–2.1 nM) in women, 300 to 1200 ng/dL in men, but varies somewhat among laboratories. Plasma free (or bioavailable) testosterone is about 50% more sensitive than total testosterone in detecting hyperandrogenemia. However, there are many pitfalls in testosterone assays at the low levels of women, reliable testosterone assays are not available to many physicians, and assaying the free testosterone introduces other potential sources of error. Therefore it is reasonable to begin the evaluation with a total testosterone determination if a free testosterone test in a reliable specialty laboratory is not available to the practitioner.
- I. Androgen resistance is characterized by a frankly male plasma testosterone level when sexual maturation is complete, male karyotype (46, XY), and absent uterus. External genitalia may be ambiguous (partial form) or normal female (complete form).
- J. The differential diagnosis of hyperandrogenism is shown in a later algorithm.
- K. Vaginal aplasia in a girl with normal ovaries may be associated with uterine aplasia (Rokitansky-Kustner-Hauser syndrome). When the vagina is blind and the uterus aplastic, this disorder must be distinguished from androgen resistance. If the external genitalia are ambiguous, it must be distinguished from other disorders of sex development (intersex).
- L. Secondary amenorrhea differential diagnosis is presented in Fig. 16.43.

hCG, Human chorionic gonadotropin.

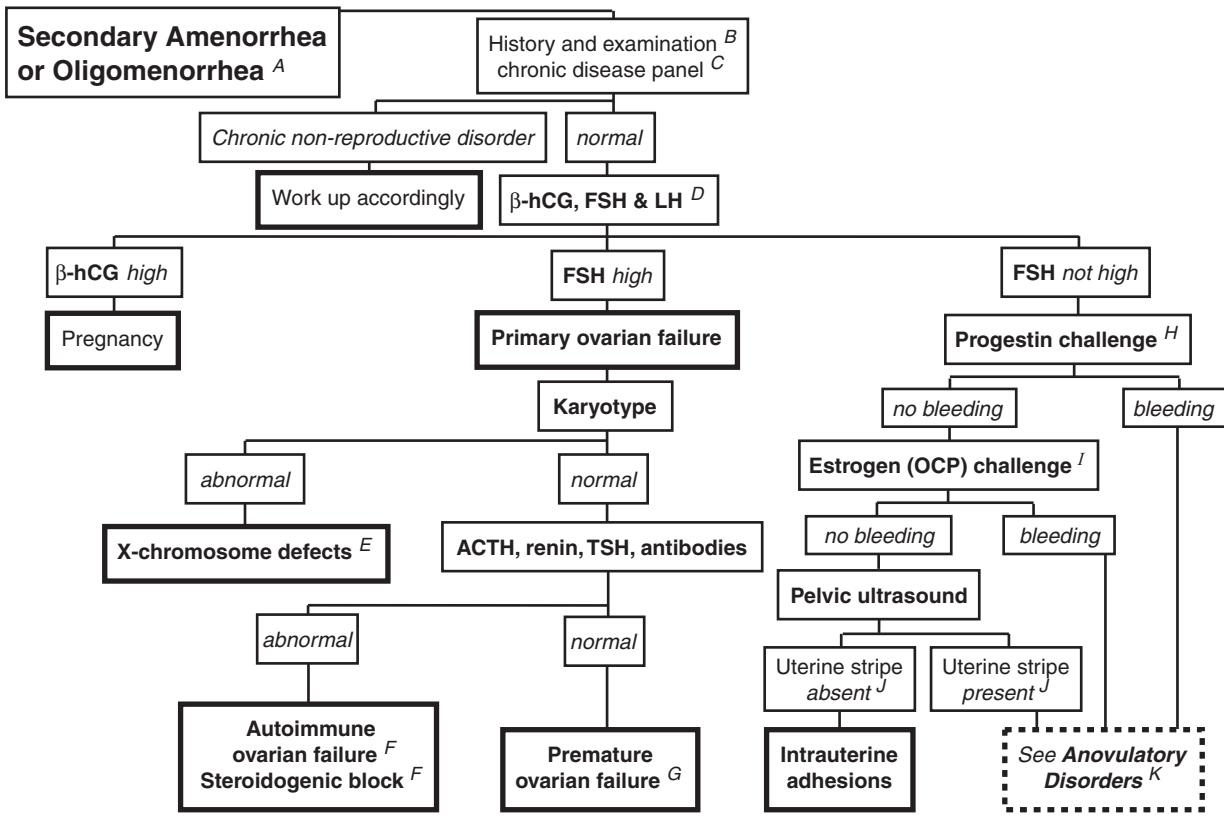


Fig. 16.44 Differential diagnosis of secondary amenorrhea or oligomenorrhea. (Modified from Rosenfield, R.L. (2003). Menstrual disorders and hyperandrogenism in adolescence. In: Radovick, S., MacGillivray, M.H. eds. Pediatric Endocrinology a Practical Clinical Guide. Totowa, N. J., Humana Press, Inc., p. 451–478. With permission.)

Footnotes:

- A. Mature secondary sex characteristics are characteristic because the occurrence of menarche indicates that a substantial degree of development of the reproductive system has taken place; however, their presence does not rule out subsequent regression of gonadal function.
- B. Diverse disorders of many systems cause anovulation. The history may reveal excessive exercise, symptoms of depression, gastrointestinal symptoms, radiotherapy to the brain or pelvis, or rapid virilization. Physical findings may include hypertension (forms of congenital adrenal hyperplasia, chronic renal failure), short stature (hypopituitarism, Turner syndrome, pseudohypoparathyroidism), abnormal weight for height (anorexia nervosa, obesity), decreased sense of smell (Kallmann syndrome), optic disc or visual field abnormality (pituitary tumor), cutaneous abnormalities (neurofibromatosis, lupus), goiter, galactorrhea, hirsutism, or abdominal mass.
- C. In the absence of specific symptoms or signs to direct the workup, evaluation for chronic disease in a sexually mature adolescent typically includes complete blood count and differential, sedimentation rate, comprehensive metabolic panel, celiac panel, thyroid panel, cortisol and insulin-like growth factor-I levels, and urinalysis.
- D. "Pediatric" gonadotropin assays sensitive to 0.15 U/L or lower are critical to the early diagnosis of many anovulatory disorders. Luteinizing hormone (LH) enters into the differential diagnosis of hypogonadotropic anovulatory disorders, which is outlined in Fig. 16.45.
- E. Patients missing only a small portion of an X-chromosome may not have the Turner syndrome phenotype. Indeed, among 45,X patients the classic Turner syndrome phenotype is found in less than one-third (with the exception of short stature in 99%). Ovarian function is sufficient for about 10% to undergo some spontaneous pubertal development and for 5% to experience menarche. If chromosomal studies are normal and there is no obvious explanation for the hypogonadism, special studies for fragile X premutation and autoimmune oophoritis should be considered.
- F. Autoimmune ovarian failure may be associated with tissue-specific antibodies and autoimmune endocrinopathies, such as chronic autoimmune thyroiditis, diabetes, adrenal insufficiency, and hypoparathyroidism. Nonendocrine autoimmune disorders may occur, such as mucocutaneous candidiasis, celiac disease, and chronic hepatitis. Rare gene mutations causing ovarian insufficiency include steroidogenic defects that affect mineralocorticoid status (17-hydroxylase deficiency is associated with mineralocorticoid excess and lipid adrenal hyperplasia with mineralocorticoid deficiency) and mutations of the gonadotropins or their receptors. Ovarian biopsy is of no prognostic or therapeutic significance. LH is disproportionately high in steroidogenic defects or autoimmune disease specifically affecting theca cells.
- G. The history may provide a diagnosis (e.g., cancer chemotherapy or radiotherapy). Other acquired causes include surgery and autoimmunity. Chromosomal causes of premature ovarian failure include X-chromosome fragile site and point mutations. Other genetic causes include gonadotropin-resistance syndromes, such as LH or follicle-stimulating hormone (FSH) receptor mutation and pseudohypoparathyroidism. A pelvic ultrasound that shows preservation of ovarian follicles carries some hope for fertility.
- H. Withdrawal bleeding in response to a 5- to 10-day course of progestin (e.g., medroxyprogesterone acetate 10 mg HS) suggests an overall estradiol level greater than 40 pg/mL. However, this is not entirely reliable and thus in the interest of making a timely diagnosis it is often worthwhile to proceed to further studies.
- I. A single cycle of an oral contraceptive pill (OCP) containing 30 to 35 mcg ethinyl estradiol generally suffices to induce withdrawal bleeding if the endometrial lining is intact.
- J. A thin uterine stripe suggests hypoestrogenism. A thick one suggests endometrial hyperplasia, as may occur in polycystic ovary syndrome.
- K. The differential diagnosis of other anovulatory disorders continues in Fig. 16.45.

ACTH, adrenocorticotrophic hormone; hCG, Human chorionic gonadotropin; TSH, thyroid-stimulating hormone.

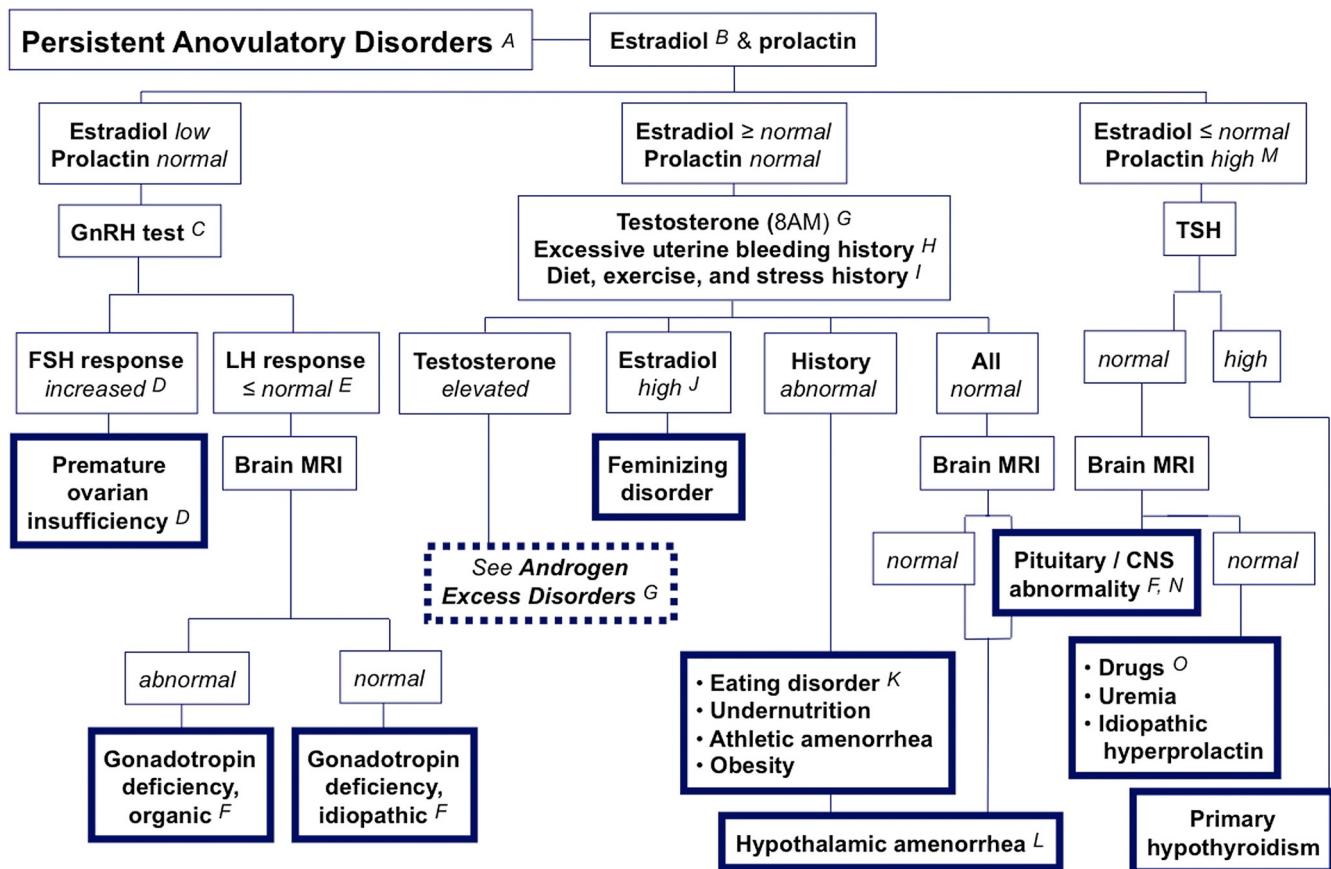


Fig. 16.45 Differential diagnosis of persistent anovulatory disorders. (Modified from Rosenfield, R.L. (2003). Menstrual disorders and hyperandrogenism in adolescence. In: Radovick, S., MacGillivray, M.H. eds. Pediatric Endocrinology a Practical Clinical Guide. Totowa, N. J.: Humana Press, Inc., p. 451–478. With permission.)

Footnotes:

- Anovulatory disorders should be considered in any girl with persistent unexplained amenorrhea or oligomenorrhea, irregular menstrual bleeding, short cycles, or excessive menstrual bleeding. Physiological adolescent anovulation, a transient variation of normal, is the most common cause of menstrual dysfunction in the early postmenarcheal years. The workup in this algorithm progresses from negative studies in the preceding algorithm.
- Once breast development has matured, the breast contour does not substantially regress if hypoestrogenism develops. Hypoestrogenism is suggested if plasma estradiol is persistently under 40 pg/mL in a "pediatric" assay sensitive to less than 10 pg/mL. However, a single estradiol level may be misleading because of cyclic or episodic variations.
- Gonadotropin-releasing hormone (GnRH) testing is usually performed by assaying luteinizing hormone (LH) and follicle-stimulating hormone (FSH) before and 0.5 hour after the administration of 1 mcg/kg GnRH intravenously. GnRH agonist testing may alternatively be performed by administering 10 mcg/kg leuprolide acetate subcutaneously and assaying LH and FSH at 3 to 4 hours to assess gonadotropin reserve and at 18 to 24 hours to assess the ovarian steroid response to endogenous gonadotropin release.
- Baseline gonadotropin levels may be normal as the ovary begins to fail, as in early menopause, but an exaggerated FSH response to GnRH and subnormal estradiol response to the gonadotropin elevation induced by acute GnRH agonist challenge are characteristic. See also Premature Ovarian Failure in preceding figure.
- LH responses to GnRH may vary from nil to normal in gonadotropin deficiency: normal LH and FSH responses in the presence of hypoestrogenism indicate inadequate compensatory hypothalamic GnRH secretion.
- Gonadotropin deficiency may be congenital or acquired, organic or functional. Congenital causes include midline brain malformations or specific genetic disorders, such as Prader-Willi syndrome, Laurence-Moon-Biedl syndrome, or Kallmann syndrome. Kallmann, the association of anosmia with gonadotropin deficiency, occurs in both the X-linked and autosomal-recessive forms. Special magnetic resonance imaging (MRI) views often demonstrate absence of the olfactory tracts. Acquired gonadotropin deficiency may be secondary to a variety of organic central nervous system (CNS) disorders, varying from hypothalamic-pituitary tumor to radiation damage to empty sella syndrome. Autoimmune hypophysitis is a rare disorder, sometimes accompanying a polyendocrine deficiency syndrome. The prototypic form of functional gonadotropin deficiency is anorexia nervosa. Idiopathic hypogonadotropic deficiency may sometimes occur in families with anosmia, suggesting a relationship to Kallmann syndrome.
- Plasma free (or bioavailable) testosterone is about 50% more sensitive than total testosterone in detecting hyperandrogenemia. However, there are many pitfalls in testosterone assays at the low levels of women, reliable testosterone assays are not available to many physicians, and assaying the free testosterone introduces other potential sources of error. Therefore it is reasonable to begin the evaluation with a total testosterone determination if a free testosterone test in a reliable specialty laboratory is not available to the practitioner. Simultaneous assay of 17-hydroxyprogesterone is indicated in subjects at high-risk for congenital adrenal hyperplasia, such as Ashkenazi Jews. Initial assessment for differentiating among the causes of androgen excess is outlined in Fig. 16.49.
- Excessive ("dysfunctional") uterine bleeding not controlled by progestin or oral contraceptive pill therapy additionally requires a pelvic ultrasound examination (for genital tract tumor or feminizing tumor), coagulation workup (which includes platelet count, prothrombin time, thromboplastin generation test, bleeding time, and von Willebrand factor), and consideration of the possibility of sexual abuse.

