13

REPRODUCTIVE ENDOCRINOLOGY

INTRODUCTION

Phylogeny is the evolutionary process of speciation, i.e., the development of new species. A *species* is defined as the population of individuals capable of producing fertile offspring. These definitions mean that new species arise and become established when the reproductive function of a population diverges from its ancestors. Because this precludes exchange of genetic material between the two populations, additional (nonreproductive) genetic and biologic differences may arise. However, due to the very nature of speciation, the least conserved of all biologic functions is reproduction.

This book is primarily focused on *human* endocrine physiology. This chapter is no exception. However, like most life sciences, endocrine research heavily relies on experimental animal models. Quite understandably, the clinical applicability of animal research in the field of reproductive endocrinology is far more restricted than in any other area of endocrinology. For this reason, we must frequently rely on human disease entities for explaining the normal regulatory processes. In spite of major advances in our understanding of human reproductive endocrinology, the explanation of several physiologic processes remains circumstantial at best.

Reproductive endocrinology involves the most intricate and complex regulatory system. We first discuss the biosynthetic pathways of sexual steroids in both sexes. This topic will be followed by the reproductive endocrinology of the adult male. The next subject, the adult female, has various reproductive states: menstrual cycle, pregnancy, and lactation. Finally, we discuss and compare the ontogeny of reproductive endocrine function in males and females: sexual differentiation, puberty, and menopause. Because of the complexity and amount of information related to reproductive function, the learning objectives are listed separately for each major area.

THE BIOSYNTHESIS, MECHANISM OF ACTION, AND METABOLISM OF SEXUAL STEROIDS

OBJECTIVES

1. Review the biosynthesis of adrenocortical steroids (see Chap. 12) and steroid hormone receptors (see Chap. 5).
2. Identify the cells involved in the gonadal biosynthesis of sexual steroids. Discuss the interplay between *granulosa* and *theca interna* cells in steroidogenesis before and after luteinization.
3. Describe the roles and cellular targets of *gonadotropins* as the main regulators of gonadal steroidogenesis. Compare and contrast the roles of *luteinizing hormone* (LH) and *follicle-stimulating hormone* (FSH) in males versus females in steroidogenesis and gametogenesis.
4. Discuss the contribution of peripheral (extragonadal) conversion in the biosynthesis of sexual steroids: identify the enzymes, their isoforms, sites of expression, tissue-specific regulation, and their roles in local versus systemic action.
5. Discuss the actions of *testosterone* in males without peripheral conversion and its actions after being converted either into *dihydrotestosterone* or into *estradiol* (E_2). Identify the laboratory parameter most closely related to the activity of *5α-reductase* and *hirsutism* in females.
6. Discuss the molecular relationship between *sex hormone-binding globulin* (SHBG) and *androgen-binding protein* (ABP). Describe the mechanism of prostatic androgen receptor activation via the SHBG receptor.
7. Discuss the mechanism of degradation and elimination of sexual steroids, and its consequences on the oral administration of these lipophilic hormones.

Sexual Steroids Are Synthesized by the Leydig Cells in Males and by the Cooperative Function of Granulosa and Theca Cells in Females

The two main functions fulfilled by the gonads are *gametogenesis* (production of germ cells) and *hormonogenesis*. The hormones produced by the gonads play an essential role in supporting all aspects of reproduction. These hormones influence other physiologic functions, such as mineral and electrolyte homeostasis, fuel and protein metabolism, adiposity, and muscle mass. In addition to the gonads, the adrenal cortex contributes to the pool of circulating androgens. *The relative importance of adrenal androgens is greater in females than in males*, whose predominant androgen source is the testis.

In the testis, the physiologic source of all steroid hormones is the *Leydig cell*, which is found in the connective tissue stroma near the seminiferous tubules and the fenestrated capillaries (Fig. 13-1). The Leydig cells primarily secrete *testosterone* and small amounts of *17β-estradiol* (E_2). *Leydig cells* show the characteristic structural features of steroid hormone-

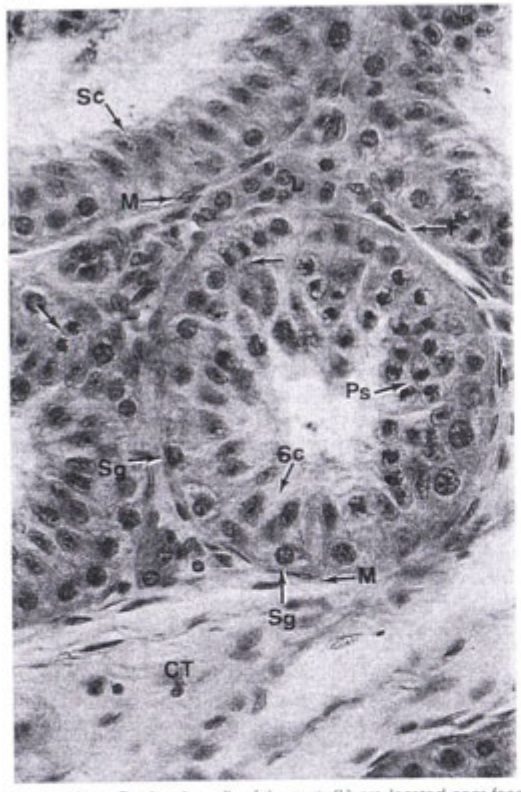


Figure 13-1. The Leydig cells of the testis (L) are located near fenestrated capillaries in the interstitium of the seminiferous tubules. The seminiferous tubules are lined by Sertoli cells (Sc) resting on a basal lamina. The tubules are encircled by the myoid cells (M). Within the tubular epithelium, various developmental forms of sperm are seen in this section, including spermatogonia (Sg) and primary spermatocytes (Ps). The arrows indicate spermatogia in mitosis. CT, connective tissue; F, fibroblast. (*Source:* Fig. 18-5, p 275 in Berman I: *Color Atlas of Histology*. Stamford, CT, Appleton & Lange, 1993.)

producing cells: they have extensive smooth endoplasmic reticulum (sER), tubulovesicular mitochondria, and several cytoplasmic lipid droplets. *Reinke's crystalloids* are cytoplasmic and sometimes intranuclear inclusions specific for Leydig cells and their female equivalents, the *hilar cells* (see the section on The Ovary (Adnexum)). The function of the crystalloids is obscure; their numbers increase with age and their appearance depends on functional androgen receptors expressed by the Leydig cells. Thus, they

are absent from the Leydig cells of patients with *androgen insensitivity syndrome* (androgen receptor defect).

In the ovary, the *maturing ovarian follicles* and (after ovulation) the *corpus luteum* are the major steroidogenic tissues. The maturing ovarian follicle consists of two adjacent steroidogenic cell populations: the epithelial *granulosa cells*, and the mesenchyme-derived *theca interna* cells (Fig. 13-2; see details in the section on the female reproductive system). The corpus luteum develops from the ovarian follicle upon ovulation. Its steroidogenic cells are derived from their preovulatory counterparts and are termed *granulosa lutein* and *theca lutein cells*, respectively (Fig. 13-3). The main secreted sexual steroid hormone before ovulation is E_2. The main steroid hormones produced by the corpus luteum are *progesterone* and E_2. During pregnancy, progesterone and *estriol* (E_3) are the main steroid products of the fetoplacental unit. Thus, even though the masculine and feminine secondary sexual characteristics are related to androgens and estrogens, respectively, *the most distinctive hormone between males and females is progesterone*, which is secreted in significant quantities only by the corpus luteum and the placenta.

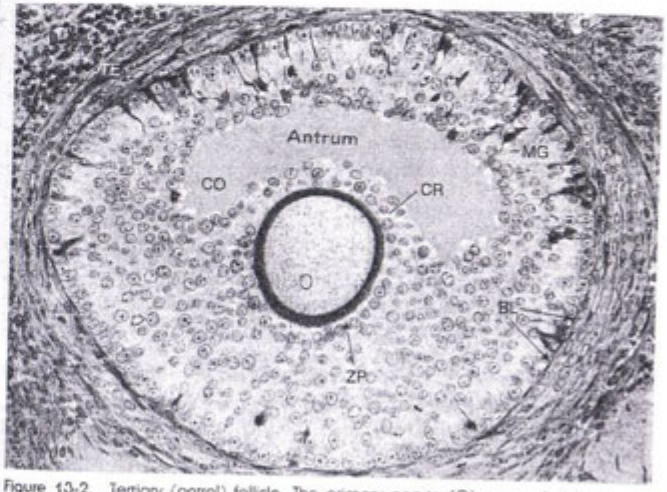


Figure 13-2. Tertiary (antral) follicle. The primary oocyte (O) surrounded by the zona pellucida (ZP) and corona radiata (CR) is found in the developing cumulus oophorus (CO). The follicular antrum is bordered by multiple layers of granulosa cells as membrana granulosa (MG). The vascular theca interna (TI) is separated from the avascular MG by a basal lamina (BL). These two cell populations contribute to sex steroid synthesis. The theca externa (TE) is not involved in hormone production. Compare with Fig. 13-15 (developing follicles). (*Source:* Modified from Fig. 32-13, p 860 in Fawcett DW: *Bloom and Fawcett's Textbook of Histology*. 11th ed., Philadelphia, Saunders, 1986.)

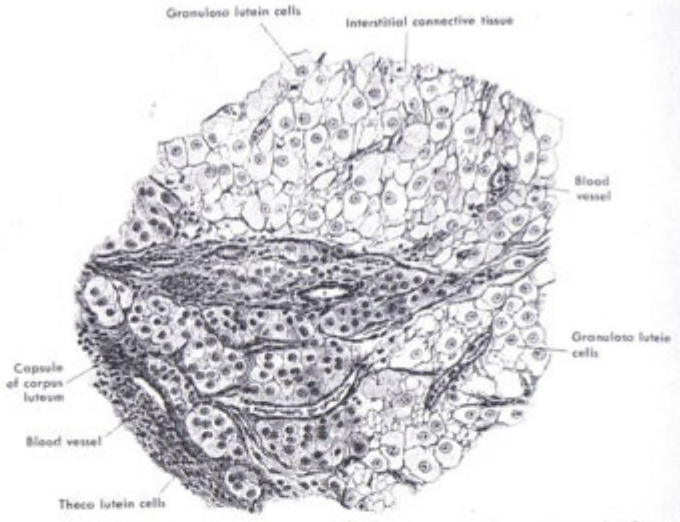


Figure 13-3. Corpus luteum. The image shows a small area of the convoluted surface of the corpus luteum at high magnification. After ovulation, the granulosa cell layer became vascularized, and connective tissue stroma developed. Both the granulosa and the theca interna cells are "luteinized" and function as granulosa lutein cells and theca lutein cells, respectively. The two cell populations essentially retain the proportions and relative positions found in the preovulatory follicle. (*Source:* Modified from Fig. 32-21, p 868 in Fawcett DW: *Bloom and Fawcett's Textbook of Histology*, 11th ed., Philadelphia, Saunders, 1986.)

In females, normal sex hormone production is achieved by the *coordinated* function of the granulosa and theca cells both before and after ovulation (Fig. 13-4):

- Theca interna and theca lutein cells express P450scc and produce pregnenolone, which is converted into progesterone by the preferentially Δ^4 steroidogenic pathway. Progesterone produced by the theca interna and the theca lutein cells is mainly used as a precursor of androgen synthesis involving P450c17.
- Theca interna cells express *aromatase* (P450arom, see below), the enzyme that converts androgens into estrogens. Upon ovulation/luteinization, aromatase expression ceases and theca lutein cells cannot secrete estrogens.
- The granulosa and granulosa lutein cells are unable to produce androgens because they lack the P450c17 enzyme. Thus, they must rely on androgens produced by the theca interna and theca lutein cells, respectively, and convert the androgens into estrogens by their aromatase activity. These cells are the most important *immediate* sources of E_2.

- In contrast with theca interna cells, the granulosa cells lack the P450scc enzyme and are unable to synthesize pregnenolone from cholesterol. After ovulation, granulosa *lutein* cells express P450scc and synthesize pregnenolone. Due to the continued absence of P450c17:
  - pregnenolone may only proceed to the production of progesterone. Thus, the main source of progesterone secreted by the corpus luteum is the granulosa lutein cell.
  - the granulosa lutein cells still rely on theca cell-derived androgenic precursors for their estrogen synthesis.

An intricate cooperation among various fetal, placental and maternal cells is involved in the generation of estrogens present in pregnant females, which is reminiscent of the cooperation between granulosa lutein and theca lutein cells. Similar to the granulosa lutein cells, the syncytiotrophoblast cells of the placenta may synthesize progesterone (but not androgens) de novo (see The Fetoplacental Unit). Most actions of androgens, estrogens and progesterone are mediated by their respective intracellular receptors involving a genomic action (see Chap. 5).

After Puberty, Steroidogenesis Is Regulated by LH in Males, and by Both FSH and LH in Females

Before puberty, the gonads secrete very low quantities of sexual steroids. This is in part related to the *basal, gonadotropin-independent steroid synthesis and secretion* of Leydig cells and of the maturing follicles (see the Female Reproductive System, below). The low levels of gonadal sexual steroids play an essential role in inhibiting gonadotropin secretion in prepubertal children, whose hypothalamus is exquisitely sensitive to negative feedback regulation by sexual steroids. This explains why, in *Turner's syndrome* (45,X gonadal dysgenesis) patients, who have *streak gonads* without follicles, plasma concentrations of gonadotropins are elevated in comparison with healthy children (see Fig. 13-43).

After puberty, the main determinants of gonadal steroid hormone secretion are the pituitary gonadotropins. The rate-limiting step of steroidogenesis in the gonads is the *LH-* or *human chorionic gonadotropin* (*hCG*)-stimulated and protein kinase A (PKA)-mediated induction of *steroidogenic acute regulatory protein* (StAR). LH receptors mediate this action in all gonadal cells that express P450scc: Leydig cells, theca interna, theca lutein, and granulosa lutein cells.

In males, the only target of LH is the Leydig cell, the source of all testicular steroid hormones. The only target of FSH is the nonsteroidogenic *Sertoli cell*, which provides the epithelial lining of the seminiferous tubules. The Sertoli cells secrete the protein hormone *inhibin B* (see Regulation of the Gonadotropin–Gonad Axis in Postpubertal Males) in response to FSH. As we shall see, the Sertoli cell is developmentally homologous with the granulosa cells, which explains the similarities in their endocrine function.

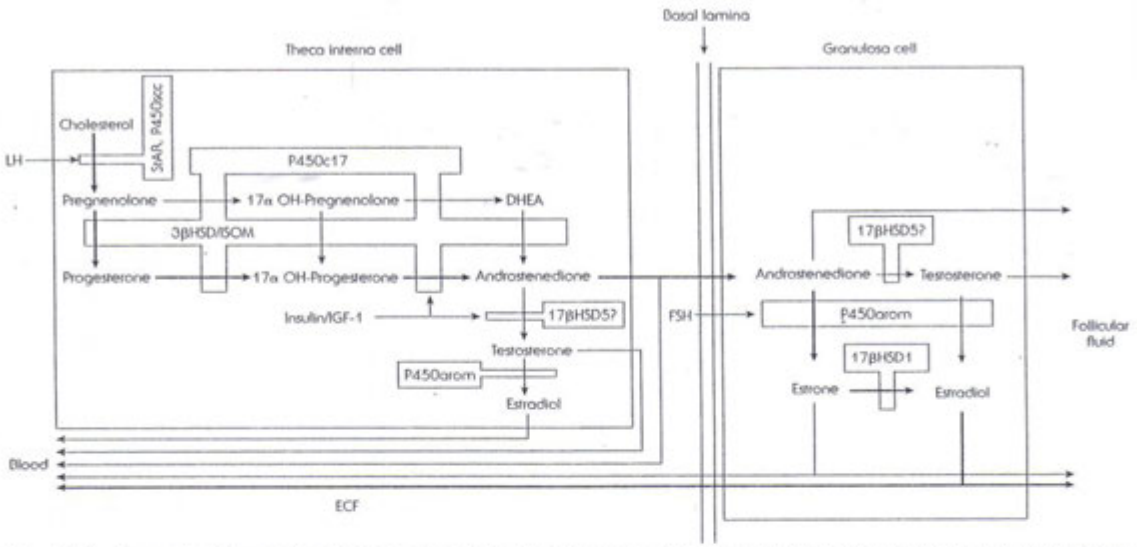


Figure 13-4. Cooperation between theca interna and granulosa cells in steroidogenesis in *preovulatory* follicles (A) and in the corpus luteum (B). The ovary produces small but physiologically relevant amounts of testosterone; however, the isoenzyme of 17β-hydroxysteroid dehydrogenase (17βHSD) converting androstenedione to testosterone is uncertain. As indicated by the thickness of the arrows, estradiol is preferentially produced by the androstenedione–estrone pathway involving aromatase (P450arom) and 17βHSD1. In nondominant follicles, FSH receptors are downregulated, androgens accumulate in the follicular fluid, and atresia ensues. Note that after ovulation of the dominant follicle, two parallel steroidogenic pathways operate leading to the secretion of estradiol and progesterone. LH not only leads to luteinization of the granulosa cells but becomes the regulator of their steroidogenic activity.

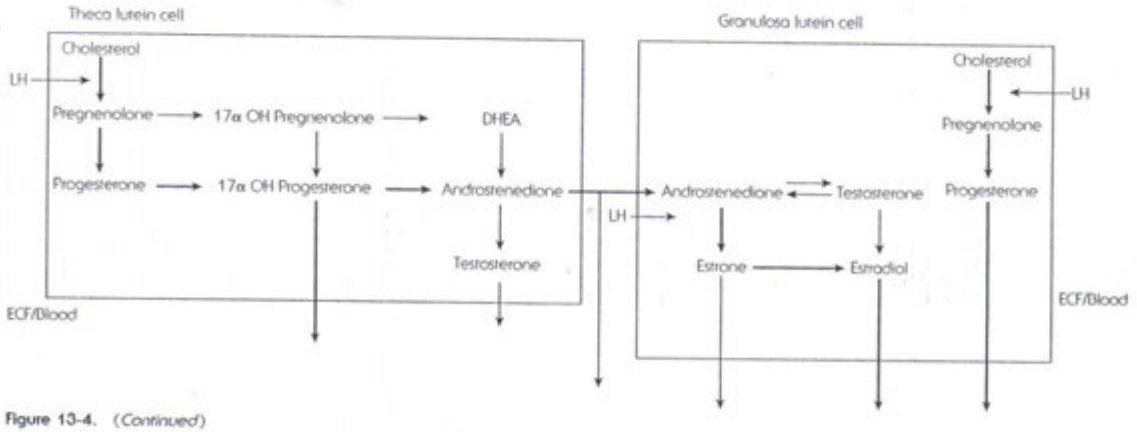


Figure 13-4. (*Continued*)

Although FSH is an important stimulator of *spermatogenesis* (see Spermatogenesis), it has no direct stimulatory action on either the de novo synthesis or the conversion of steroid hormones.

In contrast, in females FSH is the physiologic stimulus of estrogen secretion before ovulation. The only target of FSH in females is the granulosa cell. FSH stimulates the proliferation of granulosa cells, the secretion of *inhibin B*, and the expression of *aromatase*. *At advanced stages of follicular maturation, granulosa cells also express small numbers of LH receptors, which are important in luteinization.* The *preovulatory surge of LH* initiates luteinization in the theca interna and granulosa cells and causes ovulation, the development of the corpus luteum, and the completion of the luteinization process. Starting with the appearance of the *luteinized* granulosa and theca cells (i.e., shortly before ovulation), LH stimulates streoidogenesis in both cell populations. In granulosa lutein cells, LH stimulates aromatase activity, progesterone and *inhibin A* secretion. Because LH increases the secretion of androgens by the adjacent theca lutein cells, the increased aromatase activity in granulosa lutein cells results in increased estrogen secretion.

Dehydroepiandrosterone and Androstenedione Are Processed by Isoenzymes of 17β-Hydroxysteroid Dehydrogenase and a Single Aromatase Enzyme (P450arom)

We followed the biosynthesis of androgens to dehydroepiandrosterone (DHEA) and androstenedione in the adrenal cortex (see Fig. 12-8). In contrast with the adrenal cortex, which preferentially secretes DHEA over androstenedione (i.e., preferential Δ^5 pathway), the biosynthesis of androgens in the gonads follows the Δ^4 pathway. Thus, the gonads secrete relatively small quantities of DHEA, especially in females (see Fig. 13-4). The two key enzymes involved in the further processing DHEA and androstenedione in the gonads and peripheral (extragonadal) locations are

- the isoenzymes of *17β-hydroxysteroid dehydrogenase* (17βHSD), which convert androgens and/or estrogens either into their less or into more potent forms (Table 13-1); and
- a single *aromatase* enzyme (P450arom), which converts C19 steroids (androgens) into C18 steroids having an aromatic A-ring (estrogens).

The 17βHSD1, 17βHSD3, and 17βHSD5 isoenzymes constitute an *activator subfamily* that produces more potent sex steroids from substrates having lower biologic activities.

- *17βHSD1* is an *estrogen-specific* enzyme that produces 17β-estradiol from estrone. This isoenzyme is present in the main sources of circulating estrogens: the granulosa and granulosa lutein cells of the ovary and the trophoblast cells of the placenta.
- The expression of *17βHSD3* is *required for normal testicular androgen secretion* both in utero and after puberty. The main substrate of 17βHSD3 is androstenedione, which is converted into testosterone. The congenital absence of 17βHSD3 results in *male pseudohermaphroditism* (Box 13-1 and Sexual Development).
- The physiologic importance of *17βHSD5* is uncertain. Hepatic 17βHSD5 might be an important determinant of circulating testosterone levels in women by conversion of androstenedione produced by the adre-

Table 13-1 17β-Hydroxysteroid Dehydrogenase Isoenzymes

Type	Gene	Chromosomal localization	Preference for substrate(s) and product(s)	Tissue distribution
1	17βHSD1	17q11-21	Estrone to estradiol (18OH-steroid-specific); produces more active estrogens by a reductive reaction	Primarily in the ovary (granulosa cells, granulosa lutein cells), placenta, mammary gland
2	17βHSD2	17q11-21	Estradiol to estrone ≈testosterone to androstenedione; possesses 20α-HSD activity: converts 20α-dihydroprogesterone to progesterone; normally unidirectional oxidative function; limits estrogen effects on endometrium by oxidizing estradiol into estrone	Liver, secretory endometrium, placenta, small intestine, prostate
3	17βHSD3	9q22	Androstenedione to testosterone > DHEA to 5-androstenediol > estrone to estradiol; mainly produces more active androgens by a reductive reaction	Testis
4	17βHSD4	5q21	Estradiol to estrone, androst-5-ene-3β,17β-diol to DHEA; unidirectional oxidative function	Liver, heart, prostate, testis
5	17βHSD5	10p14-15	Androstenedione to testosterone; low activity	Liver, adrenal gland, prostate, ovary (?)

BOX 13-1 Hermaphroditism and Pseudohermaphroditism

In Greek mythology, Hermaphroditos was a son of Hermes and Aphrodite who possessed both male and female external genitalia. Today, the medical term *true hermaphroditism* refers to the condition when both ovarian and testicular tissues are present in the same individual. This rare condition is associated with a highly variable presentation of the external genitalia. In *pseudohermaphroditism* the gonad is either male or female (*not* both); the external genitalia and the gonad are mismatched (the external genitalia are either ambiguous or appropriate for the *opposite* gonadal sex). The gender in these cases is designated by the gonadal sex. Thus, male pseudohermaphrodites have testes (and female-like external genitalia), and female pseudohermaphrodites have ovaries (and male-like external genitalia).

nals. 17βHSD5 is a minor enzyme in the adrenal cortex, which explains the usually negligible adrenal testosterone production. The theca and granulosa cells may convert androstenedione to testosterone, but the type of 17βHSD involved has not yet been elucidated; 17βHSD5 is a candidate for this function.

The 17βHSD2 and 17βHSD4 isoenzymes mainly function as unidirectional *inactivators* of potent sex steroids.

- *17βHSD2 is equally potent in decreasing the activity of E_2 and testosterone. Its function is to limit the action of these hormones on their major target cells such as the endometrium, liver, and prostate.* In combination with aromatase, 17βHSD2 in the placenta protects the fetus from maternally derived testosterone.
- The functions of *17βHSD4* in the liver and the prostate are similar to those of 17βHSD2. However, 17βHSD4 is less active converting its androgenic than its estrogenic substrate. Thus, 17βHSD4 *mainly protects against estrogen receptor stimulation.*

Aromatase is a membrane-anchored heme glycoprotein found in the sER. Aromatase is associated with the ubiquitous *NADPH-cytochrome P450 reductase*, which transfers reducing equivalents from NADPH to *any* microsomal cytochrome P450. The aromatase reaction uses 3 moles of oxygen and 3 moles of NADPH for every mole of C19 steroid metabolized. The first two oxygens oxidize the C19 methyl group by standard hydroxylation mechanisms. The third oxidative reaction is a *peroxidative attack* on the C19 methyl group, which (by cleaving the methyl group as formic acid from the steroid frame) leads to the aromatization of the A-ring. This is the only known reaction in vertebrates that introduces an aromatic ring into a molecule.

Aromatase is encoded by the *CYP19* gene localized on chromosome

15q. It is 27.4 cM telomeric to the closely linked *CYP11A* and *CYP1A1* genes. *CYP11A* encodes the mitochondrial P450scc enzyme (see Chap. 12). *CYP1A1* encodes an *aryl hydrocarbon receptor*-regulated microsomal monooxygenase, which is involved in hepatic detoxification processes.

The *CYP19* gene consists of 10 exons and uses multiple promoters in a tissue-specific manner (Table 13-2); various first exons are regulated by these promoters. Because the AUG start codon is located on exon II, this arrangement results in a heterogeneity of the transcripts only in their 5′ untranslated regions, and the protein encoded by the various transcripts is identical. The usage of different promoters allows a tissue-specific regulation of the aromatase activity.

Depending on gender and reproductive stage, significant contribution to circulating estrogens by aromatase activity is provided by the gonads and/or certain peripheral (extragonadal) tissues. In males, under most physi-

Table 13-2 Tissue-Dependent Regulation of Aromatase Expression via Multiple Promoters

Tissue	Main promoter(s)	Main regulator(s)
Placenta	I.1	Retinoids acting via RXR/RAR; SF-1 is absent
Ovary	II	FSH, granulosa cells; LH, theca interna and granulosa lutein cells (cyclic AMP/CREB; action is SF-1-dependent)
Leydig cells of the testis	II (LH-induced) ? (SRY-induced)	LH (cyclic AMP/CREB; action is SF-1-dependent), SRY
Adipose tissue (preadipocytes, mesenchymal stromal cells in adipose tissue)	I.4 >> I.3, II	Glucocorticoids + class I cytokines (e.g., oncostatin-M, LIF, IL-6, IL-11) via the JAK1–STAT3 pathway and the GAS element; TNF-α, involving sphingomyelinase and an AP1 site; PGE_2 via cyclic AMP, PKC and CREB
Preadipocytes in breast tissue near breast cancer cells	II, I.3	PGE_2 via cyclic AMP/CREB and PKC
Bone, epiphyseal cartilage	I.4 >> I.3 > I.6	Glucocorticoids and class I cytokines, IL-1β, TNF-α
Brain (appears to be region-specific usage of promoters)	1f > II, I.4	Androgens; PKC and PKG pathways, α_1-adrenergic agonists, substance P, cholecystokinin, natriuretic peptide
Pituitary gland	Similar to brain (?)	Androgens (?)

ABBREVIATIONS: SF-1, Steroidogenic factor-1, a transcription factor that is an orphan member of the steroid hormone receptor superfamily; JAK, Janus kinase (a receptor-associated tyrosine kinase); STAT, signal transducer and activator of transcription (a phosphorylation-dependent transcription factor); GAS, γ-interferon-activated sequence (a cis-acting element); RXR, retinoid X receptor; RAR, retinoic acid receptor; FSH, follicle-stimulating hormone; LH, luteinizing hormone; SRY, a transcription factor encoded by the sex-determining region of the Y chromosome; LIF, leukemia inhibiting factor; IL, interleukin; TNF-α, tumor necrosis factor α; CREB, cyclic AMP response element-binding protein.

ologic circumstances, 80% of 17β-estradiol and 98% of estrone in plasma is derived from peripheral conversion of androgens primarily in adipose tissue. In contrast, in females during the menstrual cycle direct ovarian secretion is the main source of circulating estrogens. The specific C18 steroid produced in each tissue depends on the presentation of the C19 steroid.

- Although *Leydig cells* primarily produce testosterone, they also secrete small quantities of estrogens (mainly 17β-estradiol) because of the presence of aromatase. *Supranormal production of estrogens by Leydig cells occurs when their LH receptors are hyperstimulated.* This is the cause of LH-induced *gynecomastia* (female-breastedness), which affects 60 to 70% of *adolescent males*, and the cause of hCG-induced gynecomastia in *testicular choriocarcinoma* patients. Unlike in most animal species, the Sertoli cells of adult men do not express significant aromatase activity in vivo. However, Sertoli cell *tumors* may express aromatase, which may lead to feminization (including gynecomastia).
- Similar to the testis, the ovary may produce 17β-estradiol from testosterone. However, the preferred route of ovarian production of 17β-estradiol involves the aromatization of androsetenedione followed by the conversion of the resulting estrone into 17β-estradiol by 17βHSD1. The aromatase activity is very high in the granulosa cells, and normally the follicular secretion of androgens (mainly as androstenedione) is minimal. Overt stimulation of insulin and/or IGF-1 receptors of theca cells, however, increases the secretion of androgens, including testosterone, as seen in *polycystic ovary disease* (see Regulation of the Ovarian Cycle: The Hypothalamic–Pituitary–Ovarian Axis).

The *peripheral tissues* that significantly contribute to *circulating* estrogens by their aromatase activities include adipose tissue and the placenta.

- *Preadipocytes*, rather than mature adipocytes filled with a triglyceride droplet, *are the sites of aromatase expression*. However, *the amount of preadipocyes in the body is proportionate to the degree of adiposity*. Aromatase activity in preadipocytes displays a regional distribution: higher aromatase activity is present in the adipose tissue of the *buttocks* and *thighs* than in the abdominal subcutaneous fat or in the (nontumorous) breast. The cytokine-dependent expression of aromatase in adipose tissue requires the mandatory presence of glucocorticoids. The age-dependent increase of these cytokines explains the age-dependent increase of adipose tissue aromatase activity in both sexes even in the absence of obesity.
  - In females, adipose tissue mainly produces estrone from androstenedione primarily secreted by the adrenal cortex. The physiologically higher adipose tissue mass in females and its feminine distribution explain the higher peripheral aromatase activity in females compared to males. Obese postmenopausal women have an *increased risk for endometrial cancer* and a *decreased risk for osteoporosis*.

  - In males, adipose tissue converts both testicular androgens (testosterone) and adrenal androgens (androstenedione). Obesity increases the estrogen:androgen ratio in both sexes. Body mass index (BMI; see Box 10-4) is positively correlated with gynecomastia in males.
- The *trophoblasts* of the placenta mainly produce estriol from 16α-OH-DHEA, a product of the combined activities of the fetal adrenal cortex, the fetal liver and placental steroid sulfatase (see The Endocrine Physiology of the Pregnant Woman and the Fetoplacental Unit). Placental aromatase prevents the masculinization of the female fetus by androgens generated by the fetal adrenal cortex and an unidentified placental 17βHSD isoenzyme. Placental aromatase deficiency is one of the causes of *female pseudohermaphroditism* (see The Endocrine Physiology of the Pregnant Woman and the Fetoplacental Unit).

In certain peripheral tissues, the conversion of androgens into estrogens by aromatase has no significant impact on circulating levels of estrogens, but plays essential roles in several *local actions*. These *estrogen receptor-mediated actions of circulating androgens* include:

- The prevention of *osteoporosis* and mediation of *epiphyseal closure* in males (osteoblast and chondroblast aromatase).
- Participation in the *feedback* of androgens on gonadotropin secretion via the hypothalamus (conversion by brain aromatase) and the gonadotroph (conversion most probably by pituitary aromatase). Aromatase expression in the human pituitary gland has not yet been confirmed. At least in rodents, pituitary aromatase expression is significantly higher males than in females. The difference is presumed to be related to expression from androgen-induced promoter site.
- Stimulation of *breast cancer* growth. This mechanism involves a local positive feedback, whereby breast cancer cells stimulate aromatase in preadipocytes via a paracrine mechanism involving prostaglandin E_2 (PGE_2), and the locally generated estrogens stimulate the proliferation of the cancer cells. Inhibitors of aromatase such as *letrozole* are used in the treatment of breast cancer.

Testosterone May Be Converted to the More Potent Dihydrotestosterone by Two Isoenzymes of 5α-Reductase Expressed in Target Tissues

In addition to its conversion by aromatase, testosterone is subject to conversion into *dihydrotestosterone* (DHT) by two isoenzymes of *5α-reductase* (see Fig. 12-8; Table 13-3). Almost all circulating DHT is generated in peripheral tissues. Pharmacologic evidence obtained in men suggests that the *type II* (*finasteride-sensitive*) *5α-reductase* generates three times as much DHT as the type I (MK-386-sensitive) isoenzyme. The skin (dermal fibroblasts, keratinocytes, hair follicles, sebaceous and apocrine sweat glands)

Table 13-3 5α-Reductase Isoenzymes

Type	Chromosomal localization	Tissue distribution	Inhibitor(s)
5α-reductase type I	5p	*Sebaceous glands, apocrine sweat glands,* epidermal keratinocytes, dermal papilla cells, fibroblasts, dermal papillo cells, fibroblasts from genital and nongenital skin, brain, rat Leydig cell	MK-386, LY191704
5α-reductase type II	2p	*Prostate,* epididymis, seminal vesicles, fetal genital skin, *inner root sheath of hair follicles,* fibroblasts from normal adult genital skin, gonadotropin feedback (brain?)	Finasteride

NOTE: Italicized are the clinically most important features.

is the main source of circulating DHT; the cutaneous production is complemented by the prostate in men. The distribution and activity of 5α-reductase associated with hair follicles displays racial differences: in general, the activity is highest in Caucasians especially of Mediterranean origin, and the lowest in Orientals. Unlike testosterone, *DHT is not a substrate for aromatase*, and therefore, the biologic actions of DHT are not mediated by estrogen receptors.

DHT is the most potent activator of the androgen receptor; it is about 2.5 times more potent than testosterone. Certain actions of androgens, such as the masculinization of external genitalia and the development of benign prostatic hypertrophy, have an absolute requirement for 5α-reductase activity. Although not mandatory, 5α-reductase activity significantly contributes to the proper embryonic development of the prostate, descent of the testes, phallic growth, male-pattern balding (in individuals with genes predisposing for baldness), the development of terminal body hair, pubic and underarm hair, *hirsutism* (excessive terminal hair growth usually with a male pattern observed in females), and the activity of sebaceous and *apocrine* sweat glands. Other androgenic actions are mediated by testosterone per se irrespective of peripheral conversion, such as the embryonic development of Wolffian duct-derived structures and their postpubertal secretory activity; the pubertal growth of the larynx (deepening of the voice); the anabolic effect on eythropoiesis and muscle (increased muscle protein and lean body mass, positive nitrogen balance); inhibition of breast development; the stimulation of spermatogenesis; libido; and possibly the sexual orientation toward females. The various mechanisms of actions of androgens in males are summarized in Table 13-4.

DHT acts mainly in an *intracrine* manner, i.e., it activates the androgen receptor within the 5α-reductase-expressing cell. These cells also express

Table 13-4 Physiologic Actions of Androgens in Males Without and With Peripheral Conversion of Testosterone

Testosterone-mediated actions without peripheral conversion	Absolute requirement for 5α-reductase activity	Significant but nonmandatory involvement of 5α-reductase activity	Aromatase-dependent actions
			Mostly estrogen receptor-mediated genomic actions
Mostly androgen-receptor mediated genomic actions			
Embryonic development and postpubertal secretory activity of Wolffian duct-derived structures: epididymis, vas deferens, seminal vesicle	Masculinization of external genitalia: labioscrotal fusion, *normal* phallic development	Proper embryonic development of prostate	Epiphyseal closure
Laryngeal growth (Adam's apple)	Development of benign prostatic hypertrophy	Phallic growth	Prevention of osteoporosis
Stimulation of erythropoiesis (mainly via erythropoietin)	—	Descent of the testes	
Anabolic effect on muscle	Male-pattern distribution of hair (mustache, beard, chest, escutcheon)[a]	Male-pattern balding (with predisposing genetic background)	Prevention of Alzheimer's disease (direct trophic action in the CNS)
Decreased HDL		Ambisexual hair growth (pubic, underarm)	Fluid retention
Stimulation of spermatogenesis	—	Activity of sebaceous glands	—
Inhibition of breast development	—	Activity of apocrine sweat glands	—
Behavioral responses: libido, aggresion, sexual orientation toward females	—	Erection	—
—	—	Feedback regulation of gonadotropin secretion	Feedback regulation of gonadotropin secretion

[a] The assignment of male-type hair distribution into this column is based on the typical clinical picture of type II 5α-reductase deficiency in postpubertal male pseudohermaphrodites, who develop ambisexual hair, but no male escutcheon, beard or mustache. Male-type hair distribution, however, could also qualify as only partially dependent on 5α-reductase activity.

ABBREVIATIONS: CNS, central nervous system; HDL, high density lipoprotein.

Table 13-5 Assessment of 5α-Reductase Activity

5α-androstane-3α,17β-diol glucuronide (normal concentration range in serum)	Potential causes of supranormal concentration	Potential causes of subnormal concentration
Prepubertal children: 10–60 ng/dL (0.21–1.28 nM) Adult male: 260–1500 ng/dL (5.54–31.95 nM) Adult female: 60–300 ng/dL (1.28–6.39 nM)	Hirsutism, acne, conditions associated with virilization such as certain types of congenital adrenal hyperplasia and polycystic ovary syndrome	5α-reductase deficiency, non-Caucasian race (adult males)

3α-hydroxysteroid dehydrogenase (3αHSD; see Fig. 12-8). 3αHSD catalyzes the *reversible* reduction of DHT to *5α-androstane-3α,17β-diol* (a weak androgen). The equilibrium between the reductive and oxidative activities of 3αHSD is an important factor in the regulation of intracellular levels of DHT and androgen receptor stimulation. There are at least three isoenzymes of 3αHSD (designated as types 1 through 3).

Although DHT is primarily an intracrine/paracrine hormone, DHT also enters the circulation and is present in plasma in significant quantities (about 10% of testosterone levels); thus, DHT may exert androgenic action on 5α-reductase negative tissues. The liver expresses type 2 3αHSD, which is important in the inactivation of *circulating* DHT. The 5α-androstane-3α,17β-diol generated in the liver and by peripheral tissues is glucuronidated by the liver and excreted by the kidneys. *Plasma levels and urinary excretion of 5α-androstane-3α,17β-diol glucuronide* (3α-diol-G) *are the best markers for assessing peripheral formation of DHT* (Table 13-5).

In Addition to Being the Carrier Protein of Androgens and Estrogens in Plasma, SHBG Acts on a Membrane Receptor in a Steroid Hormone-Dependent Manner

As lipophilic compounds, androgens, estrogens, and progesterone circulate in plasma mainly in association with carrier proteins (see Chap. 4 and Table 4-2).

Plasma SHBG (mainly produced by the liver) and testicular *androgen-binding protein* (ABP, secreted by Sertoli cells into the lumen of the seminiferous tubules) are homodimeric glycoproteins with a single steroid-binding site. SHBG and ABP are products of a single gene (chromosome 17p12-13), which is expressed in several tissues including liver, testis, brain, endometrium, and placenta. The transcripts and amino acid sequences of *plasma* SHBG and *testicular* ABP are identical and differ only in their oligosaccharides. The SHGB gene is widely expressed in the brain, where several transcripts result from differential exon utilization; their biologic functions are unknown.

The glycosylation of SHBG and other hormone-binding globulins in the liver is increased by estrogens, which leads to their prolonged half-life and accumulation in plasma (Fig. 13-5). Due to the higher affinity of androgens than estrogens to SHBG, the increased plasma concentration of SHBG shifts the estrogen:androgen ratio of the free (biologically active) hormones toward estrogens. This mechanism contributes to the protection of the developing female fetus from the masculinizing action of maternal androgens. Cirrhosis of the liver is associated with an increased glycosylation of SHBG, which (by the above mechanism) results in estrogen excess and gynecomastia in males.

Plasma SHBG and testicular ABP are modular proteins comprised of an N-terminal steroid-binding and dimerization domain, and a C-terminal domain containing a highly conserved consensus sequence for glycosylation. The C-terminal domain may be required for recognition of cell surface receptors. The *SHBG receptor* has been partially characterized. Because its action on cell function is mediated by activation of adenylyl cyclase and PKA, it is presumed to be a heptahelical transmembrane receptor.

Only the unliganded SHBG/ABP may bind with its cognate receptor: *prior* binding of steroid ligands prevents interaction of SHBG with its receptor. Binding of unliganded SHBG results in a mild increase of cytosolic cyclic AMP. Whereas prior steroid ligand binding of SHBG prevents the activation of the SHBG receptor, the cyclic AMP-increasing effect of the receptor-bound SHBG is markedly enhanced by the *subsequent* binding of certain steroid ligands (Fig. 13-6). The increased cyclic AMP results in a PKA-mediated phosphorylation and the modulation of several signal transduction mechanisms, including *androgen-independent activation of the androgen receptor*. The steroid ligands that may bring about this alternate route of androgen receptor activation in the prostate include 5α-androstane-3α,17β-diol (a degradation product of DHT) and 17β-estradiol. This explains how estrogens cause *benign prostatic hypertophy* by an androgen receptor *dependent* mechanism.

Progesterone, as a C21 steroid, does not bind to SHBG but circulates in association with *cortisol-binding globulin* (CBG, a member of the serpin family; see Chap. 12) and albumin.