(Legend continuous on next page)

- I. The equivalent of 20 miles per week or more is generally required before body fat stores fall to the point where amenorrhea occurs. Physical or psychosocial stress may cause amenorrhea.
- J. The normal range for estradiol over the menstrual cycle is wide: values over 95 pg/mL usually indicate the preovulatory or luteal phase, but are compatible with a feminizing disorder.
- K. Low body fat caused by mild forms of stress disorders (anorexia nervosa, bulimia nervosa, and athletic amenorrhea) may be associated with acquired functional hypothalamic amenorrhea rather than frank gonadotropin deficiency. The low body fat content of athletic amenorrhea may not be reflected by weight for height because of high muscularity. Dual-photon absorptiometry scan may be useful in documenting body fat below 20%. Patients with anorexia nervosa may become amenorrheic before or when weight loss begins, indicating an important psychological component to the etiology. Obesity is also associated with anovulatory cycles and raises the possibility of Cushing syndrome.
- L. Hypothalamic amenorrhea is a diagnosis of exclusion. It is a form of partial gonadotropin deficiency in which baseline estrogen secretion is normal but a preovulatory LH surge cannot be generated. It may result from organic CNS disorders or be functional, caused by stress, undernutrition or obesity, diverse types of endocrine dysfunction, chronic disease, or idiopathic. It may be difficult to distinguish from hyperandrogenemia.
- M. Hyperprolactinemia is heterogeneous in its presentation. Some have normoestrogenic anovulation, which may be manifest as hypothalamic anovulation, hyperandrogenism, dysfunctional uterine bleeding, or short luteal phase. On the other hand, some are hypoestrogenic; these do not have galactorrhea.
- N. Large hypothalamic-pituitary tumors or other types of CNS injury cause variable pituitary dysfunction, which may include complete or partial gonadotropin deficiency and various manifestations of hypopituitarism (including secondary hypothyroidism). If they interrupt the pituitary stalk, hyperprolactinemia ensues. Hyperprolactinemia may also be caused by prolactinomas.
- O. Drugs, particularly neuroleptics of the phenothiazine or tricyclic type, may induce hyperprolactinemia.

Imaging studies are important ancillary measures. Pelvic ultrasound may demonstrate hypoplastic ovaries, endometrial disorders, or polycystic ovaries. MRI of the hypothalamic-pituitary area is important in the workup of gonadotropin deficiency, hyperprolactinemia, and hypothalamic anovulation. MRI may not be necessary if the patient presents with a history suggesting functional hypothalamic amenorrhea but is indicated in those cases with associated symptoms of headache, vomiting, vision change, alteration in thirst or urinary frequency, an abnormal neurological examination, or evidence of other pituitary hormonal abnormalities.⁹⁹⁶

Anorexic patients require psychiatric evaluation and consideration of brain tumor and partial bowel obstruction. Diet fadism and athletic addiction may be difficult to distinguish from anorexia nervosa. Constitutionally thinness is a variant of normal with normal menses and a distinctive hormonal profile.¹⁰⁰⁹ It is unclear whether the superior mesenteric artery syndrome is a primary disorder that mimics anorexia nervosa or is a complication of it.¹⁰¹⁰

Management

Underlying disorders must be treated appropriately. For example, tumors require surgery and/or radiotherapy.

For prolactinoma, dopaminergic treatment is the initial treatment of choice unless the patients' condition or eyesight is critical.^{991,1011} Hyperprolactinemia will be maximally suppressed within 1 month and the menstrual cycle normalized within 3 months by an effective dopaminergic agonist regimen. Cabergoline 0.5 to 1.0 mg once or twice weekly will usually control galactorrhea and shrink prolactinomas.^{987,1012}

To minimize nausea, it is best to start with a low dose at bedtime. Bromocriptine does not activate the serotonin 5-hydroxytryptamine (2B) receptor, the proposed mechanism through which cabergoline is thought to stimulate cardiac valve dysfunction in the elderly, and so bromocriptine does not seem to be associated with an increased risk of cardiac valve regurgitation, and may be considered as an alternative to cabergoline treatment, albeit a less effective one. The usual bromocriptine maintenance dose is 0.25 to 0.5 mg twice daily. After 2 years of treatment, if the prolactin level is normal and there is no tumor evident on MRI, dopamine agonist may be tapered and possibly discontinued.¹⁰¹³ Prolactin levels are then measured to monitor for recurrence.

Anorexia nervosa is best managed by an experienced multidisciplinary team. Refeeding is the first priority, and once steady weight gain is evident the psychodynamic issues can be addressed.¹⁰¹⁴ Family therapy of medically uncomplicated cases of anorexia nervosa on an outpatient basis generally yields the best results, with good improvement in over half of patients. Inpatient care is needed if there is severe bradycardia, hypotension or orthostasis, or electrolyte imbalance,⁹⁹⁶ and may be needed if there is failure of weight gain during outpatient therapy. Menses generally resume when psychotherapy is effective and body fat is restored to normal (see Fig. 16.42). The induction of menses by estrogen-progestin replacement is usually injudicious because it provides a false sense of recovery and does not yield the recovery of bone loss that occurs with weight gain.¹⁰¹⁵ However, in those adolescents with low bone density who have not had return of menses after a reasonable trial of nutritional and psychological intervention (perhaps 6–12 months), short-term treatment with transdermal estradiol and cyclic oral progestin (not oral contraceptives or ethinyl estradiol) can be considered to protect the patient's bone health.⁹⁹⁶ Although the acute episode can usually be successfully treated, there is a high rate of ongoing psychiatric disability and medical complications. Anorexia nervosa "by proxy" has been described in the offspring of former patients.¹⁰¹⁶

There are two aspects of therapy that are uniformly involved in managing hypogonadism—psychological support and hormone administration. Patients with delayed development that is a variation of normal should be reassured that there is nothing wrong, only a delay in timing of the onset of puberty. The wide normal variation in the pattern and time of the pubertal growth spurt should be explained in detail and the girl should be informed of her predicted eventual height. The majority of children with delayed puberty do not have overt psychological symptoms. Complex compensations and sublimations obviously occur. However, peer group pressures may make adjustment to sexual infantilism especially difficult when the age of 13 years is approached,¹⁰¹⁷ and a poor self-image may lead to social withdrawal and feelings of hopelessness. Physical immaturity may prolong psychological immaturity. A 6- to 12-month course of physiological sex hormone therapy at this time may help alleviate these anxieties. The physician should discuss the fact, when the evidence favors it, that the odds are overwhelmingly in favor of the "timer in the subconscious area of the brain" eventually turning on. When this will happen can be approximated from the skeletal age. One should not

hesitate to advise more intensive psychological counseling if it becomes apparent that the concern about puberty is but one aspect of a more general maladjustment. Ultimately, the decision as to whether to undertake treatment for delayed puberty is up to the patient and her family.

Assure the teenager with an organic basis for hypoestrogenism that feminization will occur, although in response to appropriate hormone treatment. Some genetic forms of gonadotropin deficiency are actually reversed by sex steroid therapy.²³⁵ However, most will require lifelong hormone replacement therapy. It should be kept in mind that attainment of normal breast development in the girl with panhypopituitarism requires replacement of GH and cortisol deficits. It is difficult however to induce secondary sex characteristics in some patients with systemic chronic inflammatory disease, such as lupus erythematosus.

In patients in whom short stature is an important concern, as in Turner syndrome, growth potential must be considered before undertaking estrogen replacement. GH therapy improves the adult height potential of patients with Turner syndrome, especially when started as soon as growth failure becomes apparent.¹⁰¹⁸ GH therapy in the United States is generally initiated at the US Food and Drug Administration (FDA)-approved dose of 0.375 mg/kg-wk. If adult height is likely to be unsatisfactory, concomitant treatment with oxandrolone 0.03–0.05 mg/kg/day should be considered.⁵⁷¹ Clitoromegaly is ordinarily negligible on this dosage; liver function should be monitored.

Two controlled studies have shown that pubertal estrogen replacement therapy is safe and efficacious in maximizing growth potential and age-appropriate feminization when started as young as 11 to 12 years of age using very low estrogen doses, far below those that are available by prescription, in conjunction with GH therapy in Turner syndrome.^{1020,1021} The estrogen content of oral contraceptive pills (OCPs) is supraphysiological for induction of breast development or for linear growth.

We favor use of one of the following hormone replacement regimens.^{571,1022} Whichever form of estrogen is used, pubertal development and growth should be monitored every 6 months, with bone age determinations at 6- to 12-month intervals to avoid unanticipated loss of growth potential.

IM depot estradiol in a starting dose of 0.2 mg/month will usually induce breast budding; the dose should be increased by 0.2 mg every 6 months.¹⁰²⁰ A midpubertal dose of 1.0 to 1.5 mg monthly, which is half the adult replacement dose, typically induces menarche within 1 year. An alternative oral regimen begins with 5 mcg/kg micronized estradiol (Estrace[®], 0.25 mg for a 50 kg girl) daily; the adult replacement dose is 1 to 2 mg/day.¹⁰²³

Transdermal estradiol is a convenient, physiological form of therapy¹⁰²⁴ that appears to have long-term health advantages over commonly used oral estrogens,^{1025,1026} but there are little data on their use for inducing puberty. We suggest starting transdermal feminization with 14 mcg daily for 1 week per month, a marginally feminizing dose that is in accord with current guidelines,^{571,1018} and escalating at 6-month intervals to an adult dose at 3 years. A suggested protocol for female pubertal induction for hypogonadal patients is provided in Table 16.7.¹⁰²³

For girls with hypogonadism and an intact uterus, cyclic progestin should be added after 2 years of estrogen therapy or when bleeding begins to occur at unpredictable times. A simple regimen is to use 100 mg of micronized progesterone (Prometrium[®]) at bedtime for 7 to 14 days during the second to third week of estrogen therapy or equivalent doses of medroxyprogesterone acetate (5–10 mg/day) or norethindrone acetate (5 mg/day). This will bring about normal menstruation during

TABLE 16.7 A Pubertal Transdermal Estradiol Replacement Regimen Beginning at 11 Years of Age^a

Age	Estradiol Dose
0–6 months	14 mcg, day 1–7 each month
6–12 months	14 mcg, day 1–14 each month
1–1.5 years	14 mcg, day 1–21 each month
1.5–2 years	25 mcg, day 1–21 each month
2–2.5 years	37.5 mcg, day 1–21 each month
2.5–3 years	28-day cycle: 50 mcg day 1–21 and Prometrium [®] 100 mg ^b day 12–21 every 28 days OR Continuous: 50 mcg day 1–14, then Combipatch [®] (50 mcg estradiol/0.14 mg norethindrone) day 16–28 every 28 days
3–3.5 years	28-day cycle: 75 mcg day 1–21 and Prometrium [®] 100 mg day 12–21 every 28 days OR Continuous: 75 mcg day 1–14 then Combipatch [®] (50 mcg estradiol/norethindrone) day 16–28 every 28 days
3.5–4 years	28-day cycle: 100 mcg day 1–21 and Prometrium [®] 100mg day 12–21 every 28 days OR 100 mcg day 1–14 then Combipatch [®] (50 mcg estradiol/norethindrone) day 16–28 every 28 days
>4.0 years	Continue regimen or offer oral contraceptive pill

^aIn children ≥13 years old, consider starting with 25 mcg for 2–3 weeks monthly and increasing the dose at shorter intervals (e.g., 3 months).

^bIf inadequate bleeding, increase Prometrium to 200 mg day 12–21 or change to Combipatch 50 mcg estradiol/0.25mg norethindrone. (Modified from Klein, K.O., Rosenfield, R.L., Santen, R.J., et al. (2018). Estrogen replacement in Turner syndrome: literature review and practical considerations. *J Clin Endocrinol Metab*, 103, 1790–1803.)

the week preceding resumption of estrogen therapy. The addition of progestin will decrease the risk of endometrial hyperplasia and endometrial carcinoma, but premenstrual symptoms should be anticipated.

Once optimal height is achieved, most patients prefer to switch to combined oral contraceptive pills (OCP) as a convenient form of estrogen-progestin therapy. The pills containing the lowest dose of estrogen that will result in normal menstrual cycles are advisable. The potential risks of oral contraceptives must be kept in mind when counseling adolescents.¹⁰²⁷ The lowest estrogen dosages currently available in combination contraceptive pills in the United States contain 20 mcg (Mircette[®]) to 30 mcg (Yasmin[®]) ethinyl estradiol. Low-dose androgen replacement has been controversial, but may confer benefits to body composition, cognition, bone mineralization, and libido.¹⁰²⁸

Hypogonadotropic patients can achieve ovulation with gonadotropin therapy. Hypothalamic GnRH deficiency can be successfully treated by pulsatile GnRH.^{91,1029} Recently, kisspeptin has been considered as a therapeutic modality for the treatment of CHH; however, its short half-life has hampered efforts. Hence kisspeptin analogs were designed to decrease proteolytic degradation and renal clearance. These analogs induced direct excitatory action on GnRH neurons, advanced puberty, and synchronized ovulations in sheep. The potential for treatment of reproductive disorders in humans remains undefined.¹⁰³⁰ Because several genes in the GnRH signaling system are expressed in the gonads, some hypogonadotropic patients have primary defects in gonadal function.^{931,1031} Induction of ovulation is best carried out by a gynecologist specializing in reproductive endocrinology.

Fertility preservation is possible in some patients with primary ovarian failure. Oocyte cryopreservation for later in vitro

fertilization is feasible in pubertal girls after ovulation induction.^{887,890,909,1032} The girls must be pubertal for successful ovulation induction. In addition, cryopreservation of embryos requires sperm, likely limiting the appropriateness of this in pediatric patients. Cryopreservation of ovarian tissue^{922,1033} is a promising technique that may be useful for fertility preservation in prepubertal girls with ovarian failure, although this is currently an investigational approach. Finally, activation of residual follicle growth in autografts has recently been accomplished in adults with POI, with successful pregnancy after embryo transfer.¹⁰³⁴ Female germline stem cell research holds promise as a future fertility treatment.¹⁰³⁵ Up-to-date information for physicians and patients can be found through the Oncofertility Consortium (www.myoncofertility.org).

In girls with Turner syndrome, fertility preservation using either oocyte or embryo cryopreservation are currently only of use in those who have undergone spontaneous puberty. These girls should be counseled about these possibilities for fertility preservation.¹⁰³⁶ In these girls, monitoring AMH levels may be useful as a marker of ovarian function.¹⁰³⁷ On the other hand, primordial follicles have been found in ovarian tissue collected for cryopreservation from girls with mosaic or nonmosaic Turner syndrome, although the likelihood of finding follicles was much lower in those with a 45,X karyotype (10.7%) than in those with 46, XX/45,X mosaicism (86%).¹⁰³⁷ As noted earlier, however, ovarian cryopreservation remains an investigational procedure. In the rare spontaneous pregnancies that have occurred in women with Turner syndrome, the rate of congenital and chromosomal abnormalities in the fetus is approximately 50%,¹⁰³⁷ which must be considered when using autologous oocytes from any approach. Finally, women with Turner syndrome are at high risk of fetal and maternal pregnancy complications because of uterine anomalies, carbohydrate intolerance, and potential cardiovascular complications including aortic rupture. (For discussion of other aspects in the care of girls with Turner syndrome see Chapter 17.)

Nonhypoestrogenic Menstrual Disturbances

Hypothalamic Anovulation

Hypothalamic anovulation causes menstrual disturbances in sexually mature women through a deficiency in GnRH secretion that is too subtle to cause frank hypoestrogenism. The neuroendocrine system stimulates ovarian estrogen secretion to a level normal for an early- or midfollicular phase female, but follicular development is inadequate for a normal dominant follicle to emerge. Amenorrhea or oligomenorrhea may result. However, in some patients, sufficient estrogenization occurs to cause dysfunctional uterine bleeding, which is discussed in the next section.

Reduced LH pulsatility¹⁰³⁸ and/or failure to generate a mid-cycle LH surge^{1039,1040} are characteristic. The pathophysiology seems to be mediated primarily by undernutrition and/or CRH excess. Negative energy balance may be present even in patients of normal, but less than average, weight and fat stores.^{497,981} Leptin deficiency is an important determinant of the decreased LH pulsatility. Ghrelin may play a role in LH inhibition.¹⁰⁴¹ The anovulation of psychic or physical stress seems to involve CRH excess.⁹⁸² In the brain, CRH releases β-endorphin from proopiomelanocortin, and the endorphin in turn inhibits GnRH release. Naloxone blockade of opioid action normalizes gonadotropin secretion.⁹¹ In the pituitary, CRH increases the set-point for ACTH release. This brings about a new steady state of increased cortisol secretion. Further ACTH response to CRH is blunted by the negative feedback of this cortisol excess. The result is a mildly cushingoid cortisol rhythm. Cortisol excess itself can contribute to the amenorrhea by inhibiting the

response to GnRH,¹⁰³³ as well as antagonizing some sex hormone actions. Adrenal androgens are elevated in competitive athletes who maintain body fat stores.¹⁰⁴²

Causes. Functional hypothalamic amenorrhea (FHA) describes hypothalamic anovulation that is unexplained by an identifiable organic CNS cause or chronic disease.⁹⁹⁶ These patients have a normal or slightly low serum LH, normal FSH, and estradiol in the early-mid follicular phase range (20–50 pg/mL). It is caused by energy deficit (because of weight loss and/or vigorous exercise) or psychological stress or a combination thereof. As with patients with anorexia nervosa and the athletic or psychogenic types of hypothalamic anovulation, they often have significant hypercortisolemia. However, there are patients with FHA who cannot be identified as having one of these causes. Some 24% of such women in one series had a history of delayed menarche.⁷²⁰ A primate model indicates that hypothalamic anovulation develops in stress-sensitive individuals from an innocuous combination of mild stress and mild caloric restriction.¹⁰⁴³ Heterozygosity for genes associated with Kallmann syndrome has been identified as a predisposing factor in 13% of cases; more than half of this subgroup had a family history of hypothalamic amenorrhea or delayed puberty.⁷²⁰

Athletic amenorrhea is the term given to hypothalamic anovulation associated with excessive exercise and low body fat stores. Running 20 miles or more per week is associated with increased incidence of amenorrhea.¹⁰⁴⁴ The female athletic triad consists of menstrual disturbance, eating disorder, and osteoporosis.¹⁰⁴⁵ Primary or secondary amenorrhea, oligomenorrhea, or short luteal phase are common in athletes.¹⁰⁴⁶ Ovarian function decreases approximately in proportion to the amount of physical activity and dietary restriction. Weight-bearing exercise is only partially protective of the effects of hypoestrogenism on weight-bearing bone. There is concern that amenorrheic athletes may be left with a permanent deficit in bone mass.¹⁰⁴⁷ Weight loss to 10% below ideal body weight and body fat less than 12% are risk factors for amenorrhea. BMI does not accurately reflect body fat stores in athletes because of their muscularity.¹⁰⁴⁸ Energy balance seems to be more critical than low body fat stores in mediating the anovulation.^{1046,1049} Menarche may occur or menses resume when the athlete's activity level suddenly decreases and before weight gain occurs. Other factors also contribute to cause amenorrhea. Nutritional deficiencies may coexist. Chronic undernutrition may suppress thyroid function as in anorexia nervosa.⁹⁸² Athletic amenorrhea resembles anorexia nervosa in patients' obsession with weight control.^{1046,1050}

Psychogenic amenorrhea from severe psychic stress has long been known (e.g., "boarding-school amenorrhea").¹⁰⁵¹ The onset of psychogenic amenorrhea may be identified as being associated with a discrete event, but the ovarian dysfunction tends to be long-lasting. Subtle nutritional deficits contribute.⁴⁹⁷

Epilepsy causes menstrual disturbances that seem to result from abnormal neuroendocrine regulation independently of drug treatment.¹⁰⁵²

Pseudocyesis is an extremely rare form of psychogenic amenorrhea that is caused by persistence of the corpus luteum. This syndrome tends to occur in infertile women with an overwhelming desire for pregnancy and conversion hysteria. Prolactin and LH excess appear to mediate this rare syndrome.¹⁰⁵³

Differential Diagnosis. Disorders outside the neuroendocrine-gonadal axis may cause or mimic hypothalamic anovulation. These include pregnancy, nutritional disturbance, glucocorticoid excess, disturbed thyroid function, drug abuse, chronic illness, hyperprolactinemia, and ectopic gonadotropin secretion.

Pregnancy must be excluded in all sexually mature adolescents with amenorrhea. An elevation of the serum β -hCG level is the earliest laboratory sign.¹⁰⁵⁴ Placental hCG initially drives constant overproduction of estrogens and progestins by the maternal corpus luteum, then production of estrogen and other sex steroids shifts to the fetoplacental unit and suppresses maternal pituitary gonadotropin release.

Optimal fat mass is necessary for normal gonadotropin levels in sexually mature women, and both obesity and under-nutrition suppress gonadotropins; thus the gonadotropin response to relative adipose mass seems biphasic.³⁰⁸ Obesity is associated with blunted LH pulse amplitude that is partially attributable to increased LH clearance rate. Overproduction of estrogen from plasma precursors in adipose tissue⁴⁵⁷ may play a role in suppressing LH pulsatility.¹⁰⁵⁵ The extent to which sleep disruption may contribute to LH suppression is unclear.¹⁰⁵⁶ The effect of undernutrition seems to be mediated by factors related to energy balance, as discussed in the section Hypothalamic Anovulation.

Cushing syndrome (glucocorticoid excess) of any etiology causes anovulation by inhibiting the gonadotropin response to GnRH.¹⁰³³ Thyroid hormone deficiency suppresses gonadotropin release via gonadotrophin-inhibitory hormone,¹⁰⁵⁷ and interferes with gonadotropin action on the ovary.¹⁰⁵⁸ Drug abuse with tetrahydrocannabinol, ethanol, or opiates causes hypothalamic anovulation.^{1059,1060} Cocaine causes menstrual irregularity by suppressing gonadotropin secretion through mechanisms that include depletion of dopaminergic stores, resulting in hyperprolactinemia, and stimulation of CRH release.^{992,1061} Inflammatory illness acutely disrupts the estradiol-induced LH surge,¹⁰⁶² and chronic illness causes gonadotropin deficiency, which may be mediated partly by undernutrition and partly by cytokines.^{193,965} Disorders as diverse as diabetes mellitus and iron overload all impact GnRH secretion.^{1063,1064} Chronic renal failure causes complex dysfunction of the reproductive system, including poor clearance of gonadotropins and prolactin in the presence of inhibition of gonadotropins by a nondialyzable factor.¹⁰⁶⁵

Hyperprolactinemia occasionally causes secondary amenorrhea without frank hypoestrogenism.¹⁰⁶⁶ This situation probably results from a mild diminution in FSH secretion that only inhibits the emergence of a dominant follicle and, therefore, ovulation.

Postpill amenorrhea has been a term applied to the amenorrhea that sometimes follows the long-term use of hormonal contraceptives. This in the past was attributed to oversuppression, but oversuppression should not be expected to be the case with the current generation of oral contraceptives.³³⁴ About one-third of patients with secondary amenorrhea after discontinuation of estrogen and progestin-containing pills have a history of previous menstrual disturbance and ongoing menstrual problems.¹⁰⁶⁷ Another third can expect spontaneous remission of the amenorrhea. About half of the remainder of cases will have resolution of their menstrual disturbance after induced pregnancy. The most common cause of postpill amenorrhea is probably hyperprolactinemia because over 20% of such cases have galactorrhea. How often this antedates ingestion of the contraceptive pill is unknown. Menses may be restored in normoprolactinemic cases by dopaminergic treatment, which suggests that in such cases there is excessive pituitary prolactin secretion that is too subtle to be detected by measurement of serum levels.¹⁰⁶⁸ The anovulation resulting from depot-medroxyprogesterone acetate contraception is related to the extremely slow rate of absorption and metabolism of this steroid; menses return when the blood levels of this progestin fall below the threshold for suppression of the LH surge,¹⁰⁶⁹ and only rarely has it been associated with disturbed prolactin secretion.¹⁰⁷⁰

Gonadotropin or hCG secretion by a tumor can cause normoestrogenic or hyperestrogenic anovulation.^{1071,1072} In one

LH-producing tumor, sex steroid levels were normal; the lack of virilization was attributed to ovarian desensitization to LH,¹⁰⁷¹ while in another, virilization occurred, which was attributed to preexisting polycystic ovary syndrome-hyperthecosis and extreme LH elevation.¹⁰⁷³ Other hyperestrogenic disorders that cause anovulatory bleeding are discussed under Precocious Puberty.

Hypothalamic anovulation is ordinarily a diagnosis of exclusion. The medical evaluation should be performed as discussed in the preceding section, with particular attention to the possibilities of emotional stressors, excessive exercise, the use of birth control pills or other drugs, and state of health. The physical examination should be particularly directed to the state of nutrition, the possibilities of intracranial or systemic disease, galactorrhea, thyroid dysfunction, glucocorticoid excess, hirsutism, and obesity. If this workup is negative, an MRI of the hypothalamic-pituitary area is indicated. Hypothalamic anovulation may be documented by demonstrating subnormal LH pulse frequency, but this is not generally practical. Leptin levels tend to be low but nondiagnostic.⁹⁸¹ The response to a GnRH agonist test is normal but seems to lack the normal priming response to repeat testing.¹⁰⁷⁴ Excessive uterine bleeding from hypothalamic anovulation must be distinguished from that caused by other causes (see next section).

Management. Many patients with hypothalamic anovulation will benefit from nutritional counseling. Diet faddists and athletes should be advised about the necessity of optimal body energy reserves for the maintenance of normal menstrual cycles (see Fig. 16.42). The teleological significance of this may be pointed out, namely, that inherent in the evolutionary process is the inhibition of pregnancy in times of inadequate food supplies. Ongoing psychological counseling is advisable for patients who cannot change their dietary or exercise patterns because of an abnormal body image. Estrogen replacement only partially corrects bone mineralization unless nutrition is optimized.^{996,1075} However, if spontaneous menses do not return after a 6- to 12-month trial of improved nutrition, modification of the patient's exercise regimen, and/or psychological counseling, short-term use of transdermal estradiol and cyclic oral progestins (not oral contraceptives or ethynodiol) should be considered in those with low bone density to protect the patient's long-term bone health.⁹⁹⁶ Obese girls should be advised that there is a substantial possibility that reduction to a normal weight will result in restoration of menses and improved probability of fertility.

Mature teenagers whose amenorrhea is unexplained should be assured that they have a high likelihood of fertility with appropriate endocrinological treatment. However, such treatment is unlikely to be of any benefit to them until such time as they desire to become pregnant. Meanwhile, the main objective of therapy is to normalize the endometrial cycle by periodic progestin administration. For this purpose, progestin (micronized progesterone 100–200 mg orally at bedtime for 14 consecutive days) usually is effective in inducing withdrawal periods. During the first few years after menarche, it is reasonable to administer this treatment on alternate months to allow detection of late maturation of a regular menstrual cycle.

Induction of an ovulatory cycle has been reported to occasionally result in resumption of spontaneous normal menses.¹⁰⁶⁷ An ovulatory cycle can normally be induced by the administration of clomiphene citrate once nightly for five doses. If treatment is successful, menses generally occurs about 1 month from commencement of the treatment. One should start with the 50-mg dose because larger doses may cause hyperstimulation of the ovaries with the development of ovarian cysts. For this reason, one should perform an ultrasound examination to

rule out cystic ovaries before going successively to 100 to 150 mg dosage. This treatment is not generally recommended in the teenage years, however. Dopaminergic therapy has been reported to be successful in causing the resumption of ovulation in postpill amenorrhea, modest undernutrition, and other unexplained cases of secondary amenorrhea. Otherwise, induction of ovulation is best left to the endocrinological gynecologist to supervise at such time as the woman wishes to conceive. The vast majority of patients with no obvious cause for their secondary amenorrhea will become pregnant after appropriate treatment with estrogen, clomiphene, dopaminergic agonist, human menopausal gonadotropins, or pulsatile GnRH therapy.

Excessive Uterine Bleeding Caused by Ovulatory Dysfunction

Causes. Heavy uterine bleeding may be abnormally frequent, as indicated by intervals less than 21 days, excessively prolonged, as indicated by menstrual flow that lasts more than 7 days, or heavy, as indicated by soaking more than one pad or tampon every 2 hours, passing large clots, or experiencing a sensation of gushing (Box 16.2).⁷⁴¹ It is usually caused by bleeding from a hyperplastic endometrium.⁸⁸² It is a manifestation of ovulatory dysfunction,¹⁰⁷⁶ which may result in anovulatory or immature ovulatory cycles.^{735,736,738} This “dysfunctional uterine bleeding” (DUB) is most often a manifestation of physiological adolescent anovulation (Box 16.7). Hyperandrogenism, particularly polycystic ovary syndrome, and coagulopathy are the most common causes of severe anemia, which can be life-threatening. Less common causes are hypothalamic anovulation, estrogen-

producing cysts or tumors, hypothyroidism, hyperprolactinemia, and incipient premature ovarian failure. The workup should therefore include measurement of serum androgen, estradiol, prolactin, thyroid, gonadotropin levels and an evaluation for bleeding disorders.

Luteal Phase Defects

A minimum serum level of progesterone must be generated by the corpus luteum for the endometrium to sustain sufficient development to support implantation.^{1077,1078} A lesser level is a cause of “luteal insufficiency” as a cause of infertility. Luteal insufficiency may present as short cycles (<21 days) with frequent menstrual bleeding or as infertility with normal menstrual cycles in which the short luteal phase follows a prolonged follicular phase (see Fig. 16.35). It is normal during the early post-menarcheal years.⁷³⁸

A major determinant of normal corpus luteum formation and function is optimal development of the corpus luteum predecessor, the dominant follicle. Luteal insufficiency¹⁰⁷⁷ may arise from subtle deficiency of inhibin, LH or FSH during the follicular phase, an inadequate preovulatory LH surge, or corpus luteum unresponsiveness to LH.^{1077,1079,1080} These abnormalities all cause incomplete emergence of a dominant follicle and the subsequent formation of an inadequate corpus luteum. Luteal insufficiency is common during early postmenarcheal cycles,^{736,738} where it is considered to be caused by ovulatory immaturity (see Fig. 16.35) and may otherwise be the result of hyperprolactinemia,¹⁰⁸¹ obesity,¹⁰⁵⁵ hypothalamic amenorrhea, or hyperandrogenism.

Daily measurements indicate that the minimum peak serum progesterone level normally reached during the middle of the normal luteal phase ranges from 900 to 3000 ng/dL (28–95 nmol/L).^{1077,1082} Both cyclic and hourly variations in progesterone secretion make it difficult to document the minimally adequate level. Thus current clinical practice commonly defines evidence of ovulation as a progesterone level over 300 to 500 ng/dL (9–15 nmol/L),^{735,1083} approximately sufficient to raise the basal body temperature.¹⁸⁸ The lower of these cutoffs is about twice the normal maximum the day before ovulation,^{110,188} and the highest of these does not necessarily indicate a normal mature ovulatory cycle. However, a progesterone value greater than 165 ng/dL in an adolescent indicates that ovulation has occurred, although not necessarily a mature luteal phase.⁷³⁸

Differential Diagnosis. DUB must be distinguished from the other causes of abnormal genital bleeding listed in Box 16.7.^{882,1076,1084,1085} The possibility that it is pregnancy related must be considered and a pregnancy test performed. Sexual abuse is a prime consideration in recurrent vaginal bleeding. Genital tract or feminizing tumors characteristically cause bleeding that cannot be controlled with cyclic progestin or estrogen-progestin therapy. Abnormally heavy menstrual flow in adolescents is often idiopathic (“essential menorrhagia”); it is theorized to result from imbalance of vasodilating and vasoconstricting prostaglandin action on the endometrium. However, pathological causes must be considered because bleeding disorders are present in about 20% of adolescents with menorrhagia requiring hospitalization and in 50% of those presenting at menarche. Patients requiring hospitalization for abnormal bleeding should have a platelet count, prothrombin time, partial thromboplastin time, bleeding time, and von Willebrand factor level performed.^{1084,1085} Transvaginal ultrasound, which is not often feasible in the virginal adolescent, is as reliable as hysteroscopy in determining whether or not the endometrial cavity is normal.

Failure of serum progesterone to rise over 500 ng/dL during the luteal phase is diagnostic of corpus luteum insufficiency with 71% accuracy.¹⁰⁷⁸ However, a higher progesterone level than this must be sustained to transform the endometrium sufficiently to support implantation.

BOX 16.7 Differential Diagnosis of Excessive Genital Bleeding in Adolescence

- Ovulatory dysfunction (“dysfunctional”) uterine bleeding
 - Physiological anovulation (perimenarcheal)
 - Hyperandrogenism
 - Polycystic ovary syndrome
 - Hyperestrogenism
 - Feminizing tumor
 - Hypothyroidism
 - Hypothalamic anovulation
 - Hyperprolactinemia
 - Chronic disease
 - Incipient premature ovarian failure
 - Luteal phase defects
- Pregnancy-related uterine bleeding
 - Threatened, missed, or incomplete abortion
 - Molar pregnancy
 - Ectopic pregnancy
- Uterine tumor, polyp, adenomyosis
- Coagulopathy
- Endometrial
 - Idiopathic (“essential menorrhagia”)
 - Intrauterine device
- Iatrogenic
 - Breakthrough bleeding (intrauterine device or contraceptive pills)
- Vaginal bleeding
 - Trauma
 - Tumor
 - Foreign body
 - Infection

(From Rosenfield, R.L., Barnes, R.B. (1993). Menstrual disorders in adolescence. *Endocrinol Metab Clin North Am*, 22, 491.)

Management. Nonsteroidal antiinflammatory agents may lessen DUB. An estrogen-progestin oral contraceptive with 35 mcg ethinyl estradiol is first-line treatment to stop dysfunctional bleeding that is acute or associated with anemia. For active bleeding, the dosage is advanced rapidly until bleeding stops, up to 4 times daily, and then sustained for 7 days. Treatment is then stopped for 5 days, and the patient warned that heavy withdrawal bleeding with cramps may occur. Therapy with a low-dose pill, given as for contraception, is then begun to prevent recurrence of dysfunctional bleeding and is continued for about 3 cycles. Hemoglobin should be monitored and supplemental iron prescribed.