The Degradation Products of Sexual Steroids and Progesterone Are Conjugated with Glucuronic Acid or Sulfate in the Liver and Mainly Excreted in the Urine

The degradation of sexual steroids and progesterone is similar to that of corticosteroids. Thus, more hydrophilic derivatives are formed that are not bound by plasma proteins and are therefore readily filtered by the kidney. The degradation is primarily performed by the liver. The rapid and extensive breakdown during a single passage through the hepatic circulation explains

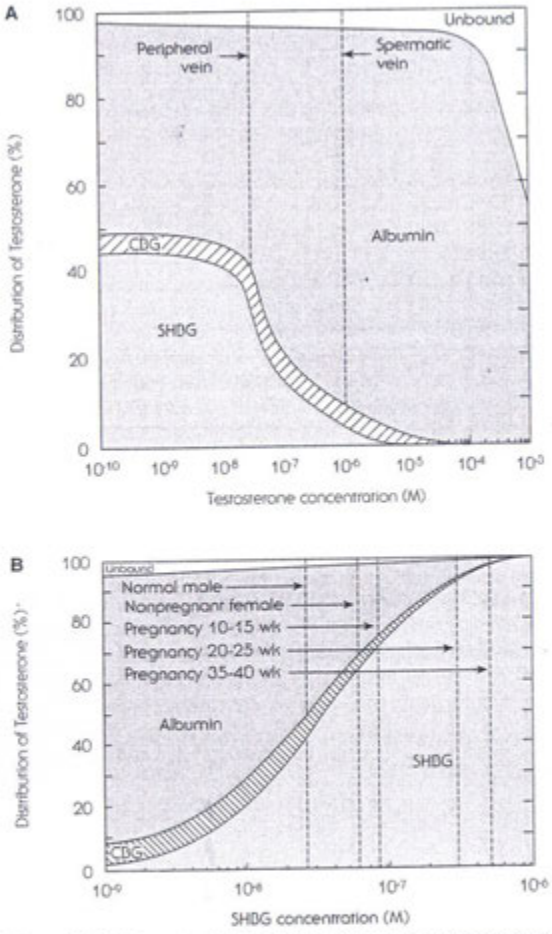


Figure 13-5. Computer-simulated effects of increasing concentration of testosterone (A) and sex hormone-binding globulin (SHBG; B) on the distribution of testosterone in plasma. Note the logarithmic scale of the concentrations. A. The high concentration of testosterone in spermatic vein nearly saturates the binding capacity of plasma SHBG, but leaves most binding sites of plasma SHBG unoccupied in the systemic circulation. With increasing concentrations of testosterone, the proportion of albumin-bound and free testosterone increases, provided the concentrations of carrier proteins remain unchanged. B. The increasing concentration of SHBG decreases free testosterone levels in plasma. Because the affinity of SHBG for estrogens is lower than for androgens, increasing plasma SHBG has less impact on free estrogens. Thus, the estrogen : androgen ratio shifts toward estrogens with increasing plasma

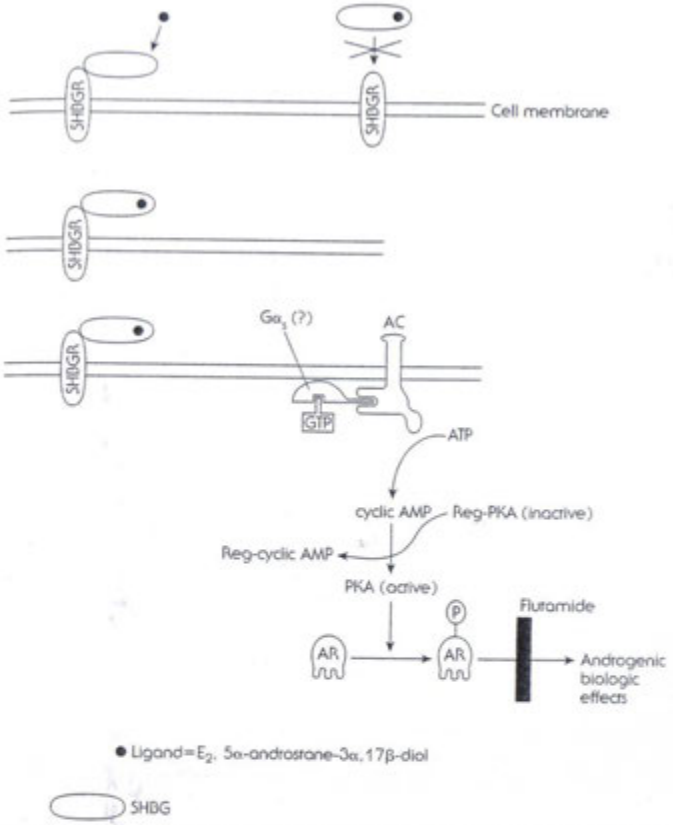


Figure 13-6. Model of signaling via the sex hormone-binding globulin receptor (SHBGR). The SHBGR is not activated by preformed SHBG-ligand complexes. Binding of unliganded SHBG to its receptor results in a weak activation of protein kinase A (PKA), which is significantly augmented by subsequent binding of the ligand, such as estradiol (E_2) or 5α-androstane-3α,17β-diol (the degradation product of dihydrotestosterone). The activation of PKA is presumed to involve a trimeric G-protein mechanism (see details in Fig. 5-4). PKA activates the androgen receptor (AR) by phosphorylation. Thus, E_2-provoked benign prostatic hypertrophy can be antagonized by antiandrogens, such as flutamide. Reg, regulatory subunit of PKA; AC, adenylyl cyclase.

Figure 13-5. (*Continued*) SHBG. Compared to men, women especially during pregnancy have significantly elevated plasma SHBG. CBG, cortisol binding globulin. (*Source*: Figs. 4 and 5 (with slight modification) from Dunn JF et al: Transport of steroid hormones: Binding of 21 endogenous steroids to both testosterone-binding globulin and corticosteroid-binding globulin in human plasma. J Clin Endocrinol Metab 53:58–68, 1981.)

that natural sexual steroids and progesterone (unlike their synthetic analogues) are not suitable as oral drugs. The degradation of androgens may follow several routes:

- aromatization followed by degradation of the resulting estrogens;
- degradation of DHT by 3αHSD;
- degradation of testosterone through androstenedione. This mechanism may include the action of 17βHSD2 (and 17βHSD4) in the liver (see above). Androstenedione is then reduced into any of three closely related compounds: *etiocholanolone*, *epiandrosterone*, or *androsterone*, which are conjugated with glucuronic acid via their 3-hydroxyl groups and end up in urine as the main *17-ketosteroids* (Fig. 13-7).

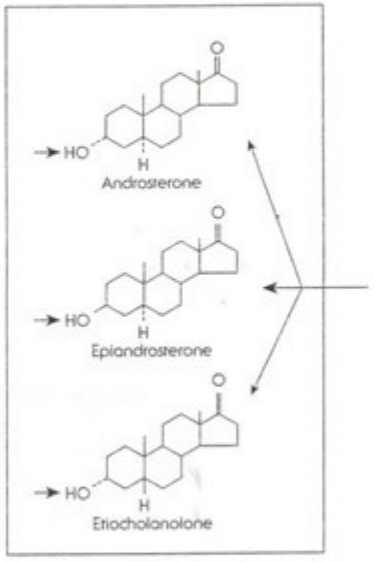


Figure 13-7. The metabolism of sexual steroids and progesterone. The reactions shown are mainly performed by the liver. The end products are hydrophilic, do not bind with SHBG, and are excreted with urine. Note that the degradation of dihydrotestosterone is neglected (see Fig. 12-8). The color arrows indicate targets of glucuronidation, the black arrowheads show targets of sulfation. The central precursor in the degradative pathway of androgens is androstenedione, which is either broken down into 17-ketosteroids, or becomes aromatized. Estrone plays a similar central role in the degradative pathway of estrogens as androsterenedione does for androgens. It may be metabolized into 3-sulfated 16-hydroxyestrone, double-conjugated estriol, or catecholestrogens which are inactivated by catechol-O-methyltransferase (COMT, compare with Fig. 12-5). A simplified flowchart of the rapid degradation of progesterone is shown on the right.

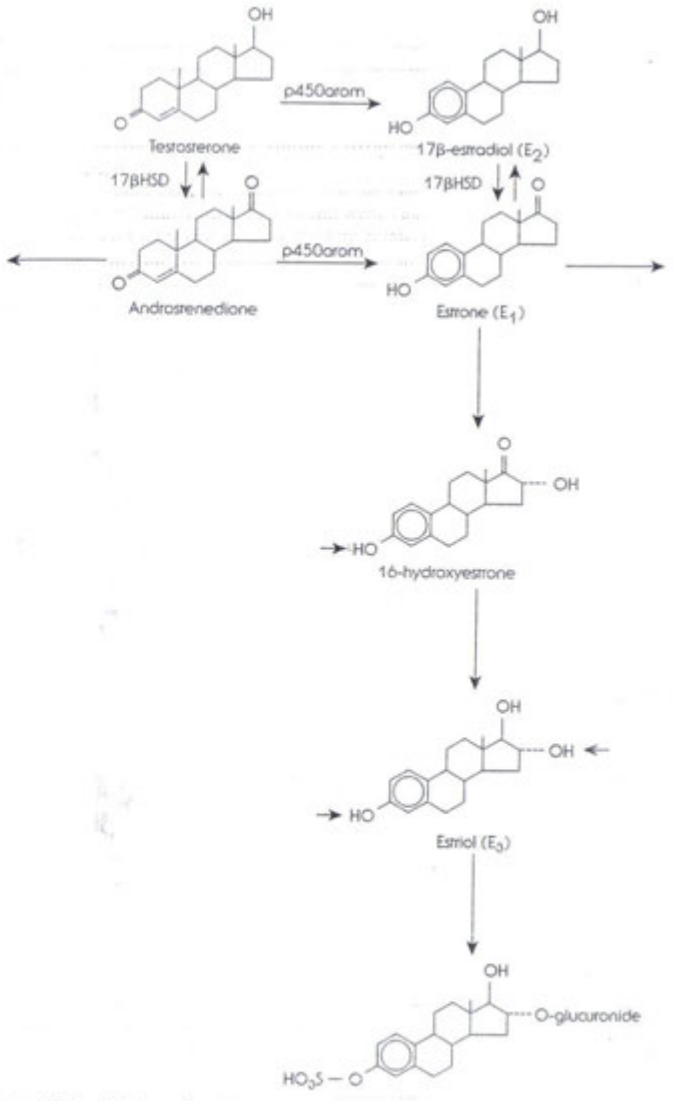


Figure 13-7. *(Continued)*

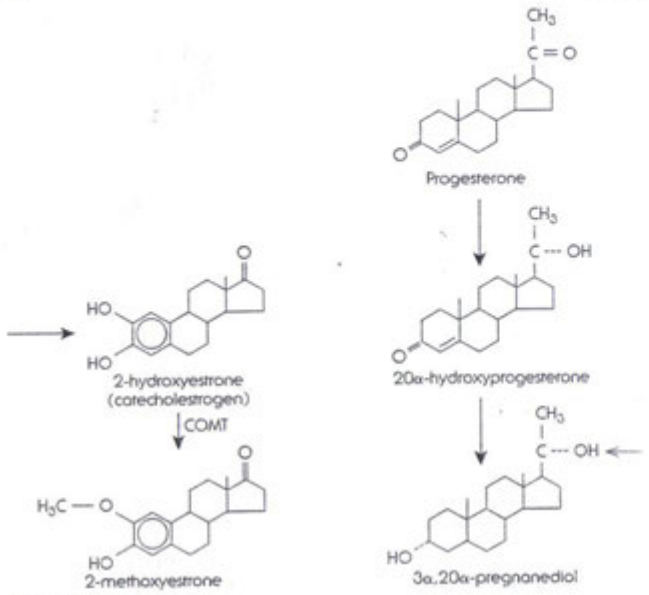


Figure 13-7. (*Continued*)

In adult men, about one-third of urinary 17-ketosteroids are derived from testosterone, and two-thirds from the less potent adrenal androgens, DHEA and androstenedione. In adult women, the ovarian contribution to *total urinary 17-ketosteroids* is minimal, compared to the amount derived from adrenal androgens. This explains why the normal urinary ketosteroid production in women is about two-thirds of that in men. It is important to note, however, that ovarian contribution to *the total androgenic activity in the circulation* is significant. The explanation for the difference lies in the fact that testosterone as well as androstenedione are metabolized into 17-ketosteroids, but testosterone has more androgenic activity. The ovaries significantly contribute to the circulating pool of testosterone by direct secretion from theca cells.

The inactivation of 17β-estradiol (the most potent estrogen) starts in the liver with conversion to estrone by 17βHSD2 and 17βHSD4. Some 17β-estradiol and estrone is directly conjugated and excreted. Estrone is mainly converted either into biologically active *catecholestrogens* (2- or 4-hydroxyestrone) or 16α-hydroxyestrone (see Fig. 13-7). The catecholestrogens are processed by the catechol-O-methyltranferase (COMT) enzyme,

which is involved in the degradation of catecholamines. The 16α-estrone is converted to estriol, which is excreted as estriol 3-sulfate,16-glucuronide. Plasma and urinary *estriol is the main estrogen during pregnancy* (see The Endocrine Physiology of the Pregnant Woman and the Fetoplacental Unit).

The breakdown of progesterone is extremely rapid. The main route of degradation involves two successive reduction reactions to 20α-hydroxyprogesterone and pregnanediol. The latter is excreted as *pregananediol-20-glucuronide* (see Fig. 13-7).

THE MALE REPRODUCTIVE SYSTEM

OBJECTIVES

1. Discuss the anatomy of the testis, the excurrent duct system, and the male accessory glands. Describe the main functions of each organ. Identify the sources of semen and preseminal fluid. Discuss the coagulation system of semen and its relationship with the *prostate-specific antigen* (PSA).
2. Discuss the stages and the timeframe of *spermatogenesis*. Identify the relationship between developing sperm and the blood-testis barrier, and the functions Sertoli cells in spermatogenesis. Discuss the hormonal regulation of spermatogenesis; identify hormonal targets, sources, and the importance of high local concentrations of testosterone.
3. Discuss the composition of semen, sperm count, the parameters of sperm quality, *azoospermia*, and the mechanism of the regulation of spermatogenesis by temperature.
4. Describe the structure of the penis and the endocrine regulation of its growth. Explain the mechanism of erection, the role of parasympathetic nerves, nitric oxide, and its relationship with androgens.
5. Discuss ejaculation, its phases, its regulation by sympathetic nerves, and its relationship with erection and orgasm.
6. Discuss the regulation of the hypothalamic–pituitary–testicular axis in adult (postpuberral) males. Discuss in detail: *gonadotropin-releasing hormone* (GnRH), pituitary *gonadotropins*, testicular hormones (including steroids and the members of the *transforming growth factor* β [TGF-β] family), their secretory patterns, receptors, cellular targets, and the mechanisms of feedback action. Distinguish *pulse frequency* and *pulse amplitude* of the pulsatile release of GnRH, and identify hypothalamic mechanisms regulating these parameters. Identify mechanisms whereby the pulse amplitude of LH may dissociate from changes in the pulse amplitude of GnRH. Discuss differential regulation of LH and FSH secretion, the role of pulse frequency, and the downregulation/desensitization of GnRH receptors. Describe the impact of *hyperprolactinemia* and *leptin* on the hypothalamic–gonadal axis.
7. Discuss selected pathologic conditions, such as *Kallmann's syndrome*, *McCune–Albright syndrome*, and *testotoxicosis*.

Anatomic Overview of the Male Reproductive System

Testis The *testis* (*orchis*) is an egg-shaped organ (see Fig. 13-8). At the time of birth, the testicles are normally found in the scrotum; their absence from the scrotum is termed *cryptorchidism* (cryptic orchidism = hidden testicles), a condition *associated with increased risk for testicular malignancies*. The scrotal temperature is about 2 to 4°C lower than the normal core body temperature, which is essential for normal spermatogenesis and fertility.

Inside the scrotum, the outermost sheath enveloping each testicle is the *cremasteric fascia*, which also serves as a sheath and attachment site of the cremaster muscle. The *cremaster muscle* lifts the testis; in certain seasonal breader species, the testis is pulled back into the abdominal cavity during periods of sexual inactivity, thereby inhibiting spermatogenesis. The sheath immediately enveloping the testis is the *tunica vaginalis*, a double layer of serosal membrane derived from the peritoneum during development. *Hydrocele* is the condition when clear fluid accumulates in the tunica vaginalis. The visceral layer of the tunica vaginalis covers the dense connective tissue capsule of the testis (*tunica albuginea*), which sends septa toward the hilar region of the testis known as the *mediastinum testis*. Coils of *seminiferous* (semen-carrying) *tubules* and their connective tissue stroma occupy the space between the septa. Occasionally, ectopic vestigial adrenocortical tissue cortical tissue may be present (*"adrenal rest tissue"*) as a consequence of the close relationship between the development of the gonads and the adrenals.

The coiled seminiferous tubules, which provide approximately 80 to 90% of the testicular volume, are the site of *spermatogenesis* (see the section titled Spermatogenesis). The Leydig cells are found in the connective tissue stroma between the seminiferous tubules. Before puberty, the testes are small and have a rubbery consistency. During puberty, the testes increase in size, mainly under the influence of FSH, and their consistency becomes comparable to muscle. The consistency is due to an arrangement that could be compared to a moderately inflated tire: the material of the tire is the dense connective tissue of the tunica albuginea and the septa, which is "inflated" by the seminiferous tubules and their luminally secreted fluid. *The size and the consistency of the testes reflect testicular function and fertility* (Box 13-2).

The continuation of each seminiferous tubule near the mediastinum is devoid of germ cells. This short portion is known as the straight tubule (*tubulus rectus*). The tubuli recti coalesce within the mediastinal connective tissue to form a reticular network termed *rete testis*, whose content is drained by the *efferent ductules* of the testis to the head of the *epididymis* (see Excurrent Duct System).

The testes and the ovaries develop from the same primordial tissues

BOX 13-2 The Relationship between Testicular Size and Function

- The testicular volume (in milliliters) is calculated as $V = 0.52 \times L \times W^2$, where L = length and W = width of the testis in centimeters. The normal length ranges between 3.6 and 5.5 cm; a length of <2.5 cm usually indicates infertility and/or hypogonadism in adults. The normal testicular volume is >15 mL (18.6 ± 4.8 mL). Testicular volume in clinical practice is often estimated with the *Prader orchidometer*, which consists of a series of plastic "reference testicles" with volumes ranging between 1 and 25 mL.
- Patients with *Klinefelter's syndrome* (47,XXY; see Ontogeny of the Reproductive System) present with bilaterally small testes, which have a firm consistency. In this condition, the seminiferous tubules are hyalinized and replaced by fibrotic tissue. Postpubertal mumps may lead to a similar condition, which may be unilateral.
- In *postpubertal testicular atrophy* (e.g., due to pituitary dysfunction), the testes become smaller and soft to the touch. This condition resembles a deflated tire.
- A sudden unilateral increase in testicular size usually indicates tumor. Most testicular tumors are malignant. Similar sudden enlargement of the testicle may be due to orchitis (inflammation) accompanying mumps. The outcome of postpubertal mumps may include postinflammatory testicular atrophy (firm testicular consistency with diminished size) and decreased fertility.

(see Intrauterine Sexual Development). During their descent into the scrotum, the testes bring along their blood and lymphatic vessels through the *inguinal canal*. The *testicular* and *ovarian arteries* are direct branches of the aorta (see Fig. 12-1). The lymphatic vessels follow the course of arteries. This explains that the lymph of both the male and female gonads is filtered by *para-aortic lymph nodes*. Thus, the metastases of malignant gonadal tumors are inconspicuous. The veins of the gonads initially follow the course of the arteries and surround them as the *pampiniform plexus*, which coalesce to form a *testicular* (or *ovarian*) *vein* on each side. The venous drainage of the gonads is similar to that of the adrenal glands: the right testicular vein joins the inferior vena cava, the left testicular vein empties into the left renal vein. Due to the higher pressure in the renal vein than in the inferior vena cava, the hydrostatic pressure is higher in the left than in the right testicular vein. This has two consequences:

- the left testicle is normally positioned somewhat lower than the right testis (physiologic asymmetry);
- venous varicosities of the testicular vein (*varicocele testis*) are more frequently encountered on the left than the right side. Varicocele results

in decreased venous return, thereby contributing to elevated scrotal temperature, which may decrease fertility.

During development, both the ovaries and the testes descend from their original position. The *further* descent of the testes from this common site is an androgen-dependent process, which relies on the testosterone secretion of the fetal testes. The descent is influenced by both testosterone and DHT; in type II 5α-reductase deficient *male pseudohermaphroditism*, the testes are found variably in the inguinal canal or in the nonfused labia majora. The descent is probably mediated by the androgen-induced involution of the *gubernaculum*, a fibrous ligament that ties the inferior pole of the testis to the inner surface of the *labioscrotal swelling* (the primordium of the scrotum). During the 28th week of gestation, the testis reaches the deep inguinal ring and descends through the inguinal canal in a matter of 2 to 3 days. The descent from the superficial inguinal ring to the scrotum is completed by the 32nd week of gestation. Testicular descent may be provoked in cryptorchid boys by stimulating endogenous testosterone secretion with injections of hCG.

Due to the descent of the testes and/or the failure of the peritoneal *processus vaginalis* to close, the inguinal canal has a larger diameter in males than in females. This explains why *inguinal hernias* are far more common in males, and why females (especially children before menarche) presenting with inguinal hernias need to be evaluated for *androgen insensitivity syndrome* (testicular feminization, see Intrauterine Sexual Development).

Excurrent Duct System and Accessory Glands The excurrent duct system includes the efferent ductules, the epididymis, the vas (ductus) deferens, the ejaculatory duct, and the urethra. The efferent ductules and the epididymis are not simple drainage tubes but have important functions in the maturation of spermatozoa. The accessory glands are the seminal vesicle, the prostate, and the bulbourethral glands of Cowper, all of which open into the excurrent duct system. The seminal vesicle and the prostate provide the bulk of the ejaculate. *The development and function of these organs are androgen-dependent.* The Cowper's glands produce mucus, which lubricates the glans penis thereby aiding its intromission into the vagina (see details that follow).

Because spermatozoa do not move actively until ejaculation, their delivery must be assured by the undulating movement of the ducts. This movement is performed by the *myoid cells* of the seminiferous tubules (see Fig. 13-1) and the *smooth muscle layers* of the excurrent duct system. The excurrect duct system starts with approximately 20 *efferent ductules* (Fig. 13-8), which drain the content produced by the seminiferous tubules to the initial portion of the *ductus epididymis* found in the *caput epididymis*. The efferent ductules are lined by a pseudostratified columnar epithelium, which

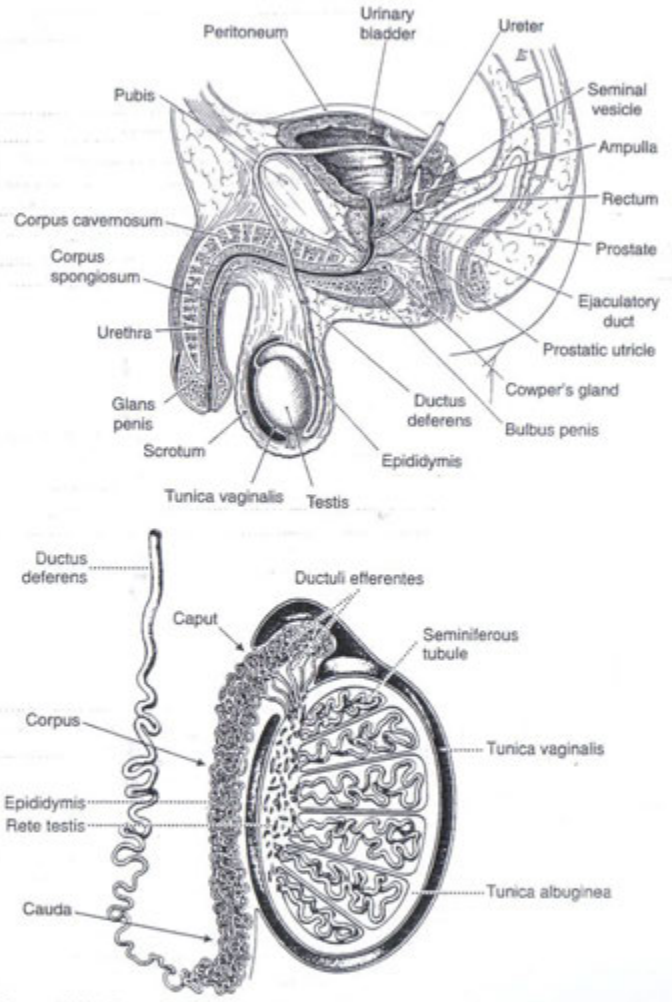


Figure 13-8. The anatomy of the male reproductive system. A. Sagittal pseudosectional diagram of the male urogenital system. B. Schematic presentation of the longitudinal section of the testis and its excurrent ducts. (*Source*: A: *modified* from Fig. 31-1, p 797 in Fawcett DW: *Bloom and Fawcett's Textbook of Histology*, 11th ed., Philadelphia, Saunders, 1986. B: *modified* from Fig. 30-3, p 932 in Dym M: The male reproductive system, in Weiss L (ed): *Cell and Tissue Biology*, 6th ed, Urban & Schwarzenberg, 1988.)

reabsorbs most of the fluid secreted by the seminiferous tubules, thereby increasing the concentration of spermatozoa.

The *epididymis* consists of three anatomic parts: *caput* (head), *corpus* (body) and *cauda* (tail). The *ductus epididymis* is a highly coiled tube with a length of 4 to 6 meters. It is lined by a *pseudostratified stereociliated columnar epithelium*. The epithelium modifies the composition of the luminal fluid both by absorption and active secretion. The secretion includes the action of the *proton pump* (H^+-ATPase) which acidifies the luminal fluid. The acidic environment is essential for both the maturation and the storage of spermatozoa. The stereocilia are large nonmotile microvilli that provide the surface area for interaction between the membrane of spermatozoa and the epithelium. As a result of this interaction, the spermatozoa

- acquire the *ability* of active directional movement;
- become "incapacitated," i.e., a receptor-masking glycoconjugate is added to their surface, which is later removed during capacitation. *Capacitation* is the process that enables the spermatozoon to fertilize the ovum (see Fertilization).

The cauda of the epididymis serves as a reservoir of mature spermatozoa. The average time between spermiation (the release of spermatozoa from the seminiferous epithelium) and ejaculation is about 12 days (range: 1 to 21 days). Unejaculated, aging spermatozoa (i.e., older than 17 to 21 days) are phagocytosed by "foam cells," presumably derived from the epithelial lining. During ejaculation, the stored spermatozoa are propelled by the wave of smooth muscle contraction starting in the ductus epididymis and continuing in the vas deferens.

The well-developed smooth muscle layers surrounding the ductus epididymis become a thick muscular wall in the vas deferens. The vas deferens is palpable under the skin before it passes through the superficial inguinal ring: it is a pencil-thick tube with a consistency similar to that of cartilage (due to the thick layer of muscle). A widely used method of irreversible male contraception is *vasectomy*, which involves the excision of a segment of the vas deferens and interposition of a fascial barrier between the occluded cut ends to prevent recanalization.

The *seminal vesicles* are located behind the urinary bladder. Their highly convoluted secretory epithelium is embedded in thick smooth muscle layers. The lumen of the gland is drained by the short *excretory duct*, which joins the vas deferens. After these two tubes merge, their continuation is termed *ejaculatory duct*. The secretory product of the seminal vesicle is rich in fructose (the primary fuel of spermatozoa in their active movement), prostaglandins, ascorbic acid, fibrinogen-like, and thrombin-like proteins (Box 13-3). The lumen of the seminal vesicle stores the secretory product, which is propelled by smooth muscle contraction at the time of ejaculation

BOX 13-3 The Coagulation System of Semen and Prostate Specific Antigen

At ejaculation, the epididymal spermatozoa become mixed with the secretions produced by the seminal vesicles and the prostate. The ejaculate immediately turns into a gel-like structure that entraps the spermatozoa. The spermatozoa become progressively motile as the gel dissolves. *Semenogelin I* and *II* are products of two separate genes on chromosome 20 with 80% identity in their primary structures. They are mainly responsible for the immediate gel formation of freshly ejaculated semen. The proteolytic degradation of coagulated semenogelins is performed by *prostate-specific antigen* (PSA, a kallikrein-like serine protease), which results in the liquefaction of semen and the progressive release of motile spermatozoa within 5 to 15 min after ejaculation. PSA is a glycoprotein composed of 93% amino acids and 7% carbohydrates, with a molecular weight of about 30 kDa. The *PSA* and the *human kidney glandular kallikrein-1 (hKGK1)* genes are tandemly located on chromosome 19q13. These two genes display a high degree of homology reflecting a common phylogenetic origin. PSA appears in the circulation especially in patients with prostate cancer, benign prostatic hyperplasia, and after palpation of the prostate. PSA is also elevated in the extremely rare cancer cases of the *Skene's periurethral glands* in women, which indicates the developmental homology between Skene's glands and the prostate. PSA is secreted by the lactating mammary gland into milk.

and flushes the spermatozoa from the downstream portion of the excurrent duct system.

The ejaculatory ducts penetrate the *prostate* and join the prostatic portion of the *urethra*. The opening of each ejaculatory duct is found on either side of the *prostatic utricule* (the remnant of the Müllerian duct) at a posterior fold of the urethral mucosa known as the *colliculus seminalis*. The prostatic glands open into the urethra mainly at the grooves on either side of the colliculus. The prostate consists of 20 to 30 tubuloalveolar glands embedded in a thick fibromuscular tissue. The urethra penetrates the anterior portion of the prostate; prostatic enlargement may cause obstruction of urine flow. Due to the close relationship between the prostate and the rectum (Fig. 13-8A), prostatic enlargement can easily be diagnosed by rectal digital examination. The prostate secretes a fluid rich in proteins such as *acid phosphatase* and *prostate-specific antigen* (PSA), a kallikrein-like protease (see Box 13-3). Similar to the seminal vesicles, the prostate stores its secretory product in the lumen and suddenly propels it into the urethra at the time of ejaculation. The high-protein secretory product may precipitate as prostatic *concretions* (*corpora amylacea*) in the glandular lumen, especially in elderly individuals.

The male urethra has three segments: the *prostatic*, *membranous*, and *penile urethra*. The membranous urethra penetrates the *urogenital diaphragm*, which contains the *external (voluntary) sphincter of the urethra*, a skeletal muscle. The *internal sphincter of the urethra* (also known as *sphincter vesicae*) is an involuntary smooth muscle surrounding the initial portion of the urethra at the bladder. At the time of ejaculation, the external sphincter opens, whereas the internal sphincter contracts. When the internal sphincter fails to contract, semen is ejected into the lumen of the urinary bladder. The condition, known as *retrograde ejaculation*, is often due to diabetic neuropathy.

The *Cowper's glands* open into the initial portion of the penile urethra. These compound tubuloalveolar mucous glands are similar to salivary glands. Under the influence of parasympathetic nerves, they secrete mucus into the urethra upon erotic arousal. Their function is to lubricate the urethra and the glans penis with *preseminal fluid* before ejaculation takes place. The female equivalents of the Cowper's glands are the Bartholin's glands (great vestibular glands).

The structure of the penis is discussed together with the mechanism of erection.

Spermatogenesis

Spermatogenesis Is a Sertoli Cell-Supported Process of Mitotic Proliferation, Meiosis, and Maturation of Spermatogonia to Produce and Release Spermatozoa The gametogenesis normally occurring in *postpubertal* males is termed *spermatogenesis*. Spermatogenesis takes place in the convoluted seminiferous tubules. The tubular wall consists of *germ cells* associated with a simple columnar epithelium of *Sertoli cells* and surrounded by a basal lamina and a few layers of contractile *myoid cells*, whose function is to propel the tubular fluid by undulating peristaltic movement (see Fig. 13-1).

The Sertoli cells form the *blood-testis barrier*, which involves tight junctions and the expression of the P-glycoprotein (see also Chap. 4). Tight junctions are typically found near the apical surface of epithelial cells. Although in a geometric sense the Sertoli cell tight junctions are closer to the basal surface, in a functional sense they still demarcate the apical and basolateral plasma membrane surfaces. The space between adjacent Sertoli cells is divided by the tight junctions into an *abluminal* (away from the lumen) and a *luminal* (or adluminal [toward the lumen]) *compartment*, which are occupied by the various developmental stages of spermatogenesis. The premeiotic *spermatogonia* are found in the abluminal compartment bordered by the basal lamina of the seminiferous tubules. As the spermatogonia divide and detach from the basal lamina, tight junctions are organized at their basal aspect and dissolved at their apical aspect. Each *cohort* of

cells derived from a single spermatogonium and entering meiosis remains interconnected by *cytoplasmic bridges* until the latest stage of spermiogenesis, when their excess cytoplasm becomes *collectively* shed. The cytoplasmic bridges assure synchronous development of spermatozoa in any patchlike area of the seminiferous tubules. Postmeiotic cells (*spermatocytes*, *spermatids* and *spermatozoa*), which express "foreign" antigenic epitopes, are located only in the luminal compartment, where they are inaccessible for immunologic surveillance.

Spermatogenesis involves four key elements:

- *Spermatocytogenesis* is the proliferation of spermatogonia by mitosis. The proliferation of the stem cells, known as *type A dark (Ad) spermatogonia*, yields type Ad, Ap (pale) and *type B spermatogonia*. *Preleptotene primary spermatocytes* arise by division of type B spermatogonia, which heralds the second phase of spermatogenesis.
- *Meiosis* is the process whereby the four chromatids (two for each chromosome) present in the diploid primary spermatocytes segregate into four daughter cells (*spermatids*) by two successive divisions. The first division (i.e., that of the primary spermatocytes) reduces the chromosome number to a haploid set ($n = 23$), the second division (i.e., that of the *secondary spermatocytes*) results in the separation of the *sister chromatids*.
- *Spermiogenesis* is the maturation of spermatids into *spermatozoa* that takes place in the apical folds of the plasma membrane of Sertoli cells (see details below).
- *Spermiation* is the release of spermatozoa from their attachment to Sertoli cells.

The process of spermatogenesis from spermatogonia to spermiation takes place over a period of 64 to 74 days. Based on the cross-sectional appearance of the seminiferous epithelium, six stages of its spermatogenic cycle can be distinguished (Fig. 13-9).

The Process of Spermiogenesis Is Characterized by a Progressive Condensation of the Nuclear Chromatin Structure, the Elongation of the Nucleus, and the Development of the Acrosome, Flagellum, and Mitochondrial Sheath Throughout the process of spermiogenesis, the maturing spermatids are attached to the Sertoli cell membrane by specialized junctions. The process of spermiogenesis consists of four phases:

- The *Golgi phase* involves two major maturational events that determine the anterior and posterior poles of the developing spermatozoon:
  - The Golgi complex generates the *acrosomal vesicle*, which is a glycoprotein-rich membrane-cased organelle positioned near the nuclear envelope. Its position signals the future *anterior pole*.
  - The two centrioles (diplosome) of the spermatid migrate to the opposite side of the nucleus (*posterior pole*), and the distal

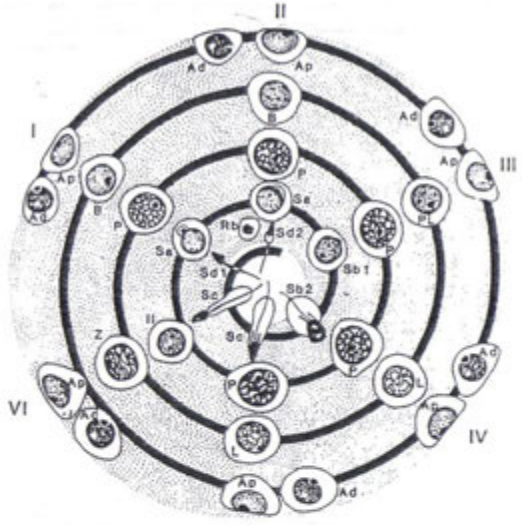


Figure 13-9. Schematic diagram of the constellations of developing germ cells based on which six stages of the spermatogenic cycle in the seminiferous tubule can be identified. The spiral indicates the time required from division of stem cells (dark type A spermatogonia [Ad]) to spermiation. The spiral portrays a total time of about 74 days, and each complete turn of the spiral indicates 16 days. The six constellations (labeled with Roman numerals) are due to the kinetics of the process. Note for example that type B spermatogonia (B) enter meiosis as preleptotene primary spermatocytes (PL) at stage III, but complete the first meiotic division 1-½ turns (~21–22 days) later as pachytene (P) primary spermatocytes at stage V. By this time, the next wave of primary spermatocytes arrived to the leptotene stage of meiosis (L). Secondary spermatocytes (II) complete the second meiotic division within 8 hours; their short-lived nature is indicated by their single spot on the spiral timeline at stage VI. Ap, pale type A spermatogonia; Z, zygotene primary spermatocytes; S, spermatogonia at various stages of spermiogenesis. (*Source:* From Kerr JB: Functional cytology of the human testis. Bailliere's Clin Endocrinol Metab 6:235–250, 1992.)

centriole initiates the development of the tail as the *axonemal complex*.

- During the *cap phase*, the acrosomal vesicle flattens and spreads over the anterior half of the condensing nucleus as the *acrosomal cap*. The axonemal complex continues to grow.
- The *acrosome phase* starts with the repositioning of the spermatids. Up until this phase, the anterior pole of the spermatid was oriented toward the lumen of the seminiferous tubule, and now becomes deeply embedded into the membrane folds of the Sertoli cell with its anterior pole oriented toward the basal lamina. This provides space for the elongation of the *flagellum* (a modified cilium) developing from the axonemal complex.

- The proximal centriole attaches to the nucleus and grows nine coarse fibers that connect the nucleus with the flagellum. This structure developing from the proximal centriole is known as the *connecting piece* or *neck region*.
- The *acrosome* develops: the space between the juxtanuclear acrosomal cap and the anterior plasma membrane diminishes; the cytoplasm is displaced posteriorly.
- With the posteriorly placed cytoplasm, mitochondria migrate to form a *mitochondrial sheath* around the coarse fibers in the *middle piece* of the flagellum.
- The distal portion of the flagellum consists of the *principal piece* and the *end piece*.

- During the *maturation phase*, the excess cytoplasm of the interconnected spermatids is collectively shed and becomes phagocytosed by the Sertoli cells.

The spermatozoa thus acquire key elements for their function (Fig. 13-10):

- *Haploid, supercoiled chromatin*. Half of the cells carry the X, the other half carry the Y sex chromosome. Unlike most somatic cells, gametes have a heterogenous genome due to meiotic crossing over and the random assignment of maternal and paternal chromosomes to each gamete.
- *Acrosome*, which contains enzymes necessary for the penetration of cervical mucus, the mucoid component of the corona radiata, and the zona pellucida of the oocyte.
- *Flagellum*, which provides active, fast, and independent movement.
- *Mitochondria*, which form a sheath in the neck region and provide adenosine triphosphate (ATP) for the flagellar movement. The GLUT5 transporter in the cell membrane of spermatozoa enables fructose uptake from semen upon ejaculation (see also Chap. 9).

Spermatogenesis Is Regulated by FSH, Testosterone, and Locally Acting Humoral Factors Spermatogenesis is a gonadotropin-dependent process. FSH stimulates spermatogenesis only indirectly: the sole target of FSH is the Sertoli cell which (among other responses) secretes ABP into the lumen of the seminiferous tubules. LH stimulates testosterone secretion by the Leydig cells. The concentration of testosterone in the testis is about 200-fold higher than in plasma; this is due to the local production and the testosterone-sequestering action of ABP. Testosterone stimulates spermatogenesis in part via an androgen receptor-mediated action on the Sertoli cells. *The locally high concentration of testosterone is a mandatory requirement of normal spermatogenesis*. An unusual and important feature is that the high local testosterone concentration does not suppress androgen receptor expression in the Sertoli cells, the peritubular myoid cells, or the Leydig cells. One of the functions of ABP is to mediate the membrane-receptor

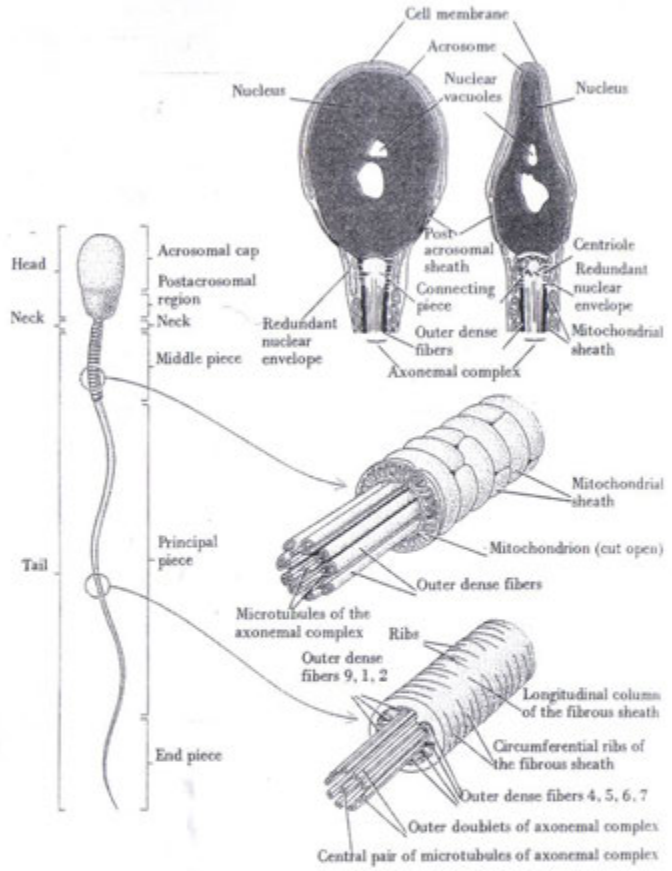


Figure 13-10. Structural features of the human spermatozoon. The regions of the spermatozoon are indicated on the left. Key features of the head (viewed in its major and minor dimensions), the middle piece and the principal piece are illustrated on the right. Note that the cell membrane covering the middle and principal pieces is not shown. (*Source:* Fig. 2-11, p 646 in Ross MH et al: *Histology: A Text and Atlas*. 3rd ed. Baltimore, Williams & Wilkins, 1995.)

(nongenomic) actions of testosterone, which include an increased protein secretory activity by spermatocytes but no effect on spermatids. Because the germ cells do not express androgen receptors, this effect is distinct from that involved in benign prostatic hypertrophy (see Fig. 13-6).

FSH stimulates the *proliferation* as well as the *secretory activity* of the Sertoli cells. The Sertoli cells secrete *inhibin B*, an inhibitor of pituitary FSH-β synthesis. Inhibin and the structurally related activin (see Regulation of the Gonadotropin–Gonad Axis in Postpubertal Males) have important local actions. *Inhibin antagonizes the proliferative action of FSH on the Sertoli cells*, thereby limiting the FSH-induced growth of the seminiferous tubules.

Additional locally acting factors mainly produced by the Sertoli and Leydig cells have been implicated in the regulation of spermatogenesis. One of the few identified factors that has a crucial role in supporting spermatogenesis is *transferrin*, which is secreted into the lumen of the seminiferous tubules by the Sertoli cells. Testicular transferrin acts as an iron shuttle system to transport ferric ions around the tight junctions to the germ cells inside the blood-testis barrier. The ferric ions are essential for the production of mitochondrial cytochromes. Spermatogenesis is defective in transferrin-deficient mutant mice. The concentration of seminal fluid transferrin is proportional to sperm production in humans.

The Normal Ejaculate Contains 80 to 120 Million Spermatozoa Per Milliliter and Has a Volume of 2 to 5 mL After 1 to 3 Days of Sexual Abstinence Human semen is a white opalescent fluid with a pH close to that of plasma (pH 7.35 to 7.50). Most of its volume is the product of the seminal vesicle (60%) and the prostate (20 to 30%). These secretions counterbalance the acidic pH of the epididymal fluid.

Sperm count and *quality* are essential for fertility. After a sexual abstinence of at least 1 to 3 days, the normal sperm count is 80 to 120 $\times$ 10^6 per milliliter of semen, with up to 20% morphologically abnormal and/or immotile spermatozoa. Some reports suggest that in Western countries sperm quality (including sperm count, the proportion of normal spermatozoa, and motility indices) has declined during the past decades. Men with sperm counts below 20 $\times$ 10^6 per milliliter are infertile, and about 50% of men are infertile with sperm counts between 20 to 40 $\times$ 10^6 per milliliter.

Whereas the seminal vesicles and the prostate may relatively rapidly (within hours) replenish their secretory products and produce ejaculates with normal volume (2 to 5 mL), spermatogenesis is unable to replenish sperm counts as rapidly. Thus, with repeated ejaculations, sperm counts decline. In the clinical evaluation of sperm, a single sample with high sperm count and quality is sufficient for excluding gonadal dysfunction. In contrast, at least three samples collected at 2- to 3-month intervals are necessary to establish the diagnosis of gonadal dysfunction because spermatogenesis and passage through the epididymis require approximately 70 to 80 days.

This time allows recovery from conditions leading to temporary decrease in spermatogenesis such as nutritional factors and fever.

The absence of spermatozoa in semen is known as *azoospermia*. This condition has multiple etiologies, such as Klinefelter's syndrome, vanishing testes syndrome, ductal obstruction, or *Sertoli cell-only syndrome*. In Sertoli cell-only syndrome, mutations of genes encoding RNA-binding proteins have been implicated. An *azoospermia factor* (AZF) is present in the euchromatic region of the long arm of the Y-chromosome (Yq11), where clusters of two gene families, *RBM* and *DAZ*, have been identified.

- The *RNA-binding motif* (RBM) *genes RBM1, RBM2, RBM3* and the closely related *cold-inducible RNA-binding protein* (CIRP) are members of the *glycine-rich RNA-binding protein* (GRP) *family*. In contrast with RBMs, CIRP is autosomal (19p13.3). CIRP is an 18-kDa *cold-shock protein* that plays an essential role in cold-induced suppression of cell proliferation by prolonging the G1 phase of the cell cycle. In cultured somatic cells, the levels of CIRP mRNA and protein increase after a temperature downshift from 37°C to 32°C. Experimental overexpression of CIRP in cells cultured at 37°C decreases cell proliferation. In cultured human cells, RBM3 is also induced by cold stress. CIRP is expressed in all cell types of the seminiferous epithelium, except in elongated spermatids. *CIRP expression is downregulated in varicocele patients, and may be a major component of temperature-related infertility*. It has been proposed that CIRP is involved in diverting male germ cells from mitotic toward meiotic division.
- A cluster of *deleted in azoospermia* (DAZ) *genes* encode proteins that are found only in late spermatids and in sperm tails. A *DAZ-like autosomal* (DAZLA) also known as DAZ-homologue (DAZH) *gene* maps to 3p24. DAZLA/DAZH is also expressed in male germ cells. It has been proposed that the DAZ cluster on the Y chromosome arose from the autosomal DAZLA/DAZH during evolution. The autosomal localization of DAZLA/DAZH and CIRP may explain why the Y chromosome is involved in the pathogenesis of only a fraction of idiopathic male infertility.

Regulation and Function of the Penis

Erection The size of the penis varies with the age, endocrine status, and erectile state. Penile length is measured on the dorsal aspect of the penis from the symphysis to the tip of the glans. In postpubertal men, the average *flaccid* length is 8.8 cm, the *stretched* length is 12.4 cm (10th percentile, 11 cm; 90th percentile, 16 cm) and the *erect* length is 12.9 cm. In contrast with the flaccid length, the stretched length is closely correlated with the erect length and is the better clinical indicator of genital development. Men with a stretched or erect length of <7.5 cm are candidates for penile lengthening.

At birth, the stretched dorsal penile length is normally >2.0 cm. Phallic growth is promoted by the GH–IGF-1 axis and testosterone both in utero and during pubertal growth. Accordingly, male patients with GH deficiency, GH resistance (Laron dwarfism), or hypogonadism present with *microphallus*. The microphallus of hypogonadal infants can be treated with hCG, which usually induces a rapid growth (up to 0.75 cm in 5 days) by increasing testosterone production. It is unknown what causes the cessation of penile growth after puberty; conflicting reports have been published regarding a decreased phallic expression of androgen receptors in postpubertal men.

On a cross section, the penis consists of three areas: two dorsal *corpora cavernosa* surrounded by a dense connective tissue layer (*tunica albuginea*), and a ventral *corpus spongiosum* that is devoid of a tunica albuginea.

- The *corpus spongiosum* houses the penile portion of the urethra, starts at *bulbus penis* at the inferior (superficial) surface of the urogenital diaphragm, and ends as the *glans*. The urethra normally opens at the tip of the glans. The ventrally positioned external orifice of the urethra is known as *hypospadias*, which is due to incomplete fusion of the urogenital folds (see Intrauterine Sexual Development).
- Each *corpus cavernosum* takes its origin from the pubic bone as the *crus penis*; its distal end is covered by the glans.

The corpora cavernosa and the corpus spongiosum consist of sinusoid systems lined by endothelial cells. Blood enters the sinusoids from the centrally positioned *helicine arteries* (branches of the *profunda penis* artery). Erection depends on the increased arterial blood flow into the corpora cavernosa of the penis. The necessary flow to provoke erection ranges between 80 and 120 mL/min. During erection, the corpora cavernosa become enlarged rigid columns, whereas the corpus spongiosum (including the glans) enlarges but remains pliable. This difference prevents compression of the urethra and permits the passage of semen during ejaculation. The rigidity of the erect corpora cavernosa is related to the increased influx of blood, the presence of the tunica albuginea, and veno-occlusion. Venous blood is primarily drained from the *subtunical venous plexus* (positioned on the *inner* surface of the tunica albuginea) by the *emissary veins* that traverse the tunica albuginea *at an angle*. The sudden influx of blood into the sinusoids has an effect comparable to inflating a tire with air. The *subtunical venous plexus* becomes pressed against the tunica albuginea and the emissary veins become compressed within the tinuca albuginea, which result in decreased venous efflux. The congestion of blood maintains erection.

Erection is induced by a *nitric oxide* (NO) mechanism (see also in Chap. 5). *Neuronal type NO synthase* (NOS) is expressed by the *parasympathetic nervi erigentes* under the stimulatory influence of testosterone. The nerves reach the penis escorting the profunda penis artery within the inter-

nal pudendal neurovascular bundle. When action potentials reach the axon terminals, NOS is activated by the influx of Ca^{2+}, and NO diffuses from inside the axon to the cytoplasm of neighboring vascular smooth muscle cells. This mechanism is similar to the endothelium-dependent vasorelaxation induced by acetylcholine (see Fig. 5-7) except that a distinct NOS isoenzyme is involved, which resides within the axon rather than the endothelium. NO activates the soluble guanylyl cyclase, and the increased cytoplasmic cyclic GMP concentration provokes smooth muscle relaxation, i.e., vasodilatation. Cyclic GMP causes vasorelaxation by several mechanisms that involve decreasing cytoplasmic concentration of Ca^{2+}, dephosphorylation of light chain myosin (the target of the contractile action of Ca^{2+}), and opening K^+-channels. *Sildenafil citrate* (Viagra) enhances (rather than causes) erection by inhibiting the degradation of cyclic GMP by type 5 cyclic phosphodiesterase (see Chap. 6). The parasympathetic nervi erigentes also synthesize vasoactive intestinal peptide (VIP), a potent vasodilatator agent, as a neurotransmitter. The physiologic role of VIP in erection is uncertain.

The preganglionic fibers of the erectile parasympathetic nerves originate from the sacral plexus (S_{2-4}). The preganglionic nerves receive input from penile mechanoreceptors as a spinal reflex arch and from corticospinal fibers that mediate *psychosexual* activation of the erectile mechanism, including *sleep-associated* (also known as nocturnal) *penile tumescence*, which occurs 4 to 8 times each night. The penile mechanoreceptors also send signals through the spinal cord to cortical sensory areas.

Alpha adrenergic sympathetic activation, such as stress, is an inhibitor of erection due to its vasoconstrictor effect on the helicine arteries. *Because ejaculation involves activation of sympathetic nerves, ejaculation usually results in the physiologic termination of erection.* For this reason, premature ejaculation is often perceived as an erectile dysfunction.

The regulation of bulbourethral gland secretions involves parasympathetic mechanisms. Preseminal fluid appears in the urethra during erection, usually when it is associated with erotic arousal.

Androgens enhance libido, the frequency of sexual acts, and sleep-associated erections. Its involvement in erections produced by erotic images or situations shows significant variations among subjects. Although postpubertal castration decreases libido and spontaneous erectile function, erotic images may continue to produce erections sufficient for intercourse. It is noteworthy that erotic images provoke physiologic sexual arousal irrespective of the subject's moral/ethical attitude toward viewing such images.

Erectile dysfunction (impotence) may have psychogenic and organic causes. *In contrast with organic erectile dysfunction, in psychogenic impotence the sleep-associated tumescence is preserved.* The organic causes of impotence include:

- Any endocrine disease that leads to decreased androgen production, such as Kallman syndrome, Klinefelter's syndrome, hyperprolactinemia, acromegaly, and hyper- and hypofunction of the thyroid or the adrenal glands.
- Cirrhosis of the liver via SHGB-mediated decrease in free testosterone and relative estrogen excess.
- Diabetic neuropathy by hyperglycemia-related activation of the polyol pathway. Other neuropathies may also be involved, such as multiple sclerosis.
- Drugs, mainly psychotropic agents and drugs used in the treatment of prostate cancer: estrogens, anti-androgens, 5α-reductase inhibitors, the GnRH agonist leuprolide, or progesterone. Several antihypertensive drugs may interfere with sexual function, including erection.
- Systemic illnesses such as cancer, chronic renal failure or chronic obstructive pulmonary disease.

The Ejaculation of Semen The ejaculation of semen is a spinal reflex involving two distinct components:

- *Emission* is the process of moving all components of semen into the urethra by a coordinated smooth muscle contraction of the vas deferens, seminal vesicle, and prostate under the influence of *sympathetic* noradrenergic nerves acting on α_1-adrenergic receptors. The preganglionic fibers originate from the upper lumbar segments of the spinal cord. The postganglionic fibers are located in the genital branch of the genitofemoral nerve, which escorts the vas deferens. The nerves of the seminal vesicle and the prostate are derived from the inferior hypogastric plexus.
- *Ejaculation proper* is the ejection of semen out of the urethra. This process involves the coordinated action of several skeletal muscles and the smooth muscle internal sphincter of the urethra. The urogenital diaphragm, the external sphincter of the urethra, and the *bulbospongiosus muscle* (a superficial perineal muscle surrounding the bulbus of the corpus spongiosum) perform involuntary reflexogenic rhythmic contractions. These contractions, together with the peristaltic waves of the vas deferens, result in fractionated ejections of semen. The simultaneous contraction of the internal urethral sphincter prevents retrograde ejaculation into the bladder.

The first fraction of ejaculated semen contains the highest sperm count, indicating that the contraction of the vas deferens slightly precedes that of the accessory glands, and that the secretions of the accessory glands tend to flush out the spermatozoa from the urethra.

Similar to erection, ejaculation is provoked by the stimulation of the penile mechanoreceptors. Partial inhibition of sensory nerve function may delay ejaculation. Thus, topical application of lidocain or similar local anesthetics may be used for the treatment of premature ejaculation. The stimulation of mechanoreceptors on the dorsal aspect of the penis tends to prolong erection before provoking ejaculation. In contrast, the stimulation of mechanoreceptors on the ventral aspect of the penis and the glans hastens the onset of ejaculation.

Ejaculation is normally accompanied by *orgasm*, a sensation of pleasure that is prolonged for about 1 minute by continued mechanical stimulation of the glans. Orgasm is followed by a *refractory period*, when males are incapable of orgasm. The mechanism of the refractory period is unknown. Orgasm is in part due to the release of endogenous opioids in the hypothalamus and the limbic system. Certain drugs may result in the dissociation of orgasm from ejaculation. Orgasm is associated with the release of several hormones such as oxytocin (OT) and prolactin (PRL) in both sexes.

Regulation of the Gonadotropin-Gonad Axis in Postpubertal Males

The regulation of testicular function includes the following main components:

- Hypothalamic GnRH also known as *luteinizing hormone-releasing hormone* (LHRH) acting on its cognate receptor expressed by pituitary gonadotroph cells.
- Pituitary gonadotropins: LH and FSH acting on their specific receptors expressed by Leydig and Sertoli cells, respectively.
- Testicular hormones:
  - steroid hormones: *testosterone* and its derivatives,
  - polypeptide hormones: *inhibin*, *activin*, *follistatin*. Note that activin and follistatin are important autocrine/paracrine regulators *also in the pituitary gland*.
- SHBG, a determinant of the free (biologically active) concentration of androgens and estrogens.

Various models describe the regulation of the hypothalamic–pituitary–gonad axis in both males and females. Due to conflicting findings, the existing models often differ in the details. In general terms, the regulation of testicular function can be summarized as follows (Fig. 13-11):

- Pulsatile hypothalamic GnRH stimulates the secretion of both FSH and LH from the anterior pituitary gland. The pulsatile secretion of GnRH and LH display high concordance. FSH pulsatility is less pronounced and may appear dissociated from the LH pulses.
- LH stimulates testosterone secretion from the Leydig cells. Free (non-SHBG-bound) testosterone exerts biologic actions (see Table 13-4), including a negative feedback on the secretion of gonadotropins both at the hypothalamic and the pituitary level. *Most of the negative feedback action is mediated by estrogen receptors after the local aromatization of testosterone into 17β-estradiol, but full negative feedback also requires androgen receptor-mediated action.*
- *Progesterone* is not secreted in significant quantities in males, and thus (unlike in females) does not participate in the physiologic regulation of gonadotropin secretion. However, admin-

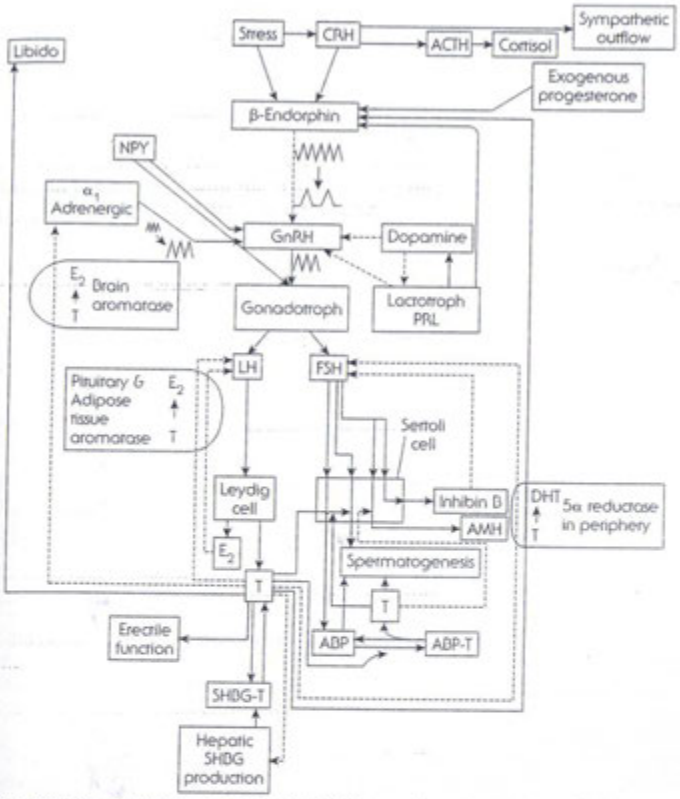


Figure 13-11. Postpubertal regulation of the GnRH-gonadotropin-testicular axis. Solid arrows indicate stimulation/increase; dashed arrows indicate inhibition/decrease. The bidirectional arrows show exchange between free and protein-bound testosterone. SHBG, sex-hormone-binding globulin; ABP, androgen-binding protein. Only free steroid hormones mediate genomic effects. The gonadotroph-Leydig cell axis involves lutreinizing hormone (LH)-induced secretion of testosterone (T) and its feedback is mainly mediated by estradiol (E_2) generated by aromatase in the hypothalamus. The gonadotroph-Sertoli cell axis is based on follicle stimulating hormone (FSH)-induced secretion of inhibin B, which provides a feedback only at the pituitary gland. The gonadotroph-Sertoli cell axis receives input from Leydig cells: T acting on pituitary androgen receptors inhibits secretion of FSH. Note that gonadotropin-releasing hormone (GnRH) does not receive direct negative feedback. Instead, neurons regulating either GnRH pulse amplitude or pulse frequency (as shown near the arrows) receive the direct negative feedback input. Spermatogenesis is stimulated by FSH only indirectly via its actions on the Sertoli cell. T promotes spermatogenesis mainly by a similar Sertoli cell-mediated indirect action, and in part by a nongenomic action on spermatocytes. ABP maintains local levels of T about 200-fold higher than in the circulation. This is important for both spermatogenesis and the suppression of anti-Müllerian hormone (AMH). The mechanisms of suppressed GnRH-gonadotropin-gonad axis by excessive secretion of prolactin (PRL) is displayed. This mechanism is shared by excessive growth hormone (GH) secretion.

Table 13-6 Normal Values of the Hormones of the Pituitary–Testicular Axis in Adults[a]

Compound	Normal value
FSH	1.42–15.4 mIU/mL
LH	1.24–7.8 mIU/mL
Inhibin B	≈120 pg/mL
Testosterone	280–1100 ng/dL
Dihydrotestosterone	30–85 ng/dL
17β-estradiol	1.0–5.0 ng/dL
SHBG	59–472 μg/dL
Progesterone[b]	13–97 ng/dL
Urinary 17-ketosteroids	10–25 mg/d

[a] Except for 17-ketosteroids, all values refer to plasma/serum.
[b] Not involved in the physiologic regulation of the pituitary–testicular axis.
ABBREVIATIONS: FSH, follicle-stimulating hormone; LH, luteinizing hormone; SHBG, sex hormone-binding globulin.

istration of exogenous progesterone has a potent GnRH-inhibiting action just as in females (see Regulation of the Ovarian Cycle: The Hypothalamic–Pituitary–Ovarian Axis) and may be used for the suppression of the pituitary-testicular axis.

- FSH stimulates the Sertoli cells, which provide feedback action mainly by secreting inhibin B. Inhibin acts on the pituitary gonadotroph cell to selectively suppress FSH synthesis.
- Activin of mainly pituitary origin is a functional antagonist of inhibin and may selectively stimulate FSH. The biologic activity of activin is limited by follistatin of pituitary origin, which binds activin with a high affinity and prevents its binding to the activin receptor.

The normal values involved in the pituitary–testicular axis are shown in Table 13-6. We now discuss the components of this regulatory system in more detail.

GnRH Neurons Develop in the Olfactory Placode and Migrate to the Medial Basal Hypothalamus Under the Influence of Anosmin, a Cell Adhesion Protein Missing in Kallmann's Syndrome Patients Two different forms of GnRH have been identified in humans (Box 13-4). The classic form of GnRH (now also called GnRH-I) is a decapeptide encoded by a gene on chromosome 8p21-8p11.2. The anatomic distribution of GnRH neurons displays significant species specificity. In humans, the GnRH neurons that project to the *median eminence* are mainly found in the *arcuate nucleus* of the hypothalamus. Fewer GnRH neurons are located in the *medial preoptic area* (the main location of GnRH neurons in rodents). During ontogeny, GnRH neurons develop in the *olfactory placode*, and

BOX 13-4 GnRH Genes in Humans

The GnRH (also referred to as GnRH-I) expressed from chromosome 8p21-p11.2 has the primary structure of *mammalian type* GnRH, and is a well-established regulator of reproductive function. A distinct gene on chromosome 20p13 encodes GnRH-II, a decapeptide of unknown physiologic function. The genomic and mRNA structures of GnRH-II parallel those of GnRH-I. Outside the brain, GnRH-II is expressed at up to thirtyfold higher levels than GnRH-I, particularly in the kidney, bone marrow, and prostate. In the brain of rhesus monkeys, GnRH-II mRNA is expressed mainly in the midbrain, hippocampus and discrete nuclei of the hypothalamus, including the suprachiasmatic, supraoptic, paraventricular and arcuate nuclei.

migrate via the olfactory bulb to their final destination under the influence of anosmin, the protein product of the *KAL* gene (chromosome Xp22.3). *Anosmin* is a 680-amino-acid cell surface-attached glycoprotein homologous with the family of *neural cell adhesion molecules* (N-CAMs). Anosmin regulates the migration of GnRH cells as well as the axons of the olfactory epithelium, including those originating from the *vomeronasal organ* (i.e., the pheromone-sensing olfactory area, see Regulation of the Ovarian Cycle: The Hypothalamic–Pituitary–Ovarian Axis). Deletions of the *KAL* gene result in *Kallmann syndrome*, an X-linked GnRH-deficient *hypogonadotrophic hypogonadism* associated with anosmia (absence of the sense of smell). The condition mainly affects males but cases of Kallman syndrome in females have also been reported. In a significant portion of reported cases, Kallmann syndrome is associated with *unilateral renal agenesis* and cerebellar dysfunction (nystagmus, ataxia, and/or mirror movements) probably reflecting the lost local actions of anosmin at these sites. A cluster of genes is found on Xp22.3 (next to the pseudoautosomal region), including the *chondrodystrophia punctata*, *steroid sulfatase* and *KAL* genes. The close apposition explains the association between Kallman syndrome, *X-linked ichthyosis* (absence of steroid sulfatase; see The Endocrine Physiology of the Pregnant Woman and the Fetoplacental Unit) and chondrodystrophia punctata.

The GnRH Pulse Generator Is Modulated by Hypothalamic Neurons and Peripheral Hormones The GnRH neurons have an *intrinsic* rhythmic activity referred to as the *GnRH pulse generator*, which assures pulsatile secretion of GnRH into the pituitary portal circulation. In postpubertal men, the GnRH and GnRH-driven LH pulses occur with a period time of 90 to 140 min. Hormones modulating the *frequency* of pulsatile LH secretion by definition act at the hypothalamic level. Hormones modulating the *amplitude* of LH pulses may act either at the hypothalamic and/or the pituitary level; their site of action is problematic to ascertain in human studies.

Altered pituitary LH release in response to a fixed GnRH challenge indicates a pituitary site of action.

GnRH activity is modulated by stimulatory and inhibitory neurons (see Fig. 13-11):

- *Stimulatory input* to the pulsatile GnRH release are provided by *neuropeptide Y* (NPY, a neurotransmitter related to pancreatic polypeptide) and *noradrenergic neurons* acting on α_1-*adrenergic* receptors. The release of NPY and norepinephrine (NE) is intrinsically pulsatile; their pulses either precede or occur simultaneously with GnRH pulses. Centrally administered antagonists of NPY and NE suppress GnRH pulses in primates indicating that these transmitters may either entrain or override the intrinsic rhythm of GnRH neurons.
- *Inhibitory input* to the GnRH neurons is provided by
  - *GABAergic neurons* acting on *$GABA_A$ receptors*. The *tonic* inhibitory action of these GABAergic neurons is involved in suppressing gonadotropin secretion before puberty.
  - A subset of NPY neurons apparently inhibits GnRH release. The activity of these NPY neurons is inhibited by circulating leptin (see following).
  - POMC-positive neurons producing the opioid peptide *β-endorphin*. These neurons have been implicated in stress/CRH-mediated inhibition of gonadotropin secretion and the negative feedback action of androgens (DHT) and progesterone, which *decrease the frequency of GnRH pulses*. The negative feedback of estrogens does not involve the opioid pathway. Consistent with this, *estrogens decrease pulse frequency only if sufficient amounts of androgens (or progesterone) are present.*
  - *Dopaminergic* neurons.

Certain *circulating hormones* may influence the activity of GnRH neurons in the arcuate nucleus.

- The negative feedback action of *sex steroids* is in part mediated by a hypothalamic action. Because the GnRH neurons lack the receptors for sexual steroids and progesterone, the negative feedback involves indirect mechanisms. As pointed out, the negative feedback of androgens is mainly mediated via estrogen receptors after local aromatization.
  - Stimulation of *estrogen* receptors decreases the *pulse amplitude* of GnRH. The reports on the effect of estrogens on the pulse frequency are conflicting. It appears that *in the presence of androgens (or progesterone)*, estrogens decrease GnRH pulse frequency (see above). *Clomiphene citrate* (an estrogen antagonist used for *ovulation induction*) increases both the frequency and amplitude of LH pulses in both sexes, albeit its effect on pulse frequency in females is inconsistent. The pulse amplitude is primarily suppressed via inhibiting the *noradrenergic* input to GnRH.

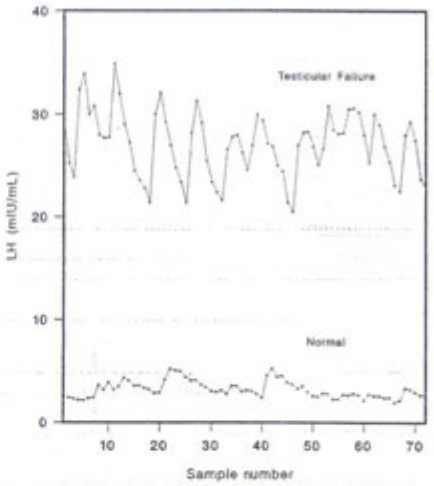
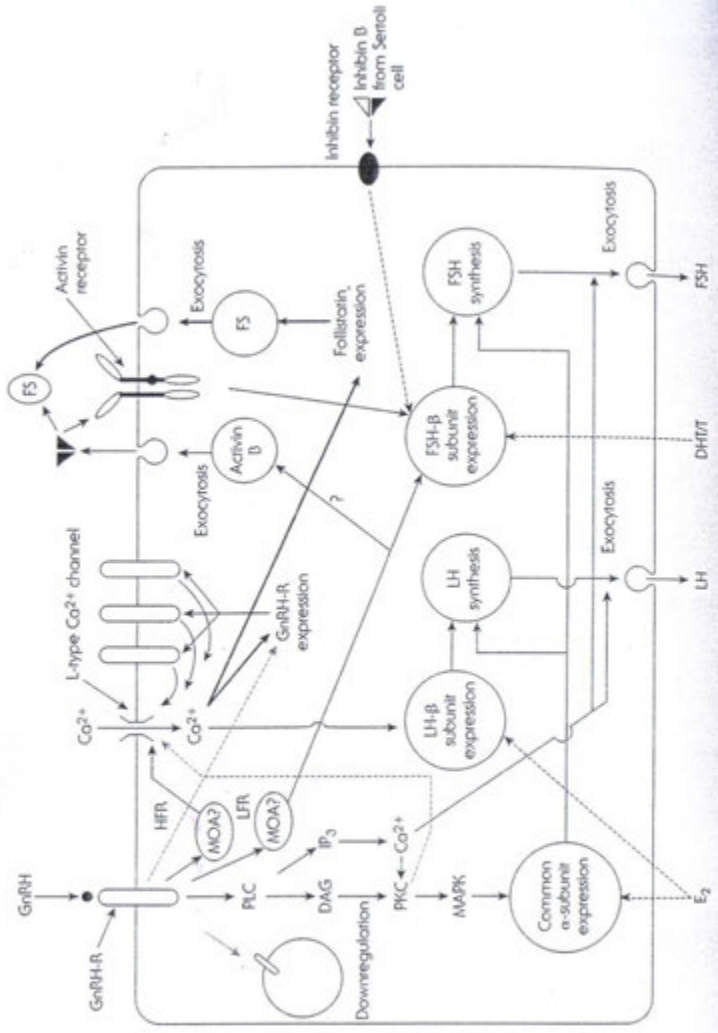


Figure 13-12. LH levels in serum samples drawn every 10 minutes for 12 hours beginning at 8:00 A.M. in a normal 21-year-old man and in a 35-year-old man with bilateral cryptorchidism. The cryptorchidism led to testicular failure, and loss of negative feedback: note the increased pulse amplitude as well as pulse frequency. The high-amplitude pulses start from an elevated baseline. (*Source:* Fig. 131-11, p 2387 in Winters SJ: Clinical disorders of the testis, in De Groot LJ (ed): *Endocrinology*, 3rd ed., Philadelphia, Saunders, 1995.)

- *Progesterone* (which is *physiologically* relevant only in females), and DHT (which is *physiologically* relevant only in males), mainly exert their hypothalamic negative feedback action via endogenous opioids by decreasing the *frequency* (but not the amplitude) of GnRH pulses.

Removal of sex steroid feedback by castration increases the frequency and the amplitude of LH pulses (Fig. 13-12). The increased amplitude of LH pulses in castrated individuals also involves enhanced pituitary responses to fixed quanta of GnRH.

- High levels of PRL and GH (cross-reacting on PRL receptors) inhibit GnRH secretion. Thus, *hyperprolactinemia and acromegaly may cause amenorrhea in women and hypogonadism/impotence in men.* Three mechanisms have been identified: direct action of PRL on GnRH neurons and indirect inhibition of GnRH via the release of either dopamine or opioids. In women, these mechanisms are involved in the suppression of GnRH and the menstrual cycle during pregnancy (in part by PRL, in part by placental lactogens) and by PRL during lactation.
- *Leptin* is an adipose tissue-derived hormone that signals the state of nutrition and energy (lipid) reserves to the hypothalamus. Leptin is a required, albeit in itself an insufficient, positive signal of GnRH secretion, which serves as a metabolic gate to the reproductive system. The leptin



mechanism involves the GnRH-inhibiting subset of NPY neurons. *Leptin assures that the energy-demanding reproductive function proceeds only if adequate energy stores are available.* Commencement of puberty requires a minimal level of adiposity and the associated increase in plasma leptin levels. After puberty, when lipid stores are severely depleted (e.g., in certain athletes), the decrease in leptin shuts down reproductive function via GnRH.

The GnRH Receptors of Pituitary Gonadotrophs Are Rapidly and Extensively Dowregulated/Desensitized by Continuous Exposure to GnRH

Both the N- and C-termini of GnRH decapeptide are involved in receptor binding. This is due to a β-turn in the secondary structure of GnRH, which creates a hairpin loop bringing the two termini in close proximity. In response to GnRH pulses, the GnRH receptors are activated, leading to cosecretion of *LH* and *FSH* by pituitary gonadotroph cells. GnRH pulses stimulate both the *release* and the *biosynthesis* of gonadotropins.

GnRH acts on the *GnRH-receptors* encoded by chromosome 4q13.2-13.3. Several *cis acting elements* have been identified in the GnRH receptor gene that regulate its expression, including AP-1 sites and response element sequences for glucocorticoid, progesterone, thyroid hormones, and CREB (cyclic AMP response element binding protein). The GnRH receptor is a member of the G-protein-linked heptahelical transmembrane receptor family. The GnRH receptor is coupled with $G\alpha_{q11}$, and activates the *PLC-β pathway*, which involves IP_3-Ca^{2+} and DAG-PKC-MAP kinase (Fig. 13-13). The activation of GnRH receptors results in the opening of *L-type (voltage-gated) Ca^{2+}-channels* and an influx of *extracellular* Ca^{2+}. The increased intracellular Ca^{2+} is essential for the exocytosis of secretory granules, that is, the *release* of gonadotropins. The ratio of LH and FSH within the secretory granules is determined by the differential regulation of their biosynthesis (see under next heading on p. 496).

GnRH or its long-acting *agonist* peptide analogues (such as *leuprolide*) *inhibit* the secretion of gonadotropins when administered in a *continuous* (as opposed to pulsatile) manner. The inhibitory action is due to *receptor*

Figure 13-13. Differential regulation of lutreinizing hormone (LH) and follicle stimulating hormone (FSH) in the gonadotroph cell. Solid arrows indicate stimulation/increase; dashed arrows indicate inhibition/decrease. The effects of continuous exposure to gonadotropin-releasing hormone (GnRH) are shown in color. The precise mechanism of action (MOA) of high frequency (HFR; 1/hour) and low frequency (LFR; 1 per 3–4 hours) GnRH pulses are uncertain. HFR GnRH pulses activate L-type Ca^{2+}-channels, induce preferential expression of the LH-β subunit, increase GnRH receptor (GnRH-R) expression/numbers, and stimulate follistatin (FS). The increased GnRH-R numbers lead to favored production of LH over FSH, increased GnRH-sensitivity and responsiveness, and amplify the LH pulse amplitude. Activin B is an autocrine positive regulator of the FSH-β subunit; its action can be prevented by autocrine FS (a competitive binder of activin) and antagonized by Sertoli cell-derived inhibin B. The expression of the gonadotropin subunits is inhibited by sexual steroids. Note that in reality LH and FSH molecules are packaged together in the secretory vesicles. PLC, phospholipase C; PKC, protein kinase C; DAG, diacylglycerol; MAPK, mitrogen activated protein kinase; T, testosterone; DHT, dihydroT; E_2, estradiol.

downregulation (internalization) and *desensitization*. GnRH receptor desensitization involves

- the inhibition of the L-type Ca^{2+}-channels probably due to their PKC-mediated phosphorylation. This mechanism explains in part why the biosynthesis of the LH β-subunit is preferentially inhibited upon prolonged GnRH receptor stimulation (see below).
- the inhibition of GnRH receptor gene expression.

The *GnRH test* is used in the evaluation of hypothalamic–pituitary–gonad axis. An acute challenge with an intravenous GnRH bolus injection (100 μg over 15 seconds) results in an age- and sex-dependent gonadotropin response. The normal response in adult men includes a more rapid and larger increase of LH and a delayed FSH increase with lower amplitude.

The Biosynthesis of LH and FSH Is Regulated Differentially by the Frequency of GnRH Pulses, Activin, Follistatin, Inhibin, and Sex Steroids

The pituitary gonadotropins are glycoprotein hormones structurally similar to thyroid-stimulating hormone (TSH) and hCG. As such, they share a *common α subunit* (localized on chromosome 6), and have *hormone-specific β-subunits* (see Chap. 10). Although the α-subunit is required for receptor binding, the β-subunit provides the receptor-specificity. The β-subunit of FSH is localized on chromosome 11. A single LH β-subunit and several highly homologous hCG β-subunit genes and pseudogenes are localized on chromosome 19. The β-subunits of LH and hCG recognize the same LH-receptor. Nevertheless antibodies distinguish the epitopes of the hCG and LH β-subunits (Box 13-5). The expression of the α-subunit gene is under multihormonal control, which varies in a cell type-specific manner in pituitary gonadotropes, thyrotropes, and the placenta, and is coordinated with the expression of different β-subunit genes. The expression of the α-subunit gene in the pituitary gonadotroph is stimulated by GnRH, inhibited by estrogens, and remains unaffected by a direct thyroid hormone action. The GnRH postreceptor signaling mechanisms exert a differential impact on the expression of the α- and β-subunit genes (see Fig. 13-13). The activation of the

- DAG-PKC-MAP kinase pathway selectively induces the expression of the *common α-subunit* gene in gonadotrophs.
- L-type (voltage-gated) Ca^{2+}-channels results in a selective expression of the *LH-β* subunit.

The molar ratio of LH and FSH in the secretory granules of the gonadotrophs is variable. This is achieved by the differential regulation of the biosynthesis of the β-subunits of LH and FSH. The frequency of the GnRH pulses has a differential effect on LH and FSH:

BOX 13-5 β-Subunit-Specific Immunoassay for the Detection of hCG

Absence of menses in women with previously established menstrual cycle is known as *secondary amenorrhea.* (*Primary amenorrhea* is diagnosed in a 16-year-old or older female, who never had a menstrual bleeding [see Table 13-9]. Note that the terms "primary" and "secondary" in this developmental setting are used in a context different from the one referring to the site in a regulatory system, such as the term "primary hypothyroidism.") The most prevalent causes of secondary amenorrhea are pregnancy and (typically after the age of 45 to 52 years) menopause. Both conditions are accompanied by increased concentration of LH-like biologic activity in urine. An outdated pregnancy test relied on the demonstration of this activity: male frogs released sperm upon injection with urine obtained from a pregnant woman. However, the test was relatively insensitive, and did not help the differential diagnosis between pregnancy and menopause. The immunologic demonstration of β-hCG in urine or plasma circumvents both problems. Measurements of β-hCG in postpartum women are essential in the diagnosis of *choriocarcinoma.*

In men, measurement of β-hCG is useful in the diagnosis and monitoring of certain testicular malignancies: all cases of *choriocarcinoma,* about 50% of *teratocarcinoma* and 5 to 10% of *seminoma* cases test positive for hCG. In *infantile embryonic carcinoma* (a yolk sac tumor of the testis usually seen in children under 3 years of age) another pregnancy-associated protein, *alpha fetoprotein* (AFP), appears in the plasma of most patients.

- FSH-β gene expression is highest when gonadotrophs are stimulated by *low-frequency* pulses of GnRH, becomes relatively suppressed when GnRH is applied at higher frequencies, and absolutely suppressed upon continuous exposure to GnRH. *Activin*, a member of the TGF-β family, is a dimeric protein closely related to inhibin (see page 500). *Activin B* by an autocrine mechanism increases the concentration of FSH-β (but not LH-β) mRNA in the gonadotroph cells. Rodent experiments suggest that the higher GnRH pulse frequencies induce the production of pituitary *follistatin*, which binds activin and prevents its FSH-stimulating action.
- The LH-β gene is stimulated when GnRH is applied at higher frequencies and amplitudes. Because sexual steroids (mainly androgens and, in females, progesterone) inhibit the frequency of GnRH pulses, their *hypothalamic* feedback is a more effective suppressor of LH than FSH secretion. In addition, the LH-β gene is suppressed by a direct pituitary action of estrogens. Testosterone provides an *estradiol-mediated* physiologic feedback suppression of LH secretion in men. The main site of conversion by aromatase is presumed to be the pituitary gland, although systemic levels of estradiol (mainly determined by adipose tissue) appear to be sufficient to inhibit LH secretion by a pituitary action.

The mechanisms involved in the differential stimulation of FSH and LH by GnRH pulse frequency are only partially understood. GnRH receptor expression is upregulated at higher GnRH pulse frequencies. When GnRH receptor concentration in the plasma membrane is increased, LH is preferentially synthesized over FSH in response to GnRH. In addition to the GnRH pulse frequency, additional mechanisms are involved in modulating the FSH/LH ratio in the secretory granules:

- The inhibin/activin system is the main regulator of FSH-β expression. The action of pituitary activin B and follistatin as autocrine/paracrine regulators has been discussed. *Inhibin B*, a product of the testicular Sertoli cells delivered to the pituitary gonadotrophs by blood plasma, selectively inhibits the expression of FSH-β by a direct action. Because secretion of inhibin B is stimulated by FSH, this mechanism represents a separate, FSH-specific feedback loop (Fig. 13-11). In castrated men and in Klinefelter's syndrome patients whose Sertoli cell population is progressively destroyed, circulating levels of FSH are elevated. Because the Leydig cell population is less affected than the Sertoli cells in Klinefelter's syndrome, FSH becomes disproportionately more elevated than LH. The disproportionate increase is in part due to the different half-lives of LH and FSH (see following).
- *Testosterone* and/or DHT, by acting on androgen receptors of the pituitary gland, may suppress the expression of FSH-β.

Due to Its Shorter Half-Life, Plasma LH Displays a More Pulsatile Secretory Pattern than FSH Under the influence of GnRH pulses, the pituitary gonadotropins are secreted in a pulsatile manner. LH and FSH circulate in plasma as free (unbound) hormones with initial half-lives of 30 min and 1 to 3 h, respectively. Within this range, the more heavily sialylated FSH has longer half-life but a decreased biologic activity. Due to its short half-life, plasma LH displays high-amplitude fluctuations ranging from very low to high concentrations. In contrast, plasma FSH levels are more stable and display lower amplitude fluctuations. Due to the difference in plasma half-lives and the variable FSH/LH ratio in the secretory vesicles, the secretory profiles of LH and FSH may appear dissociated in serially collected blood plasma samples.