Cyclic progestin can be used as an alternative to the OCP to prevent recurrent dysfunctional bleeding in a patient who is not sexually active. Micronized progesterone 100 to 200 mg/day for 1 week is given at 3- to 4-week intervals. After the third-month, therapy is stopped and the patient is observed for 1 to 2 months for spontaneous bleeding. If none occurs the progestin can be given every other month (e.g., medroxyprogesterone 5–10 mg) for 7 to 14 days to prevent recurrent dysfunctional bleeding. If in the progestin-free month a normal spontaneous menstrual period occurs, progestins are withheld in the subsequent month to determine if the patient has developed regular ovulatory cycles.

Hemodynamic instability due to severe and/or acute blood loss is an indication for hospitalization and treatment with an antifibrinolytic drug (e.g., aminocaproic acid 100 mg/kg or tranexamic acid 10 mg/kg) begun IV, and intravenous fluids and blood products as necessary. Premarin® can be administered in a dose of 25 mg intravenously every 3 to 4 hours for 3 to 4 doses. When medical management fails, a bleeding diathesis or uterine structural abnormality should be considered (see later). If heavy bleeding persists, in spite of hormonal and anti-fibrinolytic treatment, surgical intervention is indicated by a gynecologist.^{1084,1085}

Unexplained ("essential") menorrhagia is treated much the same way as dysmenorrhea. OCP therapy will decrease menstrual blood loss by about 50% in these women. Antiprostaglandins, such as naproxen 500 mg twice a day, decrease blood loss nearly as effectively.

Perimenstrual Symptoms

Dysmenorrhea. Uterine cramping is characteristic of normal ovulatory cycles, apparently as the result of prostaglandin release within the endometrium upon the withdrawal of progesterone. Pain with menses becomes a source of morbidity in 14% of adolescents.¹⁰⁸⁶ When pain is acute and qualitatively different from the usual menstrual pain, ectopic pregnancy must be considered.^{1054,1087} An ectopic pregnancy often causes vaginal bleeding that occurs 2.5 weeks later than the time of the expected next menstrual period and is typically light. However, the bleeding may be heavy and so resemble an episode of dysfunctional uterine bleeding. Ectopic pregnancy is usually diagnosable by a combination of ultrasonography, a serum β-hCG level over 1000 IU/L, and a progesterone level of less than 2500 ng/dL.

In patients with chronic pelvic pain unresponsive to anti-prostaglandins or OCP psychological overlay is possible, but attention should be directed to the possibility of endometriosis, uterine outlet obstruction, gynecological tract masses, or the poorly defined entity of *vulvodynia*.¹⁰⁸⁸ Ultrasonography and laparoscopy may be indicated to further evaluate these patients. Endometriosis is an estrogen-dependent disorder that accounts for approximately half of the cases of chronic pelvic pain in teenagers.¹⁰⁸⁹ Genetic factors and congenital obstruction of the genital tract predispose to endometriosis,

and aberrant estradiol formation in endometrial stroma has been incriminated in the pathogenesis.¹⁰⁹⁰ GnRH agonist therapy is approved to provide symptomatic relief, but adolescents are at particular risk for bone loss, and progestin therapy may be an effective alternative.

Dysmenorrhea may be ameliorated by antiprostaglandin therapy. Naproxen (275 mg 4 × daily [QID] after a 550 mg loading dose) has been shown to be superior to aspirin (650 mg QID) or placebo when begun 2 days before the anticipated onset of menses.¹⁰⁹¹ The OCP is an alternative that will relieve dysmenorrhea in about 90% of cases by reducing endometrial mass.⁸⁸² Because smoking, alcohol intake, and excessive weight are risk factors, lifestyle counselling is advisable.

Premenstrual Syndrome. This is the term applied when cyclic mood changes confined to the second half of the menstrual cycle become debilitating.¹⁰⁹² It is often disruptive to women's personal, social, and occupational function. If symptoms of marked mood swings, depressed mood, anxiety, and irritability occur, it is classified as premenstrual dysphoric disorder.¹⁰⁹³ Neuropsychiatric symptoms may include epilepsy¹⁰⁹⁴ and bizarre behavior.¹⁰⁹⁵ These seem to represent aberrant responses to normal cyclic hormonal changes.¹⁰⁹⁶ Subnormal activation of the hypothalamic-pituitary-adrenal axis in response to progesterone has been found.¹⁰⁹⁷ Some evidence indicates that variation in the degree of progesterone metabolism to neuroactive steroids affects the severity of symptomatology.¹⁰⁹⁸ Oral contraceptive therapy with the anti-mineralocorticoid progestin drospirenone is indicated if psychotropic therapy is unsuccessful. Downregulation of pituitary gonadotropin secretion by GnRH agonist therapy is efficacious, but its usefulness is limited by the side effects of estrogen deficiency. The relationship of premenstrual syndrome to other luteal phase symptomatology, such as recurrent fever and autoimmune symptoms, may be related to hyperresponsiveness of cytokines to progesterone.^{1099,1100}

Hyperandrogenism in Adolescence

Hyperandrogenism of a mild to moderate degree is the most common cause of persistent normoestrogenic menstrual abnormality and/or hirsutism. Hyperandrogenism developing during adolescence is usually caused by polycystic ovary syndrome (PCOS), but the differential diagnosis of hyperandrogenism includes other ovarian or adrenal disorders, abnormal peripheral formation of androgen, and drugs (see Box 16.8).^{40,1101}

Causes

The definition of PCOS has evolved since its description by Stein and Leventhal as a syndrome of amenorrhea and polycystic ovaries, often accompanied by hirsutism, acne, and/or obesity.¹¹⁰² Over recent decades, internationally accepted diagnostic criteria were developed for adults based on various combinations of otherwise unexplained hyperandrogenism, anovulation, and a polycystic ovary, which are all encompassed by Rotterdam consensus criteria.⁴⁰ These criteria generate four phenotypes, which are here listed in order of the decreasing diagnostic specificity of the phenotypes (Box 16.9) that underlies the controversies in the acceptance of the milder types. Insulin resistance, obesity, and LH excess, although not diagnostic features of the syndrome, are common and contribute to its pathogenesis; like hyperandrogenism, their severity has generally proven to correlate with specificity. In 2015 consensus diagnostic criteria for adolescent PCOS were developed by international pediatric subspecialty societies: these are an

BOX 16.8 Causes of Adolescent Hyperandrogenism

Physiological Adolescent Anovulation

Functional Gonadal Hyperandrogenism

- PCOS: Primary functional ovarian hyperandrogenism (common form of polycystic ovary syndrome)
- Secondary polycystic ovary syndrome
 - Virilizing congenital adrenal hyperplasia
 - Adrenal rests of the ovaries
 - Syndromes of severe insulin resistance
 - Acromegaly
 - Epilepsy ± valproic acid therapy
- Ovarian steroidogenic blocks
- Disorders of sex development
- Chorionic gonadotropin excess

Functional Adrenal Hyperandrogenism

- PCOS: Primary functional adrenal hyperandrogenism (uncommon form of polycystic ovary syndrome)
- Virilizing congenital adrenal hyperplasia
- Other glucocorticoid-suppressible functional adrenal hyperandrogenism
- Prolactin excess
- Cortisone reductase (and apparent cortisone reductase) deficiency
- Apparent dehydroepiandrosterone sulfotransferase deficiency
- Glucocorticoid-resistant functional adrenal hyperandrogenism
 - Cushing syndrome (endogenous: adrenal hyperplasia or neoplasm)
 - Glucocorticoid resistance

Peripheral Androgen Overproduction

- Idiopathic hyperandrogenism
- Obesity
- Portohepatic shunting

Virilizing Drugs

Androgenic Drugs

(Modified with permission from Buggs, C., Rosenfield, R.L. (2005). Polycystic ovary syndrome in adolescence. *Endocrinol Metab Clin North Am*, 34, 677–705.)

age- and stage-appropriate modification of the “National Institutes of Health criteria” (i.e., otherwise unexplained persistent hyperandrogenic anovulation) (see Box 16.9).^{124,1101,1103}

The adolescent consensus criteria attempt to strike a balance between under- and overdiagnosis of the syndrome. Normal adolescents often have menstrual cycles that are abnormal by adult standards, so anovulatory criteria must be age- and pubertal stage-appropriate (see Box 16.2). Persistence of the menstrual abnormality is required to avoid misdiagnosing physiological adolescent anovulation as PCOS. Unlike adult standards, mild hirsutism is not a criterion for hyperandrogenism because half of mild hyperandrogenism without menstrual abnormality is a normal variant.⁶⁴⁷ Polycystic ovary morphology (PCOM) is not a criterion because many normal adolescents meet adult PCOM criteria and adolescent norms are not well defined.

Clinical Manifestations. PCOS in adolescents resembles that in adults, with similar clinical and endocrinological heterogeneity. The cardinal symptoms typically begin when mature gonadotropin levels are achieved, in the perimenarcheal stage, so PCOS has been documented in children as young as 10 years of age. Cases with “ovulatory PCOS” have a subtle ovulatory abnormality that escapes notice until presentation

BOX 16.9 Diagnostic Criteria for Polycystic Ovary Syndrome**ADULT DIAGNOSTIC CRITERIA (ROTTERDAM)****Otherwise Unexplained Alternative Phenotypes:**

- A. Phenotype 1 (“Classic polycystic ovary syndrome [PCOS]”/“Stein-Leventhal syndrome”)^a
 - a. Clinical and/or biochemical evidence of hyperandrogenism
 - b. Evidence of oligo-anovulation
 - c. Ultrasonographic evidence of a polycystic ovary
- B. Phenotype 2 (Essential National Institutes of Health Criteria)^a
 - a. Clinical and/or biochemical evidence of hyperandrogenism
 - b. Evidence of oligo-anovulation
- C. Phenotype 3 (“Ovulatory PCOS”)
 - a. Clinical and/or biochemical evidence of hyperandrogenism
 - b. Ultrasonographic evidence of a polycystic ovary
- D. Phenotype 4 (Nonhyperandrogenic PCOS)
 - a. Evidence of oligoanovulation
 - b. Ultrasonographic evidence of a polycystic ovary

ADOLESCENT DIAGNOSTIC CRITERIA**Otherwise Unexplained Combination of:**

1. Abnormal uterine bleeding pattern
 - Abnormal for age or gynecological age
 - Persistent symptoms for 1–2 years
2. Evidence of hyperandrogenism
 - Persistent testosterone elevation above adult norms in a reliable reference laboratory is the best evidence
 - Moderate-severe hirsutism is clinical evidence of hyperandrogenism
 - Moderate-severe inflammatory acne vulgaris is an indication to test for hyperandrogenemia

(Modified and reprinted with permission from Rosenfield, R.L. (2015). The diagnosis of polycystic ovary syndrome in adolescents. *Pediatrics*, 136, 1154–1165.)

in adulthood with unexplained infertility¹¹⁰⁴ or recurrent miscarriages.¹¹⁰⁵

Persistent symptomatic menstrual abnormality (primary or secondary amenorrhea, oligo-amenorrhea, or excessive uterine bleeding) (see Box 16.2) in a normally feminized girl may be the only manifestation of hyperandrogenism. In about one-third of cases, menstrual dysfunction occurs in the absence of cutaneous manifestations.

An abnormal menstrual pattern usually constitutes evidence of oligoanovulation. The menstrual pattern should be interpreted in the context of the patient’s gynecological age (see Box 16.2), as discussed earlier in the section Physiological Adolescent Anovulation. Although persistence of menstrual abnormality for 2 years is required for diagnosing PCOS in an adolescent (see Box 16.9), a provisional diagnosis can—and should—be made within 1 year in circumstances that require treatment.¹²⁴ PCOS is a state of relative, not absolute, infertility in which ovulation often occurs unpredictably and, on the other hand, in which menstrual regularity does not necessarily indicate ovulatory regularity.

The cutaneous signs of hyperandrogenism are variably expressed manifestations of androgen excess. They are present in about two-thirds of cases. Hirsutism is the most common pilosebaceous manifestation. However, acne, or uncommonly seborrhea or balding, may be the only pilosebaceous manifestation of hyperandrogenism.^{1106,1107}

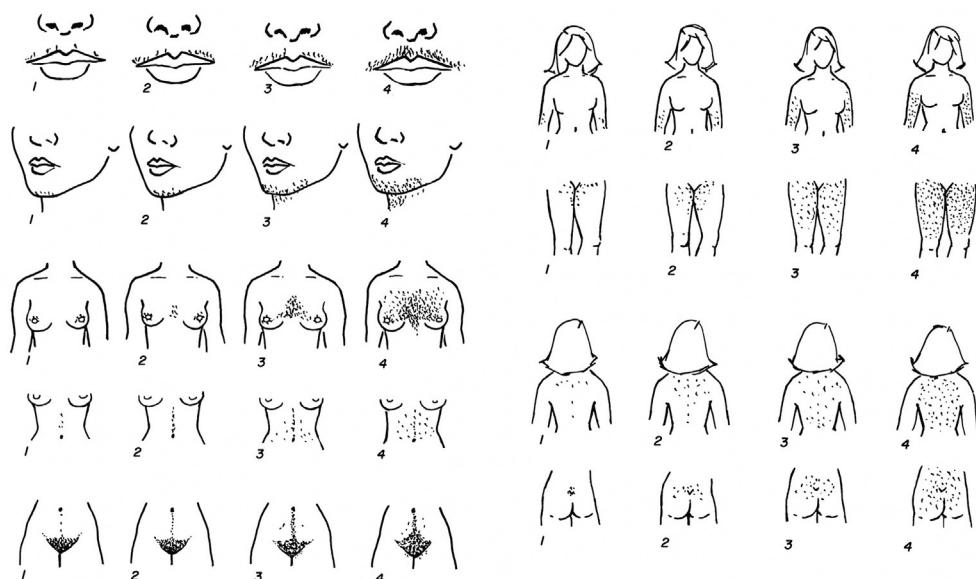


Fig. 16.46 Ferriman-Gallwey hirsutism scoring system. Each of the nine body areas that is most sensitive to androgen is assigned a score from 0 (no hair) to 4 (frankly virile), and these are summed to provide a hormonal hirsutism score. Generalized hirsutism (score ≥ 8) is abnormal in the general US population, whereas locally excessive hair growth (score 1–7) is a common normal variant that may be cosmetically important to patients. The normal score is lower in some Asian populations and higher in Mediterranean, Hispanic, and Middle Eastern populations (see text). (From Hatch, R., Rosenfield, R.L., Kim, M.H., Tredway, D. (1981). Hirsutism: implications, etiology, and management. *Am J Obstet Gynecol*, 140, 816–830. With permission.)

Hirsutism is defined clinically as excessive terminal hair that appears in a male pattern (sexual hair).⁶⁴⁷ It is commonly graded according to the hormonal Ferriman-Gallwey system, which quantitates the extent of hair growth in the most androgen-sensitive areas (see Fig. 16.46). The normal score is lower in some Asian populations and higher in Mediterranean, Hispanic, and Middle Eastern populations.

The absence of hirsutism in approximately one-third of hyperandrogenic adults appears to be because of relatively low sensitivity of their pilosebaceous unit to androgens. Thus biochemical hyperandrogenemia may be entirely cryptic, manifesting neither cutaneous signs nor anovulatory symptoms. Conversely, hirsutism without elevated circulating levels of androgen or other clinical evidence of hyperandrogenism—“idiopathic hirsutism”—accounts for approximately one-half of mild hirsutism and one-sixth of moderate-severe hirsutism.⁶⁴⁷

Although comedonal acne is common in adolescent girls, moderate-severe (>10) inflammatory acneform lesions in any area of the skin is uncommon during the perimenarcheal years and is an indication for testing for hyperandrogenemia.¹²⁴

Acanthosis nigricans, a manifestation of insulin-resistant hyperinsulinism, and associated obesity are the presenting complaints in about one-third of cases, sometimes before menstrual abnormalities develop.^{1108,1109} About one-half of PCOS patients are obese.¹¹⁰¹ The obesity occasionally begins in mid-childhood. Central body fat content is increased independently of BMI, and at least one-third of normal-weight PCOS patients have increased intraabdominal fat.^{40,1110}

Laboratory Manifestations. Persistent elevation of serum testosterone above adult norms in a reliable reference laboratory is the best evidence of hyperandrogenism (Box 16.9).¹²⁴ The problematic nature of many androgen assays is discussed later in the section Differential Diagnosis. Elevated serum free testosterone is the single most sensitive indicator of hyperandrogenemia because the bioactive portion of the serum testosterone is the free fraction.⁶⁴⁷ SHBG serum concentrations govern the fraction of testosterone that is free; they are lowered by obesity

and androgen excess itself. Androstenedione and DHEAS serum levels are often elevated in PCOS and should be measured if androgen excess is strongly suspected in spite of normal total and free testosterone levels. Serum DHT levels are of little diagnostic value.