Gonadotropins are used both in diagnostic tests and as therapeutic agents. Human recombinant FSH and LH have recently become available. The classic natural gonadotropins used in clinical practice are hCG (prepared from the urine of pregnant women) which is used as LH, and *human menopausal gonadotropin* (HMG, *menotropin*) which is used for its predominant FSH activity. The combination of HMG and hCG is used for the treatment of infertility in *hypogonadotropic hypogonadism* (Box 13-6). Cryptorchidism may be resolved by hCG treatment.

The Receptors of Gonadotropins Are Closely Related to Each Other and to the Receptor of TSH The FSH and two LH receptor genes are

BOX 13-6 Exogenously Administered Androgens and Fertility

Testosterone is a required stimulatory factor of spermatogenesis. As discussed earlier, its biologic action mandates very high concentrations *in the seminiferous tubules*, which are provided by the local production of testosterone by Leydig cells and the presence of Sertoli cell-derived ABP. When testosterone is administered exogenously (e.g., as an intramuscular depot injection) as a treatment of hypogonadism, the dosage is aimed at achieving normal *systemic* levels of testosterone. This will normalize secondary sexual characteristics (hair, libido, erectile function, volume of the ejaculate) and exert a negative feedback on pituitary LH and FSH secretion. Under these circumstances, Leydig cells will not provide the necessary high local concentration of testosterone and Sertoli cells do not receive the appropriate stimulation by FSH. Thus, whereas sexual performance is normalized by testosterone, fertility is not achieved. Exogenous testosterone may therefore be used as a male contraceptive. Its usage is not widespread because of its negative impact on lipoprotein metabolism. Administration of decreased doses of testosterone combined with progesterone has been advocated as an alternative. Due to the negative feedback on the hypothalamic–pituitary–gonad axis, body builders who abuse *anabolic steroids* (chemically modified androgens) may have decreased fertility and, due to decreased androgenic activity, may experience erectile dysfunction.

In the treatment of infertility, doses of testosterone that would increase systemic levels to the required testicular concentration are not feasible because of toxic side effects, such as *cholestatic hepatitis*.

all localized on chromosome 2p21, whereas the TSH-R gene is found on chromosome 14q31. These receptors are members of the heptahelical G-protein coupled receptor family and stimulate adenylyl cyclase activity via $G\alpha_s$.

Postzygotic activating mutations of this G-protein subunit during embryonic development result in *McCune–Albright syndrome* (Chaps. 8 and Puberty in this chapter, see Fig. 13-51), which more often affects females and usually includes precocious puberty due to ligand-independent activation of both FSH and LH receptors. In females, activation of both receptors results in follicular maturation and increased hormone production.

The activating mutation of the LH receptor, which is inherited in an autosomal dominant manner and affects segments of the receptor interacting with $G\alpha_s$, results in *male-limited familial precocious puberty* (*testotoxicosis*). The moderate ligand-independent activation of the postreceptor signaling cascade is sufficient to stimulate testosterone secretion by Leydig cells and cause precocious puberty. However, in the absence of FSH receptor stimulation and the resultant follicular growth, the ligand-independent

LH receptor stimulation is apparently insufficient to stimulate the secretion of ovarian steroids.

Loss-of-function mutations of either the FSH receptor or the FSH β-subunit result in infertility in both males and females; the presentation in females includes *primary amenorrhea* (see Table 13-9 in the section The Endometrial Cycle) indicating the mandatory involvement of FSH in follicular maturation. Males, however, do not present with hypogonadism because the LH–testosterone axis remains functional.

Members of the TGF-β Family Such as Inhibin, Activin, and Anti-Müllerian Hormone, and the Activin-Binding Protein Follistatin Play Essential Roles in Reproductive Function, the General Regulation of Mitogenesis and Morphogenesis The Sertoli cells perform several hormonal functions:

- Sertoli cells produce hormones of the TGF-β family, which include
  - *Anti-Müllerian hormone* (AMH, also known as *Müllerian inhibitory hormone* [MIH]). AMH is secreted in utero and until about 8 to 10 years of age. The age-dependent termination of AMH secretion is related to the high local concentrations of testosterone in the seminiferous tubules; circulating levels of androgens are insufficient to suppress AMH. During puberty, intratubular levels of androgens increase to high levels before a major increase in systemic circulation occurs. The AMH-suppressing effect of androgens is so potent that it completely counteracts the AMH-stimulating effect of pubertally high levels of FSH. In patients with androgen insensitivity syndrome (androgen receptor mutation), plasma AMH levels do not decrease in early puberty but significantly increase due to the unopposed action of FSH. Sertoli cells during early development do not express androgen receptors; thus, testosterone produced by the fetal testis may masculinize the external genitalia without suppressing AMH production (see details in Intrauterine Sexual Development).
  - *inhibin*, an inhibitor of FSH secretion by a direct pituitary action.
  - *activin*, a locally acting hormone structurally related to inhibin and usually opposing inhibin's actions.

Except for inhibin, which acts both locally and also as a classic endocrine hormone, *the other hormones in this group act almost exclusively as local paracrine/autocrine regulators.*

- Sertoli cells secrete ABP into the lumen of the seminiferous tubules.

Inhibin is a disulfide-linked dimeric glycoprotein hormone that exists in two forms: a shared common α *subunit* is linked to one of two distinct β *subunits* (βA or βB). *Inhibin A* and *inhibin B* consist of α-βA and α-βB heterodimers, respectively. In addition to its function as a selective inhibitor of FSH-β expression, inhibin acts as a *tumor suppressor*. Inhibin α-knockout mice develop gonadal sex-cord stromal tumors (granulosa/Sertoli cell tumors) and adrenocortical tumors. An unusual feature of these adrenocortical tumors is that they often secrete estradiol.

Activins are either homodimers (βA-βA, βB-βB) or heterodimers (βA-βB) of the β subunits. The β subunits display extensive sequence homology with TGF-β. The α subunit, which is unique to inhibins, is encoded by chromosome 2q33-qter. The β subunits, which are shared by inhibins and activins, are localized on chromosomes 2cen-q13 (βB) and 7p15-p14 (βA).

Activins function as locally acting stimulators of β-FSH expression in the pituitary gland, as *mitogens* and, similar to the related bone morphogenetic proteins, as *morphogens*. As members of the TGF-β family, the action of acitivins involves two types of single membrane-spanning receptor serine/threonine kinases (see Chap. 5). The high-affinity ligand-specific binding to a type II receptor is followed by the recruitment of type I receptors. The recruitment relies on low-affinity and less ligand-specific binding via the exposed subunit of the ligand; the ligand thus functions as a bridge leading to the formation of a receptor heterodimer. The phosphorylated type I receptor serves as a docking site and acts as the only transducer of the intracellular signaling cascade (see Fig. 5-6). The immunophilin *FKBP-12* (see insulin action in Chap. 9) interacts with the serine/threonine kinase domain of the type I receptor, and plays a key role in the downstream signal transduction events of activin and also other members of the TGF-β family. This might explain a shared biologic action of activins, insulin, and IGF-1R agonists: they all promote androgen production in various target cells, including the adrenal cortex, theca and Leydig cells.

- The type I receptors include *ActRI* (activin receptor I, also called *ALK-2*), *ActRIB* (ALK-4), and *ALK-1* (shared by activins and TGF-β), which are related to Alk 6, a bone morphogenetic protein receptor (see Chap. 8).
- Two subtypes of the type II receptors have been described: ActRII, which recognizes the βA subunit, and ActRIIB, which preferentially binds the βB subunit. ActRII, the main mediator of activin's reproductive effects, is expressed in the pituitary gland, testis (including germ cells), the epididymis/vas deferens, male accessory glands, ovary, and uterus.

Ligand-specific *inhibin receptors* have been identified in the pituitary gland and other tissues, and the cDNA of an inhibin receptor has recently been cloned from bovine pituitary glands. The inhibin receptor binds inhibin with high affinity (20–40 pM). Inhibin antagonizes the actions of activin in several target cells. Upon binding its ligand, the inhibin receptor associates with type I activin receptors (such as Alk2 and Alk4), thereby preventing the recruitment of type I receptors by ligand-activated type II or type IIB activin receptors. Inhibin receptors do not associate with type II or type IIB activin receptors. At least in vitro, inhibin may also antagonize activin

by acting on activin receptors. Inhibin A binds with the ActRII with low affinity and, in case of inhibin excess, may compete with activin. Because ActRII-bound inhibin does not recruit type I receptors, the binding of inhibin A with ActRII may antagonize the biologic action of activin.

The biologic action of activin is modulated by *follistatin*. Mature follistatin is a single-chain protein that contains four contiguous domains; three of the domains are highly similar to each other, as well as to human epidermal growth factor and human pancreatic secretory trypsin inhibitor. Follistatin is a local regulator, whose plasma concentration is relatively steady in both sexes and does not change during the menstrual cycle.

- Follistatin was originally discovered as a substance that specifically inhibits FSH. Follistatin inhibits FSH secretion by binding activin and preventing activin from binding to its (type II) receptor (see Fig. 13-13).
- Follistatin interacts with the β-subunits of activins. Because inhibin also contains a β-subunit, follistatin also binds inhibin. However, inhibin binds to follistatin with a lower affinity. In addition, the α-subunit of inhibin remains exposed. These factors may explain why follistatin does not eliminate the biologic activity of inhibin.
- Under certain circumstances, *follistatin is required for the biologic activity of activins*. Follistatin may bind to cell surface heparan sulfate proteoglycan. This *cell-surface-associated follistatin* may *present* activin to type II activin receptors by a mechanism similar to the action of the type III TGF-β receptor (betaglycan). The role of follistatin as an activin-supporting entity has been demonstrated in knockout mice. Mice deficient in the subunit βA (and thus deficient in activin A, activin AB, and inhibin A) display a phenotype shared by follistatin-deficient mice: although they survive to term, they die within 24 h of birth due to severe, multiple craniofacial abnormalities, including cleft palate, absent lower incisors and absent vibrissae. These actions exemplify the morphogenic nature of activins.

THE FEMALE REPRODUCTIVE SYSTEM

Functional Anatomic Overview of the Female Reproductive System

OBJECTIVES

1. Discuss the gross anatomy and histology of the ovary. Describe the morphologic process of follicular maturation: define primordial, primary, secondary, tertiary, and Graafian follicles. Describe the fate of the follicles after ovulation.
2. Describe the anatomic components of the female genital tract: the oviduct, the uterus, and the vagina. Compare the corpus/fundus and the cervix of the uterus. Describe the layered structure of the uterus. Identify the anatomic relationship of the ovary and the female genital tract with the peritoneum and the peritoneal cavity.
3. Discuss the female external genitalia.

The Ovary (Adnexum)

Gross Anatomy The ovaries are found close to the lateral wall of the lesser pelvis at the angle formed by the *external* and *internal iliac arteries*, and *in close association with the ureter* separated from these structures by the parietal peritoneum (Fig. 13-14). The right ovary is usually in the immediate vicinity of the *appendix*. During development, the ovary reaches its final position by a descent that is more limited than that of the testis. The location of the ovary becomes variable in parous women.

Each ovary is an approximately 1-cm thick almond-shaped organ, with a length of 3 cm and a width of 1.5 cm. The ovary is an essentially *intraperitoneal* organ as indicated by its peritoneal doubling known as the *mesovarium*, which is found on the posterior aspect of the *broad ligament* of the uterus. The portion of the broad ligament that is superior to the attachment site of the mesovarium is the *mesosalpinx*, the peritoneal doubling that reaches the *Fallopian tube* (*uterine tube*, *oviduct*, *salpinx*). At the *line of Farré*, a thin white line that encircles the ovarian attachment site of the mesovarium, the mesothelial cover of the ovary is replaced by a simple cuboidal epithelium termed *germinal epithelium*. This term is a misnomer because the germinal epithelium is not the source of germ cells; instead, the *follicular epithelium* (see Fine Structure below) is derived from it during intrauterine development.

During intrauterine development, the *inferior pole* of the ovary is tied to the dermis of the labioscrotal folds (the future labia majora) by the *gubernaculum*. The interposition of the uterus divides the fibrous gubernaculum into the caudal *round ligament* of the uterus and the more cranial *ovarian ligament*.

The *suspensory ligament* is attached to the *superior pole* of the ovary. Nerves, blood, and lymphatic vessels reach the ovary through the suspensory ligament and the mesovarium. The ovarian vessels are homologous with those of the testis and follow the same course in the retroperitoneum. The *ovarian artery* divides into a direct *ovarian* and a *salpingeal branch*, each of those forming an anastomosis with similar branches derived from the *uterine artery*; unless ligations are placed properly, this anastomosis may become the source of life-threatening bleeding during ovariectomy.

Fine Structure In prepubertal females, the surface of the ovary is smooth. Underneath the germinal epithelium, a dense connective tissue layer (*tunica albuginea*) is found, which is thinner, less organized, and mechanically weaker than its male counterpart. The tunica albuginea envelops the thick outer layer of the ovary known as the *cortex*, which *contains the ovarian follicles and their derivatives* embedded in a cell-rich connective

tissue. The tunica albuginea and the cortex form a shell that surrounds a small *medulla*, which contains highly vascular loose connective tissue. At the hilus, which is found at the mesovarium, the cortex is missing; through the hilus the nerves and vessels of the ovary directly enter (or leave) the medulla. The medulla contains variable numbers of *hilar cells*, which produce limited quantities of testosterone. The hilar cells are the female equivalents of the Leydig cells and usually contain *Reinke's crystalloids*.

The details of follicular maturation as related to the regulation of the pituitary-gonad axis are discussed in Regulation of the Ovarian Cycle: The Hypothalamic–Pituitary–Ovarian Axis. The discussion here follows the general maturational events of a single follicle destined for ovulation (Figs. 13-

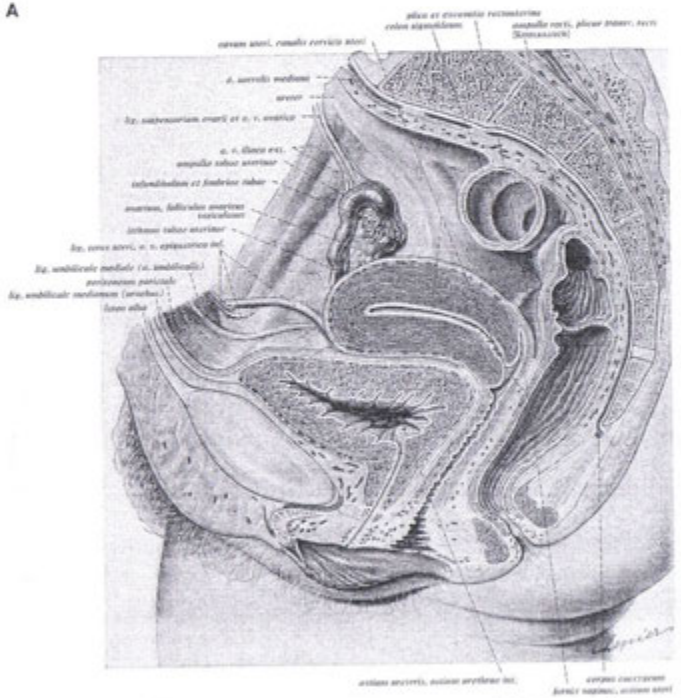


Figure 13-14. The anatomy of the female reproductive system. A. Sagittal pseudosectional diagram of the female urogenital system. B. Cutaway posterior view diagram of the internal female reproductive organs. (*Source*: A: modified from Fig. 303, p 229 and B from Fig. 300, p 226 in Ferner H, Straubesand J (eds): *Sobotta/Becher Atlas of Human Anatomy*, vol 2, 9th English ed., Urban & Schwarzenberg, 1975.)

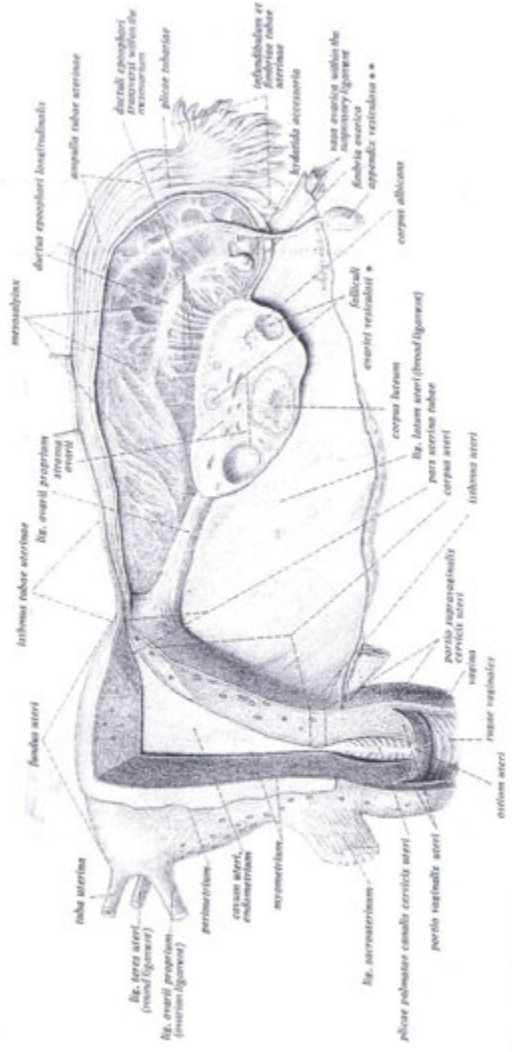


Figure 13-14. (*Continued*)

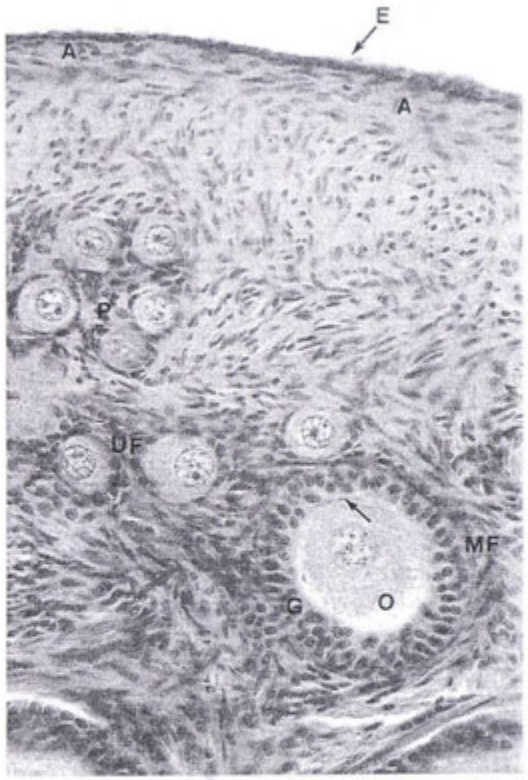


Figure 13-15. Ovarian histology displaying the early stages of follicular maturation. Primordial follicles (P) contain small primary oocytes covered by a single layer of squamous (flat) epithelium. Primary follicles (UF) have larger oocytes and the epithelium, which became cuboidal, is termed granulosa cell. Secondary follicles are also known as preantral follicles (MF). The oocyte is further enlarged, the granulosa cell layer is stratified, the basal lamina (BL) surrounding the granulosa layer is prominent, and the theca is already organized. E, the germinal epithelium covering the ovarian surface; A, Tunica albuginea; arrow, forming zona pellucida. Compare with Fig. 13-2 (antral follicle). [Fig. 19-3, p. 291 in Berman I: *Color Atlas of Histology*, Stamford, CT, Appleton & Lange, 1993.]

15 and 13-2). The diameters of the developing antral follicles can be determined by ultrasonography. This can be used in clinical practice for several purposes, such as evaluation for *polycystic ovary syndrome*, monitoring follicular development during *induced ovulation*, and in attempts to collect oocytes for *in vitro fertilization*. The terminology describing the morphologic stages

of follicular development and maturation is somewhat inconsistent in the literature. In this text the following definitions are used:

- The *primordial follicle* consists of a *primary oocyte* surrounded by a single layer of *squamous follicular epithelium*. The follicular epithelium (which later develops into *granulosa* cells) is separated from the ovarian stroma by a basement membrane, but the stroma does not yet form a recognizable *theca* around it. The growth of the follicle mandates the basal lamina/membrana limitans externa to be constantly remodeled. Primordial follicles are quiescent and are up to 40 μm in diameter. The diameter of the oocyte within the primordial follicle is 15 to 25 μm.
- The *primary follicle* is already engaged in follicular development. *The first sign of follicular development is the enlargement of the oocyte*, which grows from the original 15 to 25 μm to its maximum size of 80 to 100 μm in late secondary (preantral) follicles. The follicular epithelium assumes a *simple cuboidal* morphology and the cells are henceforth called *granulosa cells*. A *glycoprotein-rich* eosinophilic material known as the *zona pellucida* begins to be deposited between the oocyte and the granulosa cells. At this stage the ovarian stroma *begins* to organize as a fibroblast-like *theca*. These follicles are 40 to 100 μm in diameter.
- The *secondary* or *preantral follicles* develop when the granulosa cells further proliferate and become stratified. The granulosa cell layer (as a true epithelium) is *avascular*. Its basement membrane develops into a prominent *membrana limitans externa*. The granulosa cells are connected by well-developed *gap junctions*, which synchronize their function and allow the transfer of nutrients to the innermost layers. A definitive *theca* layer appears only when at least six layers of granulosa cells are present in the follicle; however, the *theca interna* and *externa* are not yet recognizable as separate layers. These follicles are 100 to 200 μm in diameter.
- The *tertiary follicles* are the antral follicles. The *antrum* (chamber) appears *within* the highly stratified granulosa cell layer and contains a hormone-rich *liquor folliculi* (follicular fluid). A number of granulosa cells are anchored in the zona pellucida and form the *corona radiata*. Outside the membrana limitans externa, the stroma is organized as concentric layers around the core of the follicle; this stromal component is termed *theca folliculi* (capsule, sheath). Unlike the epithelial granulosa layer, the mesenchymal theca is vascularized. Two cell types can be recognized in the theca:
  - the *theca interna* cells are characteristic steroid hormone-producing cells;
  - the *theca externa* cells are fibroblast-like cells, which display some contractility and are generally regarded as hormonally inactive.

The size of those tertiary follicles which are destined to regress without ovulation ranges between 200 μm and usually 5 mm (maximum 8 mm). The differential role of theca interna and granulosa cells in steroidogenesis is discussed in The Biosynthesis, Mechanism of Action and Metabolism of Sexual Steroids.

Some texts define the primary follicle as having the characteristics of both the primary and the secondary (preantral) follicles in the above

description. In that terminology, the term *secondary follicle* means antral follicle, and the term *tertiary follicle* is not used. Thus, the terms primary and secondary follicles are ambiguous and their definitions must be sought when reading an unfamiliar text.

- The single follicle in an ovarian cycle that can be recognized as the *dominant* follicle is called the *preovulatory* or *Graafian follicle*. This is typically the largest antral follicle at any given stage of the cycle. During the midfollicular phase, the dominant follicle can usually be identified by a diameter of 5 to 9 mm. By the time of ovulation, it usually reaches a diameter of 20 to 22 mm. In the Graafian follicle the oocyte with its corona radiata emerges into the antrum as the *cumulus oophorus* (ovum-carrying hill). As the time of ovulation approaches, hyaluronic acid accumulates in the interstitial space between the corona radiata cells and loosens the attachment of the cumulus oophorus with the surrounding granulosa cells. This process is known as *mucification*. At the time of ovulation, the base of the cumulus oophorus detaches from the underlying layer of granulosa cells and the oocyte is expelled with its corona radiata remaining attached to it. The preovulatory LH surge induces the completion of the first meiotic division of the oocyte, which yields the *secondary oocyte* and the first *polar body*, both of which are encased by the zona pellucida. The second meiotic division is prompted by fertilization (see Fertilization).

At the time of *ovulation*, which is induced by the preovulatory surge of LH, the entire wall of the follicle ruptures, including the (1) granulosa cell layers around the antrum, (2) membrana limitans externa, (3) theca interna, (4) theca externa, (5) general ovarian stroma, (6) tunica albuginea, and (7) germinal epithelium. This mechanism has several consequences:

- The oocyte/corona radiata is expelled into the peritoneal cavity.
- Due to its sensory innervation, the rupture of the tunica albuginea is painful.
- After puberty, the originally smooth surface of the ovary becomes progressively scarred because ovulation repeatedly disrupts the tunica albuginea; the germinal epithelium regenerates and covers the sometimes deep fissures of the ovarian surface.
- Between the membrana limitans externa and the germinal epithelium, all layers are vascularized. Thus, ovulation results in a small bleeding that fills the antral space previously occupied by the liquor folliculi. This modified follicle-containing clotted blood is known as the *corpus hemorrhagicum*.

The luteinization of the granulosa cells is *initiated* by the preovulatory LH surge. After ovulation, the granulosa lutein cells become vascularized, which leads to the *completion* of their luteinization process. The granulosa lutein cells, theca lutein cells, and the associated microvasculature form the corpus luteum. The function of these cells in steroidogenesis is discussed in The Biosynthesis, Mechanism of Action and Metabolism of Sexual Steroids. *The corpus luteum* has a defined life-span: unless rescued by stimulation of LH-receptors by hCG (i.e., in pregnancy), it *involutes and undergoes apoptosis in 14 ± 2 days after being formed*. When this happens, the cell debris is removed by macrophages, and the space occupied by the corpus luteum is filled with hormonally inactive fibrotic scar tissue: this is known as *corpus albicans* (white/glistening body). Eventually the corpus albicans is eliminated by degradation of the collagenous deposit.

The Female Genital Tract The female genital tract consists of a pair of Fallopian tubes (oviducts), the uterus, and the vagina (see Fig. 13-14). Except for the lower two-thirds of the vagina, which is derived from the *urogenital sinus*, the female genital tract develops from the *Müllerian* (*paramesonephric*) *ducts* (see details in Intrauterine Sexual Development). The Fallopian tube is intraperitoneal, the uterus is mainly intraperitoneal and in part *infra*peritoneal. The vagina is below the peritoneal sac.

The *Fallopian tubes* are 10- to 13-cm long visceral canals that develop from the *nonfused* (cranial) portion of the Müllerian duct. The Fallopian tube has four segments:

- The *infundibulum* is a funnel-shaped expansion of the abdominal end of the oviduct, which is surrounded by narrow irregular processes called *fimbriae*. The *ovarian fimbria* is attached to the ovary near the suspensory ligament. Due to their smooth muscle, the fimbriae are motile and may engulf the ovary at the time of ovulation, thereby aiding the attachment of the expelled ovum (covered with the corona radiata) to the inner surface of the Fallopian tube.
- The *ampulla* is the longest portion of oviduct. The highly folded mucosal surface of the ampulla is the usual site of fertilization.
- The *isthmus* is the narrowing of the Fallopian tube, which constitutes about one third of the length of the canal near the uterus. The folds of the oviduct diminish at the isthmus.
- The *intramural* (within the wall) *uterine part* is a short portion of the canal widening toward the uterine cavity, where it joins the uterus at the junction of the fundus and corpus.

The smooth muscle wall of the oviduct performs an active undulating movement. The mucosa of the oviduct is similar to that of the uterus. On occasion, implantation may take place in the oviduct (*ectopic pregnancy*). Neither the mucosa nor the space are sufficient for supporting normal development, and these tubal pregnancies abort amidst a rupture that may lead to life-threatening bleeding. The epithelial lining of the Fallopian tube contains *secretory* (peg) and *ciliated cells*. The active beating of cilia keeps a thin layer of mucous film flowing toward the vagina. This *mucous escalator* serves three major functions:

- keeps the mucosal lining germ-free by constant flushing;
- contributes to the orientation of the movement of spermatozoa, thereby aiding fertilization;
- carries the developing zygote (which is incapable of active movement) to its site of implantation.

The *uterus* is a modified oviduct, which (together with the upper one-third of the vagina) develops by the fusion of the caudal portion of the Müllerian ducts. Anatomically the uterus is an approximately pear-shaped organ, which has two main portions:

- The *corpus uteri* is the broader upper part of the uterus. The convex anterosuperior surface of the corpus between the joining oviducts is the *fundus*. The corpus surrounds the *uterine cavity*, a flat triangular space. The base of this triangle is the fundus, the downward-pointing tip of the triangle is the *internal orifice* of the cervical canal. The length of the corpus in nulliparous women is about 4.5 cm; in parous women, the corpus is longer (~5.5 cm).
- The *cervix uteri* is the lower, narrow cylindrical part of the uterus, which surrounds the *cervical canal*. The cervical canal is about 2.5 cm long both in nulliparous and parous women. Its *internal orifice* opens into the uterine cavity at the *isthmus uteri*. The cervix has two anatomic portions:
  - The *supravaginal portion* is cranial to the insertion of the vaginal fornix into the uterine cervix.
  - The *vaginal portion* (*portio vaginalis*) protrudes into the vagina. The *external orifice* of the cervical canal opens at the center of the vaginal portion; its shape varies with the parous state from round opening (nulliparous) to a transverse horizontal narrow slit (multiparous).

The *uterine cavity* is surrounded by three layers of the corpus and fundus of the uterus:

- *The endometrium* is the mucosal layer that consists of a simple columnar epithelium and an underlying connective tissue stroma (*lamina propria*). The simple tubular *uterine glands* span the entire thickness of the endometrium, and are lined by nonciliated as well as ciliated columnar epithelium identical with those of the surface. Unlike the cervical mucosa, the apical layers of the endometrium (*stratum functionale*) in the corpus/fundus region are shed during menstruation, leaving behind the *stratum basale,* a thin layer of lamina propria without surface epithelium and the blind ends of uterine glands. The stratum functionale is supplied by *spiral arteries* of the endometrium; the stratum basal receives a separate, parallel supply from the *basilar (straight) arteries*. The entire surface epithelium regenerates from the remnants of the glands, which explains the identical morphology of the glandular and surface epithelia. The endometrium is the site of implantation. Its involvement in the formation of the placenta is discussed in Implantation and the Placenta.
- The *myometrium* is the thickest layer of the uterine wall and consists of smooth muscle and vasculature. The enlargement of the uterus during pregnancy mainly involves *hypertrophy* of existing smooth muscle cells, but *hyperplasia* (proliferation of the smooth muscle cells) also contributes. This muscle is important in delivering the newborn. The permanent enlargement of the corpus in parous women is due to the incomplete postpartum involution of the myometrium. In older, especially multiparous women, the myometrium often contains benign smooth muscle tumors (*leiomyoma*). Because the blood vessels reach the endometrium via the myometrium, these tumors may cause irregular menstruation.
- The *perimetrium* is the connective tissue (within the broad ligament and the subserosal layer) and the associated mesothelium lining of the peritoneal cavity. The nerves, blood vessels, and lymphatics reach the uterus through the perimetrium mainly via the broad ligament. The arterial supply is provided by the *uterine artery*, a branch of the internal iliac artery.

The layers are modified in the *uterine cervix*.

- The *cervical mucosa* contains large, expanded branched glands. These glands produce the *cervical mucous plug* that is rich in hyaluronic acid and serves as a barrier against bacterial invasion. Because the acrosome of spermatozoa contains *hyaluronidase*, they can penetrate the mucous plug. Although the cervical mucosa is not shed at the time of menstruation, it shows important cycle-dependent functional changes that affect the amount and consistency of the cervical mucus, and influence fertility. The mucosal glands are sometimes obliterated leading to the development of *Nabothian cysts*, which are usually asymptomatic accidental findings in clinical practice. At the external orifice, the simple columnar epithelial lining of the cervical canal abruptly changes into the stratified squamous epithelium of the portio vaginalis uteri. The *Papanicolaou (Pap) smear* used in the screening for *cervical cancer* (a malignant tumor of the epithelium of the cervical canal probably caused by human papilloma virus) is taken from the external orifice, where shed cells of the cervical canal appear as a part of normal epithelial turnover.
- In lieu of the myometrial layer, the cervix has a thick dense connective tissue wall. This structure is important for parturition: contraction of smooth muscle would narrow the birth canal. Near parturition, the dense connective tissue is softened by the action of *relaxin* (see Box 13-11 in The Endocrine Physiology of the Pregnant Woman and the Fetoplacental Unit). This phenomenon is known as *cervical ripening*, which enables the cervix to dilate, "disappear," and allow the passage of the fetus.
- The peritoneal cover of the uterus leaves the uterine surface at the insertion of the anterior and posterior vaginal fornices.

The *vagina* is an approximately 9 cm long, flattened muscular canal that connects the uterine cervix with the opening of the urogenital diaphragm. The anterior wall of the vagina is closely associated with the urethra. Through the rectum and the posterior wall of the vagina the portio vaginalis can be palpated. In pregnant women this association can be used in the assessment of cervical dilation without increasing the risk of infection of the fetal membranes. The superior end of the vagina forms an anterior and a posterior fornix. These are in association with two recesses of the peritoneal sac: the vesicouterine and the rectouterine recesses. In cases of ruptured tubal pregnancy, blood accumulates in the *rectouterine recess* [*of Douglas*], which can be detected by needle aspiration upon piercing through the posterior vaginal fornix.

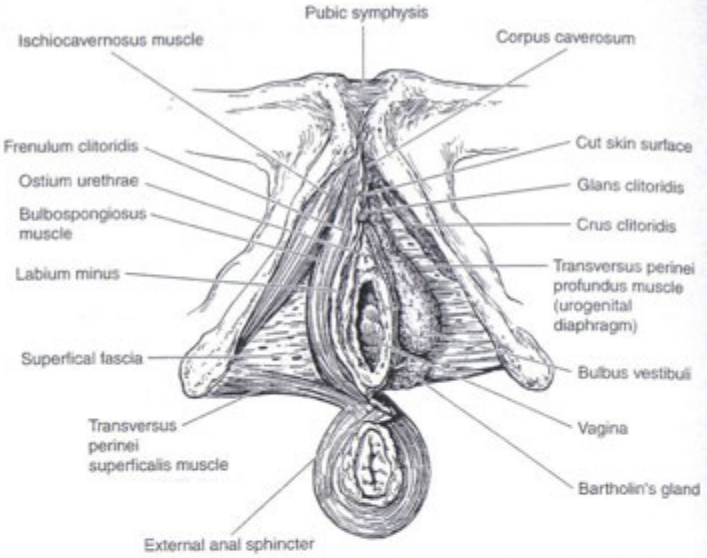


Figure 13-16. Anatomy of the female external genitalia. The skin including the labia majora has been removed. The cut diagram shows the bulbus vestibuli underneath the bulbospongiosus muscle. The transversus perinei superficialis represents the posterior (in this figure, inferior) edge of the urogenital diaphragm. The voluntary sphincter of the urethra is embedded in the tissue layer of the urogenital diaphragm (not shown). (*Source:* Modified from Fig. 297, p 223, in Ferner H, Staubesand J (eds): *Sobotta/Becher Atlas of Human Anatomy*, vol 2, 9th English ed., Urban & Schwarzenberg, 1975.)

The mucosal lining of the vagina is devoid of glands. The moist environment is provided by the cervical glands, the vestibular glands, and (during sexual arousal) filtration of fluids due to vascular congestion in the vaginal wall (*transudate*). The vagina must resist harsh mechanical forces, such as childbirth and intercourse. The mechanical resistance is achieved by the adaptation of three constituents:

- Stratified squamous epithelium.
- A thin layer of lamina propria that decreases the shearing effect of tangential mechanical forces.
- Thick layers of smooth muscle.

The Female External Genitalia Physical examination may reveal ambiguous external genitalia and provide evidence of endocrine dysfunction. The female external genitalia consist of three main components (Fig. 13-16):

- The *clitoris* is the homologous organ of the penis. Its *crura* and *corpora cavernosa* are erectile and correspond to those of the penis. The urethra opens at the base of the *glans clitoridis*.
- The *labia minora* (the inner longitudinal pair of skin folds) border the *vestibule*. They develop from the *urogenital folds* without fusion, and are homologous with the skin of the penis. The labia minora are covered by a thin, hairless, highly pigmented epidermis. In their deeper tissue layer is found the *bulbus vestibuli*. This erectile tissue is homologous with the bulbus penis (the root of the corpus spongiosum), but unlike its male counterpart, it is a paired organ on either side of the vestibule. The bulbus vestibuli continues anteriorly into the *frenulum clitoridis*, and fuses with the contralateral bulbus to form the glans. The *greater vestibular (Bartholin's) gland*, which is the female equivalent of the Cowper's gland is located posterior to the bulbus and opens into the vestibule.
- The *labia majora* (the outer longitudinal pair of skin folds) develop from the *labioscrotal swellings* without fusion and are homologous with the scrotum. They contain adipose tissue that spreads to the anterior surface of the symphysis pubis and forms the *mons pubis*. At puberty, pubic hair grows on the mons and the outer surface of the labia majora.

The vagina opens to the vestibule through the *urogenital diaphragm*. On the external surface of the diaphragm, on either side of the vestibule, a triangle is formed by a group of *superficial perineal muscles*. These include the *bulbospongiosus, ischiocavernosus,* and *trasversus perinei superficialis* muscles.

The Menstrual Cycle

OBJECTIVES

1. Discuss the regulation of the ovarian cycle: the hypothalamic–pituitary–ovarian axis. Identify the relationship between the *ovarian cycle* and the *endometrial cycle*. Identify the time-course of the plasma levels of gonadotropins and ovarian hormones during the menstrual cycle, and the feedback mechanisms operating during the *follicular phase, preovulatory surge* of gonadotropins (positive feedback), and *luteal phase*. Compare and contrast the feedback regulation of gonadotropin secretion in males and females.
2. Discuss *oogenesis*, the gonadotropin-independent, and the gonadotropin-dependent phases of follicular growth and maturation. Discuss the timeframe of these processes, and the fate of the follicular pool during the lifespan of women. Discuss the roles of *Wilms' tumor 1* (WT1) and *growth differentiation factor-9* (GDF-9) in follicular growth. Define the terms *cohort, recruitment, selection,* and *dominance*. Compare and contrast bi-ovarial and mono-ovarial phases of feedback regulation during the menstrual cycle. Discuss the mechanism of *dizygotic twinning*.
3. Identify the role of *kit* and *kit ligand* in the LH-regulated resumption of meiosis. Discuss the mechanism of ovulation, and the role and regulation of proteases

in the process. Describe postovulatory neovascularization and its role in the completion of luteinization.

4. Describe the ovarian contribution to circulating androgens in healthy women. Discuss *hyperthecoses* and the *polycystic ovary syndrome*. Discuss hormonal contraceptives and their mechanism of action. Discuss ovulation induction.
5. Discuss the *pheromonal* regulation of the menstrual cycle.
6. Discuss the biologic actions of androgens, estrogens, and progesterone in cycling women. Describe the regulation of the endometrial cycle and the mechanism of *menstrual bleeding*, including the involvement of *prostaglandins* and *matrix metalloproteinases*. Describe the main types of abnormal menstrual bleeding and the endocrine background of *breakthrough bleeding*.
7. Discuss cyclic changes in the vagina and the uterine cervix/cervical mucus. Describe the normal flora of the vagina and its relationship with vaginal pH. Discuss the *maturation index*, *Spinnbarkeit*, and *fern*ing.
8. Discuss the mechanism of *premenstrual syndrome*.

Regulation of the Ovarian Cycle: The Hypothalamic–Pituitary–Ovarian Axis

The Phases of the Endometrial Cycle Are Dictated by the Phases of the Ovarian Cycle The regulation of the hypothalamic–pituitary–ovarian axis in postpubertal females typically follows a 28-day cycle (normal range 25 to 35 days). *Menstruation* (menses; from the Latin *mensa*, month) is the shedding of the *stratum functionale* of the endometrium, which is accompanied by mainly arterial bleeding. *The onset of menstrual bleeding is designated as the first day of the cycle.* Two cycles are distinguished:

- The *ovarian cycle* is related to the gonadotropin-dependent maturation of the ovarian follicles, including the formation and demise of the corpus luteum. The ovarian cycle has two phases:
  - The *follicular phase* is characterized by the absence of a functional corpus luteum, and the gonadotropin-dependent follicular maturation leading up to ovulation. The maturing follicles mainly secrete *17β-estradiol* (E_2) and *inhibin B*, but plasma progesterone remains very low. The length of the follicular phase is variable, and this variability accounts for the overall variability of the length of the menstrual cycle.
  - The *luteal phase* starts with the development of the corpus luteum. The characteristic and determinant hormone of the luteal phase is *progesterone*. However, the corpus luteum also secretes *17β-estradiol* and *inhibin A*. The length of the luteal phase is relatively invariable (14 ± 2 days) and determined by the life-span of the corpus luteum.
- The *endometrial cycle* is the cyclic change of the endometrium, which is essentially dictated by the ovarian hormones and the ovarian cycle. Three phases are distinguished:

BOX 13-7 Diagnostic Use of Progesterone

Exogenously administered progesterone or its analogs such as *medroxyprogesterone* can be used diagnostically for evaluating endogenous estrogen secretion. The test is useful as an inexpensive initial evaluation that does not require direct hormone measurements. Unlike hormone measurements, this biologic test takes tissue responsiveness into consideration. In *nonpregnant* women who present with *secondary amenorrhea*, progesterone is administered for 5 to 7 days. If menstrual bleeding occurs upon withdrawal of progesterone, it indicates the prior stimulation of the endometrium by sufficient exposure to endogenous estrogens. Sufficient (i.e., biologically relevant) amounts of estrogens are produced only in a gonadotropin-dependent manner. If menstrual bleeding does not occur upon withdrawal of progesterone, the cause of decreased estrogen action is identified by further tests.

Most contraceptive pills contain a combination of synthetic estrogen and progestin. In each 28-day cycle, tablets are taken for 21 days, followed by a 7-day pause of hormone exposure. Thus, the "menstrual" bleeding that follows is essentially a progesterone-withdrawal bleeding. This can be used as a test of uterine function.

- *Menstruation*. With the demise of the corpus luteum of the previous cycle, plasma levels of progesterone decrease and the endometrium is shed. Menstruation usually lasts 4 days (normal range: 2 to 7 days). The menstrual blood loss is approximately 30 mL; blood loss above 80 mL is abnormal.
- The *proliferative phase* is the estrogen-dependent regeneration and growth of the endometrial lining. The stimulation of estrogen receptors leads to proliferation of both stromal and epithelial cells. The epithelium regenerates from the basal portion of the endometrial glands left behind in the stratum basale during menstruation. *Both menstruation and the proliferative phase occur during the follicular phase of the ovarian cycle.*
- The *secretory phase* is the *progesterone-dependent* maturation of the endometrium that makes it suitable for the implantation of the blastocyst. The secretory phase of the endometrial cycle *coincides with the luteal phase* of the ovarian cycle. The action of progesterone requires the preparation of the endometrium by estrogens. Although 17β-estradiol is secreted together with progesterone by the corpus luteum, its continued presence is not required (Box 13-7).

The Feedback Regulation of the Hypothalamic–Pituitary–Ovarian Axis Varies with the Stage of the Ovarian Cycle The regulatory mechanisms functioning in adult males (Regulation of the Gonadotropin–Gonad Axis in Postpubertal Males) are significantly modified in adult females:

- In males, the negative feedback action of circulating androgens on gonadotropins is mainly (but not exclusively) exerted via estrogen receptors after *local* aromatization. In contrast, in females the *circulating* estrogens (primarily 17β-estradiol) secreted directly from the ovaries serve in lieu of locally converted androgens. The stimulation of androgen receptors is not involved in the physiologic regulation of gonadotropin secretion in females.
- If the plasma levels of estrogens are high for a prolonged period of time (17β-estradiol exceeds approximately 150 to 200 pg/mL for at least 36 h), they exert a *positive feedback* action on gonadotropin secretion. The positive feedback mechanism is abolished in male rats by perinatally secreted androgens, which alter hypothalamic structures via stimulating estrogen receptors. Conflicting data exist as to whether the positive feedback mechanism is operational in men. Under physiologic circumstances, it is definitely a unique female mechanism of gonadotropin regulation.
- In females, hormone secretion follows an approximately 28-day cycle dictated by the growth, differentiation, and apoptosis of ovarian steroidogenic tissues.
  - The cellular mass of the androgen-producing Leydig cell is relatively constant. In contrast, in females *the cellular mass of theca and granulosa cells,* which cooperatively secrete 17β-estradiol, significantly increases during the follicular phase of the ovarian cycle.
  - With the cyclic development of the corpus luteum, females acquire a short-lived steroidogenic organ that secretes *progesterone.* Progesterone has multiple actions in the regulation of gonadotropin secretion, contributing to both the positive and negative feedback actions. In males, its negative feedback action can be demonstrated on exposure to *exogenous* progesterone.

The normal values of the hormones involved in the pituitary-ovarian axis are listed in Table 13-7. The typical profile of the hormones of the hypothalamic–pituitary–ovarian axis during the menstrual cycle is displayed in Figs. 13-17 and 13-18. Feedback regulation during the follicular phase, the preovulatory surge of gonadotropins, and the luteal phase are shown in Fig. 13-19. The regulation during these three phases can be briefly summarized as follows.

During the early *follicular phase,* due to the decreased feedback by the degenerating corpus luteum of the previous cycle, plasma concentration of FSH is elevated. This increase is required to engage follicles in gonadotropin-dependent maturation (*recruitment*). The main regulatory mechanisms during the follicular phase are similar to those observed in males: 17β-estradiol by acting on both the pituitary and the hypothalamus, and inhibin B by acting only on the pituitary (suppression of FSH-β synthesis), exert a negative feedback on gonadotropin secretion (Fig. 13-19A). During the follicular phase, the *GnRH pulses occur at about 90-min intervals* in spite of increasing levels of estradiol. The absence of a decrease in pulse frequency is related to the lack of sufficient concentrations of progesterone and androgens. (Androgens normally do not participate in the feedback regulation

Table 13-7 Normal Values of the Hormones of the Pituitary–Ovarian Axis in Adults[a]

Compound	Follicular phase	Midcycle peak	Luteal phase
FSH (mIU/mL)	1.37–9.9	6.17–17.2	1.09–9.2
LH (mIU/mL)	1.68–15.0	21.9–56.6	0.61–16.3
Inhibin B (pg/mL)	139–403		60–220
Inhibin A (pg/mL)	10–35		30–70
17β-estradiol (ng/dL)	Early: 2.0–15.0 Late: 4.0–35.0	15.0–75.0	3.0–45.0
Progesterone (ng/dL)	15–70	—	200–2500
	Mostly Independent of Menstrual Cycle Phase		
SHBG (μg/dL)	118–797		
DHEAS[b] (μg/dL)	19–30 y: 29–781 31–50 y: 12–379		
Androstenedione[b] (ng/dL)	85–275 (shows midcycle peak)		
Testosterone[b] (ng/dL)	15–70 (shows midcycle peak)		
Dihydrotestosterone[b] (ng/dL)	4–22		
Urinary 17-ketosteroids[c] (mg/d)	6–14		

[a] Except for 17-ketosteroids, all values refer to plasma/serum.
[b] Circulating androgens are not involved in the physiologic regulation of the pituitary–ovarian axis.
[c] Mainly reflects urinary excretion of androgen metabolites.
ABBREVIATIONS: FSH, follicle-stimulating hormone; LH, luteinizing hormone; SHBG, sex hormone-binding globulin; DHEAS, dehydroepiandrosterone sulfate.

of gonadotropins in females.) The exponential increase of estradiol during the second half of the follicular phase occurs in spite of decreasing levels of FSH and very low levels of LH. The increasing plasma concentration of estradiol is explained by the proliferation of theca interna and (mainly) the granulosa cells of the *dominant follicle.* This follicle is dominant because of its increased responsiveness to FSH; thus, in spite of a decrease in FSH, its granulosa cells continue proliferating, and producing about the same amount of estradiol *per cell.* The very high levels of estradiol lead to the next stage of the cycle.

The *preovulatory surge* of gonadotropins is due to a *transient positive feedback* regulation by 17β-estradiol and progesterone (Fig. 13-19B). In spite of intensive research, the mechanism of the positive feedback is only partially understood. The preovulatory surge of gonadotropins (LH and FSH) is *initiated* by the prolonged and robust elevation of circulating 17β-estradiol (see Fig. 13-17). The rising LH induces luteinization and progesterone secretion by preovulatory granulosa cells. This progesterone secretion, which precedes the LH peak by 12 to 42 h (see Fig. 13-18), is mandatory for the development of the preovulatory LH surge with normal amplitude and duration. The normal LH surge is required for ovulation and the formation of the corpus luteum. The antiprogestin *mifepristone* (RU486) disrupts the generation of a normal preovulatory LH surge and prevents ovulation by inhibiting the positive feedback effect of progesterone.

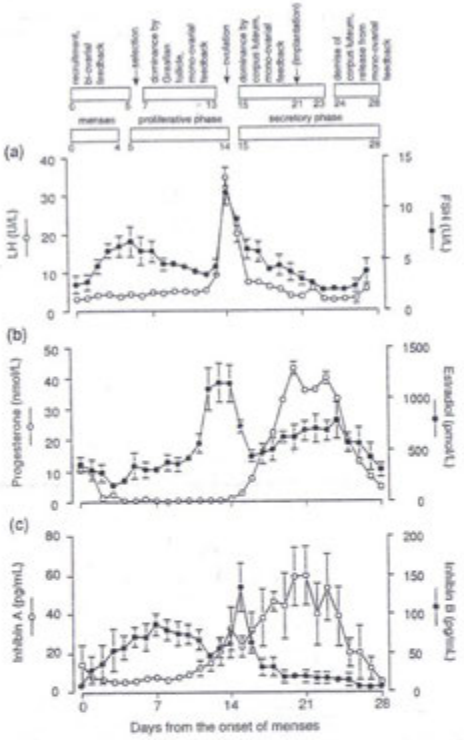


Figure 13-17. Plasma concentrations of pituitary and gonadal hormones during the menstrual cycle. Peak levels of biologically active inhibin coincide with the peak of inhibin A. (*Source:* Modified from Fig. 1, p 155 in Buffet NC et al: Regulation of the human menstrual cycle. Frontiers Neuroendocrinol 19:151–186, 1998.)

- The positive feedback by 17β-estradiol targets both the pituitary and the hypothalamus.
- The pituitary responsiveness to GnRH is increased mainly because of estradiol-induced expression of GnRH receptors. Administration of GnRH *at constant pulse frequency* to GnRH-deficient women (such as in Kallmann's syndrome) elicits normal ovulation and may result in pregnancy. This finding suggests that the primary site of positive feedback is the pituitary gland. Note, however, that the exogenous dose of GnRH may achieve concentrations corresponding to peak levels of high-amplitude GnRH pulses.

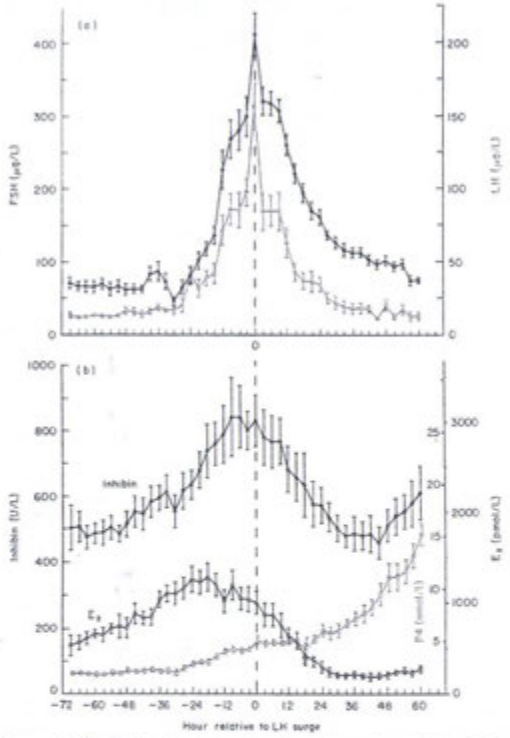
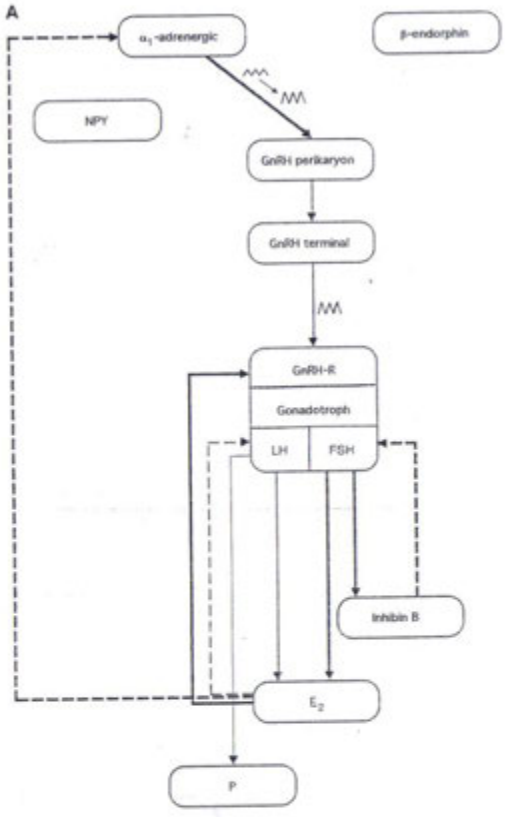


Figure 13-18. Midcycle dynamics of gonadotropin regulation. Data points were collected at 3-hour intervals; the timing of the LH/FSH peak is designated as zero. Note that the increase of LH starts about 27 hours prior to attaining peak levels (−27 h). The critical estradiol (E_2) threshold of 150 pg/mL (550 pmol/L) was reached at −69 h (i.e., 42 h before the initial rise of LH). Progesterone (P4) started to rise between −27 and −24 h in response to an initial LH stimulus. The rise in progesterone is critical in the development of the full-blown surge of gonadotropins. (*Source:* From MacLachlan RI et al: Serum inhibin levels during the periovulatory interval in normal women: Relationship with sex steroid and gonadotropin levels. Clin Endocrinol 32:44, 1990.)

- During the follicular phase, the GnRH pulse frequency does not display major changes. Each GnRH pulse is preceded by a burst of electrical activity of the GnRH neurons. During the estrogen-induced positive feedback, a *GnRH surge* is observed, which includes an *increased GnRH pulse frequency in spite of the electrical silence of the GnRH neurons*. The apparent discrepancy might be resolved by a NPY-mediated action. High levels of estrogens stimulate the secretion and pulse frequency of *NPY* via mechanisms



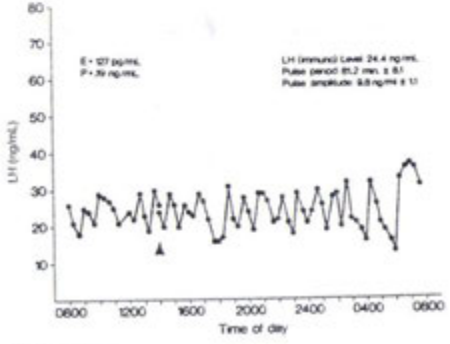


Figure 13-19.

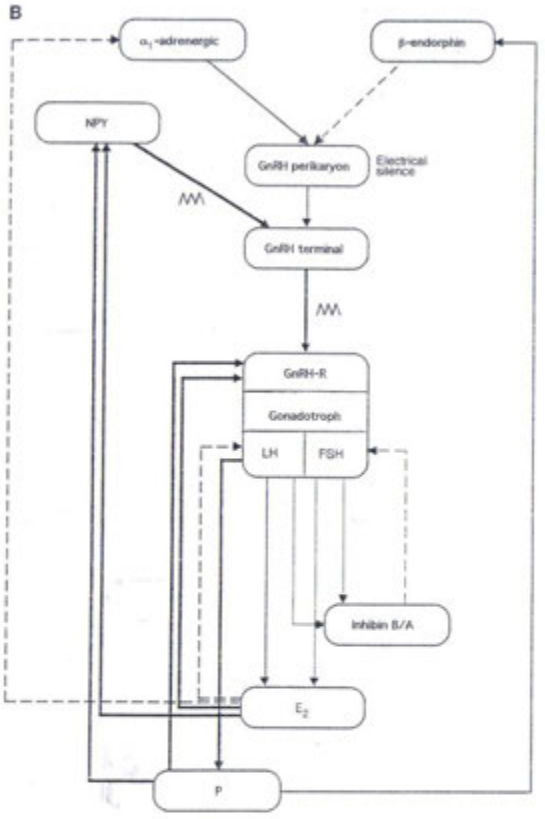


Figure 13-19. (*Continued*) Regulation of the GnRH–gonadotropin–ovarian axis during the menstrual cycle. Solid arrows indicate stimulation/increase; dashed arrows indicate inhibition/decrease. The thickness of the arrows indicates the relative importance of the mechanism. A, Midfollicular phase. B, Preovulatory surge of gonadotropins. C, Midluteal phase. Compare with the dynamic changes of plasma hormone levels shown in Fig. 13-17. Compare with the regulatory mechanisms in postpubertal men (Fig. 13-11). Secretion of estradiol involves the cooperation of theca interna and granulosa cells (see Fig. 13-4). For normal regulation, the concentration of sex hormone-binding globulin (SHBG) must not be elevated (not shown). The insets display secretory patterns of LH during the late follicular phase (panel A), and the same day of the follicular phase after administration of exogenous progesterone for 8 days (panel C). The latter condition induces an LH secretory pattern normally seen during the luteal phase. GnRH, gonadotropin-releasing hormone; GnRH-R, GnRH receptor; LH, lutreinizing hormone; FSH, follicle stimulating hormone; E_2, estradiol; P, progesterone; NPY, neuropeptide Y. (*Source:* Insets from Soules et al: Progesterone modulation of pulsatile lutreinizing hormone secretion in normal women. J Clin Endocrinol Metab 58:378–383, 1984.)

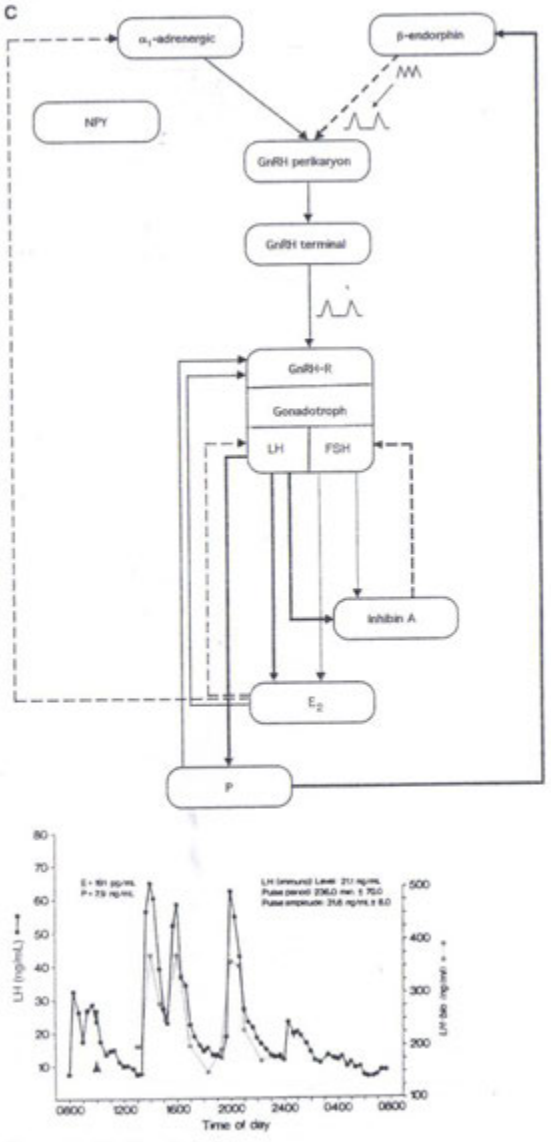


Figure 13-19. *(Continued)*

involving increased nitric oxide production and decreased opioid input. NPY acting on Y1 receptors *in the median eminence* provokes the release of GnRH from nerve terminals without evoking electrical activity of GnRH perikarya.

- The positive feedback by progesterone also involves dual targets.
  - After the priming action of estradiol, progesterone enhances pituitary sensitivity to the action of GnRH.
  - Progesterone increases the pulse frequency of NPY secretion after the priming action of estradiol. The coupling between the pulses of NPY and GnRH is maintained under the influence of progesterone.
- The termination of the preovulatory gonadotropin surge is poorly understood. The surge of pituitary gonadotropins is terminated well before the GnRH surge subsides. This may imply pituitary desensitization during the robust surge of GnRH. The declining LH and FSH, and the follicular events, result in decreased estradiol secretion, which eliminates an important signal of the positive feedback.

The regulation of gonadotropins during the *luteal phase* is similar to that of males, except that the role of androgenic action is replaced by progesterone, and that progesterone production is terminated by the demise of the corpus luteum (see Fig. 13-19C). Progesterone and 17β-estradiol acting on both the pituitary and the hypothalamus, and inhibin A acting only on the pituitary, exert a strong negative feedback on gonadotropin secretion. *GnRH/LH pulses occur at about 2- to 4-h intervals during the early luteal phase, then progressively slow down to 4- to 6-h intervals during the midluteal phase and 8- to 12-h intervals by the late luteal phase.* This pattern demonstrates the preeminence of progesterone's long-lasting inhibitory effect on the GnRH pulse generator. The potent negative feedback via decreased pulse frequency assures very low levels of LH, which leads to *luteolysis* (the demise of the corpus luteum), unless the LH receptors of the corpus luteum are stimulated by hCG, i.e., pregnancy occurs. Unlike pituitary gonadotropins, the secretion of hCG is not suppressed by ovarian steroids. In the absence of hCG, luteolysis terminates the negative feedback action on the hypothalamus and the pituitary, and a new cycle begins. This is heralded by menstrual bleeding, which is due to the decrease of progesterone secretion by the degenerating corpus luteum.

From the above description it is clear that the regulation involves events in the hypothalamo-hypophyseal system and in the ovaries. A crucial element is the dynamic coordination of these two poles of the axis. In the next section, we discuss ovarian regulatory mechanisms in more detail.

Oogenesis During Embryonic Life Generates a Nonreplenishable Pool of Primary Oocytes that Are Arrested in the Prophase of the First Meiotic Division up to 50 Years Unlike spermatocytogenesis, *oogenesis* (the mitotic proliferation of oogonia) occurs only prenatally and is completed by

the 7th gestational month (*ovum*, egg [Latin]). *Oogonia*, which are derived from the *yolk sac* about 24 days after conception, eventually generate *primary oocytes* that *enter meiosis during embryonic life* and *become arrested at the diplotene stage* of the first meiotic division for up to 12 to 50 years, i.e., the range between the average ages of *menarche* and *menopause*. The completion of meiosis and the maturation of the oocyte takes place as a component of *gonadotropin-dependent follicular maturation* (see below). Follicular maturation is classified into a *gonadotropin-independent phase* that occurs continuously from embryonic life until menopause and *gonadotropin-dependent phase* that commences with puberty.

The Gonadotropin-Independent Phase Involves All Maturation Stages up to Early Antral Follicles Having a Diameter of up to 8 mm (Usually 0.5 to 2 mm) As mentioned earlier (The Biosynthesis, Mechanism of Action and Metabolism of Sexual Steroids), the prepubertal ovaries secrete very low quantities of sex steroids, which are insufficient to bring about the development of secondary sexual characteristics. Due to prepubertally enhanced hypothalamic responsiveness to sex steroids, the low concentrations are nevertheless sufficient to suppress pituitary gonadotropin secretion. This basal, gonadotropin-independent ovarian steroid synthesis and secretion requires the generation of steroidogenic theca interna and granulosa cells. Reaching the antral stage by a gonadotropin-independent follicular development assures that the cell types as well as the cell mass meet the requirements of gonadotropin-suppressing steroidogenesis.

The gonadotropin-independent maturation starts in utero as soon as the ovarian follicles are formed, and continues until the depletion of the ovarian follicular pool at menopause. After an initial rapid atresia, which decreases the number of primordial ovarian follicles from about 5–7 million to 2 million between the 7th gestational month and birth, the ovarian follicles are depleted with a predictable *half-life of about 5 to 6 years. The rate of depletion is irrespective of the reproductive stage and/or the endocrine milieu such as prepubertal life, pregnancy, or hormonal contraceptives.*

Follicular maturation is initiated by unknown local factors. The gonadotropin-independent maturation is *noncyclic*, i.e., the follicles are continuously engaged in maturation *in both ovaries*. The gonadotropin-independent growth and maturation of follicles is slow and takes approximately 250 days. At that stage, the follicles either undergo *atresia* (apoptosis), or (after puberty) some may be rescued by pituitary FSH and enter the gonadotropin-dependent phase (Fig. 13-20).

- Preantral follicles develop from primordial follicles over the course of about 180 days, and represent the last stage of maturation at which follicles do not undergo apoptosis.
- The first stage in the development of tertiary follicles is the last gonadotropin-independent process. This is known as the *phase of slow*

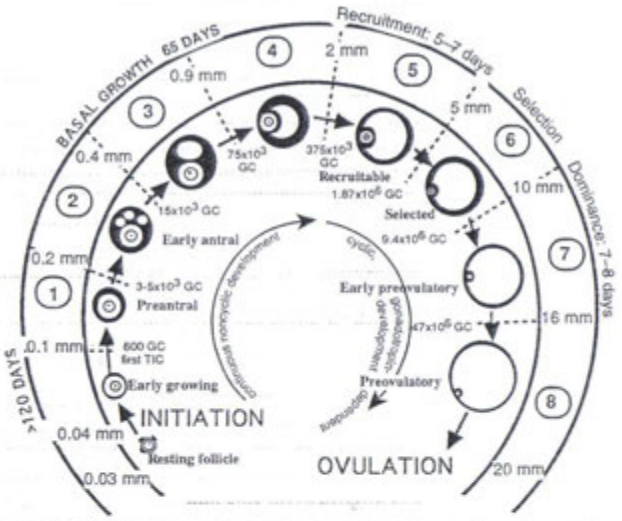


Figure 13-20. The scheme of gonadotropin-independent and gonadotropin-dependent follicular growth. The numbers in circles refer to "classes" of follicles not detailed in this text. Before puberty, follicular growth may proceed to recruitable follicles that, in the absence of an FSH stimulus, may reach sizes up to 8 mm in diameter as detected by ultrasonography. TIC, theca interna cell; GC, granulosa cell. (*Source:* Modified from Fig. 3 in Gougeon A: Regulation of ovarian follicular development in primates: Facts and hypotheses. Endocrine Rev 17:121–155, 1996.)

(basal) growth: the early antral follicles usually grow from about 200 μm to 2 mm (maximum, 8 mm) in diameter *over the course of about 65 to 70 days*. After puberty, about 35% of apoptosis (atresia) occurs at this stage of development. *Before puberty, this is the last stage of development and all follicles become atretic.*

The regulation of the gonadotropin-independent phase of follicular growth involves the *WT1* transcription factor and *GDF-9*.

- The *WT1* gene found on chromosome 11p13 (whose mutation is a cause of Wilms' tumor) encodes a zinc finger protein with transcriptional *repressor* activity on several growth factor and growth factor receptor genes, including the inhibin α subunit. Ovarian WT1 mRNA is expressed exclusively in the follicular epithelium of primordial follicles and the granulosa cells of primary and secondary follicles. WT1 mRNA levels decrease during follicle growth. *WT1 may inhibit the initiation of follicular growth.*
- GDFs are members of the TGF-β family (see Box 8-1). Follicular development is arrested at the stage of primary follicles in GDF-9 knockout mice, indicating a *mandatory role of GDF-9 in follicular development be-*

yond the primary stage. GDF-9 has been localized in the oocyte of primary, secondary, and preantral follicles. Growth of preantral follicles is enhanced by treatment with GDF-9 in vitro.

Several growth factors as well as their receptors are expressed by primary follicles, but not by the quiescent primordial follicles. These growth factors, such as IGF-1, IGF-2, EGF, and the closely related TGF-α may exert paracrine/autocrine effects and promote follicular growth.

Gonadotropin-Dependent Follicular Maturation Involves the FSH-Mediated Recruitment of a Cohort of Early Antral Follicles and the Selection of a Dominant Follicle, Which in Turn Causes Apoptosis of the Other Follicles in the Cohort by Feedback Suppression of FSH The *gonadotropin-dependent phase* starts with the second phase in the development of tertiary follicles, includes all subsequent maturation stages of follicular development, and ends with the involution of the corpus luteum. Thus, whereas the gonadotropin-independent phase required about 250 days, the gonadotropin-dependent growth and maturation is accomplished within a single ovarian cycle.

The gonadotropin-dependent phase occurs starting with puberty: it is *cyclic* and initially involves a *cohort* of FSH-dependent/FSH-recruited follicles from *both ovaries* during each menstrual cycle. A single follicle selected from either one of the two ovaries establishes *dominance* during the cycle and proceeds to ovulation. Between menarche and menopause, approximately 350 to 400 follicles mature to *Graafian follicles* and expel their oocytes in the process of ovulation. With *menopause*, the nonrenewable pool of primordial follicles becomes exhausted. Thus, nearly all of the 5 to 7 million follicles die in the apoptotic process of follicular atresia. The development of tertiary follicles has three distinct stages.

- As mentioned, the phase of slow growth is the last gonadotropin-independent phase. Although the granulosa cells at this stage express receptors for FSH, the follicles grow up to 8 mm in diameter even in the absence of FSH. However, their rescue from apoptosis is dependent on the presence of FSH.
- *Recruitment is the FSH-mediated rescue of a quasi-synchronous group of 1 to 15 follicles known as the cohort. Recruitment begins on the first day of the menstrual cycle (onset of menses) when FSH levels are elevated, and is completed by day 5 to 7 of the same cycle*. The follicles of the recruited (rescued) cohort are present in both ovaries, and there is no difference in the estradiol production by the two gonads. Recruitment means that the follicles have become sensitive to the action of gonadotropins, and their need to be rescued implies that they are *gonadotropin-dependent*. If FSH levels are low at the time when the follicle reaches this stage of development, it undergoes apoptosis. About 50% of atresia occurs at this stage of development in postpubertal women.

- *Selection* is the process whereby a single follicle of the recruited cohort *becomes dominant*, destined to ovulate and form the corpus luteum. Selection usually occurs at a follicular size of 2 to 5 mm. Typically the largest, fastest growing follicle is selected and dominates, probably because it has the highest level of FSH receptor expression. Experimental ablation of the largest follicle during the stage of recruitment has no impact on the cycle because another follicle can take over and reach dominance. However, once the selection has occurred and dominance is established, ablation of the largest (dominant) follicle prevents ovulation until the cycle is repeated.
- The dominant follicle is also known as *preovulatory* or *Graafian follicle*. *Dominance* is defined as the period of growth of a single follicle from its selection until its ovulation. This phase corresponds with days 7 to 14 of the menstrual cycle, during which the dominant follicle grows from about 5–8 to 20–22 mm in diameter. The granulosa cell population grows over 125-fold in an exponential manner, which (together with the growth of the theca interna) explains the rapid rise of 17β-estradiol in plasma (see Fig. 13-17). Once dominance is established, the estradiol secretion from the two ovaries becomes asymmetrical: *only the ovary containing the dominant follicle secretes significant amounts of estradiol and inhibin*. This also means that the negative feedback regulation of gonadotropin secretion that was accomplished jointly by the two ovaries during the first 7 days of the menstrual cycle is replaced by a *mono-ovarial* (*monofollicular*) *feedback*. The mono-ovarial feedback by estradiol includes both negative and positive feedback actions on pituitary gonadotropins. The negative feedback exerted by estradiol and inhibin from the dominant follicle leads to atresia of the other follicles in the cohort because the other follicles have lower expression levels of FSH receptors than the dominant follicle (see the concept of spare receptors in Chap. 5). This also means that the expression of aromatase in nondominant follicles declines, and the increasing concentrations of nonconverted local androgens induce atresia. The mechanism assures not only monofollicular feedback but also monofollicular ovulation (Box 13-8).

Gonadotropin-Dependent Follicular Maturation and Ovulation Results in the Cyclic Formation and Demise of the Corpus Luteum As discussed, about 36 to 48 hours after the onset of the preovulatory LH surge, the prolonged stimulation of LH receptors causes several events:

- The primary oocyte resumes and completes the first meiotic division. The function of LH is to suspend the mechanism responsible for meiotic arrest. *Kit* is a protooncogene receptor tyrosine kinase (RTK) expressed by oocytes. Two alternatively spliced *kit ligand* transcripts encoding 248- and 220-amino acid membrane-associated kit ligand proteins have been demonstrated in ovarian tissue and granulosa cells. The ligand-induced stimulation of kit (due to the interaction between the oocyte and the follicular epithelium) is responsible for meiotic arrest. LH suppresses the expression of *kit ligand* by granulosa cells.
- The LH surge halts the further proliferation of granulosa cells.
- The granulosa and theca interna cells become luteinized, which

BOX 13-8 Dizygotic Twinning

Heredity, older maternal age, and increased parity are associated with dizygotic twinning. In general, the cause of dizygotic twinning is that supranormal FSH prevents the apoptosis of more than one follicle. In hereditary dizygotic twinning, FSH levels are elevated because of the primary increase of stimulating mechanisms that regulate pituitary gonadotropin secretion. In most nonhereditary conditions, such as recovery from hypothalamic amenorrhea and older age, excessive FSH secretion occurs as a result of diminished ovarian feedback. Thus, dominance is shared by two (or sometimes more) follicles, and the two dominant follicles together provide sufficient suppression of FSH to cause apoptosis of the other rescued follicles in the cohort. This mechanism is operational in species normally giving birth to multiple sets of twins. In clinical practice, supraphysiologic doses of exogenous FSH are utilized for *induced ovulation* with the intent of collecting multiple ova for *in vitro fertilization*.

leads to increased secretion of progesterone mainly by the granulosa lutein cells (see Figs. 13-4 and 13-18). This involves an increase in cytoplasmic cyclic AMP concentration exceeding those achieved by the stimulation of FSH receptors.

- The Graafian follicle ruptures involving inflammatory mechanisms and leads to ovulation. The LH surge induces cyclooxygenase-2 (COX-2). In response to the preovulatory surge of gonadotropins, the granulosa cells secrete *tissue plasminogen activator* (tPA) into the follicular fluid, which activates plasminogen. The resulting plasmin may activate latent *matrix metalloproteinases* (MMPs). These enzymes degrade the perifollicular matrix and break down the collagen fibers, which provide the strength to the follicular wall. The increased synthesis of the protease inhibitors *plasminogen activator inhibitor-1* (PAI-1) and *tissue inhibitor of metalloproteinases-1* (TIMP-1) in the *theca* of growing follicles protects them from enzymes diffusing from neighboring ovulatory follicles.

The rupture of the follicle leads to the formation of the *corpus hemorrhagicum*. Marcophages and mesenchymal cells invade the corpus hemorrhagicum, which leads to rapid vascularization of the entire structure, including the formerly avascular granulosa cells. The *neovascularization* requires vascular endothelial growth factor (VEGF) mainly produced by luteinized granulosa and theca cells in an LH-dependent manner. The fenestrated endothelium makes the already luteinized granulosa cells accessible to low density lipoprotein (LDL) cholesterol as the precursor of pregnenolone synthesis. At this time, the luteinization of granulosa cells becomes complete: the *granulosa lutein* cells give the bulk of the *corpus luteum* (yellow body). Unlike granulosa cells, granulosa lutein cells express the P450scc (see The Biosynthesis, Mechanism of Action and Metabolism of Sexual Steroids), which is indicated by the change of mitochondrial

morphology from shelflike to tubulovesicular cristae. The theca interna cells differentiate into lipid droplet-rich *theca lutein* cells that form a narrow layer on the lobulated surface of the corpus luteum (see Fig. 13-3).

As discussed, the steroid hormones of the corpus luteum suppress the secretion of LH and FSH by inhibiting the GnRH pulse generator and the mainly LH-driven inhibin A suppresses the synthesis of FSH-β in the pituitary gonadotrophs. The low levels of LH result in the demise of the corpus luteum in 14 days. This event removes suppression mainly from FSH secretion. The increased FSH leads to recruitment of a cohort from the pool of follicles that is continuously maturing in a gonadotropin-independent manner, and the cycle is repeated.

The Ovaries Contribute to Circulating Androgens In women, androgens are not involved in the physiologic regulation of gonadotropin secretion. However, they are important in the development of sexual hair and body odor during puberty (underarm and pubic hair, apocrine sweat glands) and contribute to the maintenance of libido.

As discussed, the adrenal cortex is the main source of androgens and their degradation products (such as the 17-ketosteroids) in women. The significance of the ovarian contribution to the pool of circulating androgens is exemplified by the scanty development of sexual hair in Turner's syndrome patients, whose streak gonads are in essence hormonally inactive.

The source of ovarian androgens is primarily the *theca interna* cell population of developing *antral* follicles (Box 13-9). The contribution of hilar cells varies but is usually negligible. Because the theca interna cells produce these androgens as precursors of estrogen secretion, it is understandable that ovarian androgen secretion follows a pattern that parallels the profile of estradiol during the menstrual cycle. In contrast, adrenal androgen secretion shows no relationship with the menstrual cycle, but displays a circadian rhythm dictated by ACTH.

Due to the preferentially Δ^4 pathway, the ovaries contribute minimally to circulating DHEA and DHEAS. This is indicated by the lack of menstrual cycle-dependent changes in circulating DHEA and DHEAS. Androstenedione (a weak androgen) has the highest production rate among ovarian androgens. However, in terms of normal androgenic *action*, only testosterone and dihydrotestosterone are relevant. Under physiologic conditions, up to 300 μg testosterone is produced daily. On the average, 50 to 70% of the testosterone is generated by *peripheral conversion* of androstenedione by 17β-hydroxysteroid dehydrogenases. Because the adrenal gland has negligible ability to synthesize testosterone, the rest must be produced directly by the ovaries. The absolute concentration of androstenedione and testosterone, and the relative contribution of the ovary to both circulating androgens is increased during the preovulatory LH surge.

At the same *total* plasma concentration of testosterone, the concentration of *free* testosterone is lower in women than in men because of the

BOX 13-9 Polycystic Ovary Syndrome and Stromal Hyperthecosis

In *polycystic ovary syndrome* (PCOS also known as *Stein-Leventhal syndrome*), follicular development does not result in ovulation and formation of the corpus luteum. Instead, the hyperplastic theca interna cells of the aberrant cystic follicles (*follicular hyperthecosis*) produce overt quantities of androgens. The supranormal androgen secretion exerts a negative feedback on the hypothalamic–pituitary–gonad axis, and suspends the ovarian and menstrual cycle. In addition, male-type distribution of hair growth develops (hirsutism). Amenorrhea and hirsutism are therefore the leading features of this condition. As mentioned, PCOS patients are insulin-resistant and at least 40% present with obesity. The compensatory increase of insulin may normalize plasma glucose concentration. However, stimulation of insulin and IGF-1 receptors of theca interna cells increases androgen production mainly by inducing the expression of P450c17 and its 17,20-lyase activity.

In *stromal hyperthecosis*, the ovarian cortical stromal cells assume the morphology and function of theca interna cells. The condition, which results in the uniform enlargement of the ovaries and hypersecretion of androgens, is typically seen either in postmenopausal women or as a part of the presentation of PCOS.

higher plasma levels of SHBG (see Fig. 13-5). SHBG limits the availability of testosterone to target tissues, which may express 5α-reductase and produce DHT. A shift in the estrogen:androgen ratio toward androgens may cause a decrease of SHBG and increased biologic availability of testosterone. The increased androgenic activity may have mild clinical manifestations (hair growth, acne) before a clear elevation of total testosterone is observed.

Hormonal Contraception Prevents Ovulation by Suppressing Gonadotropin Secretion Most orally active hormonal contraceptives contain a combination of synthetic estrogen and progestin. All preparations provide negative feedback suppression of gonadotropin secretion by an exogenous source. *The exogenous source of ovarian hormones fulfills the role of the dominant follicle, except that the preovulatory rise of estrogen is omitted.* The exogenous estrogens do not reach the levels necessary to provoke positive feedback, and prevent the rise of endogenous estrogen production by suppressing follicular growth via inhibiting FSH. Since the introduction of the first oral contraceptives, the dose of estrogen has been reduced to avoid severe side effects, such as thromboembolism and liver tumors (Table 13-8). The lower doses, however, occasionally prove insufficient to take over the role of the dominant follicle in providing the negative feedback

to FSH. The efficacy of ovulation prevention is lower during first cycle of contraceptive pills because the recruitment may have occurred during the luteolysis of the previous (spontaneous cycle). To imitate the normal cycle, the pills are administered for 21 days followed by either a 7-day break or 7 days of hormonally inactive tablets. A fixed dose of both agents is used in *monophasic preparations*. *Sequential preparations* are designed to mimic the estrogen and progesterone pattern of the normal menstrual cycle. *Biphasic preparations* contain a fixed dose of estrogen, and after 10 days of a lower dose of progestin, the progestin dose is increased for the next 11 days to mimic the luteal phase. *Triphasic preparations* essentially follow the same concept with more graded doses of steroids. The progestin content of the pills also contributes to contraception by altering cervical mucus, making it less permeable for spermatozoa.

Progestins alone may also be used as oral contraceptives. However, in the absence of the negative feedback provided by estrogens, progestins are less effective in suppressing the GnRH pulse generator. Thus, progestin-only "minipills" are less effective in preventing pregnancy. However, unlike estrogen-containing pills, which suppress established milk production (*galactopoiesis*, see Functional Development of the Breast), these low-dose progestin pills are compatible with lactation and may be prescribed to breastfeeding women.

The observations with oral contraceptives indicate that steroid-mediated feedback is sufficient for suppressing pituitary gonadotropin secretion and preventing ovulation. Thus, the feedback provided by inhibin appears to be a redundant mechanism.

Hormonal contraception can be achieved with subcutaneous implants of superactive *agonists of GnRH*. This approach is similar to the treatment of prostate cancer and relies on the extensive downregulation and desensitization of the GnRH receptors.

Ovulation May Be Induced by Estrogen Antagonists, Pulsatile Administration of GnRH, or Sequential Administration of FSH and LH *Ovulation induction* is used in the treatment of infertility. The common problem associated with ovulation induction is that the precise control of endogenous mechanisms is not matched by these exogenous hormonal interventions. This often leads to multiple ovulations and twin pregnancies.

Clomiphene citrate is an estrogen receptor antagonist that *prevents the negative feedback action* of endogenous estrogens, thereby inducing gonadotropin secretion. The action of clomiphene citrate requires an intact hypothalamic–pituitary unit. In conditions such as Kallmann's syndrome, idiopathic GnRH deficiency or posthypophysectomy states alternative means must be utilized.

The pulsatile administration of GnRH does not require an intact hypothalamic GnRH, only an intact pituitary gonadotroph cell population. This method, however, necessitates specialized equipment.