A unique type of functional ovarian hyperandrogenism (primary FOH) can be documented in 85% of PCOS cases by specific tests of ovarian androgenic function.⁴⁰ The GnRH agonist test and hCG tests evaluate the gonadal response to, respectively, endogenous gonadotropin release or exogenous administration of the LH analogue hCG. In two-thirds of PCOS patients, these tests show a distinctive pattern of ovarian steroidogenic hyperresponsiveness, of which 17-OHP hyperresponsiveness is the best marker; there is no evidence of a steroidogenic block, and, indeed, estradiol is significantly hyperresponsive. The dexamethasone androgen-suppression test (DAST)—predicated on the principle that residual concentrations of androgens after suppression of adrenal function by glucocorticoid administration ordinarily arise from the ovary—shows elevated testosterone post-DAST in 80% of PCOS patients: this includes most with 17-OHP hyperresponsiveness to gonadotropin stimulation and two-thirds of those who lack it. These tests are indicated only in the case of diagnostic uncertainty.

A related adrenal steroidogenic defect is found in 25% to 50% of PCOS: primary functional adrenal hyperandrogenism (FAH).⁴⁰ This is indicated by DHEA hyperresponsiveness to ACTH; this correlates ($r = 0.7$) with DHEAS elevation. Isolated FAH is the sole steroidogenic defect in about 5% of PCOS.

The identification and clinical significance of PCOM presents quandaries.¹²⁴ Ultrasonographically, PCOM has been defined in adults by consensus criteria as an ovary with a volume greater than 10.0 cc by a simplified formula or a small antral follicle (2–9 mm diameter) count of 12 or more per ovary. However, these criteria have become problematic in young adults, particularly because the newer high-definition vaginal imaging techniques show that small antral follicle counts up to 24 are normal. Adult PCOM criteria are especially problematic when applied to adolescents. For one thing, an

accurate antral follicle count cannot be defined by the abdominal ultrasonographic approach necessary in virginal adolescents. Furthermore, current data suggest that ovarian volume and antral follicle count are slightly higher in adolescents than in adults. Consequently, one-third to half of normal adolescents meet adult criteria for PCOM. Until further research establishes definitive criteria, current evidence suggests that a mean ovarian volume of 12 cc (or single ovary >15 cc) be considered enlarged in adolescents, and PCOM are not a diagnostic criterion for PCOS in adolescents.

PCOM is variably related to hyperandrogenism.^{40,124} On one hand, it is absent in 5% to 20% of adult PCOS. On the other, PCOM is a common finding among healthy women. When care has been taken to exclude those with PCOS features, about one-quarter of apparently normal volunteers with PCOM have mild subclinical androgenic ovarian dysfunction that is in the PCOS range; it has been postulated that these are carriers of PCOS or at risk for PCOS.

AMH levels are independently associated with PCOM, because they indicate an increased number of small growing follicles, and with hyperandrogenism.⁴⁰ Although a mildly increased AMH level is common in asymptomatic females with PCOM and has been suggested as a surrogate indicator of it, AMH elevation of twofold or more above the upper normal limit suggests PCOS with high specificity.

Serum LH and the LH/FSH ratio are increased in about half of PCOS subjects.⁴⁰ Accumulating evidence suggests that LH levels in PCOS are determined by the severity of hyperandrogenemia, moderate degrees of which stimulate LH production, and the extent of obesity, which suppresses LH levels.

Insulin resistance in PCOS is significant independently of obesity.⁴⁰ Insulin resistance interacts with obesity and age to cause metabolic syndrome, which confers risk for cardiovascular disease,^{40,111} sleep-disordered breathing,^{1112,1113} and nonalcoholic fatty liver disease.¹¹¹⁴

Approximately one-half of PCOS patients have an abnormal degree of insulin resistance, and about one-half of these have metabolic syndrome. Insulin resistance, as determined by euglycemic clamp, is present in 50% of obese adolescents with PCOS compared with BMI-matched controls¹¹¹⁵ (75% compared with normal-BMI controls) and about one-half of such girls have metabolic syndrome.¹¹¹⁶ Adolescents with PCOS are thus at increased risk for glucose intolerance.¹¹¹⁷ They are also predisposed to the pancreatic beta-cell dysfunction of type 2 diabetes mellitus.¹¹¹⁸ Dyslipidemia prevalence is generally low, but it is related to the degree of obesity and insulin resistance.^{1116,1119–1121}

Pathophysiology. PCOS ordinarily seems to be caused by intraovarian androgen excess (see Fig. 16.47).⁴⁰ Primary FOH accounts for the vast majority (about 85%) of PCOS (Fig. 16.48).^{40,1101} Functionally typical PCOS, constituting two-thirds of cases, is caused by typical FOH, which occurs because of a unique type of steroidogenic dysfunction, dysregulation of steroidogenesis. A related dysregulation of adrenal dysregulation, FAH, occurs in both typical and atypical FOH.¹¹²²

Typical FOH is characterized by generalized steroidogenic hyperresponsiveness to LH, of which 17-OHP is the most consistent marker. One-third of PCOS is functionally atypical, in that the 17-OHP abnormality is not demonstrable. One-half of these have functionally atypical FOH, identifiable by subnormal androgen suppression by dexamethasone. In the remainder, which constitutes a small minority (15%) of PCOS cases, an ovarian source for androgen excess, is not demonstrable. The androgen excess of these latter functionally atypical PCOS cases appears to arise from isolated FAH in about one-third of cases and from obesity in most of the remainder. These are exceptions to the general rule that modest extraovarian androgen excess does not interfere with ovarian function.

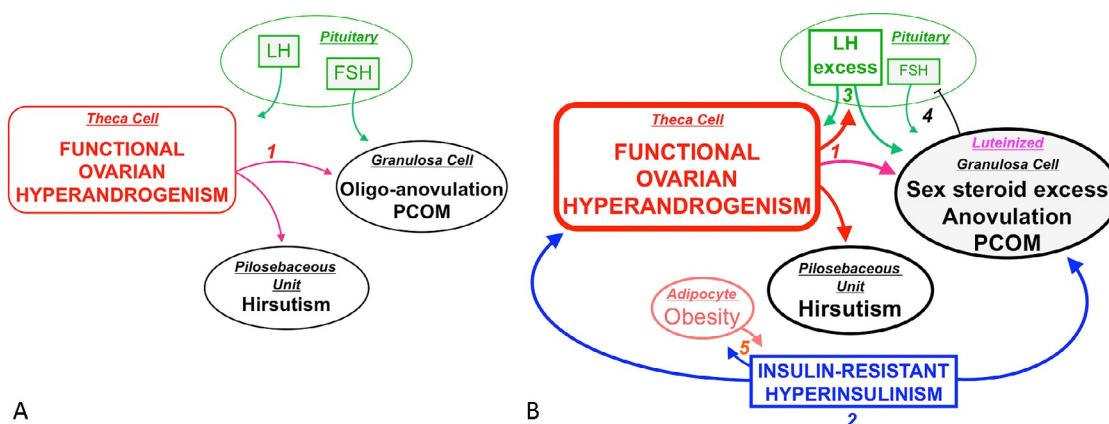


Fig. 16.47 Unified minimal model of polycystic ovary syndrome (PCOS) pathophysiology. Panel A: Functional ovarian hyperandrogenism (FOH) is present in nearly 90% of PCOS and can account for all the cardinal clinical features of the syndrome: hyperandrogenemia, oligoanovulation, and polycystic ovaries (step 1). Pituitary luteinizing hormone (LH) secretion is necessary to sustain the ovarian androgen excess, but it is not sufficient to cause it. Panel B: About one-half of patients with FOH have an abnormal degree of insulin-resistant hyperinsulinism (step 2). Insulin-resistant hyperinsulinism acts on theca cells to aggravate hyperandrogenism, synergizes with androgen in prematurely luteinizing granulosa cells, and stimulates fat accumulation. The increased hyperandrogenemia provokes LH excess, which then acts on both theca and luteinized granulosa cells to worsen hyperandrogenism (step 3). LH also stimulates luteinized granulosa cells to secrete estradiol (step 4), which suppresses follicle-stimulating hormone (FSH) secretion. These hyperinsulinism-initiated changes in granulosa cell function further exacerbate polycystic ovary morphology (PCOM) and further hinder ovulation. Obesity increases insulin resistance, and the resultant increased hyperinsulinism further aggravates hyperandrogenism (step 5). Boldness and enlarged font represents greater severity. Both FOH and insulin resistance typically have an intrinsic basis. This model does not exclude the possibility that the unknown intrinsic ovarian defects that underlay the ovarian steroidogenic dysfunction also involve granulosa cell folliculogenesis and other systems as well. The figure also does not depict other associated defects, such as the functional adrenal hyperandrogenism that often accompanies the ovarian hyperandrogenism and the contribution of excess adiposity to peripheral androgen production and gonadotropin suppression. (Modified from Rosenfield, R.L., Ehrmann, D.A. (2016). The pathogenesis of polycystic ovary syndrome (PCOS): the hypothesis of PCOS as functional ovarian hyperandrogenism revisited. *Endocr Rev*, 37, 467–520. With permission.)

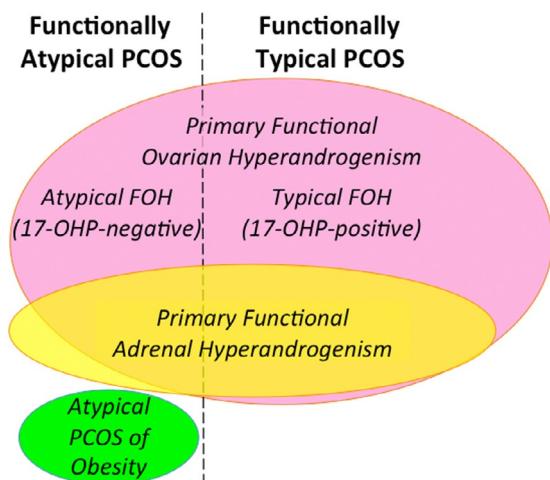


Fig. 16.48 Functional categorization of polycystic ovary syndrome (PCOS) in relationship to source of androgen and its prevalence. Most PCOS (85%) is caused by primary functional ovarian hyperandrogenism (FOH); about 30% of these have an associated primary functional adrenal hyperandrogenism. About two-thirds of PCOS have functionally typical PCOS, that is, they have typical FOH, which is characterized by hyperresponsiveness of 17-OHP to a GnRHag or hCG test. The remaining one-third of PCOS cases have functionally atypical PCOS, lacking 17-OHP hyperresponsiveness. Most of these have functionally atypical FOH, in which ovarian androgen excess is indicated by a dexamethasone androgen-suppression test. A small number (5%) are caused by isolated functional adrenal hyperandrogenism. In a minority of cases, the source of androgen cannot be identified as ovarian or adrenal: most of these have the functionally atypical PCOS of obesity (5%); the source of the androgen is unexplained (idiopathic) in less than 20% of the functionally atypical PCOS group ($\geq 5\%$ of PCOS). 17-OHP, 17-Hydroxyprogesterone. (Modified and reproduced with permission from Rosenfield, Polycystic ovary syndrome in adolescents. In: Rose BD, ed. UpToDate: 2014. Waltham, MA, www.uptodate.com/index.)

Dysregulation of steroidogenesis seems to result from imbalance among the various intrinsic and extrinsic factors involved in the modulation of trophic hormone action.⁴⁰ Within the ovary, there appear to be flaws in the processes that normally coordinate androgen and estrogen secretion in response to gonadotropin stimulation (see Fig. 16.19). Theca cells from polycystic ovaries of classic PCOS patients in long-term culture have an intrinsic steroidogenic dysregulation that can account for these steroidogenic abnormalities: these cells constitutively overexpress most steroidogenic enzymes, most prominently at the level of 17-hydroxylase and 17,20-lyase activities, both properties of P450c17, which are the rate-limiting steps in the biosynthesis of testosterone precursors. As in the ovary, dysregulation of local steroidogenic regulatory processes within the adrenal cortex appears to cause a characteristic type of primary FAH in which excessive dehydroepiandrosterone is formed in response to ACTH as a by-product of cortisol secretion.

As a consequence of dysregulated steroidogenesis, PCOS theca cells do not undergo the normal homologous desensitization to LH with downregulation of steroidogenesis in response to excess LH stimulation. Therefore, they are hypersensitive to LH stimulation. "Escape" from homologous desensitization to LH excess appears to be the basis for the distinctive pattern of steroidogenic hyperresponse to GnRH agonist or hCG testing, which is characterized by disproportionate

hyperresponsiveness of 17-OHP relative to other ovarian steroids without evidence of a steroidogenic block.

Granulosa cell functions are also defective in PCOS.⁴⁰ Folliculogenesis of small follicles is excessive, which has been attributed to androgen excess, although a primary granulosa cell defect cannot be ruled out, accounting for excess AMH production. Inhibin-B hyperresponsiveness to FSH also appears to aggravate thecal androgen secretion via a paracrine action and also contributes to tamping down FSH production.

The reason for the high prevalence of obesity in PCOS is not entirely clear.⁴⁰ Obesity increases insulin resistance, and in turn the insulin-resistant hyperinsulinism seems to enhance obesity because insulin signaling in human subcutaneous adipose tissue is intact in PCOS. The mechanisms by which obesity causes insulin resistance are also unclear, but they include deficient insulin-sensitizing adipokines, such as adiponectin, and excessive proinflammatory cytokines, such as TNF-alpha, which both aggravate insulin resistance and hyperandrogenism, that are secreted by unique adipose tissue macrophages.^{40,1123} Hyperandrogenism also sensitizes circulating mononuclear cells to secrete inflammatory cytokines in response to glucose and saturated fat ingestion. Visceral fat contributes more to PCOS insulin resistance than does abdominal fat because its lipolytic response to catecholamines, the major lipolytic stimulus in man, is uniquely enhanced.⁴⁰

Insulin resistance is an inconsistent, aggravating factor in the pathogenesis of FOH that is present in about half of cases (Fig. 16.47, panel B). It is usually mild, particularly in the developmental (adolescent) phase of the syndrome. It does not account for the intrinsic theca cell dysfunction characteristic of ordinary PCOS and only plays a *primary* pathogenic role in the development of the PCOS that complicates severe or extreme insulin-resistance syndromes.

The insulin resistance of PCOS is selective for the metabolic effects of insulin.⁴⁰ This creates a paradox in which the hyperinsulinemia compensatory for resistance to the glucose-metabolic effect of insulin, nevertheless, elicits excess insulin action in some tissues. Thus the steroidogenic, mitogenic, and protein-metabolic actions of insulin remain intact.

This insulin-resistant hyperinsulinism (hyperinsulinism) aggravates the ovarian and adrenal steroidogenic dysregulation of PCOS.⁴⁰ It is closely associated with the anovulation of PCOS. Insulin, like IGFs, modulates gonadotropin and ACTH action. Consequently in excess it synergizes with them. In the ovary insulin synergizes with LH by increasing theca cell LH receptor site expression to cause "escape" from desensitization. Consequently, hyperinsulinism enhances theca cell androgen production and theca and stromal cell hyperplasia. Insulin excess also synergizes with androgen to prematurely luteinize granulosa cells by inducing LH receptor expression, thus enhancing estrogen, progesterone, and inhibin-B production and arresting dominant follicle development.

Hyperinsulinism also seems to play a role in mediating the development of obesity in PCOS.^{40,1124} The transcription factor KLF15 seems to play an important role in mediating insulin stimulation of both adipogenesis and testosterone formation.⁵⁶³

PCOS is gonadotropin dependent; gonadotropins are necessary for the expression of gonadal steroidogenic enzymes.⁴⁰ However, LH excess does not seem to ordinarily be the fundamental cause of the hyperandrogenism. LH excess is variable (found in 50%–75% of cases) and seems to depend on androgen-obesity balance, which, respectively, raise and lower gonadotropin levels. Homologous desensitization normally limits the androgenic response to LH elevation: thus LH excess alone seems unlikely to cause the hyperandrogenism of PCOS, although it may aggravate it in PCOS, where intrinsic ovarian dysfunction causes "escape" from desensitization. Evidence is accumulating that androgen excess causes LH excess by

interfering with progesterone-estrogen negative feedback effect on LH secretion. Nevertheless, the possibility of a primary role for LH excess remains, particularly in the PCOS that is secondary to congenital virilizing disorders.