Table 13-8 The Physiologic Actions of Estrogens and Progesterone in Postpubertal Cycling Women*

Target	Estrogens	Progesterone
Fallopian tube	Increases the numbers of ciliated cells in the oviduct	After an initial upregulation of ciliary bearing activity, decreases the numbers of ciliated cells
Endometrium	Regeneration and proliferation of the endometrium	Inhibition of endometrial proliferation and induction of maturation to secretory endometrium
Uterine cervix	Increased production of low-viscosity cervical mucus with high content of hydrated hyaluronic acid	Decreased production of cervical mucus; viscosity of the mucus increases
Vagina	Increased proliferation, thickness, glycogen accumulation, and cornification of the epithelium; increased numbers of surface cells in vaginal smears	Increased contribution of polymorphonuclear leukocytes and parabasal epithelial cells in vaginal smears
Breast	Maintenance of connective/adipose tissue and turgor; minimal cyclic development of mammary ductal epithelium	Minimal cyclic development of alveolar epithelium
Bones	Prevention of osteoporosis (see Table 8-7)	?
Brain	Prevention of Alzheimer's disease	Increases body temperature by a hypothalamic action; increased ventilatory response to CO_2
Liver/metabolism	Increased production of vitamin K-dependent clotting factors (II, VII, IX, X) Increased glycosylation (and synthesis?) of SHGB, TBG, and CBG leading to increased plasma levels of carrier proteins Increases HDL, decreases LDL, with slight decrease of total cholesterol; slightly increases triglycerides	Mainly glucocorticoid-like effects: induces insulin resistance leading to increased insulin secretion
Electrolytes	Enhances movement of ECF from the intravascular to the interstitial compartment thereby activating the renin mechanism and leading to fluid retention	Decreased Na^+-retention by antagonizing aldosterone on the mineralocorticoid receptor (becomes prominent in pregnancy)

Table 13-8 (*Continued*)

Target	Estrogens	Progesterone
Smooth muscle	Sensitizes uterine smooth muscle to the action of oxytocin (relevant in pregnancy)	Decreases the contractility of smooth muscles in general: may cause constipation (GI smooth muscle), decreased uterine contractility, and contributes to the development of venous varicosities by vascular smooth muscle relaxation (mainly during pregnancy)
Pituitary/hypothalamus	Direct pituitary action: increased PRL gene expression; induces lactotroph hyperplasia Via the hypothalamus: increased synthesis and secretion of PRL in part by inhibiting the tuberoinfundibular dopaminergic activity	Upregulation of GnRH receptors in estrogen-primed pituitary Coupling the pulsatile release of NPY with that of GnRH during the preovulatory surge of gonadotropins; luteal phase, inhibits the pulse frequency of the GnRH pulse generator via endogenous opioid mechanism
Skin, sebaceous gland	Looser consistency of sebum, decreased formation of acne	—

* The regulation of gonadotropins is not included in detail.

ABBREVIATIONS: SHBG, sex hormone-binding globulin; TBG, thyroxine-binding globulin; CBG, cortisol-binding globulin; HDL, high density lipoprotein; LDL, low density lipoprotein; ECF, extracellular fluid; GI, gastrointestinal; PRL, prolactin; NPY, neuropeptide Y; GnRH, gonadotropin releasing hormone.

The gonadotropins used for ovulation induction are HMG (menotropin) and hCG, which provide the FSH and the LH-like activities, respectively. These hormones need not be administered in a pulsatile manner to induce ovulation. Menotropin-induced follicular development may be monitored by ultrasonography. Ovulation is usually induced with hCG when the lead Graafian follicle reaches the diameter of at least 16 to 18 mm but less than 20 to 22 mm.

The Menstrual Cycle May Be Entrained by Pheromonal Signals

Pheromones are signaling molecules that communicate information *between individuals*. In mammals, most pheromones are odorous substances emitted by *apocrine sweat glands* of an individual (mainly the underarm, mammary, and perianal glands in humans), and provoke behavioral and/or physiologic changes in other individuals via a specialized olfactory epithelium known as the *vomeronasal organ*. As discussed related to the development of GnRH neurons, the olfactory epithelium (including the vomeronasal organ)

is directly connected to the anterior hypothalamus and the limbic system, an area of the brain involved with emotions and sexual function.

Human studies indicate the existence of a functioning vomeronasal organ–hypothalamic connection that regulates the gonadotropin–gonad axis in postpubertal women. Body odor collected on underarm cotton pads was wiped on the upper lip of recipient women for 6 h per day. When the donor was in her follicular phase, the follicular phase of the recipients became shorter and ovulated earlier. Axillary odors of women on the day of ovulation and the next 2 days delayed ovulation of recipients in their follicular phase. As mentioned, the function of the apocrine sweat glands, and presumably pheromone secretion, is androgen-dependent. The cyclic nature of ovarian androgen production may explain the phase-dependent pheromone composition of underarm sweat. Such phase-advancing and phase-delaying effects of pheromones may explain the *menstrual synchrony* experienced by women living in close relationship, such as roommates or members of a sports team.

The Effects of Ovarian Hormones During the Menstrual Cycle The systemic effects of ovarian hormones produced during the menstrual cycle are listed in Table 13-8. Estrogen and progesterone are secreted in a sequential manner during the menstrual cycle. This is important for those actions of progesterone, which are exerted only in estrogen-primed tissues. The estrogen priming includes the induction of progesterone receptors. Conversely, progesterone decreases estrogen receptors in several target tissues.

In addition to the cyclic nature of their action, ovarian steroids exert chronic effects, which on the whole are irrespective of cyclicity. Examples of this type of action include the osteoporosis-preventing action of estrogens and the maintenance of normal sexual hair by ovarian androgens.

In addition to hirsutism, androgenic hyperactivity in women often presents as seborrhea and/or acne due to overactive sebaceous glands. Estrogens exert a direct anti-acne action on the sebaceous glands by decreasing the viscosity of sebum. Some women experience acne during their menstrual bleeding. This is related to the nadir of plasma estrogen during the cycle, which leads to a peak androgen : estrogen ratio.

The Endometrial Cycle The phases of the endometrial cycle are discussed on page 514. Here we discuss selected mechanisms involved in the endometrial cycle. Abnormal menstruation often indicates endocrine dysfunction. The main types of abnormal menstrual bleedings are summarized in Table 13-9.

Menstrual bleeding is triggered by the decrease of progesterone exposure of an estrogen-primed endometrium. The bleeding is mainly caused by MMPs and prostaglandin-mediated ischemia, although additional factors such as the liberation of lysosomal proteases may play important roles.

Table 13-9 Abnormal Menstruation

Terminology	Manifestation/definition
Primary amenorrhea	Menarche (first menstrual bleeding) has never occurred and the patient is >16 years old.
Secondary amenorrhea	Absence of menstrual period after menstrual cycles have already occurred. Amenorrhea is usually diagnosed if menstruation is absent for at least 6 months.
Oligomenorrhea	Menstrual periods occur more than 35 days apart.
Polymenorrhea	Menstrual periods occur too frequently (<3 weeks apart).
Hypomenorrhea (cryptomenorrhea)	Unusually light menstrual flow.
Dysmenorrhea	Excessively painful menstruation.
Menorrhagia (hypermenorrhea)	Too heavy (>80 mL) or prolonged (>6 days) menstruation.
Metrorrhagia	Bleeding that occurs between menstrual phases within a cycle. Includes midcycle breakthrough bleeding. May be due to endometrial or cervical cancer.
Menometrorrhagia	Bleeding that occurs at irregular intervals.

- The degradation of the stromal extracellular matrix in the endometrium involves mechanisms similar to those causing follicular rupture during ovulation. Upon progesterone withdrawal, endometrial stromal cells express the mRNA of MMP-1, -2, -3, and -9, and secrete them as inactive proproteins that are subsequently activated by plasmin. Most of these MMPs are also induced by IL-1 and TNF-α, suggesting that progesterone suppresses MMP expression by suppressing locally acting cytokines. The endometrial expression of TIMP-1 and -2 (the inhibitors of MMPs) is unchanged during the menstrual cycle. Thus, decrease of progesterone induces matrix degradation by altering the balance between MMPs and their inhibitors.
- Similar to glucocorticoids, progesterone inhibits the synthesis of prostaglandins. With the luteolytic decrease of progesterone, the production of $PGF_{2\alpha}$ increases. The stratum functionale is supplied by the spiral arteries, which (unlike the straight arteries supplying the stratum basale) respond to these mechanisms with a sustained and strong vasoconstriction leading ischemia. The prostaglandins cause cramps related to smooth muscle contraction of the myometrium. These muscle contractions contribute to the expulsion of the sloughed off endometrium from the uterine cavity through the cervical canal.

The etiology of *breakthrough bleeding* is different from that of bona fide menstrual bleeding. Breakthrough bleeding occurs when the estrogen-stimulated endometrial growth yields such a thick mucosal lining that its innermost layers are not properly supplied by blood and undergo an ischemic necrosis. This mechanism implies that in the absence of progesterone (which normally stops endometrial proliferation), further growth is limited

by the blood supply. During puberty, several of the initial "menstrual" cycles involve breakthrough bleeding. Pubertal development involves a gradual decrease in the hypothalamic sensitivity to the feedback action of gonadal steroids. This results in a gradual increase in gonadotropin secretion. Thus, FSH secretion may be sufficient to recruit/rescue early antral follicles, induce their growth, and increase estrogen production, but insufficient to reach the threshold of plasma estradiol necessary for provoking the preovulatory LH surge. The consequence is the absence of both ovulation and corpus luteum formation. In the absence of progesterone, the unopposed estrogen action leads to overproliferation of the endometrium and breakthrough bleeding. Thus, these breakthrough bleedings are associated with anovulatory cycles. Some women experience "*midcycle spotting*." This is essentially a breakthrough bleeding that occurs just before ovulation due to overt estrogenization of the endometrium.

The *unopposed action of estrogens* (i.e., absence of progesterone) is a risk factor for the development of endometrial cancer. Obesity is a hyperestrogenic state due to the conversion of adrenally derived androstenedione into estrone by adipose tissue aromatase. Obesity therefore predisposes postmenopausal women to endometrial cancer. This explains the clinical practice to supplement estrogen with progestin in the prevention of postmenopausal osteoporosis.

Unlike the menstrual cycle of primates, which is characterized by shedding the endometrium upon decreased progesterone exposure, most mammals follow an *estrous cycle*. Estrus coincides with ovulation, and is characterized by sexual receptivity ("heat"). The mechanism of midcycle breakthrough bleeding is involved in the bloody vaginal discharge seen in dogs during their estrus.

The Vagina and the Uterine Cervix Under the influence of estrogens, the vaginal epithelium thickens and accumulates glycogen. This has several important consequences:

- The color of blood in the capillaries of the lamina propria shows through the thin vaginal epithelium of prepubertal girls. This red color turns pink after puberty due to the estrogen-induced epithelial thickening.
- The glycogen-rich surface cells are continuously shed, die, and release their content. This glycogen serves as the nutrient of *lactobacillus acidophilus* (also known as *Döderlein's bacilli*, which are used in active yogurt cultures), which is the normal flora of the postpubertal vagina. The activity of these bacteria turns the pH of the vagina acidic (pH 3.8 to 4.2), which protects against colonization by other bacteria.
- Vaginal cytology reveals three types of epithelial cells in postpubertal women: *parabasal cells* (small, round, thick noncornified cells with a large nucleus), *intermediate cells* (large, polyogonal cells with a relatively *large nonpyknotic nucleus*), and *superficial cells* (large, polygonal, flat, cornified cells with a *small pyknotic nucleus*). All cell types have a single nucleus.

The *maturation index* is the percentage of these cells in the vaginal smear preparation. Prepubertal and postmenopausal vaginal smears have a preponderance of parabasal cells. Normally superficial (>30 to 50%) and intermediate cells are dominant during the reproductive years. Whereas estrogens increase, progestins decrease the contribution of superficial cells. Due to other influencing factors (such as vaginal infections and personal hygiene), the maturation index is not a reliable measure of estrogen production and is now rarely used in clinical practice.

The quality and quantity of the cervical mucous plug is regulated by estradiol and progesterone and varies with the stage of the ovarian cycle.

- *Spinnbarkeit* is the elasticity of the mucus, which is examined by dropping a sample of cervical mucus on a glass slide, covering it with another slide, then stretching the mucus by lifting the upper slide until the thinning thread of mucus breaks.
- *Ferning* (arborization) refers to the microscopic pattern of mucus dried on a glass slide and is the consequence of the composition of cervical mucus (Fig. 13-21).

Estradiol promotes the secretion of copious amounts of watery mucus rich in hyaluronic acid. The production of this type of mucus peaks at peak levels of estradiol, i.e., at the time of the preovulatory surge of gonadotropins. The preovulatory midcycle mucus shows the most prominent ferning. During the follicular phase the Spinnbarkeit of mucus increases from about 2 cm (early follicular phase) to 14 cm (midcycle); the maximum Spinnbarkeit coincides with maximum ferning. This midcycle mucus is easily penetrated by spermatozoa.

Progesterone decreases the amount and increases the viscosity of the cervical mucus. The Spinnbarkeit decreases to its minimum (2 to 3 cm) within days after ovulation. The ferning pattern disappears and the mucus becomes highly cellular. This type of mucus represents a significant barrier against penetration by spematozoa. As noted, progestins present in oral contraceptives decrease fertility in part by altering the character of cervical mucus.

Premenstrual Syndrome The recurrent mood and physical disturbances associated with the luteal phase of the menstrual cycle is known as the premenstrual syndrome (PMS).

- The mood symptoms include *negative affect* (depression, irritability, emotional lability), *food cravings*, and insomnia.
- The physical symptoms include *water retention* (bloating, ankle edema) and *pain* (headaches, breast tenderness).

The severity of these symptoms shows significant individual variability. About 5 to 10% of cycling women present with moderate to severe symp-

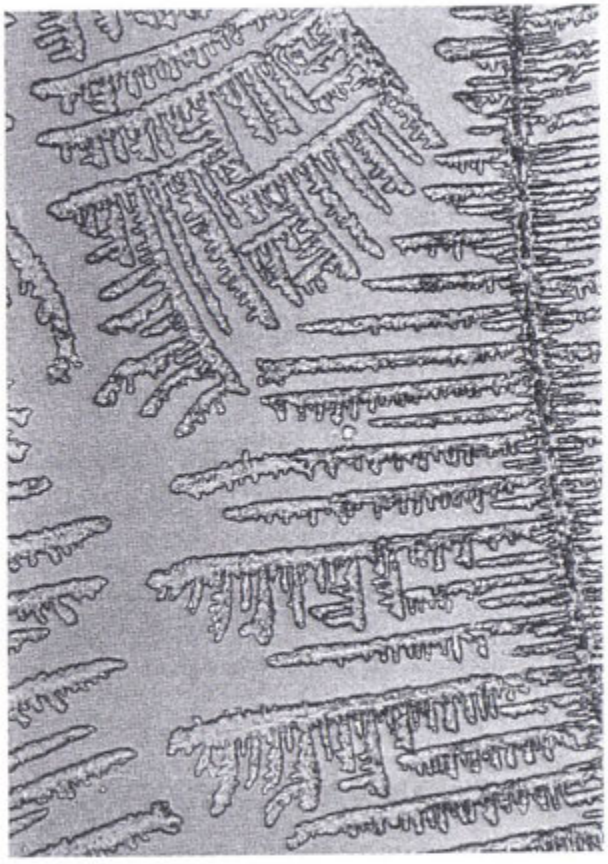


Figure 13-21. Fernlng: microscopic image of dried cervical mucus obtained from a woman on the day prior to ovulation. The extensive fernlng pattern is consistent with high Spinnbarkeit and high E_2 levels. (Source: Fig. 14-9, p. 321 in Moore WT, Eastman RC (eds): *Diagnostic Endocrinology*, 2nd ed., St. Louis, Mosby, 1996.)

toms. The underlying cause of PMS is most probably a cyclic dysregulation of calcium homeostasis. PMS has a long-term association with the development of osteopenia/osteoporosis. Women presenting with PMS have lower plasma ionized calcium levels than women without PMS. The consequence is a cycle-dependent secondary hyperparathyroidism with an immunoreactive PTH peak during midcycle. PTH does not fluctuate during the menstrual cycle in women without PMS. Changes in plasma ionized calcium are reflected in the cerebrospinal fluid (CSF), and this may result in altered neuronal activity and mood. Ionized calcium acts upon the calcium-sensing receptors (CaR) in the thick ascending limb of the loop of Henle, where it antagonizes the action of antidiuretic hormone (ADH) on sodium reabsorption. By this action, increased ionized calcium decreases the corticomedullary osmotic gradient and may cause diabetes insipidus-like polyuria. Conversely, when ionized calcium is decreased (such as in women with PMS), fluid is retained. The etiology of the deranged calcium homeostasis is uncertain. However, the involvement of ionized calcium in the pathomechanism of PMS is supported by the finding that dietary calcium (1200 mg/d) or magnesium (200 mg/d) supplementation alleviates the symptoms of PMS in most patients.

Pregnancy

OBJECTIVES

1. Discuss the female aspects of intercourse, including arousal, orgasm, and its relationship with oxytocin secretion.
2. Describe fertilization. Discuss the timecourse of events from ejaculation to fertilization. Identify the mechanisms involved in *capacitation*, the interaction between spermal *fertilization antigen-1* and *zona pellucida receptors* (ZP3), *acrosomal reaction*, and *block to polyspermy*. Discuss the fusion of pronuclei, and its relationship with *trophoblastic disease*. Identify the mechanisms underlying the immunosuppressive action of semen.
3. Discuss implantation, the involvement of *leukemia inhibiting factor* (LIF), and *epidermal growth factor* (EGF). Describe the stage of embryonic development at the time of implantation, and the interaction between the trophoblast cells and the endometrial lining. Define the difference between embryonic/fetal age (postfertilization time) and gestational weeks counted from the last menstrual period (LMP).
4. Discuss human placentation, and the structure and development of the placenta. Describe the main functions of the placenta and the types of its endocrine function including the hormones synthesized, degraded, transported, or modified. Discuss the mechanisms leading to shallow placentation and *preeclampsia*. Describe the mechanisms preventing the rejection of the placenta/fetus as an *allograft*.

5. Discuss the regulation and role of the corpus luteum maintaining pregnancy. Identify the timing of placental development when the corpus luteum becomes expendable. Discuss the central role of hCG in placental endocrine function, including its impact on maternal and fetal targets. Identify markers of luteal function and discuss *relaxin*. Discuss steroidogenesis within the framework of the *feroplacental unit*. Compare and contrast the fetoplacental unit with the function of granulosa lutein and theca lutein cells. Identify the role of the fetal adrenal in placental estrogen production, and the role of the placenta in corticosteroid production by the fetal zone of the adrenal cortex. Discuss *X-linked ichthyosis* and its relationship to placental estrogen production.
6. Discuss the causes and impact of altered endocrine function during pregnancy: the consequences of increased estrogens and progesterone, the nature of increased mineralocorticoid secretion, the Cushingoid features of pregnancy related to excess glucocorticoid production, the increased GH-like biologic activity and IGF-1 in plasma. Discuss calcium homeostasis during pregnancy. Describe the functions of the maternal pituitary gland during pregnancy: gonadotropins, PRL, GH, TSH, and ACTH. Discuss insulin during pregnancy, the concept of *accelerated starvation*, and the mechanisms potentially leading to *gestational diabetes mellitus*.
7. Discuss parturition, the *placental clock*, the role of placental corticotropin-releasing hormone (CRH) and its binding protein, prostaglandins, the *Ferguson reflex*, and oxytocin. Identify the role of estrogens and progesterone in uterine contractility. Discuss potential mechanisms of *tocolysis* to prevent premature delivery and pharmacologically induced uterine contraction to control postpartum bleeding.
8. Compare the timecourse of placental CRH secretion and uterine contractions. Define *Braxton-Hicks contractions*. Describe the changes in the uterine cervix during prelabor and labor.

Intercourse Pregnancy starts with fertilization, which usually occurs subsequent to intercourse. Upon erotic arousal, the Bartholin's glands provide lubrication to aid the intromission of the penis. The bulbus vestibuli, the glans clitoridis and the corpora cavernosa are erectile tissues that swell with blood.

Orgasm is induced by a combination of stimuli:

- Tactile stimulation of the clitoris (especially the glans), the labia minora, and the *anterior* wall of the vagina. The existence of the *Grafenberg spot* (G spot, supposedly a small circumscribed hypersensitive orgastic area of the anterior wall) is uncertain.
- Activation of stretch receptors located in the *upper* portion of the vagina.
- Stimulation of the nipple and the areola of the breasts.
- Reinforcing olfactory, visual, and auditory stimuli.

Orgasm involves the activation of the sympathetic system, which results in rhythmic contractions of the vaginal wall. In addition, 3 to 15 involuntary rhythmic contractions of the bulbospongiosus and ischiocavernosus muscles are provoked by somatic motor fibers of the pudendal nerve. These contractions of smooth and skeletal muscles are similar to those observed in males. On the average, orgasm lasts approximately 30 seconds. Unlike in males, repeated orgasms may be achieved without a refractory period. Some females experience "ejaculation" during orgasm. The composition of the ejaculate is urine-like and does not support an origin from the Skene's paraurethral glands (the female prostate-equivalent).

It is notable that the mechanical stimuli leading to orgasm coincide with the stimuli of *oxytocin* secretion (see also Parturition and Lactation). During orgasm, oxytocin is released from the posterior pituitary gland. Oxytocin may cause contraction of the uterine smooth muscle. Upon relaxation, the uterine cavity expands and the expansion generates a slight vacuum that aspirates sperm and delivers spermatozoa faster to the site of fertilization. This mechanism, however, is not essential for successful fertilization.

Fertilization At the time of ejaculation, semen is deposited in the vagina near the external orifice of the cervical canal. The rapid clotting of semen prevents its outflow. The spermatozoa migrate from the liquefying semen to the cervical mucus. This movement is directed by pH: spermatozoa avoid the acidic pH of the vagina and are attracted by the slightly alkaline (pH 8) mucus. With the aid of hyaluronidase, spermatozoa penetrate the abundant, low viscosity midphase cervical mucus. In part aided by uterine contraction-induced suction, in part by active flagellar movement, spermatozoa find their way to the ampullary portion of the Fallopian tube, where fertilization usually occurs. Spermatozoa reach the ampulla within 5 to 10 minutes after ejaculation. Of the approximately 200 to 300 $\times 10^6$ ejaculated spermatozoa, about 200 to 300 reach the vicinity of the ovum.

Spermatozoa are capable of fertilizing the egg for up to 48 to 72 h after ejaculation. The ovum (at this stage, secondary oocyte) can be fertilized for about 24 to 48 h after ovulation. The time limits of fertilization serve as the basis of the traditional albeit unreliable "calendar method" of birth control. The efficacy of this method can be improved by regular monitoring of morning body temperature, which rises by approximately 0.5 to 0.7°C as an effect of progesterone, indicating the timing of ovulation.

A single spermatozoon out of the about 200 to 300 million present in the ejaculate fertilizes the ovum. The process of fertilization involves several steps:

- The spermatozoon must reach and attach to the zona pellucida. To achieve this goal, the hyaluronidase content of the spermatozoon is needed for penetrating the mucified intercellular space of the corona radiata.
- During the penetration of the corona radiata, the final steps of *capacitation* occur. This is in part supported by a nongenomic action of

progesterone produced by the corona radiata granulosa lutein cells. In the process of capacitation, epididymal glycoproteins that have masked binding sites of plasma membrane proteins are removed from the surface of the spermatozoon.

- Upon capacitation, the spermatozoon binds to the zona pellucida. The interaction involves several receptor proteins making the spermatozoon-zona pellucida interaction relatively species specific. A crucial factor supporting this process is a 51-kDa protein receptor tyrosine kinase in the spermatozoon membrane termed *fertilization antigen-1* (FA-1), which specifically reacts with the zona pellucida receptor *ZP3*. Affinity-purified antibodies targeting FA-1 completely block the sperm-zona pellucida interaction. Because FA-1 is a unique protein (its sequence is unrelated to other known protein families) and its expression is restricted to sperm, FA-1 is a potential target of male contraceptives.
- The binding of FA-1 with ZP3 results in the activation of the tyrosine kinase domain of FA-1 leading to autophosphorylation and tyrosine phosphorylation of other proteins. These events activate voltage-gated Ca^{2+}-channels and ultimately trigger the *acrosome reaction*, which includes the release and activation of *acrosin*, a trypsin-like enzyme needed for the penetration of the zona pellucida.
- Only the head of the spermatozoon fuses with the oocyte. The mitochondrial sheath of the middle piece, the centrioles, and the flagellum are left behind. Thus, mutations of mitochondrial DNA (such as mitochondrial diabetes) are passed on to the next generation only by the mother.
- The fusion triggers the *second meiotic division* of the oocyte, which leads to the formation of the mature oocyte and the *second polar body*. Note that the polar bodies contain minimal cytoplasm due to the inequal cytokinesis of these meiotic divisions.
- Fusion by a spermatozoon head triggers a cascade of events that block *polyspermy*, i.e., prevent the fusion with multiple spermatozoa. These events include
    - *Depolarization* of the oolemma (also known as *fast block to polyspermy*).
    - *Cortical reaction*. The oocyte contains cortical granules. The cortical reaction is their exocytosis, which is provoked by the increased cytoplasmic concentration of Ca^{2+}. The Ca^{2+} influx is due to the initial depolarization of the membrane.
    - The *zona reaction* is carried out by the exocytosed cortical granule proteins. This includes the proteolytic degradation of ZP3, and the crosslinking of proteins on the surface of the zona pellucida that yields the *perivitelline barrier*.
- The male and female *pronuclei* fuse, thereby reconstituting the diploid karyotype. This normally diploid cell is the *zygote* (Box 13-10).
- The mitotic proliferation of the zygote proceeds within the confines of the zona pellucida to yield a *morula* then a *blastocyst*. During this process, the developing embryo travels on the mucous escalator of the Fallopian tube toward the site of implantation in the endometrium near the opening of the oviduct.

BOX 13-10 Hydatidiform Mole

Hydatidiform mole is a proliferative *trophoblastic disease* characterized by the transformation of the conceptus into a mass of grape-like clusters. The "grapes" are aberrant, edematous chorionic villi. In the classic form, the entire conceptus is transformed (*complete mole*). In *partial mole*, the trophoblastic proliferation is focal and fetal parts develop. Ninety percent of complete moles develop from zygotes having a *uniparental, paternally derived diploid karyotype:* the oocyte loses the female pronucleus, and the 23 paternal chromosomes of a single fertilizing spermatozoon are duplicated. In contrast, partial moles are due to an imperfect block to polyspermy: two spermatozoa fertilize the egg yielding a triploid karyotype. Molar pregnancies typically present with a uterine growth exceeding the expected size for gestational age. Due to the abundance of trophoblasts, maternal plasma and urinary hCG levels are supranormal. The moles may have locally malignant character (invasive mole) or may give rise to distant metastases by hematogenous spread (*choriocarcinoma*). This condition may also arise in postpartum women after a normal pregnancy. Similar to the choriocarcinoma of the testis, the condition is characterized by the secretion of hCG. Monitoring plasma hCG is used for the assessment of tumor mass during chemotherapy.

In general, foreign cells (such as spermatozoa and viral infected cells) are eradicated by the killing activity of T cells and natural killer (NK) cells of the immune system. This cytotoxicity is stimulated by interleukin (IL)-12 and partially inhibited by IL-10. Human semen contains high concentrations of prostaglandins, especially PGE and 19-OH PGE. Either human seminal plasma or synthetic PGE/19-OHPGE increases the IL-10/IL-12 ratio. The seminal fluid prostaglandins thus can effect a cytokine-mediated switch away from a cell-mediated immune response. This effect, mediated via the antigen-presenting *Langerhans cells* of the genital mucosa, induces a state of non-responsiveness to sperm antigens in the female reproductive tract. It has been proposed that the induction of anergy to sperm antigen is needed for maintaining the fecundity of the female during repeated exposure to sperm. Although this immune system modulation benefits fertility, the response to infective agents present in semen, especially human immunodeficiency virus (HIV), will also be diminished and may play a critical role in the pathomechanism of sexually transmitted diseases.

Implantation and the Placenta

Implantation Requires Signaling to the Trophoblast by Uterine Leukemia Inhibiting Factor (LIF) Ovulation occurs on about day 14 of the ovarian cycle followed by fertilization within 24 to 48 h. The rapidly proliferating zygote forms a *morula*, which transforms into the *blastocyst*. The *trophoblast* cells are the surface cells of the blastocyst, which eliminate

the remnants of the zona pellucida and function as the active embryonic participants of implantation process.

Implantation requires signaling between the uterine epithelium and the trophoblast cells of the embryo.

- Mutant mouse embryos, which lack the EGF receptor, fail to attach to the endometrial epithelium, indicating that the EGF receptor is necessary for producing an implantation-competent embryo.
- LIF is a crucial factor in the uterine-trophoblast interaction. Knockout mice unable to express LIF in the endometrium fail to support implantation. Their blastocysts, however, are viable and, when transferred to wild-type pseudopregnant recipients, they can implant and develop to term. Recent evidence indicates that abnormal expression of LIF, or the related cytokine IL-6 in the endometrium may underlie some forms of human infertility.

Normally, human pregnancy lasts 280 days (40 weeks) *from the last menstrual period* (LMP). On the average, the actual pregnancy (i.e., *from fertilization*) is 2 weeks shorter. In clinical practice, the point of reference is the LMP. Embryology texts typically describe the early developmental events using fertilization as the point of reference ("fertilization days").

The implantation of the embryo begins 5 to 6 days after fertilization, i.e., on about the 21st day of the cycle. This event may be accompanied by a bleeding due to the trophoblastic invasion of the endometrium, and may be mistaken for an early-onset (albeit unusually light) menstrual bleeding, especially in women with irregular cycles.

Implantation coincides with peak production of progesterone by the corpus luteum, which has prepared the appropriately decidualized secretory endometrium for implantation. Even before implantation is complete, the anatomic arrangement is established to secrete hCG (the LH-like glycoprotein hormone product of the *syncytiotrophoblast*) directly into the maternal circulation, which is the key step in maintaining the corpus luteum, progesterone secretion, the endometrial lining, and thus pregnancy. The implantation process is complete by the 11th day after fertilization. The implantation of the blastocyst eventually leads to the development of the chorion and the placenta.

The Placenta Is a Transient Multifunctional Organ Consisting of Maternal and Fetal Components The placenta is often described as the interface between maternal and fetal tissues, which functions as an *exchange organ*: it provides nutrients and oxygen to the fetus, and eliminates the byproducts of metabolism from the fetus. The placenta also functions as a *barrier*, which prevents passage of certain molecules (such as hydrophilic hormones) and blood cells between the fetal and maternal compartments (see also Chap. 4). Indeed, a crucial role of placental structure is *keeping the maternal and fetal intravascular fluid compartments separated*. The interface or barrier is primarily provided by the *trophoblast* cells that belong to the *fetal components* of the placenta (see below). The *maternal component* of the placenta is the *decidua* (decidualized endometrial stroma), which has no barrier function.

In many ways, the placenta functions as if it were an incomplete twin serving the fetus as an accessory gastrointestinal tract, lung, and kidney. The placenta has to fulfill two main additional roles:

- The placenta is a major *immunologic organ*. It is an interface between genetically distinct individuals, and as such it must prevent immunologic rejection of the fetus by the mother (see page 550). The trophoblastic barrier also prevents the transfer of most immunoglobulins, except IgG. Whereas the transfer of maternal IgGs is important in obtaining *passive immunity* against infectious agents as a preparation for adaptation to extrauterine life, it may also be harmful. Examples of the deleterious effects of IgG transfer include
  - Rh blood group-specific antibodies of an Rh-negative mother entering the circulation of the Rh-positive fetus may cause potentially fatal *erythroblastosis fetalis*.
  - TSH receptor-specific antibodies of Graves' disease mothers may cause *congenital hyperthyroidism*. The transfer of TSH receptor-blocking antibodies may cause *congenital hypothyroidism*.
- The placenta is a complex *endocrine organ* (Table 13-10) that coordinates the metabolism of the fetus and the mother, prepares the mother's body for lactation, regulates growth and several developmental processes of the conceptus, and is the main determinant of the onset of parturition. As an endocrine organ, the placenta
  - synthesizes hormones and hormone-binding proteins de novo and delivers them to the fetal and/or maternal intravascular fluid compartment;
  - produces hormones by processing precursors derived either from maternal or from fetal sources;
  - transports hormones between the maternal and fetal compartments;
  - degrades hormones, thereby altering maternal endocrine function and/or protecting the fetus from undue exposure to maternal and fetal hormones;
  - serves as a target of hormones mediating regulated transport mechanisms.

To understand these placental functions, we first briefly review the most important aspects of the development and structure of the placenta (Fig. 13-22).

Due to the invasive character of the trophoblast cells, the entire blastocyst penetrates the uterine epithelial lining and becomes encapsulated by the endometrial *stroma*. The blastocyst has an *inner cell mass* that develops into the *embryo proper*, and an outer cell mass that is the trophoblast. By

Table 13-10 The Main Endocrine Functions of the Placenta

Hormone group	Hormones synthesized de novo	Hormones produced by processing	Hormones transported through the placenta	Hormones degraded by the placenta
Steroids	Progesterone	Estriol	Progesterone, estriol, non-aromatizable androgens	Androgens by aromatase; cortisol by type II β-HSD
Thyroid hormones	—	—	T_4, T_3 (minimal but relevant transfer from mother to fetus)	T_4, T_3 by type III deiodinase
Monoamines	—	—	—	By MAO-A, MAO-B, and COMT (requires uptake–1 mechanism)
Calcium homeostasis	PTHrP	Calcitriol, 24,R,25-$(OH)_2D_3$	Vitamin D and its derivatives	Calcitriol by 24-hydroxylase
Insulin and related hormones	IGF–1, IGF–2, relaxin (by decidual cells)	—	—	Insulin by insulinase
Growth factors	EGF, TGF–α, FGF, PDGF, TGF–β, activin, inhibin, follistatin	—	—	—
Anterior pituitary related hormones	hCG, hCS (hPL), hGH–V, PRL (by decidual cells); ACTH	—	—	—
Posterior pituitary related hormones	OT	—	—	—
Hypothalamic neurohormones	CRH, CRH–BP, GnRH, TRH, GHRH, SRIF, NPY, β–endorphin	—	TRH	—
Cytokines	TNF–α, IL–1, IL–2, IL–6, IL–8, IFN–γ	—	—	—

ABBREVIATIONS: MAO, monoamine oxidase; COMT, catecho-O-methyltransferase; IGF, insulin-like growth factor; EGF, epidermal growth factor; TGF, transforming growth factor; FGF, fibroblast growth factor; PDGF, platelet-derived growth factor; hCG, human chorionic gonadotropin; hPL, human placental lactogen; hCS, chorionic somatomammotropin; hGH-V, human growth hormone variant; PRL, prolactin; ACTH, adrenocorticotropic hormone; OT, oxytocin; CRH, corticotropin-releasing hormone; CRH-BP, CRH binding protein; GnRH, gonadotropin-releasing hormone; TRH, thyrotropin-releasing hormone; GHRH, growth hormone-releasing hormone; SRIF, somatostatin; TNF, tumor necrosis factor; IL, interleukin; IFN, interferon.

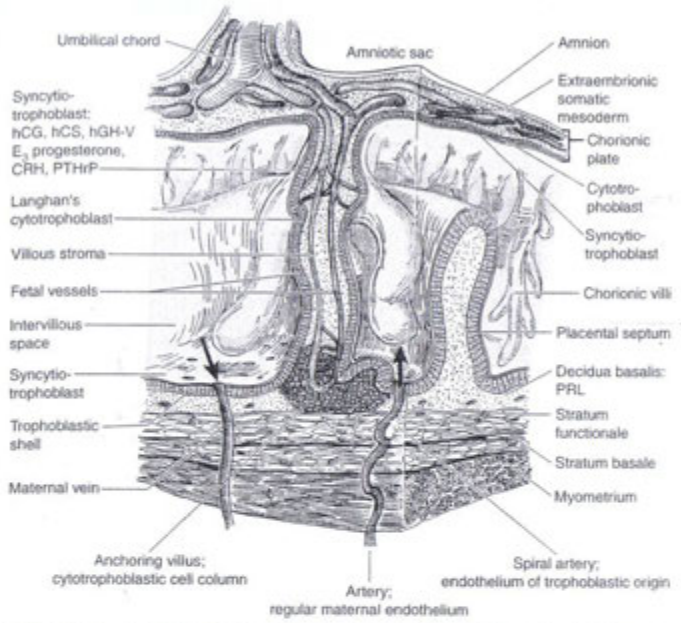


Figure 13-22. Placenta. The fetus is developing in the *amniotic sac*. Its blood vessels communicate through the *umbilical cord* to the *chorionic plate* and the *chorionic villi*. In early pregnancy, the chorionic villi are covered by two layers of trophoblast cells. The *Langhans cytotrophoblast*, which serves as a stem cell for developing the *syncytiotrophoblastic* surface layer, becomes depleted later in pregnancy. At the tip of the *anchoring villi*, the syncytiotrophoblast is absent, and the extravillous (peripheral) cytotrophoblast cells form a compact *cell column* that spreads to form the *trophoblastic shell*. These extravillous trophoblasts are in contact with the maternal decidua cells in the *decidua basalis*. The decidua basalis and the trophoblastic shell form *placental septa*, which demarcate the cotyledons (lobules) of the placenta but do not reach the chorionic plate. Modified extravillous cytotrophoblasts invade the maternal blood vessels as endothelium and intermingle with maternal endothelial cells. Maternal blood enters the intervillous spaces from *spiral arteries*, exchanges materials with feral blood through a barrier of the villi, and leaves via *maternal veins*. The villous barrier includes two layers of trophoblast cells, the trophoblast basal lamina, the villous connective tissue stroma, endothelial basal lamina and the feral endothelium. Maternal blood and decidua cells are in contact with the foreign trophoblastic tissue antigens. The trophoblast and the decidua are hormone-producing cells. (*Source:* Modified from Fig. 23-20, p 456 in Junqueira LC et al: *Basic Histology*, 6th ed., Norwalk, CT, Appleton & Lange, 1989; originally appeared in Duplessis GDT, Haegel P. *Embriologie*. Masson, 1971; English Edition Springer Verlag/Chapman & Hall/Masson, 1972.)

the 13th day of development, *extraembryonic somatic mesoderm* appears at the inner surface of the *trophoblasts*, which together form the *chorionic sac*. The trophoblasts of the chorion are in contact with the decidualized stromal cells of the endometrium. The decidua interpositioned between the implanted conceptus and the myometrium is known as the *decidua basalis*, which serves as the route of placental blood supply. The endometrium apical to the conceptus is referred to as *decidua capsularis*; the endometrium peripheral to the site of implantation is the *decidua parietalis*.

The chorionic trophoblast already has two cell populations:

- the *cytotrophoblast* is composed of mitotic individual cells. The cytotrophoblast cells are further classified as the *Langhans* (or *villous*) *cytotrophoblast* cells which form the inner trophoblastic layer of chorionic villi during early pregnancy, and *peripheral cytotrophoblast* cells that form the *trophoblastic cell columns* and the *trophoblastic shell*.
- the *syncytiotrophoblast* is a *postmitotic* surface layer derived by the fusion of the plasma membranes of cytotrophoblast cells. This multinuclear syncitium is highly differentiated with ultrastructural hallmarks of steroidogenesis, and may only grow by fusion of additional cytotrophoblast cells until the pool of cytotrophoblasts becomes depleted. The differentiation of cytotrophoblast into syncytiotrophoblast is probably induced by exposure to relatively high oxygen tension.

The syncytiotrophoblast forms an initial chorionic shell that erodes thin-walled endometrial capillaries and veins. The *lacunae* within the chorionic shell (which are the forerunners of the *intervillous spaces*) are filled with blood from the eroded veins. By the erosion of few small arterioles, the lacunae are sluggishly perfused by maternal blood (*lacunar phase*; fertilization days 9 to 13).

At the end of the second fertilization week, chorionic villi develop as proliferating solid epithelial cords of cytotrophoblasts (*primary villi*). *Secondary villi*, defined by the presence of embryonic mesenchyme, develop a few days later and start a branching pattern of growth. By the end of the third fertilization week, blood vessels appear in the core of the villi. The development of these *tertiary chorionic villi* coincides with the development of the primitive cardiovascular system, although blood cell formation in the islands of the yolk sac does not begin until the 5th week.

During the 6th fertilization week, chorionic villi start growing in an asymmetric manner: elaborate villi are grown toward the decidua basalis and form the *chorion frondosum* (leafy chorion), whereas the chorion facing toward the decidua capsularis remains smooth (*chorion laeve*). (The correct Latin spelling is *leve*, but the misspelled version used in anatomic texts is retained here as a time-honored tradition). The largest villi of the chorion frondosum attach to the endometrium as *anchoring villi* via solid columns of cytotrophoblast cells, which spread as the trophoblastic shell and secure the attachment of the placenta to the decidua basalis (see Fig. 13-22). The

trophoblastic invasion of about 40 to 60 spiral endometrial arteries occurs at this time. The inner cell mass (by then the embryo within the amnion) is adjacent to the chorion frondosum. The allantois-associated blood vessels communicate with the vessels within the chorionic plate and the chorion frondosum; this communication elongates and develops into the *umbilical cord*.

The *cotyledons* (lobules of the placenta emerging from the chorionic plate) are clusters of branching chorionic villi separated from each other by placental septa. The *placental septa* are wedgelike protrusions of the decidua covered by trophoblastic shell. Formation of primordial cotyledons occurs during the 6th to 7th fertilization weeks. At this time, maternal circulation in the intervillous space is still sluggish with low pressure (5 to 8 mmHg). By the 11th to 12th fertilization week (about 13th week LMP), 10 to 12 large cotyledons develop that are flushed by high-pressure maternal blood (40 to 60 mmHg in the central intervillous space). This stage heralds the development of the *definitive placenta. The maturational increase in placental perfusion coincides with the peak plasma hCG concentrations.*

The name decidua (decidual membrane) refers to the fate of the endometrial lining: it falls off at the time of parturition. At parturition, the apical layer of the decidua is shed, which corresponds with the stratum functionale of the cycling endometrial lining. This is the layer supplied by the spiral arteries. The stratum basale supplied by the straight arteries is retained and the endometrium regenerates from it during the *puerperium* (early postpartum period).

Shallow Placentation Is Due to the Failure of Cytotrophoblast Cells to Express Adhesion Molecules of the Endothelial Phenotype, and Its Consequence Is Preeclampsia During the development of the placenta, cytotrophoblast cells of anchoring chorionic villi aggregate into cell columns and invade both the uterine interstitium and vasculature, thereby generating the basal plate, which anchors the fetus to the uterus and establishes maternal blood flow to the intervillous space. The *invasive character* of trophoblast cells is a result of several factors, including the expression of integrin $\alpha V\beta 3$, VE-cadherin, and MMP-9, which make trophoblasts functionally similar to osteoclast cells (see Chap. 8). Cytotrophoblasts colonizing the spiral arterioles replace the maternal endothelium as far as the luminal one third of the myometrium. The differentiating *cytotrophoblasts switch their adhesion receptor repertoire so as to resemble the endothelial cell phenotype they replace.*

- The cytotrophoblasts in cell columns display decreased expression of E-cadherin and express VE-cadherin, platelet-endothelial adhesion molecule-1, vascular endothelial adhesion molecule-1, and α_4-integrins.
- The cytotrophoblasts in the uterine interstitium and maternal vasculature express these receptors and integrin $\alpha V\beta 3$.

The phenotype switch is critical for endovascular invasion. The musculoelastic wall of the invaded spiral arterioles is replaced by a mixture of fibrinoid and fibrous tissue. These vessels become relatively large, low-resistance channels functionally similar to arteriovenous shunts. The decreased vascular resistance related to trophoblastic invasion can be demonstrated by *pulsed Doppler* ultrasound examination of the uterine *arcuate arteries* from which the spiral (and the straight) arteries originate.

In *preeclampsia*, a potentially life-threatening hypertensive disease (also known as *toxemia of pregnancy*) in which endovascular invasion is abrogated, cytotrophoblasts fail to adopt a vascular adhesion phenotype, including integrin, cadherin, and Ig superfamily members. The consequence is shallow placentation leading to placental ischemia, the release of vasoconstrictor substances, and a decrease of placental PGE_2 and prostacyclin production. Doppler examination may verify the abnormally high vascular resistance.

Preeclampsia occurs in approximately 6% of pregnant women, and mainly affects primigravida women during the last trimester. It is characterized by hypertension, edema, proteinuria, and fetal growth retardation. The endothelial dysfunction may proceed to *disseminated intravascular coagulation* (DIC) resulting in widespread organ damage (*eclampsia*).

Preeclampsia has a strong genetic component. It has been proposed that the condition is due to the mutation of a paternally imprinted, maternally active gene which must be expressed by the fetus in order to establish a normal placenta.

The Implanted Conceptus Avoids Immunologic Rejection by Trophoblastic Expression of Indoleamine 2,3-Dioxygenase (IDO), Which Rapidly Degrades Tryptophan, Thereby Suppressing T-Cell Activity All tissues of the developing fetus are immunologically foreign to the mother due to the presence of paternally determined histocompatibility antigens. The human placenta is *hemochorial*, meaning that the syncytiotrophoblast cells are in direct contact with maternal blood. The syncytiotrophoblast cells form a barrier between maternal and fetal tissues on the surfaces of *chorionic villi*, the *chorionic plate*, and the *trophoblastic shell* (Fig. 13-22). Several mechanisms have been suggested for the explanation for the maternal immunologic tolerance for the "fetal allograft." Experimental evidence obtained in mice suggests the expression of *indoleamine 2,3-dioxygenase (IDO)* by the trophoblast is essential in the prevention of immunologic rejection. T cells have a mandatory requirement for tryptophan for their proliferation. Marcophages may express IDO upon stimulation with interferon-γ (IFN-γ), and inhibit T-cell responses by depriving them of tryptophan. Murine as well as human trophoblast cells express IDO, and the systemic concentration of tryptophan in maternal plasma decreases during normal pregnancy. Inhibition of IDO activity by 1-methyl-tryptophan has no impact on the survival of syngeneic concepti, but allogeneic concepti are rejected in a rapid T-cell mediated reaction.

Tryptophan is an essential amino acid that is required for fetal protein synthesis. Tryptophan is transported across the trophoblast barrier into the fetal circulation. Thus, the expression of IDO in the trophoblast had been puzzling until the discovery of its involvement in preventing fetal rejection.

It is currently uncertain how the IDO-mediated protection fits with the *trophoblast-lymphocyte cross-reactive* (TLX) *alloantigen system*, another regulatory mechanism involved in preventing rejection. TLX (also known as *membrane cofactor protein* [MCP] or CD46 antigen) is a complement regulatory protein that protects tissues from autologous complement damage. TLX is involved in maternal allogeneic recognition during pregnancy. Antibodies to paternal TLX allotypes are produced in women suffering from secondary recurrent abortions. Because of the allotypic nature of TLX antigens, a pregnant female must be able to regulate TLX immune responses to avoid rejection of the conceptus. The proposed mechanism of specific and systemic regulation of TLX immunity is the idiotype–anti-idiotype network. Seminal plasma contains high levels of allotypic TLX antigen produced by the seminal vesicle that could prime mothers prior to fertilization. Such priming effects for pregnancy acceptance are supported by improved implantation rates upon using timed vaginal exposure to semen during in vitro fertilization.

The Endocrine Physiology of the Pregnant Woman and the Fetoplacental Unit

Implantation Rescues the Corpus Luteum from Apoptosis Resulting in the Continued Maintenance of the Endometrium Implantation coincides with peak plasma progesterone levels of the midluteal phase, i.e., the time when the function of the corpus luteum would start its spontaneous decline. However, the syncytiotrophoblast cells secrete hCG directly into the maternal circulation, which is detectable in maternal plasma within 24 h after the beginning of implantation (on about the 21st day of the cycle). The resulting maintenance of the endometrium is usually the first sign of pregnancy: women are alerted to pregnancy by missing their menstrual bleeding at the expected time. In fact, the most prevalent cause of *secondary amenorrhea* in women during the reproductive age is pregnancy. Usually the urine test for β-hCG (see Box 13-5) turns positive by the first day after the missed bleeding, (i.e. day 14 after fertilization and day 7 after implantation).

The syncytiotrophoblast cell mass at this time is very small. Plasma hCG may reach sufficient concentrations only because the half-life of hCG is unusually long among the glycoprotein hormones: about 9 to 24 h. The long half-life is due to the high sialic acid content of hCG (see also the half-life of FSH, Regulation of the Gonadotropin–Gonad Axis in Postpubertal Males). With the increasing mass of trophoblast, plasma concentration of hCG doubles approximately every 2 days, and peaks at the end of the first trimester (13th week LMP; Fig. 13-23). The time-course of plasma hCG explains several functions of hCG:

SYSTEM	HORMONE	PATTERN	AVERAGE PEAK CONCENTRATION (TIME)
Placenta and corpus luteum	Progesterone	Rises to term.	190 ng/mL (552 nmol/L) (term)
	17-Hydroxyprogesterone	Peaks at 5 weeks, then declines.	6 ng/mL (19 nmol/L) (5 weeks)
Adrenal	Cortisol	Increases to 3 times prepregnancy values at term.	300 ng/mL (0.83 μmol/L) (term)
	Aldosterone	Plateaus at 34 weeks with small rise near term.	100 ng/mL (277 nmol/L) (term)
	DOC	Increases to 10 times prepregnancy value at term.	1200 pg/mL (3.48 nmol/L) (term)
Thyroid	Total T_4	Increases during first trimester, then plateaus.	150 ng/mL (193 pmol/L)
	Free T_4	Unchanged.	30 pg/mL (38.6 pmol/L)
	Total T_3	Increases during first trimester, then plateaus.	2 ng/mL (3.1 nmol/L)
	Free T_3	Unchanged.	4 pg/mL (5.1 pmol/L)

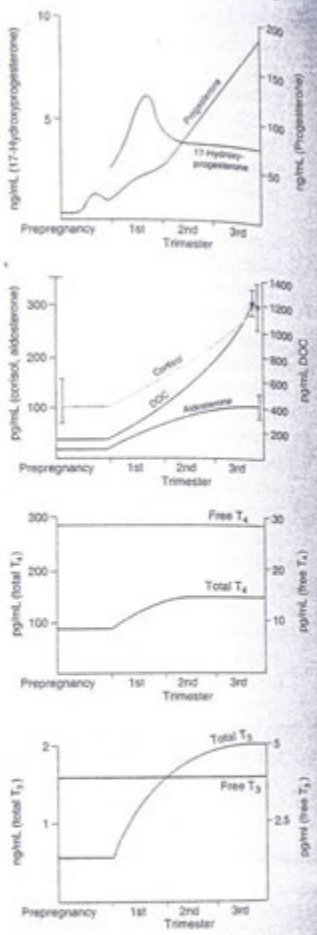


Figure 13-23. Maternal serum hormone changes during pregnancy. (*Source:* Modified from Fig. 16-1 in Taylor RN, Martin MC: The endocrinology of pregnancy, in Greenspan FS, Strewler GJ: *Basic & Clinical Endocrinology*, 5th ed., Stamford, CT, Appleton & Lange, 1997.)

SYSTEM	HORMONE	PATTERN	AVERAGE PEAK CONCENTRATION (TIME)
Anterior pituitary	LH, FSH	Low, basal levels.	
	ACTH	Unchanged.	
	PRL	Rise to term.	≈200 ng/mL (200 μg/L) (term)
Placental proteins	hCG	Peaks at 10 weeks, then decreases to a lower plateau.	5 μg/mL (5μg/L) (end of first trimester)
	hPL	Rises with placental weight.	5–25 μg/mL (5–25 μg/L) (term)
Fetoplacental estrogens	Estriol	Increases to term.	15–17 ng/mL (55–62 nmol/L) (term)
	Estradiol	Increases to term.	12–15 ng/mL (42–52 nmol/L) (term)
	Estrone	Increases to term.	5–7 ng/mL (18.5–26 nmol/L) (term)
Fetoplacental androgens	Testosterone	Rises to 10 times prepregnancy values.	≈2000 pg/mL (6.9 nmol/L) (term)
	DHEA	Falls during pregnancy.	5 ng/mL (17.3 nmol/L) (prepregnancy)
	Androstenedione	Small increase.	2.6 ng/mL (9.0 nmol/L) (term)

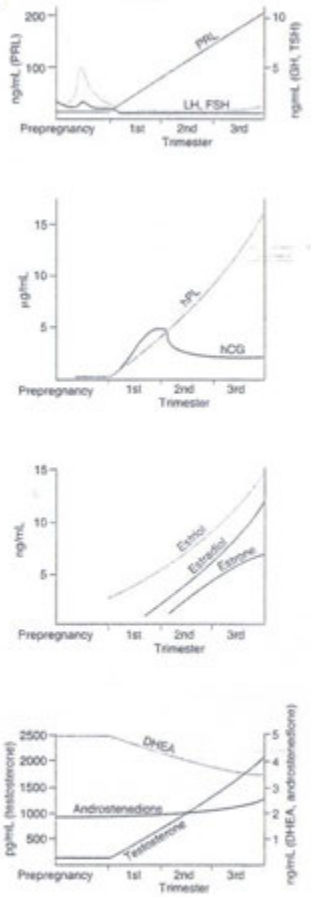


Figure 13-23. (*Continued*)

- stimulation of luteal function (see below).
- inducing "morning sickness," a condition characterized by nausea and vomiting, which usually (but not necessarily) occurs in the morning. About 50% of women experience morning sickness during pregnancy. The condition is typically most intense during the first 12 to 13 weeks of gestation LMP, i.e., the rising phase of plasma hCG. The target of hCG is the *area postrema*, the chemosensitive trigger zone of vomiting, which is a circumventricular organ not protected by the blood-brain barrier (BBB).
- regulating masculinization of the genitalia by stimulating fetal testicular androgen secretion (see Intrauterine Sexual Development). This function implies that hCG is also secreted into the fetal circulation, where its concentration is about 10% that of hCG in maternal plasma.

Chorionic gonadotropin increases *luteal hormone production*. In addition to progesterone, the theca lutein cells of the corpus luteum secrete significant quantities of *17-hydroxyprogesterone* in response to LH or hCG (see Fig. 13-4B). Similar to the granulosa lutein cells, the syncytiotrophoblast cells do not express p450c17, and thus do not contribute to circulating 17-hydroxyprogesterone (Fig. 13-24). Plasma levels of 17-hydroxyprogesterone may be used diagnostically as a marker of corpus luteum function in early pregnancy. Whereas plasma progesterone steadily increases toward term, plasma 17-hydroxyprogesterone peaks around the 7th to 8th gestational week LMP (i.e., before the peak levels of hCG are attained) followed by a decline to about two-thirds of peak levels which are maintained during the remainder of pregnancy. The same time-course is followed by another marker of luteal function, plasma concentration of *relaxin*, which is stabilized at 20% of peak levels (Box 13-11). The cause of declining of corpus luteum function is unknown. Maintenance of pregnancy requires an uninterrupted production of progesterone until parturition. A functioning corpus luteum is a mandatory requirement for the maintenance of pregnancy during the first 7 weeks. Starting with about the 7th gestational week (LMP), the placental production of progesterone becomes sufficient to maintain pregnancy in the absence of the corpus luteum. Thus, if an appendicitis involves the right ovary housing the corpus luteum of pregnancy, which necessitates removal of the ovary at appendectomy, pregnancy may normally proceed provided the event occurred after the 7th week of gestation.

The Fetoplacental Unit The fetoplacental unit refers to the mandatory cooperation between the syncytiotrophoblast and the fetal adrenal gland in the biosynthesis of several steroid hormones. The fetal liver also contributes to the normal steroidogenic process. In terms of estrogen and progesterone production, the fetoplacental unit resembles the cooperation between theca lutein and granulosa lutein cells (see Fig. 13-4B). The steroidogenic pathways of the fetoplacental unit are summarized in Fig. 13-24. The fetoplacental unit is involved in the production of three main hormonal groups: estrogens, progesterone, and corticosteroids.

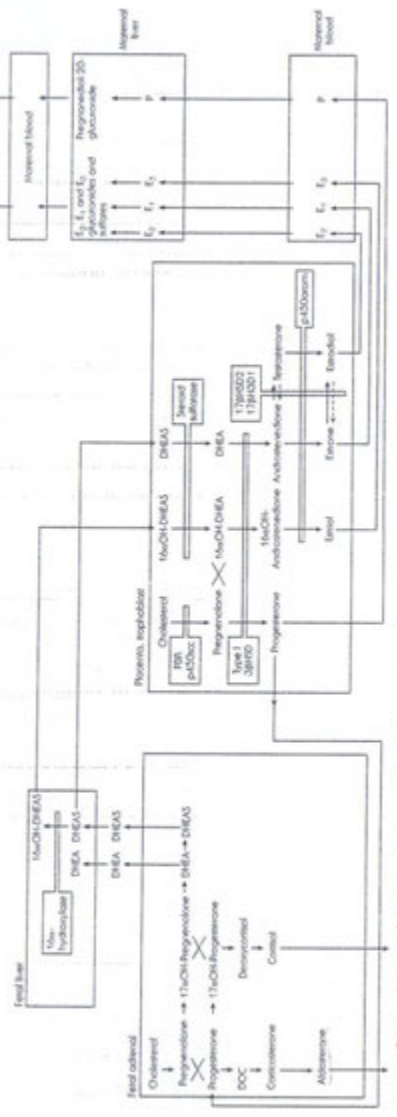


Figure 13-24. Model of the steroidogenesis by the fetoplacental unit. The isoenzyme of 17β-hydroxysteroid dehydrogenase (17βHSD) converting androstenedione to testosterone is uncertain. DHEA, dehydroepiandrosterone; DHEAS, DHEA sulfate; DOC, 11-deoxycorticosterone; E_1, estrone; E_2, estradiol; E_3, estriol; P, progesterone; PBR, peripheral type benzodiazepine receptor.

BOX 13-11 Relaxin

Relaxin is an approximately 6-kDa polypeptide hormone related to insulin and IGFs. Two relaxin genes have been identified (*H1* and *H2*); both are located on the short arm of chromosome 9 and have apparently been derived by gene duplication. The *H2 gene encodes authentic relaxin*, the function of H1 (a relaxin-like peptide expressed in the decidua) is unknown. The primary transcript of the *H2* gene undergoes alternative splicing resulting in two types of mRNAs differing only in their 3′ untranslated regions. Similar to insulin, relaxin consists of two polypeptide chains (A and B) linked by two disulfide bonds, and its C-peptide is removed. In males, the prostate secretes relaxin into the seminal fluid. The main source of circulating relaxin in females is the corpus luteum, which produces larger amounts of relaxin during pregnancy than during the menstrual cycle. Relaxin secretion is increased by hCG; the plasma level of relaxin follows the time-course of hCG during pregnancy. Additional sites of expression such as the decidua, heart, and brain may have more importance in local than in systemic actions. The name relaxin reflects the softening of the pubic ligament in guinea pigs, the biologic activity that led to the discovery of this hormone. The main biologic action of relaxin involves the regulation of the gene expression, synthesis, and secretion of matrix metalloproteinases, which are utilized in tissue remodeling during growth of the uterus, the mammary gland, the fetal membranes and the birth canal as a preparation for delivery. Relaxin is essential for cervical ripening and for the preparation of the breast for lactation, including structural changes in the glandular epithelium and the nipple. Relaxin decreases uterin motility- and induces vasodilation in several organs, including the uterus, the mammary gland, lungs and the heart. By inducing matrix metalloproteinases, exogenous relaxin may counteract various factors (such as the anticancer drug busulfan) that induce pulmonary fibrosis. Relaxin inhibits histamine release by mast cells thereby antagonizing experimental asthma. It also depresses aggregation of platelets and their release by megakaryocytes. The relaxin receptor has not yet been cloned but it is presumed to be related to the insulin receptor. The nitric oxide-cyclic GMP pathway is involved in the signal transduction of relaxin, an effect compatible with its vasodilatory action.

- *Production of estrogens* by syncytiotrophoblast from androgenic precursors provided by the fetal zone of the adrenal cortex. The effects of estrogens during pregnancy are listed in Table 13-11; their plasma levels are shown in Fig. 13-23.
  - The placenta is unable to generate 17-hydroxyprogesterone because it does not express the p450c17 enzyme (see page 554). This also means that neither DHEA nor androstenedione can be produced by the placenta. The lack of androgen production by the placenta is important in protecting the female fetus from masculinization. In contrast, the placenta protects the female fetus against masculinization by either maternal or fetal androgens because the placenta has a strong aromatase activity.
  - The fetal zone of the adrenal cortex does not express 3β-hydroxysteroid dehydrogenase (3βHSD). Thus, all the pregnenolone generated by the p450scc is processed into DHEA and its sulfate.
- *Progesterone* is synthesized de novo by the syncytiotrophoblast from cholesterol obtained from maternal plasma LDL. (The placenta is unable to synthesize cholesterol.) Placental progesterone secretion (unlike placental estrogen production) does not require the presence of a viable fetus. The syncytiotrophoblast expresses high levels of p450scc, which is also indicated by the tubulovesicular mitochondria. Pregnenolone is converted into progesterone by the type I isoenzyme of 3βHSD. In the absence of p450c17 and p450c21, progesterone is the end product of this pathway. Progesterone as a lipophilic hormone readily enters both the maternal and the fetal circulation. The effects of progesterone during pregnancy are listed in Table 13-11 and their plasma levels are shown in Fig. 13-23. In the fetus, progesterone is an important precursor of adrenocortical hormones produced by the fetal zone of the adrenal cortex.
- *Fetal* production of *glucocorticoids* and *mineralocorticoids*. In general, glucocorticoids are inhibitors of growth, and as such are kept at minimum concentrations throughout much of fetal development. Type II 11βHSD expressed by the placenta protects the fetus against exposure to maternally derived cortisol. Nevertheless the fetus produces corticosteroids. Whereas the fetal zone of the adrenal cortex does not express 3βHSD, the definitive zone expresses type II 3βHSD. During the 13th to 24th gestational weeks, the definitive zone only scarcely expresses p450c21, a mandatory enzyme for the biosynthesis of mineralocorticoids and glucocorticoids. However, the undisturbed human fetus during 16th to 20th gestational weeks actively secretes cortisol, corticosterone, and aldosterone. The apparent conflict is resolved by the processing of placentally produced progesterone by p450c21 followed by p450c11 or p450aldo, which are all expressed at low levels in the fetal zone (see Table 12-6).
  - After the 20th gestational week, the trophic action of ACTH is essential for both the fetal and the definitive zones, as indicated by their atrophy in anencephalic fetuses.
  - After about the 25th gestational week, the definitive zone grows and matures more rapidly under the influence of increasing plasma ACTH levels, and contributes to the rising levels of corticosteroids. The concomitant increase of ACTH and cortisol implies that the setpoint of the negative feedback is shifting (see also Intrauterine Sexual Development). The rise in cortisol near term promotes maturation of several organ systems such as the lungs (surfactant production) and the gastrointestinal tract (cessation of macromolecular absorption, development of enzyme systems).

Table 13-11 The Main Effects of Estrogens and Progesterone Related to Pregnancy

Target	Estrogens	Progesterone
Endometrium	—	Decidualization and maintenance of the endometrial lining
Myometrium	Sensitization to OT (promotes contractions)	Decreases uterine contractility
Extrauterine smooth muscle	—	Decreases the contractility of smooth muscles in general; may cause constipation (GI smooth muscle); contributes to the development of venous varicosities by vascular smooth muscle relaxation
Uterine cervix	—	Decreased production of cervical mucus; viscosity of the mucus increases
Vagina	Increased proliferation, thickness, glycogen accumulation, and cornification of the epithelium; increased numbers of surface cells in vaginal smears	Increased contribution of polymorphonuclear leukocytes and parabasal epithelial cells in vaginal smears
Breast	Growth and development of mammary ductal epithelium; inhibition of milk production (both lactogenesis and galactopoiesis); precipitous fall after parturition allows lactation to commence	Growth and development of alveolar epithelium; inhibition of lactogenesis but not galactopoiesis
Bones	Prevention of osteoporosis (see Table 8-7)	?
Brain	—	Increases body temperature by a hypothalamic action; increased ventilatory response to CO_2; precipitous drop at parturition may cause postpartum depression
Liver/ metabolism	Increased production of vitamin K-dependent clotting factors (II, VII, IX, X).	Glucocorticoid-antagonist and agonistic effects in the development of insulin resistance
	Increased glycosylation (and synthesis?) of SHGB, TBG, and CBG leading to increased plasma levels of carrier proteins	—
	Increases HDL, decreases LDL, with slight decrease of total cholesterol; slightly increases triglycerides	—
Electrolytes	—	Compensated state of aldosterone antagonism on the mineralocorticoid receptor

Table 13-11 (*Continued*)

Target	Estrogens	Progesterone
Pituitary/ Hypothalamus	Direct pituitary action: increased PRL gene expression; lactotroph hyperplasia	—
	Via the hypothalamus: increased synthesis and secretion of PRL in part by inhibiting the tuberoinfundibular dopaminergic activity	—
	Suppression of gonadotropin secretion and pituitary GnRH responsiveness	
Skin	Chloasma gravidarum; increased pigmentation of the areola, nipple and the vulva; looser consistency of sebum; decreased formation of acne	—

ABBREVIATIONS: OT, oxytocin; GI, gastrointestinal; SHBG, sex hormone-binding globulin; TBG, thyroxine-binding globulin; CBG, cortisol-binding globulin; PRL, prolactin.

Estriol is the main estrogenic product of the placenta. Its daily production rate is usually assessed by measuring maternal urinary estriol excretion. Subnormal estriol excretion may indicate:

- Placental insufficiency and/or fetal pathologies.
- *Aromatase* deficiency. In this case, plasma androgen levels increase, and lead to virilization symptoms in the pregnant mother as well as severe/complete masculinization of the female fetus. Virilization in pregnant women is alarming. Normally the estrogen-mediated increase in plasma SHBG decreases free androgen levels in spite of an increase in total plasma testosterone.
- *Steroid sulfatase* (arylsulfatase C) deficiency. As mentioned, the gene encoding steroid sulfatase is located near the Kallmann's syndrome gene on the short arm of the X chromosome. In the case of isolated steroid sulfatase deficiency, the decrease of estriol is not accompanied by increased levels of androgens, and virilization does not occur. The fetus, however, is born with *X-linked ichthyosis* (the name refers to fish-like scales of the skin), which occurs with a frequency of 1 in 2000 to 1 in 6000 and mainly affects hemizygotic males or homozygotic females born usually from consanguinous parents. The skin condition is due to the accumulation of cholesterol sulfate in the epidermis, which usually presents in the 2nd to 3rd month of age. Steroid sulfatase is expressed by leukocytes; the differential diagnosis of ichthyosis vulgaris and X-linked ichthyosis includes the laboratory test of leukocyte-mediated hydrolysis of DHEAS into DHEA. The test is also useful in identifying heterozygotic carrier females.

Decreased placental production of estrogens may cause abnormal patterns of labor because estrogens are important in sensitizing the uterine

smooth muscle to oxytocin. Sulfatase deficiency may cause impaired lactation because

- estrogens mediate the gestational hyperplasia and hypertrophy of the pituitary gland by promoting the growth of PRL-producing mammotroph cells; estrogens also stimulate their secretory activity, which contributes to the preparation of the breast for lactation.
- estrogens directly promote the growth of the ductal epithelium of the mammary gland.

The high levels of circulating estrogens are responsible for the development of *chloasma gravidarum* (mask of pregnancy), a cutaneous hyperpigmentation usually occurring after the 16th gestational week, which is accentuated by exposure to sunlight.

Maternal Pituitary Gonadotropin and GH Secretion Are Suppressed During Pregnancy At the time of implantation, progesterone secreted by the corpus luteum under the influence of hCG prevents menstruation. In addition, the sustained function of the corpus luteum (i.e., estradiol, progesterone, inhibin) exert strong negative feedback suppression of pituitary gonadotropin secretion. This role of the corpus luteum is then replaced by the fetoplacental unit. Plasma LH and FSH are suppressed to nearly nondetectable levels and the pituitary gonadotrophs become unresponsive to a GnRH challenge. The GnRH unresponsiveness continues into the first postpartum week.

The placenta secretes a molecular variant of GH expressed from the *GH-V* gene. During the first trimester, pituitary GH is the only measurable GH in maternal plasma, and its concentrations fluctuate in an episodic manner. From about the 15th to 17th weeks of gestation, pituitary GH is progressively replaced by placental GH-V. GH-V is responsible for the continuously rising plasma levels of IGF-1 during pregnancy. By its negative feedback action on the pituitary and the hypothalamus, IGF-1 suppresses pituitary GH secretion. Concurrently the episodic secretory pattern of GH disappears because GH-V is secreted in a continuous, non-episodic manner. The pituitary GH secretion becomes refractory to secretagogues such as hypoglycemia.

The increased GH-like biologic activity includes chorionic somatomammotropin (hCS). This hormone exerts more GH-like than PRL-like actions in humans. GH-specific antibodies may crossreact with hCS. The increased circulating GH-like biologic activity increases lipolysis thereby decreasing insulin sensitivity. Thus, it is potentially diabetogenic in spite of the direct insulin-stimulating action of hCS.

Similar to hCS, GH-like placental hormones enter the fetal circulation. GH-V and/or hCS may be involved in promoting fetal growth by stimulating fetal production of IGF-1. Chorionic somatomammotropin has been implicated as a trophic factor for the developing pancreatic islets.

During Normal Pregnancy, Free Thyroid Hormones Remain Unchanged but Their Total Plasma Concentrations Are Elevated by Estrogen-Induced Increase of Thyroxine-Binding Globulin Estrogens increase plasma concentration of thyroxine-binding globulin (TBG), which leads to an initial decrease in plasma levels of *free* T_4 and T_3. The normally functioning feedback regulation increases thyroid hormone production. Restoration of normal plasma concentrations of free T_4/T_3 is achieved at an elevated concentration of *total* T_4 and T_3. Thus, in spite of increased total T_4 and T_3, healthy pregnant women remain euthyroid.

During pregnancy, iodine turnover is increased, and the iodine demand of the fetus must be met. This may result in a usually compensated state of iodine deficiency in pregnant women especially toward late gestation. The compensation involves *euthyroidism at elevated TSH levels*, which may result in enlargement of the thyroid gland.

At very high levels (such as seen in trophoblastic diseases), hCG may crossreact with the TSH-receptor and cause true hyperthyroidism. Up to 10% of postpartum women experience lymphocytic thyroiditis, which presents with an initial hyperthyroid phase followed by a prolonged phase of hypothyroidism (see Chap. 11). The condition is usually self-limited.

The Partial Corticosteroid Agonist/Antagonist Effects of High Plasma Progesterone Are Important Determinants of Maternal Adrenocortical Function Progesterone competes with aldosterone on mineralocorticoid receptors, and functions mainly as an antagonist. This is the most likely cause of the activation of the renin-angiotensin system in pregnant women, which leads to a progressive increase of plasma *aldosterone* until reaching plateau levels toward the end of the second trimester (Fig. 13-23). The substantial increase in plasma aldosterone is best interpreted as a *compensatory* feedback mechanism in response to an endogenous receptor antagonist, which explains why neither hypertension nor hypokalemic alkalosis are present.

Maternal ACTH and Cortisol Rise Throughout Pregnancy Due to Placental Production of CRH Plasma total cortisol increases approximately threefold during pregnancy, and the increase in free cortisol is approximately twofold. The circadian rhythm of cortisol secretion is maintained throughout pregnancy, although its relative amplitude (the fluctuation expressed as a percent of the average concentration of cortisol) is blunted. Plasma ACTH also increases throughout pregnancy. The main cause of the concomitant increase in ACTH and cortisol is the physiologic ectopic production of CRH by the placenta. Placental CRH secretion increases during pregnancy (see Parturition). ACTH stimulates adrenal production of both cortisol and androgens.

Cortisol, unlike aldosterone, circulates mainly in association with CBG (cortisol-binding globulin; see Tables 4-1 and 4-2). Plasma CBG levels are

elevated by estrogens during pregnancy. This explains why the increase of free cortisol is lower than the increase of total cortisol. The increase in CBG-bound cortisol results in an increased plasma half-life of cortisol. As a C21 steroid, progesterone also binds to CBG. Progesterone competes with cortisol for the glucocorticoid receptor, ameliorates the biologic effect of increased free cortisol, and prevents the development of overt Cushingoid features.

The elevated cortisol nevertheless contributes to

- the development of abdominal striae, which may lead to permanent stretch marks;
- hyperphagia and weight gain;
- the development of gestational diabetes in susceptible individuals.

Some of the adrenal androgens may be processed by 17βHSD into testosterone. The increased production of adrenal androgens does not result in virilization for several reasons:

- increased levels of SHBG outpace the increase of androgens and free androgen levels decrease.
- placental aromatase activity rapidly converts androgens into estrogens.

The Calcium and Phosphate Demands of the Fetus Are Primarily Met by an Increase in Plasma Calcitriol of the Pregnant Mother Calcium transport into the fetus is stimulated mainly by the autocrine action of placental PTHrP (see Chap. 8). This maternal calcium loss can be replenished from two sources:

- mobilization of calcium from maternal skeletal tissues by secondary hyperparathyroidism;
- increased absorption of dietary calcium and phosphate.

Under physiologic conditions, provided that dietary calcium intake is appropriate (1.2 g/d), no significant secondary hyperparathyroidism occurs. In contrast, circulating calcitriol increases about four- to fivefold during pregnancy. Estrogens, GH, and PRL, which increase during pregnancy, are all inducers of renal 1α-hydroxylase. However, placentally expressed 1α-hydroxylase is the main determinant of increased calcitriol and increased intestinal absorption of calcium. Calcium together with the direct parathyroid action of increased levels of calcitriol prevent the development of secondary hyperparathyroidism.

The Potentially Diabetogenic Changes in Maternal Fuel Homeostasis Support Fetal Nutrition For their survival, cells depend on the constant supply of fuel present in the extracellular fluid (ECF). Regulatory processes

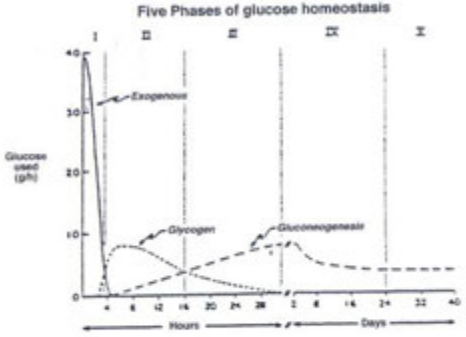
assure that the fuel supply in the ECF is continuously present in spite of the intermittent feeding of the organism. As discussed, an important feature of fuel homeostasis includes adpatational mechanisms to the fasting and the fed states. The fed state promotes fuel conservation (*anabolic state*), whereas the fasting state mobilizes stored fuel (*catabolic state*). Pregnancy complicates this regulatory process because the continuous supply of nutrients to the conceptus must be integrated with the nutritional requirements of the mother. *To prevent fetal growth retardation, amino acids and glucose must be spared for, and preferentially delivered to, the fetus during periods of fasting*. This means that maternal fuel requirements must be primarily satisfied from mobilization of FFA during fasting (Fig. 13-25).

Glucose is transported into the fetus by a concentration gradient-dependent passive transport via GLUT1. In cases of diabetes mellitus (either IDDM or *gestational diabetes mellitus* [GDM]), maternal hyperglycemia results in an increased flux of glucose into the fetus. The fetal pancreas responds to the glucose challenge with insulin secretion starting with the 15th fetal week. The ensuing fetal hyperinsulinemia delivers supranormal amounts of glucose to the intracellular fluid (ICF) for utilization (increased fuel). In addition, high insulin levels exert growth factor-like biologic effects acting on both insulin and IGF-1 receptors. These mechanisms together lead to *fetal macrosomia* (see also in Chap. 10), i.e., a birth weight of >4000 g. Macrosomia may lead to *cephalopelvic disproportion*, which necessitates cesarean section. Infants born from diabetic pregnancies often experience postpartum hypoglycemia because their increased β-cell mass produces insulin that is inappropriately high for the glucose obtained during feedings.

By the third trimester, the increase in cortisol and GH-like biologic activity in maternal plasma results in a significantly altered metabolic state, which is characterized by a *compensated state of insulin resistance*. Therefore, hyperglycemia is prevented at the expense of hyperinsulinemia. During the last trimester, plasma insulin levels are 1.5 to 2.5 times higher than in nonpregnant women. GDM develops when the increase of insulin output does not match the degree of insulin resistance. Compared to nonpregnant women, the oral glucose tolerance test during late *normal* pregnancy displays higher amplitude and prolonged increases of both plasma glucose and insulin. The increase in insulin *secretory rate* is even higher than expected from the increase in plasma levels: the half-life of insulin is shorter during pregnancy due to its placental degradation.

Although the high secretory rate of insulin suppresses glucagon production, the glucagon response to amino acids is retained. This constellation is important in minimizing use of amino acids for gluconeogenesis *during fasting* (which equals accelerated starvation during the third trimester), thereby conserving amino acids for the fetus. During hyperaminoacidemia, the surplus amino acids may be diverted to gluconeogenesis.

In Cushing's disease, hypercortisolemia suppresses the pituitary GH–IGF-1 axis. An unusual feature of late gestation is the concurrent increase



Phase	I	II	III	IV	V
Origin of blood glucose	Exogenous	Glycogen, gluconeogenesis	Gluconeogenesis glycogen	Gluconeogenesis	Gluconeogenesis
Tissues using glucose as fuel	All	All except liver. Muscle, adipose at diminished rates	All except liver, but rate is diminished	Brain/RBC/renal medulla. Small amount by muscle	Brain at lower rates. RBCs, renal medulla.
Major fuel of brain	Glucose	Glucose	Glucose	Glucose, ketone bodies	Ketone bodies, glucose

Figure 13-25. A. The sources of glucose during the feeding/fasting cycle in nonpregnant individuals. Gluconeogenesis becomes the preeminent source of plasma glucose about 16 hours after the last meal. B. Physiologic "accelerated starvation" during late pregnancy. Upon fasting, free fatty acids and ketone bodies more rapidly and more substantially increase in plasma of late pregnant than in nonpregnant women. In nonpregnant women, this coincides with an increase in gluconeogenesis that maintains euglycemia, whereas pregnant women become hypoglycemic. At the same time, plasma alanine in nonpregnant women remains unchanged, which reflects an equilibrium between gluconeogenesis and cortisol-induced mobilization of alanine from muscle tissue. In contrast, plasma alanine decreases in pregnant women because of fetal uptake. The decrease in plasma glucose is the consequence of fetal glucose uptake and subnormal gluconeogenesis. The limited gluconeogenesis in late pregnancy is mainly explained by the elimination of the main substrate (alanine) by fetal uptake. (*Source*: A: from Ruderman NB et al. in Hanson RW, Mehlman MA (eds): *Gluconeogenesis: Its Regulation In Mammalian Species*, New York, Wiley, 1976, p 518; B: Fig. 14-6 in Freinkel N: Metabolic changes in pregnancy, in Wilson JD, Foster DW (eds): *Williams Textbook of Endocrinology*, 8th ed., Philadelphia, Saunders, 1992.)

of cortisol and (placental) GH. The lipolytic effect of GH requires the permissive action of cortisol. Thus, these hormones are synergistic in mobilizing FFA and decreasing insulin sensitivity. The protein catabolic effects of hypercortisolemia, however, are offset by the protein anabolic action of the increased GH–IGF-1 axis and by hyperinsulinemia.

Parturition

The Timing of Parturition Is Determined by the Placental CRH Clock Natural birth is the physiologic end point of pregnancy, when the

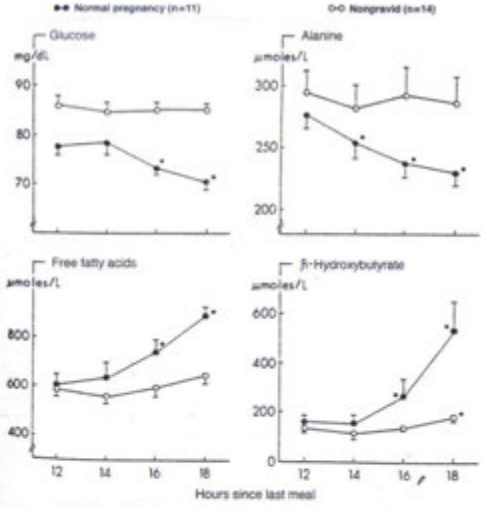
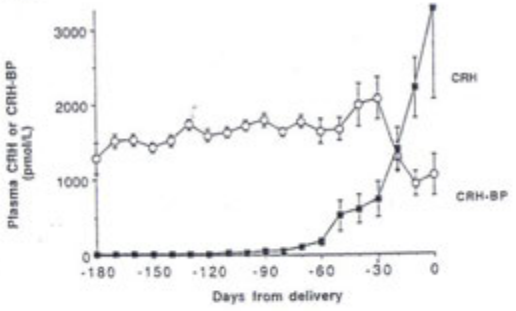


Figure 13-25. (*Continued*)

fetal membranes rupture, the amniotic fluid is lost, and the newborn is expelled from the uterine cavity by active contractions of the myometrium followed by the delivery of the placenta. Recent evidence indicates that the initiating factor of parturition is placental CRH.

CRH and CRH-binding protein (CRH-BP) are synthesized in the placenta and secreted into both the maternal and fetal circulation. Placental CRH is bioactive but causes relatively modest increases in ACTH and cortisol in the pregnant woman because of high levels of CRH-BP. CRH concentrations increase exponentially in maternal plasma as gestation advances. During the last month of pregnancy, there is a significant decrease in CRH-BP resulting in a steep rise in the concentration of free CRH both in the maternal and fetal circulation (Fig. 13-26A). Elevated concentrations of CRH, compared with gestational age-matched controls, occur in patients in preterm labor. In pregnancies complicated by preterm labor, a premature decrease of plasma CRH-BP level occurs. The exponential curve describing the CRH increase is shifted to the left in women who will subsequently deliver preterm and to the right in women who will deliver post-dates, indicating that CRH is linked to a *placental clock* that determines the length of gestation (Fig. 13-26B). Measurement of the maternal plasma CRH concentration as early as 16 to 20 weeks of gestation identifies groups of women who are destined to experience normal term, preterm or postterm delivery.

In the fetus, CRH not only targets the pituitary gland but also exerts a direct action on the fetal zone of the adrenal cortex, and stimulates



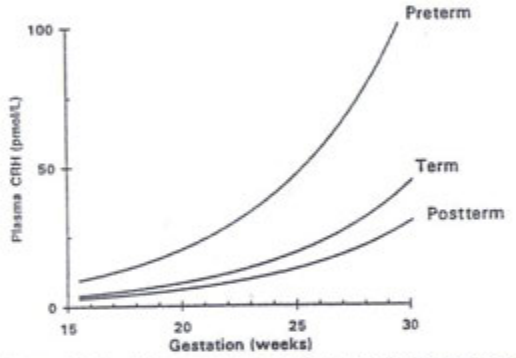


Figure 13-26. A. The concentrations of corticotropin-releasing hormone (CRH) and its binding protein (CRH-BP) in maternal plasma during the last 180 days of gestation in pregnancies ending in spontaneous term labor (37–42 weeks of gestation from the last menstrual period). B. Concentrations of CRH in maternal plasma during midgestation in women whose pregnancies ended in spontaneous preterm labor, spontaneous term labor, or postterm delivery. Premature increase of plasma CRH is a predictor of preterm labor. (*Source:* Fig. 2 and Fig. 1D in McLean M et al: A placental clock controlling the length of human pregnancy. Nature Med 1:460–460, 1995.)

preferential secretion of DHEAS over cortisol. The increased production of DHEAS provides an increased substrate load for the placenta, which results in an increase of estrogen production and an increase in the *estrogen/progesterone ratio* in spite of the sustained plasma progesetrone levels. This, in turn, increases oxytocin-receptor expression in the myometrium.

CRH-R1 is expressed in the myometrial smooth muscle and in fetal membranes; CRH-R2 is expressed at lower levels and only in the myometrium. Toward parturition, CRH-R1 is upregulated in the myometrium of the lower uterine segment, but remains unchanged in the fundus. Stimulation of the CRH-receptors in the myometrium and fetal membranes may be causally linked to parturition by several mechanisms:

- *stimulation of CRH-Rs increases the production of locally acting prostaglandins*, mainly PGE_2 and $PGF_{2\alpha}$;
- stimulation of CRH-Rs potentiates the contractile response of smooth muscle to oxytocin via a prostaglandin-dependent mechanism.