A model of the pathophysiology of PCOS is shown in Fig. 16.47.⁴⁰ The high intraovarian androgen concentration arising from the thecal cell dysfunction of FOH can cause all the essential features of the syndrome (Fig. 16.47, panel A). Excess androgen secretion causes the pilosebaceous manifestations of the syndrome. The locally very high intraovarian androgen concentrations act on granulosa cells to hinder the emergence of a dominant follicle, causing oligoanovulation. This paracrine effect of great androgen excess is coupled with its stimulation of increased proliferation of small follicles causing PCOM. Insulin-resistant hyperinsulinism aggravates PCOS severity by acting in conjunction with androgen excess to prematurely luteinize follicles, causing thecal and stromal hyperplasia. The moderately increased hyperandrogenemia causes secondary LH elevation by interfering with female hormone negative feedback; meanwhile, estrogen and inhibin-B excess inhibit FSH secretion. In the presence of the escape from homologous desensitization induced by hyperinsulinism, this LH excess amplifies the ovarian dysfunction. The hyperinsulinism also promotes adiposity, which in turn aggravates the insulin-resistant state.

In primary FAH, the steroidogenic pattern of response to ACTH resembles an exaggerated adrenarche and in the past was confused with nonclassic 3 β -HSD deficiency.⁴⁰ This FAH has been associated with mild adrenal enlargement and a small degree of autonomous adrenocortical function in some cases.^{40,1125} The pattern of adrenal secretion is compatible with dysregulation of zona reticularis steroidogenesis prominent at the level of the 17-hydroxylase/17,20-lyase activities of P450c17 and it correlates with evidence of the related ovarian dysregulation.¹¹²⁶ Nevertheless, it is the only steroidogenic abnormality in a small minority of PCOS.

The remaining PCOS cases lack evidence of steroid secretory abnormalities. Most of these are obese (Fig. 16.48), a few are idiopathic. Excess adipose tissue appears sufficient to account for hyperandrogenic anovulation and thus cause the atypical PCOS of obesity.⁴⁰ Adipocytes form testosterone from androstanedione via type 5 17 β -hydroxysteroid dehydrogenase, which is upregulated by insulin. Obesity also suppresses ovulation and LH levels; blunted LH pulse amplitude is at least in part caused by accelerated metabolism of LH. The atypical PCOS of obesity is in general characterized by mild hyperandrogenemia (normal total testosterone, mildly elevated free testosterone, normal DHEAS, low SHBG), and most have normal-size ovaries, normal AMH levels, and normal LH levels; their insulin resistance is unremarkable for PCOS.

One of the great puzzles about PCOS pathogenesis is whether hyperandrogenism might cause insulin resistance. Androgens in vitro exert some antiinsulin effects.¹¹²⁷ A variety of studies indicate that administration of oral androgens or virilizing doses of androgen to humans causes insulin resistance.^{1127,1128} The induction of modest hyperandrogenism does not do this in normal women,¹¹²⁹ but in nonhuman primates it will induce insulin resistance when combined with an obesogenic diet.^{1130,1131} However, reversal of PCOS androgen excess has not ameliorated insulin resistance in the majority of studies.¹¹²⁷

Etiology. PCOS seems to arise as a complex trait that results from the interaction of diverse genetic and environmental factors once mature gonadotropin levels are achieved peripubertally.⁴⁰ These complex interactions generally mimic an autosomal dominant pattern of inheritance with variable penetrance. Heritability of PCOS has been estimated at over 70%, based on studies in identical twin sisters. Heritable factors

include maternal PCOS and PCOM, hyperandrogenemia, and metabolic syndrome.

GWAS identified overexpression of DENND1A.V2 in most PCOS populations.^{40,1132} This previously unsuspected protein is present in theca and zona reticularis cells and is overexpressed in PCOS. It facilitates steroidogenesis; the mechanism is unknown, but it has been speculated to upregulate LH receptor signaling by affecting receptor trafficking. Induced overexpression of it in normal theca cells has reproduced this PCOS genetic and biochemical phenotype in vitro.⁴⁰ A wide variety of other gene variants with linkage and/or association with PCOS have been identified by candidate gene and molecular genetic studies, and novel markers are reported regularly.⁴⁰ Variants discovered in fibrillin 3 have been proposed to dysregulate TGF- β signaling and account for ovarian stromal hyperplasia. AMH coding variants with decreased signaling that could potentially cause the syndrome have been reported in 6.7% of PCOS patients.¹⁷³

Environmental factors include prenatal androgen exposure and both undernutrition and overnutrition.⁴⁰ Virilizing CAH is sometimes complicated by PCOS in patients whose CAH is well controlled. Congenital virilization of several animal species, including nonhuman primates, has been shown to program hyperandrogenic anovulation and insulin resistance in offspring. These observations suggest that insults to the intrauterine environment induce epigenetic changes that lead to altered gene expression and disease in later life. Because in utero androgen excess from the fetal or maternal PCOS ovary is unlikely to account for this programming in ordinary PCOS,⁴⁰ the androgen programming models rather suggest that intrauterine alterations in intermediates in androgen signaling that are common to other effector pathways, such as prostaglandins, may play a role in the pathogenesis of PCOS by altering the epigenome.^{517,587,613}

Postnatal environmental risk factors can be viewed as a second "hit," which cause a latent heritable or congenitally programmed susceptibility trait to become manifest as PCOS. Postnatal factors include acquired obesity, insulin resistance, and hyperandrogenism of nonovarian origin. The variety of pathways involved and lack of a common thread attest to the multifactorial nature and heterogeneity of the syndrome.

Moderately severe insulin-resistant hyperinsulinism characterizes two syndromes of intractable obesity in childhood that herald PCOS in adolescence: pseudo-Cushing syndrome and pseudoacromegaly.⁴⁰ Premature adrenarche seems to pose a moderately increased risk (overall approximately twofold) for developing PCOS.⁴⁰ The nature of the association is unclear. We favor the concept that premature adrenarche is sometimes an early manifestation of the steroidogenic dysregulation that underlies PCOS, although in some populations low birth weight may be the underlying risk factor.

Other Causes of Functional Ovarian Hyperandrogenism. In the early postmenarcheal years, physiological anovulation may present with mild hyperandrogenemia without clinical evidence of androgen excess in about one-quarter of cases, but the hyperandrogenic anovulation does not persist. Secondary PCOS can result from several disorders (see Box 16.8).⁴⁰ Virilizing CAH frequently causes ovarian hyperandrogenism. Three mechanisms are involved.¹¹³³ For one, poor control of adrenal hyperandrogenism causes polycystic ovaries and amenorrhea by direct effects on the ovary. Adrenal rests of the ovaries may cause polycystic ovaries and hyperandrogenism. Finally, patients with CAH, particularly those with classic CAH, are at high risk for the emergence of PCOS at puberty caused by developmental programming (see previous section Disorders of Sex Development).

All known forms of severe or extreme insulin resistance, including hereditary cases of insulin receptor mutations and acquired lipodystrophy, are accompanied by PCOS, possibly by acting through the IGF-1 signal transduction pathway to cause escape from desensitization to LH. Acromegaly itself is associated with PCOS. The antiepileptic drug valproic acid causes hyperandrogenism and polycystic ovaries, and an association of epilepsy itself with PCOS is possible.^{462,1052}

Rare causes of hyperandrogenism include primary ovarian steroidogenic blocks. These (aromatase deficiency⁹⁹⁹ and virilizing CAH caused by 3 β -HSD deficiency¹¹³⁴) cause hyperandrogenism in association with grossly polycystic ovaries and elevated LH levels. Ovarian 17-ketosteroid reductase deficiency has been reported to be responsible for a PCOS-like picture in two families, but there has been no molecular confirmation of an underlying mutation.¹¹³⁵ Functional ovarian hyperandrogenism may also result from an ovotesticular disorder of sex development. Excessive hCG stimulation mediates the hyperandrogenism of hyperreactio luteinalis and luteoma of pregnancy,¹¹³⁶ and excessive LH appeared to mediate hilus cell hyperplasia in a case of FSH-resistant ovarian follicles.¹¹³⁷ An extremely high level of hCG caused by tumor has been reported to virilize a nonpregnant woman with preexisting PCOS.¹⁰⁷³

Other Causes of Functional Adrenal Hyperandrogenism. The PCOS type of primary FAH that appears to arise from dysregulation of adrenal steroidogenesis occurs as an isolated entity, not associated with FOH, in 5% of hyperandrogenic women.⁴⁰ This may sometimes be an outcome of premature adrenarche. This type of adrenal dysfunction was previously mistaken for nonclassic 3 β -HSD deficiency, which is now known to be a rare disorder.¹¹³⁸

Less than 10% of adrenal hyperandrogenism is caused by the other disorders listed in Box 16.8. The most common of these are nonclassic CAH, which accounts for 4.2% of hyperandrogenic women worldwide, second only to PCOS (prevalence >80%) as the most common cause of hyperandrogenism in adolescent and adult females.⁶⁴⁷ The prevalence varies from 0.1% to 2% in the general US population to 4% in Ashkenazi Jews, to 10% in Mediterranean-Middle East countries.⁸⁴¹ Adrenal hyperandrogenism can on rare occasions arise from other rare congenital disorders of adrenal steroid action or metabolism, such as glucocorticoid resistance, apparent cortisone reductase deficiency, and apparent sulfotransferase deficiency.^{1139–1142}

About 40% of hyperprolactinemic women have hyperandrogenism, sometimes in association with polycystic ovaries.^{367,1143} The great majority (about 85%) of these women have galactorrhea; the combination of hirsutism, galactorrhea, and amenorrhea comprises the Forbes-Albright syndrome. Prolactin excess causes adrenal hyperandrogenism because of multiple effects of prolactin excess on adrenal androgen production and androgen metabolism.³⁶⁷ Serum prolactin values more than 25 ng/mL in hyperandrogenic women usually have an identifiable cause, rather than PCOS itself: most common are pituitary adenoma and antidopaminergic drugs.¹¹⁴⁴

Peripheral Androgen Overproduction. In about 10% of PCOS patients, an ovarian or adrenal source cannot be ascertained by thorough testing (see Fig. 16.48). This “functionally atypical PCOS” seems in most cases to be caused by obesity: excess adipose tissue has the capacity to both cause anovulation and to form testosterone from androstenedione.⁴⁰ In about 20% of such cases (<5% of PCOS), the source of the hyperandrogenemia is unexplained: this is termed *idiopathic hyperandrogenism* (as distinct from “idiopathic hirsutism”). Quirks in steroid peripheral metabolism have been suspected to be the cause.

PCOS has also been reported as a complication of portosystemic shunting.¹¹⁴⁵ Impaired steroid metabolism has been postulated as the mechanism.¹¹⁴⁶

Tumoral Hyperandrogenism. Virilizing tumors are rare, accounting for about 0.2% of hyperandrogenism; about half are malignant.⁶⁴⁷ About half are ovarian, half adrenal. Masculinizing sex cord-stromal tumors of the ovary are unusual before the teenage years. Leydig-Sertoli cell tumor (androblastoma, arrhenoblastoma) is the most common type. Virilizing granulosa-theca cell tumor (thecoma) is unusual before menopause.^{1073,1147} Dysgerminomas virilize only if they have interstitial cell elements. Lipid cell tumors tend to respond to ACTH, as well as LH and produce 17-OHP; thus they must be considered in the differential diagnosis of late-onset CAH.¹¹⁴⁸ The abnormal differentiation that underlies tumor formation typically leads to an abnormal pattern of steroid secretion with androstenedione greatly predominating over testosterone secretion.¹¹⁴⁹ However, some thecomas have been reported to predominantly secrete testosterone.^{1150,1151} Gonadoblastomas are virilizing tumors virtually confined to individuals with dysgenetic gonads with Y-chromosomal material in their genome. Some masculinizing ovarian sex cord-stromal tumors may be caused by activating mutations of stimulatory G-proteins.¹¹⁵² Leydig cell and adrenal rest tumors of the ovary are extremely rare causes of masculinization in childhood.^{20,436,1153} Adrenal virilizing tumors are rare in adolescence; their peak incidence is in early childhood and adulthood⁸²¹ (see sections Precocious Puberty and Incomplete precocity).

Androgenic Drugs. Drug-induced masculinization in adolescence is encountered most often in athletes. The medical history is important in detection because standard clinical laboratory tests for androgens are not helpful in the detection of either natural or artificial androgens.^{647,1154} This is also the case for masculinization that results from unintended contact with topical androgens used by a parent or by a sexual partner.^{647,828} Valproic acid use in epileptics raises testosterone levels and may mimic PCOS.⁴⁶²

Differential Diagnosis

Hyperandrogenism should be considered in any girl who presents with hirsutism or moderate-severe inflammatory acne, menstrual disturbance, or acanthosis nigricans with central obesity (waist circumference >88 cm) during puberty.

Hirsutism must be distinguished from locally excessive hair growth in the absence of an abnormal hirsutism score (“patient-important hirsutism”) and from hypertrichosis, the generalized excess growth of vellus hair that sometimes occurs on a hereditary basis or in patients taking glucocorticoids, phenytoins, diazoxide, or cyclosporine. Hypertrichosis is distributed in a nonsexual pattern (e.g., generalized distribution or more prominent distribution on the forehead or shoulders) and is not caused by excess androgen, although it may be aggravated by excess androgen.

The possibility of PCOS is heightened if hirsutism, treatment-resistant acne, or menstrual irregularity are associated with a history of prepubertal risk factors for PCOS—congenital virilizing disorders; premature adrenarche; intractable prepubertal obesity, particularly if associated with pseudo-Cushing syndrome or pseudo-acromegaly; or a family history of PCOS or metabolic syndrome.

Diagnostic Approach

The goals of the laboratory evaluation for hyperandrogenism are to obtain evidence of hyperandrogenemia and to determine the specific etiology. The diagnosis of hyperandrogenism is on the firmest grounds if hyperandrogenemia is demonstrated biochemically, rather than relying on hirsutism as a clinical

surrogate for it, although documentation of hyperandrogenemia can be problematic.

The evaluation starts with a thorough assessment of the clinical symptoms and signs suggestive of PCOS and for disorders that mimic it (Fig. 16.49).^{462,1101}

The degree and distribution of sexual hair growth should be identified, usually done using the Ferriman-Gallwey score (see Fig. 16.46). Moderate or severe hirsutism (hirsutism score >15) constitutes clinical evidence of hyperandrogenism in an adolescent (see Box 16.9). The history should specifically explore

whether the patient is taking medications that may mask symptoms (e.g., depilatory use) or cause hirsutism (e.g., anabolic steroids, valproate).

Moderate or severe inflammatory acne (>10 lesions in any area, e.g., face, chest, back) or acne that is persistent and poorly responsive to topical dermatological therapy suggests the possibility of hyperandrogenism.

Assessment should also include evaluation for symptoms or signs that suggest a hyperandrogenic disorder other than PCOS, including virilization (e.g., rapidly progressive hirsutism,

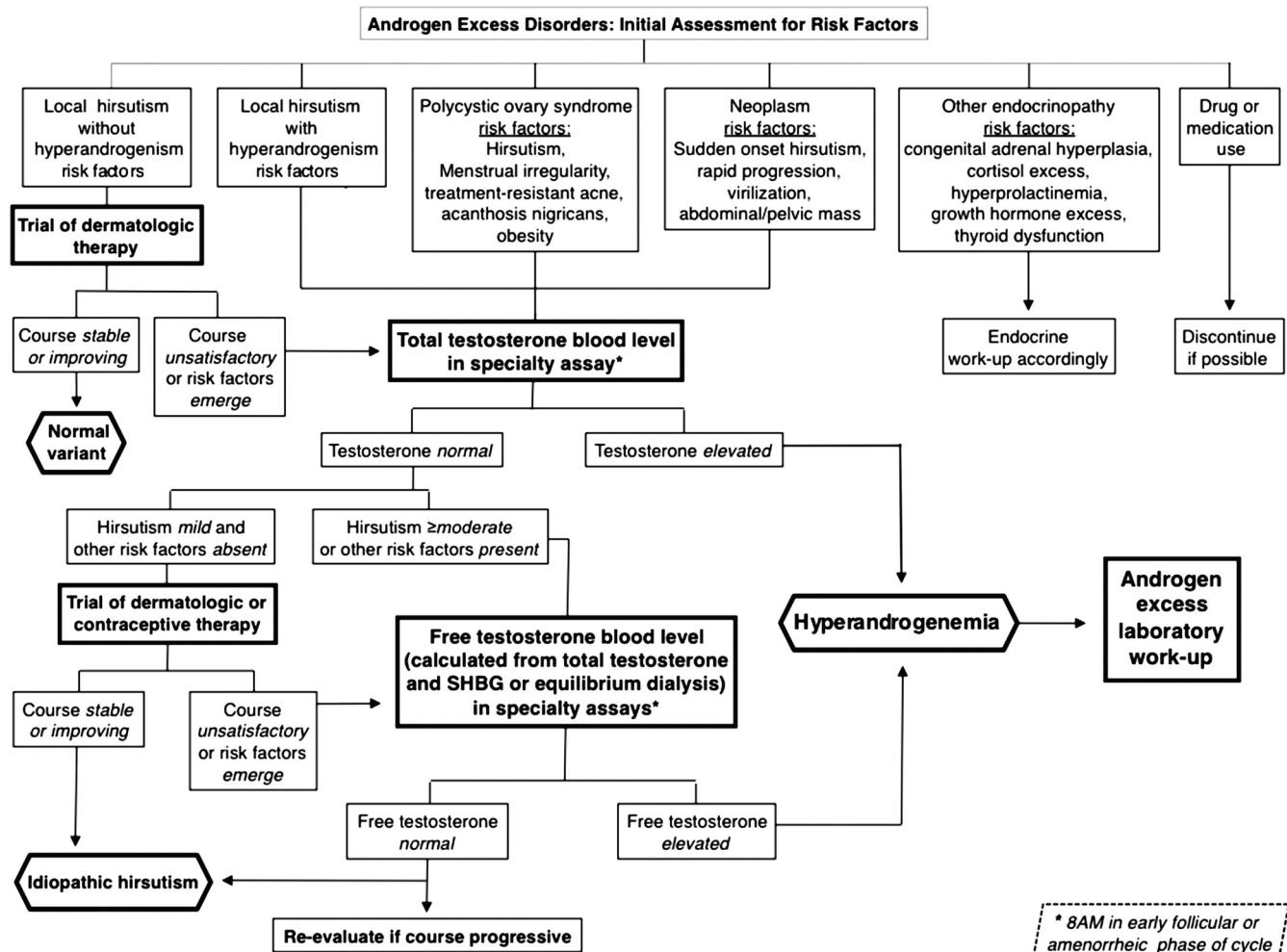


Fig. 16.49 Initial assessment of women for risk of androgen excess disorders. Patient-important localized areas of hair growth in the presence of a normal hirsutism score ("local hirsutism"), but not accompanied by other androgen excess risk factors, do not require an endocrine workup before embarking on local dermatologic therapy. In women with local hirsutism with other androgen excess risk factors or an elevated hirsutism score, androgen levels should be assessed. PCOS is the most common cause to be considered, but androgen-producing tumors, congenital adrenal hyperplasia, and diverse androgenic disorders, as shown, should be excluded. Drugs that cause hirsutism include anabolic or androgenic steroids (consider in athletes and patients with sexual dysfunction) and valproic acid (consider in neurologic disorders), which causes hyperandrogenemia. Serum testosterone is best assessed in the early morning, on days 4-10 of the menstrual cycle in eumenorrheic women or on random days in amenorrheic women, by an accurate and specific assay such as liquid chromatography/mass spectrometry. Females with mild hirsutism (score 8-15), normal total testosterone level, and no risk factors probably have idiopathic hirsutism, which may be responsive to endocrine therapy. Serum free testosterone should be measured as indicated by a specialty reference assay if the serum total testosterone is normal in the presence of risk factors or progression of hirsutism on therapy. Simultaneous assay of 17-hydroxyprogesterone is indicated in subjects at high risk for congenital adrenal hyperplasia. Some adolescents diagnosed with idiopathic hirsutism by this algorithm will have polycystic ovaries on ultrasound as adults, which is unlikely to be important unless fertility is an issue. Progression of hyperandrogenism in the presence of a normal serum free testosterone is very unusual; such patients should be thoroughly reevaluated, possibly also measuring other steroids related to androgen excess, such as 17-hydroxyprogesterone, androstenedione and DHEAS. The initial laboratory work-up for androgen excess is shown in Fig. 16.50. (Modified with permission from Martin, et al. Evaluation and treatment of hirsutism in premenopausal women: an Endocrine Society Clinical Practice Guideline. Copyright The Endocrine Society.)

clitoromegaly), abdominal mass, galactorrhea, cushingoid or acromegaloid changes, evidence of thyroid dysfunction, or a family history of hyperandrogenic disorders. Inspection of the external genitalia is indicated, but an internal pelvic examination seldom is necessary.⁹⁹⁷

If hirsutism is local or mild (hirsutism score <8) and if menses are regular with no other clinical evidence of risk factors that would suggest an underlying cause, unwanted or excess sexual hair growth can be managed by dermatological means without pursuing further laboratory evaluation.^{462,647} Unless fertility becomes an issue in adulthood for subjects with local hirsutism or idiopathic hirsutism, demonstrating polycystic ovary morphology to diagnose ovulatory PCOS is unlikely to affect management.

Laboratory assessment for hyperandrogenism begins with measurement of serum testosterone: this is the single most important androgen to evaluate (Fig. 16.49).^{124,467,647} Although other androgens are present in blood, their assessment ordinarily makes little difference in diagnosis and management if serum free testosterone is normal. Serum free testosterone is about 50% more sensitive in detecting excessive androgen production because hyperandrogenic women have a relatively low level of SHBG. There are many pitfalls in testosterone assays at the low levels found in women and children, and reliable testosterone assays are not available in most local laboratories. Assays of high sensitivity and specificity, such as are provided by postchromatographic RIA or tandem mass spectrometry by specialty laboratories, are required. Assaying the free testosterone level introduces other potential sources of error. Direct assays of the free testosterone concentration are inaccurate and should be avoided. The best methods to calculate free testosterone as the product of the total testosterone and a function of SHBG: free testosterone = total testosterone × percent free testosterone, where percent free testosterone is most accurately determined by dialysis, or alternatively calculated from the SHBG concentration.¹¹⁵⁵ The combination of a high-normal total testosterone and a low-normal SHBG yields a high free testosterone concentration.

Exclusion of hyperandrogenic disorders that mimic PCOS (see Box 16.8) is important to meet diagnostic criteria. Because PCOS accounts for 95% of hyperandrogenisms, a diagnostic strategy is required that takes into account individual patient preference, including benefits in relation to economy of cost and of time. Guidelines vary slightly, but most suggest screening for nonclassic CAH, which accounts for most non-PCOS hyperandrogenism, hyperprolactinemia, thyroid disease, and virilizing tumor, which is the most serious although rare; testing for Cushing syndrome and acromegaly are generally suggested for those with suggestive clinical features.^{124,647,1107,1156} Normal results of a simple panel of endocrine screening tests to exclude these other causes of hyperandrogenemia (see Fig. 16.50) ensures the diagnosis of PCOS with about 99% reliability.

Practice varies as to the indications for ultrasonography in girls with confirmed hyperandrogenemia. The primary purpose of ultrasonography is to screen for the rare but serious adrenal or ovarian tumor, as it is not required for diagnosis. Ultrasonography also provides the opportunity for patient reassurance and education. For many women, the diagnosis of ovarian "cysts" raises a concern about tumors, so it is reassuring to know that a tumor has not been seen.

Some tests require consideration of the time of day or stage of cycle that sampling is performed. Serum testosterone levels are 20% higher in the morning than in the afternoon and double in midcycle; norms are based on midfollicular phase controls.⁶⁴⁷ An early morning 17-OHP level over 170 ng/dL (>5.1 nmol/L) is approximately 95% sensitive and 90% specific for nonclassic CAH, but they are insensitive by

midmorning.^{647,841} The most common cause of false positives is PCOS,¹¹⁵⁷ the most serious cause is virilizing tumors.^{1148,1157} DHEAS elevations are usually caused by the FAH of PCOS; DHEAS greater than 700 mcg/dL suggests adrenal virilizing tumor or a rare type of CAH (3β-HSD deficiency). A cortisol level less than 10 mcg/dL (276 nmol/L) is reassuring evidence against endogenous Cushing syndrome unless the clinical index of suspicion is high.

The senior author's practice is to ordinarily begin the specific workup for PCOS at the initial evaluation for hyperandrogenic anovulation, an evaluation that includes testosterone (see Fig. 16.49), by adding a few tests for the common non-PCOS causes of hyperandrogenism (see Fig. 16.50).¹¹⁰¹ For practical reasons, sampling for early morning serum 17-OHP and ultrasound are ordinarily scheduled; at the same time, whatever further workup might be indicated by the initial test results (e.g., early morning free testosterone if midday testosterone was not clear-cut in the face of high clinical index of suspicion for hyperandrogenism).

A more comprehensive endocrine evaluation is sometimes indicated because the basic evaluation described earlier does not exclude rare virilizing disorders (see Box 16.8).^{647,1156} The approach to further studies to determine the source of hyperandrogenemia varies among subspecialists and with the needs of the individual patient.

Our preference is to use a DAST to attempt to make a positive diagnosis of the ovarian dysfunction of PCOS versus determining whether further workup is necessary for rare forms of CAH or other rare adrenal disorders (Fig. 16.51).^{1101,1122} Suppression of serum androgens and cortisol in response to a low-dose dexamethasone suppression test segregates patients diagnostically. Subnormal testosterone suppression with normal adrenocortical suppression indicates a source of androgen other than an ACTH-dependent adrenal one and is found in 80% of PCOS. However, tumor or other ovarian pathology must be excluded by ultrasound examination. If both cortisol and androgen suppression are subnormal, then the androgen excess may be secondary to dexamethasone noncompliance, endogenous Cushing syndrome, or glucocorticoid resistance. If testosterone suppression is normal, then ACTH (cosyntropin) stimulation testing to ruleout nonclassic CAH is recommended. If both dexamethasone and ACTH testing are normal, the most likely diagnosis is the atypical PCOS of obesity or idiopathic hyperandrogenism.

A short (4-h) DAST, as described in the Fig. 16.51 legend, suffices in the absence of high suspicion for virilizing disorder. It is helpful in distinguishing the potentially reversible pseudo-PCOS of simple obesity, in which case testosterone suppresses normally, from the persistent ovarian dysfunction of ordinary PCOS.¹¹²² However, a more prolonged course of low-dose dexamethasone (long DAST, 4 days) is required to suppress the hyperandrogenism of CAH.

Further extensive diagnostic studies beyond those indicated in Fig. 16.50 are seldom indicated unless there is reason to suspect a virilizing tumor or a disorder of sexual differentiation. Computed tomography and MRI permit the best visualization and more detailed assessment of tumors, particularly of the adrenal gland. On rare occasions, ultrasonography has been insensitive in detecting a virilizing ovarian tumor in adults.^{847,1158} Further or alternative workup may include acute GnRH agonist testing, or assessment of the response to hormonal suppression treatment to determine the source of androgen.

Management

Management of hyperandrogenism is individualized according to symptoms and patient goals—hirsutism and acne;

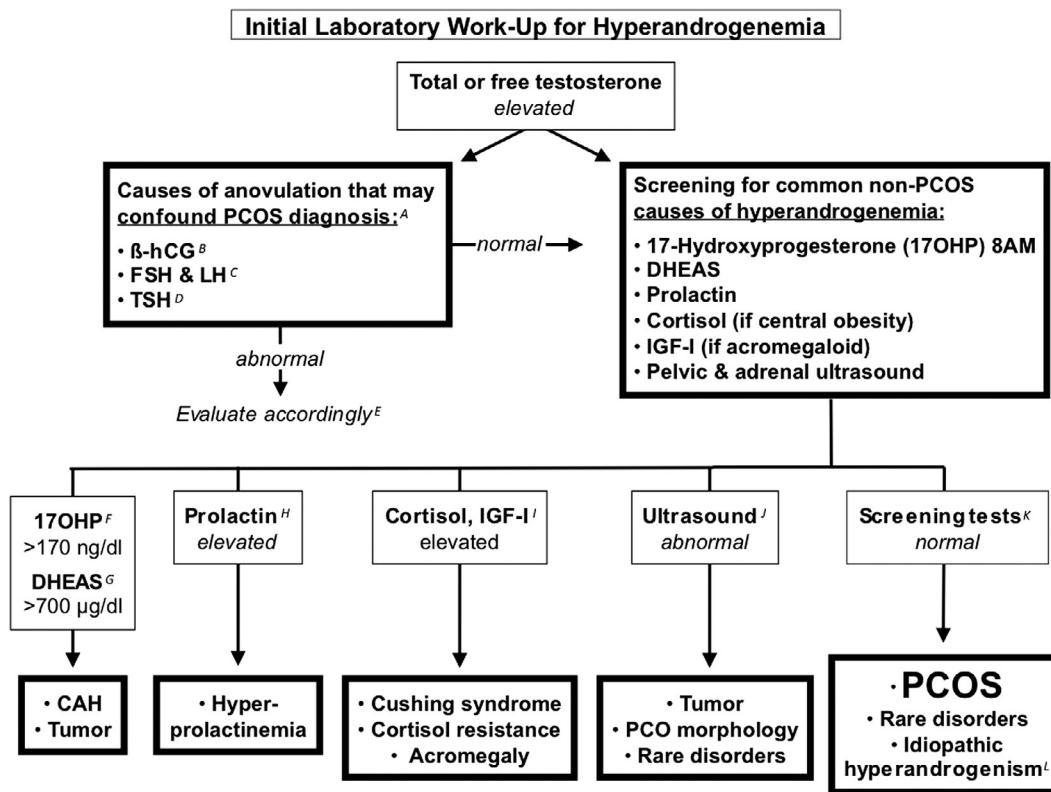


Fig. 16.50 Initial laboratory workup for cause of androgen excess. Polycystic ovary syndrome is a diagnosis of exclusion: this algorithm identifies the most common other causes of hyperandrogenemia and hyperandrogenic anovulation. (Modified from Rosenfield, R.L. (2018). Polycystic ovary syndrome in adolescence. Clinical features and diagnosis of polycystic ovary syndrome in adolescents. *UpToDate*. With permission.)

Footnotes:

- These tests for anovulatory disorders are advisable for hyperandrogenemic patients with menstrual abnormalities, because they may confound the diagnosis of polycystic ovary syndrome (PCOS).
- Pregnancy is associated with high testosterone levels.
- Slightly elevated luteinizing hormone (*LH*) and slightly low follicle-stimulating hormone (*FSH*) are common in PCOS. High *FSH* suggests primary hypogonadism. Low *LH* suggests hypogonadotropic hypogonadism.
- Thyroid dysfunction may alter the total testosterone level by altering sex hormone binding globulin. Hypothyroidism may cause multicystic ovaries and coarse hair that may be mistaken for hirsutism.
- See preceding algorithms for workup of anovulatory disorders.
- 8:0 AM 17-hydroxyprogesterone (17-OHP) over 170 ng/dL is approximately 95% sensitive and 90% specific for detecting common type (21-hydroxylase-deficient) nonclassic congenital adrenal hyperplasia (NCCAH) in anovulatory or early-midfollicular phase women. NCCAH is the most common disorder mimicking PCOS. False-positive 17-OHP elevation is found in PCOS, luteal phase women, and virilizing neoplasms.
- Dehydroepiandrosterone sulfate (DHEAS) greater than 700 mcg/dL suggests adrenal virilizing tumor or the rare 3β-hydroxysteroid dehydrogenase deficiency form of NCCAH.
- Hyperprolactinemia may cause either hypogonadotropic hypogonadism or hyperandrogenic anovulation (which is usually accompanied by galactorrhea, the Forbes-Albright syndrome).
- Optional tests are cortisol and insulin-like growth factor 1 (IGF-I). Endogenous Cushing syndrome should be considered in cases with central obesity. Plasma cortisol under 10 mcg/dL essentially rules out endogenous Cushing syndrome unless the clinical index of suspicion is high. Acromegaly should be ruled out by IGF-I screening if the patient has acromegaloid overgrowth.
- Ultrasonography screens for ovarian and adrenal tumor. Polycystic ovary morphology is supportive, but it is not specific, nor is it a criterion for, the diagnosis of adolescent PCOS. Ultrasound may detect ovotesticular disorder of sex development (true hermaphroditism) or human chorionic gonadotropin (*hCG*)-related disorders of pregnancy.
- Exclusion of the preceding disorders in a hyperandrogenic patient with menstrual dysfunction meets standard diagnostic criteria for PCOS with approximately 99% reliability. However, this workup does not identify rare adrenal disorders (e.g., rare types of congenital adrenal hyperplasia [CAH] and adrenal steroid metabolic disorders, very small tumors, such as the rare testosterone-secreting neoplasm) or idiopathic hyperandrogenism.
- Idiopathic hyperandrogenism (documented hyperandrogenemia with no demonstrable source) occurs in approximately 1% of hyperandrogenic women. This is distinct from the more common "idiopathic hirsutism" (hirsutism without other clinical evidence of hyperandrogenism). *TSH*, Thyroid-stimulating hormone.

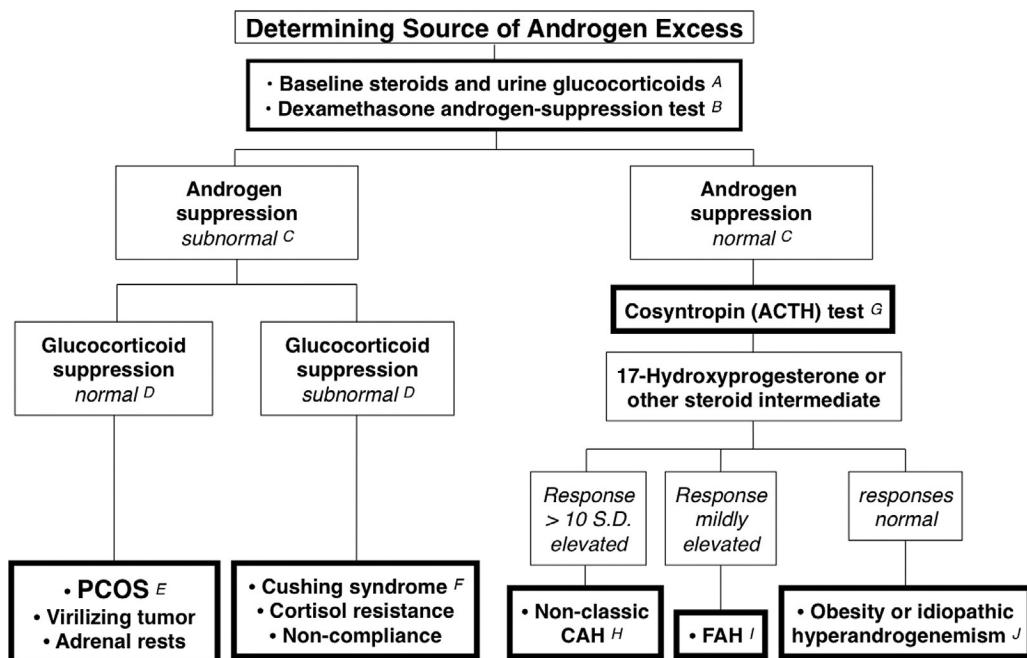


Fig. 16.51 An approach to determining the source of androgen excess. Determination of the source of excess androgen often permits a positive diagnosis of the characteristic ovarian and adrenal dysfunction of polycystic ovary syndrome (PCOS) and will elucidate rare disorders that mimic PCOS. (From Buggs C, Rosenfield RL. Polycystic ovary syndrome in adolescence. *Endocrinol Metab Clin North Am* 2005; 34:677.)

Footnotes:

- A. After obtaining an early morning blood sample for baseline steroid intermediates (e.g., 17-hydroxypregnенolone, 17-hydroxyprogesterone, 11-deoxycortisol, dehydroepiandrosterone, androstenedione) and a 24-hour urine for glucocorticoids (i.e., free cortisol and 17alpha-hydroxycorticosteroids, as well as creatinine to control for completeness of collection), a dexamethasone androgen-suppression test (DAST) is performed.
- B. A short DAST (sampling blood 4 h after a single noontime 0.5 mg dexamethasone dose) maximally suppresses total and free testosterone and 17-hydroxyprogesterone, but dehydroepiandrosterone sulfate (DHEAS) and cortisol are not maximally suppressed in comparison to the 4-day DAST. A long DAST (4 days) is a definitive test: this consists of a 4-day course of dexamethasone 0.5 mg 4 times daily before an early morning posttest blood sample on day 5.
- C. Normal androgen suppression in response to the 4-day DAST is indicated in our laboratory by total testosterone less than 28 ng/dL (1.0 nmol/L), free testosterone under 8 pg/mL (28 pmol/L), DHEAS less than 40 mcg/dL (1.0 micromol/L) (>75 % fall), and 17-hydroxyprogesterone under 26 ng/dL (0.8 nmol/L).
- D. Normal glucocorticoid suppression is indicated by serum cortisol under 1.5 mcg/dL (40 nmol/L) and urinary cortisol under 10 mcg (27 nmol) per 24 hours by the second day of dexamethasone administration.
- E. Subnormal suppression of androgens and a normal suppression of cortisol and DHEAS is characteristic of PCOS, but the rare (<1% of cases) virilizing adrenal tumor or adrenal rests should be considered on the basis of clinical features. Virilizing tumors are often characterized by an abnormal pattern of steroid intermediate elevations that is atypical for congenital adrenal hyperplasia (CAH).
- F. Subnormal suppression of both cortisol and androgen is consistent with endogenous Cushing syndrome or cortisol resistance. Poor suppression can also result from noncompliance with the dexamethasone regimen.
- G. A cosyntropin (ACTH) stimulation test is appropriate if androgen suppression by dexamethasone is normal. The ACTH test is usually performed by administering 250 mcg cosyntropin by IV push and drawing blood for steroids 30 to 60 minutes later.
- H. The diagnosis of CAH is suggested if the response to ACTH is over 10 standard deviations (SDs) above the average: this corresponds to, for example, 17-hydroxyprogesterone greater than 1500 ng/dL (45 nmol/L), 17-hydroxypregnенolone greater than 5000 ng/dL (158 nmol/L).
- I. Primary functional adrenal hyperandrogenism (FAH) (suggested by a modest rise in 17-hydroxypregnенolone or 17-hydroxyprogesterone that does not meet the criteria for the diagnosis of CAH) is sometimes the only demonstrable source of androgen excess in PCOS. A rare mimic of FAH and idiopathic hyperandrogenemia is (apparent) cortisone reductase deficiency: baseline urinary glucocorticoids consist primarily of cortisone metabolites rather than cortisol metabolites, so 17alpha-hydroxycorticosteroid excretion is elevated, but cortisol excretion is normal.
- J. When the source of hyperandrogenemic anovulation remains unexplained after intensive investigation (approximately 10% of cases), it usually appears to be caused by the atypical PCOS of obesity. Otherwise, the diagnosis is idiopathic hyperandrogenism (distinct from idiopathic hirsutism) if (apparent) cortisone reductase deficiency has been excluded.

menstrual irregularity; obesity—and the cause of androgen excess.^{706,1159,1160} Here, we will discuss the management of PCOS.

Hirsutism and Acne. Cosmetic measures are the cornerstone of care for unwanted hair.⁶⁴⁷ Bleaching and shaving suffice for many. Depilating agents and waxing treatments are useful, but prone to cause skin irritation. Repeated topical application

of eflornithine hydrochloride cream brings about significant reduction of local hirsutism within 6 to 8 weeks. It is a useful adjunct to photoepilation, providing a more rapid response. Topical benzoyl peroxide and retinoids are the cornerstone of care for acne; short-term oral tetracycline-derived antibiotics are appropriate for moderate-severe inflammatory acne.¹¹⁶¹

For hirsutism or acne that requires more than these dermatological measures, first-line treatment for both the hyperandrogenemia and menstrual abnormalities of PCOS is ordinarily OCPs, which correct both,⁶⁴⁷ with several being FDA approved for the treatment of acne. Antiandrogen treatment of cutaneous symptoms is suggested for most hyperandrogenic women before undertaking treatment with photoepilation⁶⁴⁷ or oral isotretinoin (Accutane®).

Endocrine therapy is directed at interrupting androgen production or action. This causes the pilosebaceous unit to revert toward the prepupal vellus type (see Fig. 16.29). The maximal effect on the sebaceous gland occurs within 3 months, but that on sexual hairs requires 9 to 12 months of treatment, because of the long duration of the hair growth cycle. Hirsutism requires use of endocrine treatment as long as the patient wishes to maintain improvement.

Combination OCPs, the first-line endocrine treatment for women with the dermatological or menstrual abnormalities of PCOS, act by suppressing serum androgens, mainly by inhibiting ovarian function. They also raise SHBG, which suppresses the serum free fraction of testosterone, and modestly lower DHEA sulfate levels. They normalize androgen levels by 3 weeks of therapy.

All estrogen-progestin combinations in combination with cosmetic measures are similarly effective for women with acne or mild hirsutism.⁶⁴⁷ A 7-point improvement of hirsutism score can be anticipated on average. Compared with levonorgestrel or norethindrone, low-androgenic progestins, such as norgestimate, and antiandrogenic progestins, such as drospirenone, successively better optimize serum lipid profiles, but successively increase venous thromboembolism (VTE) risk, although the risk is ordinarily low in adolescence and far less than that of pregnancy. VTE risk is also related to estrogen dose, but 20 to 35 mcg ethinyl estradiol-containing OCPs have generally favorable risk-benefit ratios. Drospirenone-containing OCPs also have antimineralocorticoid activity and thus may be associated with less weight gain than other OCPs.¹¹⁶²

Antiandrogens yield mild improvement in hirsutism beyond that attainable with OCPs.⁶⁴⁷ The combination can be expected to reduce the Ferriman-Gallwey score by 15% to 40%, although there is considerable individual variation. Antiandrogen use for this purpose is off-label because all carry the risk of causing undervirilization of the male fetus. Therefore all antiandrogens should be prescribed with a contraceptive, preferably an OCP. They may have a modest effect on the metabolic abnormalities associated with PCOS.¹¹⁶³

Spirostanolactone in high dosage is probably the safest most effective antiandrogen available in the United States.⁶⁴⁷ Guidelines recommend starting with 100 mg twice a day until the maximal effect has been achieved and then attempting to reduce the dose to 50 mg twice a day for maintenance therapy. Spirostanolactone usually is well tolerated, but it is contraindicated in patients with adrenal, hepatic, or renal insufficiency. Women are at risk of hyperkalemia if on potassium-sparing diuretics, potassium supplements, daily nonsteroidal antiinflammatory drugs, angiotensin-converting enzyme inhibitors, heparin, or such drugs. Therefore electrolytes should be monitored. Alone, it tends to cause irregular bleeding.

Other antiandrogens have been extensively evaluated to treat hirsutism and hirsutism equivalents, including cyproterone acetate, flutamide, and finasteride. Cyproterone acetate is a potent progestational antiandrogen that is used with estrogen in a reverse sequential regimen: 50 to 100 mg is given during days 1 to 10 of cycles in which estrogen is given from days 1 to 21. Flutamide is a more specific antiandrogen with efficacy similar to that of cyproterone and spirostanolactone, but it is not recommended because of a seemingly idiosyncratic risk of fatal hepatocellular toxicity.⁶⁴⁷ Finasteride, a type 1 5-alpha-reductase inhibitor, tends to be

somewhat less effective than other antiandrogens in the treatment of hirsutism and pattern balding in females. Other more potent and selective antiandrogens are in earlier stages of evaluation.^{1164,1165} Although topical minoxidil is the only medication approved for alopecia treatment, antiandrogen-OCP therapy may be superior in those with PCOS.

GnRH agonist therapy with estrogen-progestin add-back therapy should be considered for the treatment of hirsute women only if they are intolerant of OCPs or if OCPs are ineffective for severe hyperandrogenemia.⁶⁴⁷ Glucocorticoids are of limited efficacy in the management of the hirsutism of the FAH of PCOS.

Menstrual Irregularity. OCPs, discussed in some detail earlier as the usual first-line endocrine treatment of hirsutism and acne unresponsive to cosmetic measures, are also ordinarily the first-line treatment for menstrual irregularity. Beyond hygienic concerns, menstrual irregularity requires treatment because chronic anovulation is associated with increased risk of developing endometrial hyperplasia and carcinoma. Although lower estrogen doses are the safest, 30 to 35 mcg ethinyl-estradiol is often necessary in larger girls to provide menstrual regularity.

There are several limitations to the use of OCPs in the management of PCOS in adolescents. OCPs may be contraindicated in patients who are at high risk for venous thrombosis, and they should be used with caution and in the lowest estrogen dose possible in patients with migraine headaches. OCPs must be discontinued when patients desire conception. OCPs will bring growth to end in perimenarcheal girls. The long-term consequences of these agents on fertility are unknown; while there is the theoretic possibility of postpill amenorrhea, very high-dose estrogen begun in early adolescence increases the risk of primary ovarian insufficiency rather than hypogonadotropism.⁸⁸⁶

It is advisable to recheck patients after 3 months of therapy to assess the efficacy of treatment and normalization of androgen levels. To document persistence of PCOS and to curtail mistaken beliefs that the treatment is curative, it is advisable to withdraw OCPs for approximately 3 months when the patient is gynecologically mature to determine persistence of hyperandrogenic anovulation. In doing so, however, one must keep in mind that the anovulatory infertility of PCOS is relative, not absolute, so contraceptive counseling is indicated.

Progestin monotherapy is an alternative to OCPs for the control of menstrual irregularities. Micronized progesterone (Prometrium®) 100 to 200 mg daily at bedtime for 7 to 10 days induces withdrawal bleeding in the majority of patients, but some do not respond, apparently because of an antiestrogenic effect of androgen excess on the endometrium, and breakthrough bleeding is more likely than with OCPs. Progestin therapy has the appeal of permitting the detection of the emergence of normal menstrual cyclicity. However, it is ineffective for improving androgen levels or hirsutism. The perimenarcheal girl who responds well to progestin therapy can be maintained at approximately 6-week cycles to permit the detection of spontaneous menses. Side effects of progestin include mood symptoms (depression), bloating, and breast soreness. Patients must be informed that oral progestin dosed in this way is not a means of contraception.

Obesity and Metabolic Syndrome. An oral glucose tolerance test, more sensitive for the detection of prediabetes than hemoglobin A1c, and lipid panel, is suggested in obese PCOS patients.^{1103,1166} Those with metabolic syndrome should be screened for sleep-disordered breathing and fatty liver disease. We also advise screening primary relatives for metabolic syndrome and PCOS, because PCOS is closely related to parental

metabolic syndrome and maternal PCOS.¹¹⁶⁶ Women with PCOS are at significantly increased risk of depression, anxiety, and bipolar, autism spectrum, and attention deficit hyperactivity disorders and should be screened for these disorders.¹¹⁶⁷

Insulin-lowering treatments of obesity, from weight loss to drug treatment, uniformly but modestly improve hyperandrogenism. Lifestyle modification is the first-line treatment for the overweight and obesity of PCOS.^{1103,1156,1168} Calorie reduction is indicated, as with any obese subject.^{1168,1169} Obese patients experience improvement in their anovulatory symptoms in approximate proportion to the amount of weight loss.¹¹⁷⁰ Improvement in hirsutism is minimal, however.^{647,1171}

Bariatric surgery has led to improvement in hirsutism, androgen levels, and menses in the vast majority of obese adults with PCOS.¹¹⁷² Much of this seems to be caused by the atypical form of PCOS caused by obesity.^{40,1055} Bariatric surgery is suggested only for select adolescents with extremely high BMI and access to highly specialized centers.¹¹⁶⁸

Metformin is often used as an adjunct to the management of obesity and insulin-resistant metabolic abnormalities in patients with PCOS, although abnormal glucose tolerance is the only approved indication for it. It is usually administered by escalating doses over 3 to 4 weeks from 500 mg to 2000 mg in two divided doses (or as a single dose of the extended release form) daily, as tolerated, to minimize anorexia and nausea. Such effects contribute to weight loss but are also the cause of about a 15% drop-out rate. Metformin effectiveness for PCOS is minimized in the absence of weight control.¹¹⁷³ Three randomized, double-blind, placebo-controlled trials compared metformin to lifestyle counseling for 3 to 6 months in adolescents with PCOS.¹¹⁷⁴⁻¹¹⁷⁶ Metformin was found to significantly improve HDL cholesterol levels. It increased the likelihood of menses significantly in only the shortest of the three studies, with only nonsignificant tendencies to an increase in ovulatory cycles in the others. It did not lower testosterone levels or weight significantly better than lifestyle/placebo, which likely explains its inconsistent effects on insulin levels in these trials. These results are less salutary than those of open-label trials.¹¹⁰³

Available randomized trials comparing metformin to OCPs in adolescents have not been blinded, which limits their quality.¹¹⁷⁷ Although the mean suppression of free testosterone levels is greatest on OCPs the relatively small size of the studies has made it difficult to demonstrate statistical differences between treatments.¹¹⁷⁵ When metformin and OCPs are used in combination, the positive metabolic effects of metformin are blunted by the negative effects of OCPs on lipids.¹¹⁷⁸ Metformin has been reported to ameliorate the negative effect of a COC on thrombin generation.¹¹⁷⁹

It is advisable to obtain a baseline comprehensive metabolic panel to confirm normal hepatic and renal function before institution of metformin therapy. Although extremely rare, lactic acidosis is a potential complication of metformin use.

Other metabolic therapies have a place in the management of PCOS only to the extent that they may be indicated for control of coexistent diabetes mellitus or hyperlipidemia. Because of the limited efficacy and concern for weight gain and toxicity, thiazolidinediones are not generally advisable in adolescents with PCOS.¹¹⁰¹

FUTURE DIRECTIONS

Tremendous advances continue to occur in our understanding of puberty. The identification of genes involved in ovarian differentiation, the discovery of new hormones and hormone receptors, new insights into the regulation of gene transcription and signal transduction, further identification of the role of genetic factors and prenatal epigenomic imprinting on pubertal

disorders, and advances in the application of mass spectrometry to steroid assays can also be anticipated to occur in the next 5 years. We are in the midst of an explosion of information in the biological sciences. It is becoming clear that the body puts a wide but finite repertoire of hormones and growth factors to myriad and unexpected uses. Many concepts that we hold dear at this moment are at the best likely to be shown to be oversimplifications, at worst wrong. New information comes to light faster than we can assimilate it. The understanding of the interactions of the human genome with environmental factors can be expected to yield new insights into our understanding of puberty and its disorders.

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