Unlike their inhibitory effect on hypothalamic CRH, glucocorticoids stimulate placental CRH-production. However, glucocorticoids inhibit prostaglandin production.

Androstenedione infusion to pregnant monkeys leads to premature labor and live delivery. Androstenedione-induced preterm labor also increases placental CRH messenger RNA and fetal plasma peptide to concentrations observed at term in pregnant monkeys.

Clinically Observed Labor Gradually Evolves from Prelabor Uterine Activity The coordinated contraction of uterine smooth muscle cells is

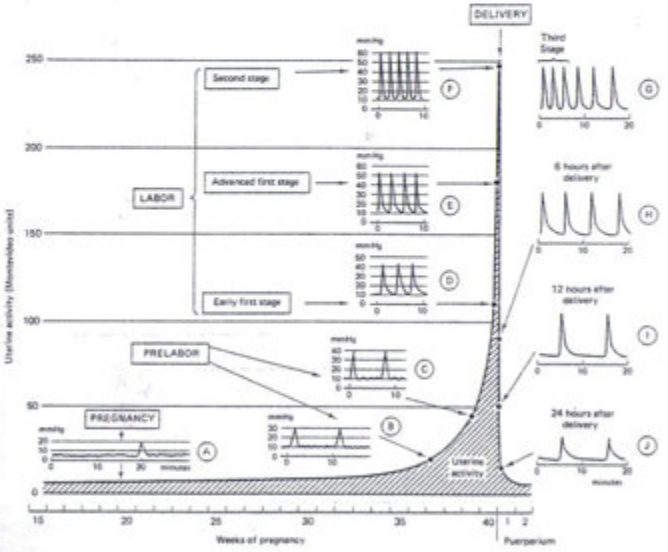


Figure 13-27. Uterine activity after the 15th gestational week. Note the exponential increase uterine activity and compare it with the time-course of CRH (Fig. 13-26). The Montevideo unit is obtained by multiplying the number of contractions/10 min with the average amplitude of the contractions measured in mmHg. Both components of the uterine activity (frequency and amplitude) increase until delivery, followed by an abrupt decrease. The puerperial activity is a functional component of postpartum uterine involution. (*Source:* Fig. 12, p 141 in Elstein M, Chantler EN: Functional anatomy of the cervix and uterus, in Philipp EE, Barnes J, Newton M (eds): *Scientific Foundations of Obstetrics and Gynecology*, 3rd ed., William Heinemann Medical Books publication distributed by Year Book Medical Publishers, Inc., 1986; Figure originally appeared as Fig. 8.12 in Caldeyro-Barcia R: *The Cervix in Pregnancy and Labour*, Churchill Livingstone, 1959.)

assured by gap junctions. During the first trimester, the uterus is relatively quiescent. *Braxton-Hicks contractions* are defined as contractions increasing intrauterine pressure by 10 to 15 mmHg and have a duration of at least 30 seconds. The intensity as well as the frequency of these contractions increases in an exponential manner following the time-course of the exponential increase in plasma CRH (Fig. 13-27). Especially during the last 5 weeks of pregnancy, the myometrial work (often referred to as *prelabor*) results in "taking up" the cervical canal: the internal os of the cervical canal expands, the lumen of the cervical canal unifies with the uterine cavity, but the diameter of the external os of the cervical canal remains narrow. The process continues the ripening of the cervix that was initiated early in pregnancy by luteal relaxin. Before the onset of labor, these changes result in the development of two functionally distinct segments of the uterus:

- The *upper segment* has a thicker and more muscular wall. Its function is the delivery of the fetus by active contractions. Uterine contractions progress from the fundus toward the lower segment.
    - The normal site of implantation and the placenta is in that portion of the endometrium, which belongs to the developing upper segment. This anatomic arrangement assures that the placenta detaches and is delivered only after fetus. Thus, while passing through the birth canal, the fetus continues to be supplied with oxygen by the placenta.
- The *lower segment* becomes progressively thinner and less muscular. If the lower segment were as muscular as the upper segment, its contractions would block the passage of the fetus. Thus, the function of the lower segment is to unify with the vagina during labor and provide a relatively passive fibromuscular birth canal. Unification with the vagina is observed as the dilatation of the external os of the cervix. The dilatation rapidly progresses during labor from about 1 to 2 cm to about 10 cm (the diameter of the head of the fetus).
    - The implantation of the placenta at the lower segment is known as *placenta previa* ("placenta ahead" [of the fetus]). Because detachment and delivery of the placenta would precede the birth of the fetus, this life-threatening condition is a mandatory indication of cesarean section.

The mother is alerted to labor by 2 to 4 relatively intense contractions per 10 minutes and/or by the rupture of the fetal membranes. The latter may present either as a sudden loss of a significant volume, or just a slow leakage of fluid.

Oxytocin Enhances the Uterine Contractions During Labor As discussed, CRH-stimulated prostaglandins are involved in the onset of labor by directly increasing uterine contractions. The resulting descent of the fetus activates stretch receptors located in the cervix and upper vaginal wall, which in turn results in the release of oxytocin from the posterior pituitary gland (*Ferguson reflex*). More importantly, both the elevated estrogen/progesterone ratio and CRH-induced prostaglandins sensitize uterine smooth muscle to oxytocin: an approximately eightfold increase in responsiveness to oxytocin develops in an exponential manner between the 20th and 39th gestational week. Oxytocin stimulates uterine production of prostaglandins; thus, the relationship between prostaglandins and oxytocin may be viewed as a positive feedback. The positive feedback is terminated by the delivery of the fetus and the placenta: plasma concentrations of CRH (due to the elimination of its source) and oxytocin (due to the lack of stretch receptor activation) precipitously drop thereby decreasing prostaglandin production.

Oxytocin [*oxy*, rapid; *tocos*, parturition] is a nonapeptide hormone (see Fig. 10-5) with a circulating half-life of about 5 to 12 min. Oxytocin is mainly eliminated by the kidney and the liver. In addition to cervicovaginal stretch receptors, oxytocin secretion is stimulated by stimulation of the clitoris or the nipple (see milk ejection reflex, The Structure of the Breast). In lactating women, oxytocin is released by psychosensory input: hearing the baby's cry, seeing the baby, or performing baby care. Similar to ADH, oxytocin secretion is increased by dehydration and stress, and decreased by ethanol. Ethanol was utilized as a *tocolytic* agent to suspend uterine contractions in premature labor.

Oxytocin enhances both the amplitude and the frequency of contractions. The oxytocin receptor, which is encoded by chromosome 3, is a heptahelical receptor coupled by $G\alpha_{q/11}$ with the PLC signaling pathway that leads to liberation of Ca^{2+} from intracellular stores. The initial increase of intracellular calcium opens calcium-activated chloride and cation channels leading to depolarization of the myometrial cells. In turn, depolarization opens voltage-dependent calcium channels and the calcium influx from the ECF results in contraction.

The traditional quantitative measure of oxytocin is the "uterine-stimulating potency" (USP) unit. One USP unit equals about 2 μg synthetic hormone. Synthetic oxytocin is utilized in clinical practice to *induce labor* and/or to *augment contraction* during labor. At low infusion rates (0.5–1 mU/min) the endogenous rhythmic uterine contractions are enhanced. The infusion can be gradually increased until the duration of contractions reaches 40 to 60 seconds occurring at 2.5- to 4-min intervals. Further increase of the dose may lead to *uterine tetany*, when the uterus does not relax between contractions. During contraction, venous efflux of the placenta through the uterine wall becomes severely compromised. The rhythmic contractions allow appropriate oxygenation of fetal blood, but prolonged contractions may result in fetal hypoxia and *cerebral palsy*. Oxytocin continues inducing uterine contractions after delivery (i.e., stage 3 of labor, see Fig. 13-27). These contractions are accompanied by vasoconstriction and vasocompression, which are important in minimizing postpartum bleeding.

When the uterine tone is weak (*uterine atony*), life-threatening bleeding may develop.

When administered at high doses, oxytocin may crossreact with V_2 receptors of ADH in the kidney and cause fluid retention similar to SIADH (syndrome of inappropriate ADH).

Oxytocin is not only a neurohormone but also a neurotransmitter:

- Oxytocin promotes maternal behavior (including acceptance of the newborn).
- In contrast with ADH, which improves memory/retrieval, oxytocin inhibits these cognitive functions by a hippocampal action. This action has been interpreted as the means to prevent recalling the intense pain associated with labor.

Adrenergic Receptor Stimulation Modulates Uterine Contraction Uterine smooth muscle cells express both α_1- and β_2-adrenergic receptors (see Table 12-4). The ratio of the two receptor types changes during pregnancy. In the nonpregnant uterus and during the last month of gestation, β_2-adrenergic receptors are dominant, and adrenaline causes uterine relaxation by increasing intracellular concentration of cyclic AMP. In clinical practice, this mechanism is utilized for tocolysis by β-adrenergic agonist drugs. In contrast, α_1-adrenergic agonists increase intracellular Ca^{2+} and induce uterine contraction. The ergot alkaloid *ergonovine*, which acts in part by activating α_1-adrenergic receptors, is used especially in cases of uterine atony to induce protracted uterine contraction; the drug must be administered only after the delivery of the fetus and the complete placenta.

The Puerperium

OBJECTIVES

1. Define the timeframe of puerperium. Identify the role of breastfeeding in uterine involution and *afterpains*. Identify the cellular mechanisms of *uterine involution*. Define *lochia*. Describe the regeneration of the uterine cervix.
2. Describe the role of the corpus luteum in the puerperal transition of gonadotropin regulation. Compare and contrast gonadotropin secretion, prolactin secretion and the return of the ovarian cycles in lactating versus nonlactating women. Discuss postpartum depression.

The Puerperium Is a 6-Week Postpartum Period During Which All Reproductive Organs Return to an Approximately Preconceptual State Immediately after delivery, the uterus weighs close to 1 kg, it has an extensive wound area (the detachment site of the placenta), and the cervix is fully dilated and often torn.

The *uterus* involutes to a weight of <100 g within 6 weeks. The involution mainly involves a decrease of the size of the hypertrophied smooth muscle upon *decreased exposure to estrogens*. However, degradation of the collagenous matrix by activated matrix metalloproteinases and tissue breakdown by infiltrating macrophages also take place. The enlarged uterine cavity shrinks to nearly its original size. Uterine contractions play an active role in this process. These uterine contractions are stimulated by *nursing-induced oxytocin*. Thus, uterine involution proceeds at a faster pace in breastfeeding women, and is accompanied by more intense *afterpains*.

Uterine involution includes regeneration of the *cervix*. Although the originally rounded shape of external os of the cervix changes permanently into a transverse horizontal slit, the regeneration is usually rapid and complete. By the end of the first week, the at least 10-centimeter dilatated cervix narrows to about 1 cm in diameter.

Uterine contractions are important in stopping the *bleeding* from the placental attachment site. One of the reasons for recommending early breastfeeding is to induce oxytocin thereby decreasing blood loss. The *detachment site* is a potential entryway for microorganisms. Before the discovery of antisepsis/asepsis by Semmelweis in the nineteenth century, a dreaded and usually fatal complication of childbirth was *puerperal fever*–sepsis that develops from an iatrogenic infection of the placental detachment site.

Due to the postpartum *decrease of progesterone*, the retained portion of the decidua is shed as *lochia rubra* (red lochia), a blood-tinged discharge containing shreds of tissue. The lochia gradually becomes serous in a few days, then mucoid during the 2nd or 3rd postpartum week. Lochial discharge ceases during the 5th week and the endometrium completely regenerates by the 6th postpartum week. The postpartum regeneration process is much slower than after menstruation, which is in part due to the *very low circulating levels of estrogens*.

After the Postpartum Involution of the Corpus Luteum, the Secretion of Gonadotropins Resumes and Leads to Ovulation About 6 Weeks After Parturition in Nonlactating Women At the time of delivery, the hormones produced by the placenta sharply decrease in the circulation. Plasma progesterone decreases to *luteal phase levels* within 24 h. As indicated by 17α-hydroxyprogesterone concentration in maternal plasma (see Fig. 13-23), the corpus luteum still functions under the influence of hCG at the time of parturition. After parturition, plasma hCG decreases in an exponential manner to follicular phase levels of LH-like biologic activity by the early 2nd postpartum week, and becomes undetectable between the 11th and 16th day postpartum. Because pituitary LH secretion remains suppressed and hCG decreases, the corpus luteum involutes with a timecourse comparable with that observed during the second half of the luteal phase (Fig. 13-28). During this time, the pituitary gland is unresponsive to exogenous GnRH, which explains low levels of FSH and pituitary LH.

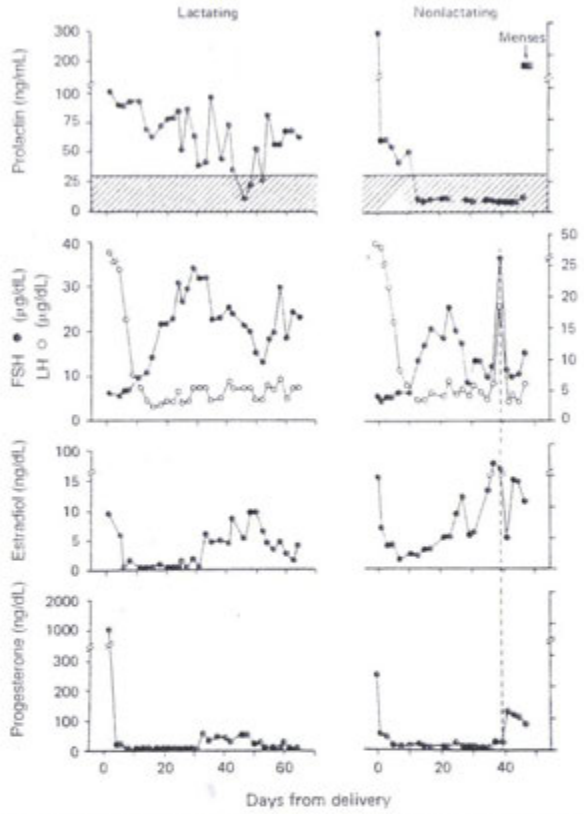


Figure 13-25. Serum concentrations of progesterone, estradiol, prolactin, FSH, and LH-like immunoreactivity during the puerperium in a lactating (left) and a nonlactating woman (right). The LH assay measured both hCG and LH. Note that estradiol fails to follow the rise in plasma FSH in lactating women indicating suppressed follicular development. In nonlactating women, first preovulatory gonadotropin surge occurs about 6 weeks postpartum. (*Source:* From Reyes FI et al: Pituitary-ovarian interrelationships during the puerperium. Am J Obstet Gynecol 114:589–594, 1972.)

- In lactating women, the stimulation of the nipple and hyperprolactinemia inhibit the GnRH pulse generator; the slow pulse frequency and the low levels of circulating inhibin allow increased FSH secretion that is not accompanied either by increased LH or by increased estradiol production. The latter may indicate a direct ovarian action of PRL to inhibit follicular growth and aromatase expression.

- In nonlactating women, the rising FSH stimulates follicular growth and estradiol production, which lead to a positive feedback/preovulatory gonadotropin surge by approximately 6 weeks postpartum. This timing coincides with the complete regeneration of the endometrial lining, including the placental attachment site. If no fertilization occurs, the first menstrual bleeding follows in 14 days.

The sharp decrease of placental steroid hormones often precipitates a usually self-limiting *postpartum depression* ("postpartum blues"). Postpartum depression coincides with the first attempts to breastfeed the infant. Unsuccessful attempts may worsen the depression, and conversely, the depression may hamper the attempts of breastfeeding.

Lactation

OBJECTIVES

1. Identify the main roles of breastfeeding.
2. Discuss the structure and development of the breast. Identify the relationship between mammary epithelium and the underlying mesenchyme, the regulation of morphogenesis, ductal growth, and lobuloalveolar development. Describe the feral, neonatal, pubertal, pregnant, and lactational phases of mammary development.
3. Discuss the relationship between normal development, growth, and differentiation of the breast and *breast cancer*. Identify hormonal risk factors and potential endocrine markers of breast cancer.
4. Discuss *prolactin* (PRL): its synthesis, secretion, regulation, biologic actions, and signal transduction mechanisms. Identify cellular sources of PRL. Discuss the hypothalamic PRL-inhibiting and -releasing factors. Describe the causes and consequences of hyperprolactinemia in nonlactating individuals. Discuss the physiologic regulators of PRL secretion (such as suckling, stress, and hydration status), and the role of PRL in lactation.
5. Discuss the composition of milk. Define the terms *lactogenesis, galactopoiesis, colostrum, mature milk, foremilk* and *hindmilk*. Identify components of milk responsible for immunologic defense of the infant. Consider the potential physiologic roles of hormones present in milk. Compare and contrast merocrine and apocrine secretory mechanisms. Discuss the regulation of milk protein gene expression, carbohydrate and lipid synthesis.
6. Discuss the relationship between the caloric and fluid requirements of the newborn, and how these relate to the composition of milk. Identify the mechanisms resulting in, and the physiologic need for, milk as an isosmotic but low-sodium fluid. Discuss calcium homeostasis during lactation and the transport of calcium into milk. Identify minerals, iodide and other trace elements in milk and discuss their delivery to the infant.
7. Discuss the regulation of milk yield by PRL, locally acting factors in the breast and the mechanism of *milk ejection*. Consider possible mechanisms of *lactational failure*, measures to improve milk yield in breastfeeding mothers and

to suppress lactation in women who do not elect breastfeeding. Discuss the process of weaning and postlactational involution of the mammary gland.

The survival of the *fetus* depends on maternal bodily functions tuned to pregnancy by the *fetoplacental unit*. Similarly, the survival of the *newborn* depends on a maternal bodily functions tuned to lactation by signals emanating from the "*neonatomammary unit*." The healthy term newborn is well prepared to adapt to certain aspects of extrauterine life such as breathing through the lungs. Several physiologic systems of the term infant (the infant born between the 37th and 42nd weeks of gestation LMP) are normally immature at birth, such as the digestive system, the kidneys, and all main regulatory systems (nervous, endocrine, and immune systems). Although the placental influences are lost in an abrupt manner, maternal influences over the developing infant are only gradually diminished as the infant gains independence:

- Breast milk is suitable for digestion and absorption by the immature digestive system.
- For its caloric value, breast milk provides the optimal proportion of water and electrolytes. This prevents fluid and electrolyte imbalance that could develop because of the narrow functional reserve of the immature renal system.
- Breast milk provides immunologic protection to the newborn against infections, thereby compensating for the immature immune system.
- Maternal behavior helps to maintain body temperature, and is crucial for the emotional and intellectual development of the infant.
- Breast milk contains hormones which may contribute to the infant's endogenous hormonal regulatory processes.

The natural point of reaching *metabolic independence* is the completion of weaning, when breast milk is replaced by adult-type food.

We now discuss the basic aspects of lactation and its regulation.

The Structure of the Breast The mammary gland is a modified sweat gland. Each breast contains 15 to 25 lobes, which are in effect separate *compound tubuloalveolar* glands that open independently on the nipple (Fig. 13-29). Before pregnancy, the terminal (lobuloalveolar, secretory) portion of the gland is rudimentary. The secretory product of each lobe is collected into a *lactiferous duct*, which has an expansion near the nipple (*lactiferous sinus*). The secretory alveoli form clusters known as the *lobules*, which are embedded in *loose, cellular, intralobular connective tissue*. The initial segment of the duct system draining the alveoli is the *intralobular duct*. Several intralobular ducts open into each *terminal (inter)lobular duct* which, by definition, drains milk from a whole lobule. Separate lobules, which are drained by distinct terminal (inter)lobular ducts, are demarcated from each other by *dense* interlobular connective tissue.

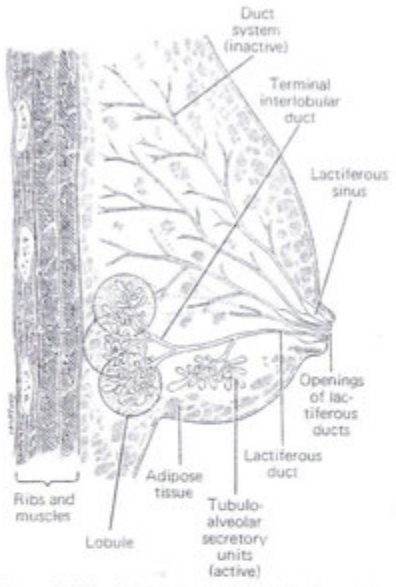


Figure 13-29. Schematic drawing of the female breast. (*Source:* Fig. 23-23 in Junqueira LC et al: *Basic Histology*, 7th ed., Norwalk, CT, Appleton & Lange, 1992.)

The acini and the duct system, until reaching the lactoferous sinus, are lined by an inner *glandular epithelium* and an outer, discontinuous layer of *myoepithelial cells* that rest on a shared basal lamina. The myoepthelial cells are typically absent from breast cancers. The acinar (alveolar) epithelium secretes milk into the lumen. The myoepithelial cells contract in response to oxytocin leading to increased intraluminal pressure and *milk ejection* (milk letdown). The loose intralobular connective tissue of the lactating mammary gland normally contains lymphocytes and plasma cells, which are responsible for producing the immunoglobulins present in milk. The duct system has both secretory and absorptive functions, and actively modifies the composition of milk.

The *nipple* is a multifunctional organ: it is a sensory organ in the milk-ejection reflex, it is the site where the lactiferous ducts open, and it functions together with the areola as *an adaptor that fits the sucking device* formed by the lips, gingiva/hard palate and tongue of the infant. The nipple is a protruding structure that consists of highly innervated modified skin. The nipple and the areola are hairless areas, whose pigmentation depends on race and is promoted by estrogens. Sparse hair may normally grow in the periareolar skin, and may become abundant in hirsutism. The thoracic *spinal*

nerves (T4–T6) communicate sensory and sympathetic (mainly vasomotor) nerves to the breast. The sensory nerves are important in signaling oxytocin release by the posterior pituitary gland, PRL release by the anterior pituitary gland via a hypothalamic action, and mediating sexual arousal. Stress-induced vasoconstriction in the breast may decrease milk production and the availability of circulating oxytocin to the myoepithelial cells. Sympathetic fibers activate smooth muscles and cause *erection* of the nipple upon exposure to cold, mechanical and/or erotic stimulation.

Most of the lymphatic drainage of the breast leads into various groups of *axillary lymph nodes*. The *apical group* is also known as *sentinel lymph nodes* because lymph from all other axillary groups is finally conveyed to them making their histologic evaluation especially important in assessing the spread of breast cancer. By the axillary nodes, the lymphatic drainage of the upper limb and the breast have a common final pathway, which explains the *lymphedema* of the upper limb following *radical mastectomy*. Lymph from the medial part of the breast is drained through the chest wall to *parasternal nodes*.

The shape and size of the female breast is due to the combination of its connective tissue fibers and adipose tissue. The connective tissue fibers spread from the interlobular dense connective tissue to the overlying dermis; these fibers together form the *suspensory ligaments of Cooper*. The suspensory ligaments also serve as septa which, together with the dermis, provide enclosed spaces for the subcutaneous adipose tissue. The adipose tissue of the breast (often referred to as the *fat pad*) accumulates under the influence of estrogen and PRL. The fat pad produces locally acting factors and is a highly active metabolic tissue. Invasive breast cancers often cause retraction of the connective tissue fibers, *dimpling* of the skin and asymmetry of the breast, which is especially obvious upon raising the arms. The cancerous infiltration of the fibers may also cause *retraction of the nipple*.

Functional Development of the Breast Carcinogenesis involves a derangement of the balance between proliferation and differentiation, which implies an intrinsic relationship between these processes during normal development. This relationship is particularly evident in the breast, as exemplified by the existence of endocrine risk factors for breast cancer that are related to the timing of normal developmental events. In the United States, women face a 1:8 lifetime risk of developing breast cancer. The following discussion focuses on the normal developmental process with references to its relationship with carcinogenesis.

The Development of the Mammary Gland Depends on Epithelial-Mesenchymal Interactions The development of several organs depends on epithelial-mesenchymal interactions, including the teeth (dental papilla), hair (dermal papilla of the hair follicle), and the sweat and mammary glands (milk lines). *The mammary gland and hair are mammalian-specific features*

shared by all mammals. The developmental regulation of their morphogenesis involves genetic switches adapted from tooth development.

- *Ulnar-mammary syndrome* is an autosomal dominant disorder characterized by developmental anomalies of posterior elements of forearm, wrist and hand, apocrine/mammary gland hypoplasia and/or dysfunction, diminished to absent axillary hair, abnormal dentition, delayed puberty in males, and genital anomalies. Mutations in *TBX3* (chromosome 12q23-q24.1), a member of the T-box gene family, cause ulnar-mammary syndrome.
- *X-Linked hypohidrotic ectodermal dysplasia* is a congenital disorder resulting in abnormal tooth, hair, and mainly eccrine sweat gland development. The condition is due to the mutations of the *EDA1* gene (the human homolog of the murine *Tabby* gene). Carrier females, who are by definition mosaics for X-linked genes, often present with difficulties with lactation.
- In mice, expression of the *Msx-1* and *Msx-2 homeobox genes* have been shown to be coordinately regulated with the bone morphogenetic protein-2 and -4 (BMP-2 and BMP-4) ligands in the developing mammary gland and teeth.
- The epidermal mesenchymal signaling involves parathyroid hormone-related protein (PTHrP; see Chap. 8). PTHrP secreted by the epithelium targets *PTH/PTHrP receptors* expressed in the underlying mesenchyme. According to experiments with knockout mice, PTHrP is required for epidermal, mammary gland and tooth morphogenesis during embryonic life. PTHrP is probably involved in the branching ductal development of the mammary gland in puberty and pregnancy.

The development of the mammary glands begins during the 4th week of fetal life, when the epidermis forms bilateral *mammary ridges* (milk lines) extending from the axillary to the inguinal regions of the developing limbs. This anatomic arrangement explains both the upper limb and the genital abnormalities in the ulnar-mammary syndrome. By the 6th week, the mammary ridges involute, except in the pectoral area, where normal breast development proceeds. Incomplete involution of the mammary ridges may results in *supranumerous mammary glands and nipples* (*polythelia*) in both sexes.

The Development of the Mammary Gland Occurs in Four Main Stages: Embryonic Morphogenesis, Pubertal Growth, Pregnancy-Induced Growth, and Terminal Differentiation During Lactation During *embryonic/fetal life*, the lobes of each breast are formed by repeated bifurcations of the primary bud. This process leads to the development of the main lactiferous ducts. Although the morphogenesis of the mammary gland proceeds in a manner independent of traditional hormonal influences, the rudimentary mammary gland of the fetus and newborn is responsive to hormones. During pregnancy, the fetal and maternal mammary glands are exposed to essentially identical hormonal environments. When the maternal

mammary gland is released from the influence of estrogens and progesterone, which inhibited lactogenesis (the onset of milk production), active secretion may proceed. Palpable mammary tissue and milky discharge ("witch's milk") normally occur in newborns of both sexes during the first few postpartum days.

After the early postpartum days, the mammary gland remains quiescent and rudimentary until puberty. During *puberty*, under the influence of ovarian *estrogens*, pituitary PRL and locally produced EGF, the duct system grows and bifurcates rapidly. GH, which has intrinsic PRL-like activity might contribute to the process. PTHrP produced by the epithelium may be required for the branching pattern of growth. The developmental process is first evident by the protrusion of the areola from its originally flat surface (primary mound, Tanner stage B2). The initiation of breast development (*thelarche*) is usually the first *noticed* sign of puberty in females. During pubertal development, adipose tissue accumulates to form a secondary mound, and finally the primary mound recesses to yield the contour of the mature breast (Fig. 13-30). When *ovulatory* menstrual cycles begin, a *progesterone-mediated* rudimentary lobuloalveolar development accompanies the cyclic growth and regression of the duct system. In the absence of pregnancy, this mechanism results in a slow overall growth of the mature breast until about the age of 30 years.

During *pregnancy*, several systemic hormones acting jointly cause rapid growth and maturation of the mammary gland. These hormones include estrogens, progesterone, PRL, hCS, GH-V, cortisol, insulin, EGF, and probably PTHrP. Under their influence, the epithelial tissue expands and becomes activated, but (mainly because estrogens inhibit terminal differentiation and progesterone inhibits lactogenesis) milk production does not commence. Estrogens and PRL cause deposition of adipose tissue leading to engorgement of the breasts, which is often accompanied by tenderness (*mastodynia*). *Mammary development by the 2nd trimester becomes sufficient to initiate milk production in cases of premature birth.*

The final stage of development is active *lactation*. During lactation estrogen and progesterone levels are low and several factors lead to *amenorrhea* (see below). Lactation is mainly regulated by PRL, but also requires insulin and cortisol. During lactation, further proliferation of the mammary epithelium is arrested, and the epithelium undergoes terminal differentiation leading to *lactogenesis* (the *onset* of milk production). During the first 1 to 3 days of lactation, milk (colostrum) production is minimal. PRL induced by the stimulation of the nipples by the sucking neonate induces further engorgement of the breasts and increased milk production. The term *galactopoiesis* refers to the *maintenance* of ongoing milk production. Progesterone inhibits lactogenesis thereby contributing to the lack of substantial mammary secretion during pregnancy. However, *progesterone does not inhibit galactopoiesis*; thus, progestin-only contraceptive pills are acceptable during lactation.

Figure 13-30. Stages of breast development according to Marshall and Tanner. Stage B1: Preadolescent—elevation of the papilla only. Stage B2: Breast bud stage—elevation of the areola as a primary mound and enlargement of the areolar diameter. Stage B3: Further enlargement of the breast and the areola, with no separation of their contours. Stage B4: Development of a secondary mound; the primary mound protrudes from the contour of the secondary mound. Stage B5: Mature stage—the areola no longer protrudes from the contour of the secondary mound. (*Source*: Van Wieringen JC et al: Growth diagrams 1965 Netherlands: Second National Survey on 0–24 Year Olds. Groningen, Netherlands Institute for Preventive Medicine NO Leiden, Wolters-Noordhoff Publishing, 1971. Marshall WA, Tanner JM: Variations in the pattern of pubertal changes in girls. Arch Dis Child 44:291, 1969.)

The CCAAT/Enhancer Binding Protein Family of Transcription Factors Plays a Pivotal Role in the Hormonal Control Determining the Proliferative vs. the Terminally Differentiated Phenotype of Mammary Epithelial Cells The ductal growth and lobuloalveolar development of *CCAAT/enhancer binding protein β* (C/EBPβ) knockout mice is rudimen-

tary even when exposed to the adequate ovarian hormones. In wild-type mice, estrogens promote ductal growth and inhibit terminal differentiation by modulating the expression of C/EBPβ isoforms.

The expression of three C/EBPβ isoforms known as the *liver-enriched activating proteins* (LAPs), and a *dominant-negative isoform* of C/EBPβ known as the *liver-enriched inhibiting protein* (LIP) are elevated throughout pregnancy. LIP can form heterodimers with LAPs and suppress their transcriptional activity, thereby preventing terminal differentiation and facilitating continued proliferation. During pregnancy, LIP expression increases over 100-fold, which exceeds the increase in the expression of LAPs leading to a low LAP/LIP ratio (<5). The drop in circulating estrogens at parturition results in a decrease of LIP to preconceptual levels, and an over 100-fold increase in the LAP/LIP ratio occurs. LAPs are apparently positive regulators of the expression of β-casein, a major milk protein: the promoter region of the β-casein gene contains a C/EBP-binding cis-acting element.

Elevated expression levels of LIP have been detected in various human and experimental breast cancers. Estrogens (provided the estrogen receptor is expressed by the cell) promote growth of breast cancer cells, and antiestrogens such as *tamoxifen* are used in the therapy of breast cancer.

The Extracellular Matrix Plays a Crucial Role in the Attainment of the Terminally Differentiated Phenotype In the normal mammary gland, *matrix metalloproteinases* are expressed when remodeling of the basement membrane is required for the physiologic processes including ductal growth during puberty/pregnancy, lobulo-alveolar development during pregnancy and involution after weaning. *Relaxin* is required for MMP expression in the mammary gland during pregnancy (see Box 13-11). Relaxin-deficient knockout mice fail to expand their mammary glands during pregnancy and their young die due to lactation failure in spite of the onset of milk secretion from the underdeveloped gland. Dysregulated expression of matrix metalloproteinases, especially *stromelysin-1*, may play a role in *tumorigenesis* by elimination of a differentiation signal normally provided by the basal lamina and in *tumor progression* by facilitating invasion and metastasis of malignant cells through degradation of the basal lamina and the extracellular matrix.

Mammary-Derived Growth Inhibitor Is an Autocrine Factor Inducing Terminal Differentiation and Lactational Phenotype *Mammary-derived growth inhibitor* (MGDI) is a member of the fatty acid-binding protein (FABP) family (see Chap. 9), and is identical with the heart type FABP. Independent of its fatty acid-binding activity and probably related to its C-terminal 11 amino acids, MGDI functions as an inhibitor of epithelial (but not stromal) cell proliferation. MDGI inhibits ductal growth and induces the formation of fully developed lobulo-alveolar structures. MDGI stimulates

its own epithelial-restricted expression and promotes milk protein synthesis. Selective inhibition of endogenous MDGI expression suppresses the appearance of alveolar end buds and lowers the β-casein level in organ cultures. MDGI and EGF are functional antagonists in their effects on cell proliferation.

Proliferating Mammary Epithelial Cells Acquire DNA Damages that Are Repaired by Various Tumor Suppressor Gene Products, Including hRAD51, BRCA1, BRCA2, and ATM Any proliferating cell is bound to acquire DNA damage. These damages activate DNA repair mechanisms, which may involve *base excision repair* or *nucleotide excision repair* of single-stranded DNA damages, or *recombination-mediated repair* of double-stranded DNA damages. These DNA repair mechanisms are essential for the genetic stability of the cell.

hRAD51 is one of at least five recombination and repair proteins that are involved in ATP-dependent DNA strand exchange reactions, such as recombination-mediated DNA repair, normal meiotic and mitotic recombination (Fig. 13-31). In its absence, the proliferating cells are hypersensitive to ionizing radiation, and develop spontaneous chromosomal abnormalities. The failed repair leads to the presence of the damaged DNA, which is a signal for *checkpoint activation* in the cell cycle and increases the activity of p53 (protein 53), a major tumor suppressor gene. In turn, p53 induces $p21^{waf1/cip1}$ which leads to cell cycle arrest, and Bax which induces apoptosis.

hRAD51 is part of a nuclear multiprotein complex, which includes RNA polymerase II (see transcription, Chap. 3), BRCA1, and BRCA2. The association between hRAD51 and BRCA2 is a direct noncovalent binding involving at least two sites; BRCA1 is only indirectly associated with hRAD51. Both *BRCA* genes were originally identified as "*br*east *ca*ncer genes" (hence their name), whose mutation is responsible for a high percentage of *familial breast cancer* cases. They behave as tumor suppressor genes: the mutation of both copies is required for the development of malignancy (Knudson's two-hit model of tumorigenesis; see also Box 10-3). Individuals carrying a germline mutation in a heterozygous form have a fifteen- to twentyfold higher risk for developing breast cancer than the general population. In these cases, the "second hit" is a somatic mutation that inactivates the only normal copy of the gene in a proliferating cell.

hRAD51/BRCA2/BRCA1 dysfunction is expected to cause cell cycle arrest and apoptosis rather than uncontrolled proliferation. However, the unrepaired DNA damage may have inactivated the p53 mechanism, and breast cancer develops. This model suggests that the mutation of *BRCA* genes results in breast cancer by indirect mechanisms.

BRCA genes are expressed in several tissues. Interestingly, their mutations are typically associated with cancers of hormone-sensitive tissues: mutations of *BRCA1* predispose to *ovarian cancer*, those of *BRCA2* to

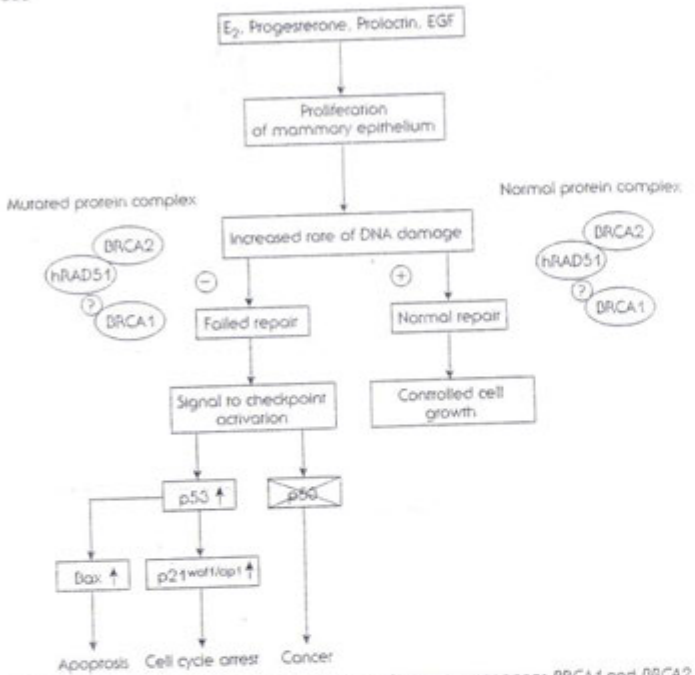


Figure 13-31. A model describing the function of breast cancer genes *BRCA1* and *BRCA2*. Note that the initial damage of the DNA is a normal feature of proliferating cells, and mammary epithelial cells regularly undergo a cycle of limited proliferation and involution as a consequence of exposure to ovarian hormones produced during the menstrual cycle. BRCA proteins are involved in the normal maintenance of genetic integrity by recombination-mediated DNA repair. (*Source:* Modified from Brugarolas J, Jacks T: Double indemnity: p53, BRCA and cancer. Nature Med 3:721–722, 1997.)

prostate cancer. A common feature of the affected tissues is the physiologic hormone-dependent proliferation of cells, and the normal function of the BRCA genes appears to be the recombination-mediated repair of the proliferation-associated DNA damage.

Another gene, whose heterozygous mutation predisposes to breast cancer by similar mechanisms, is the *ATM* gene. The homozygous form of this mutation results in *ataxia telangiectasia*, an ultimately fatal degenerative neurological disease. The ATM gene encodes a PI3-kinase-related enzyme, which is involved in DNA repair and cell cycle checkpoint regulation (see Chap. 9).

The *BRCA* and *ATM* genes are not involved in *sporadic breast cancer.*

The Postlactational Involution of the Breast Is Mainly Due to a Decrease of Circulating PRL At the time of weaning, the stimulation of the nipple gradually decreases, resulting in a decreased secretion of PRL. As a consequence, the breasts involute: the alveoli atrophy, the duct system undergoes apoptosis, and the adipose tissue regresses until approximately prepregnancy conditions are reinstated. The mechanism might also involve the autocrine inhibition of lactation by a factor accumulating in the lumen of the breasts (see The Composition of Milk and the Regulation of Milk Production).

Some aspects of the maturational changes, however, are apparently irreversible: pregnancy carried to term at an early age (up to the age of 30 years) provides relative protection against breast cancer development. Within this group of women, the risk progressively increases with age. Lactation contributes little, although statistically significant, additional protection against breast cancer. If the first pregnancy occurs after the age of 30 years, the risk for breast cancer seems to be increased. This is probably related to the hormonal stimulation of preexisting cancer. It is estimated that the clinical presentation of the breast cancer occurs only about 8 to 10 years after the development of the cancerous clone of cells.

After menopause, when ovarian hormone production ceases, the mammary epithelium and the connective tissue stroma involute substantially. This may lead to significant changes in the shape, size, and consistency of the breasts. Typically the involution is less severe in obese women, whose adipose tissue aromatase maintains higher levels of estrogens by converting adrenal androgens. It is noteworthy that obesity is associated with an increased risk for breast cancer.

Prolactin PRL, a member of the lactogenic hormone group of the helix bundle peptide (HBP) family, developed from a GH-like ancestral gene in teleost fish during evolution (see Chap. 10). The main source of plasma PRL is the lactotroph (mammotroph) cell population of the pituitary gland. Pituitary PRL is secreted in an episodic manner with a pulse frequency of about 90 min. Plasma PRL follows a bimodal circadian pattern with a diurnal and a larger nocturnal increase of pulse amplitude but not frequency. The nocturnal PRL surges are usually associated with non-REM sleep.

PRL in the circulation shows molecular heterogeneity: in addition to the monomeric (23-kDa) hormone, disulfide-linked dimers (big PRL), tetramers (big-big PRL), glycosylated, phosphorylated PRL, and PRL cleavage products are present. A 16-kDa N-terminal fragment of PRL (16K PRL) has been demonstrated to exert its effects via a distinct receptor and inhibit angiogenesis in rodents, but the mechanism might not be operational in humans. PRL circulates in plasma with a half-life of about 20 to 30 minutes. PRL is mainly eliminated by hepatic receptor-mediated internalization and proteolysis, and by renal filtration of the intact hormone.

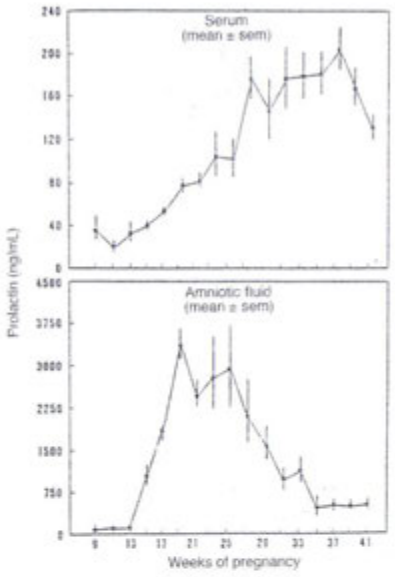


Figure 13-32. Prolactin (PRL) concentrations in maternal serum and amniotic fluid during gestation. About 50% of amniotic fluid PRL is glycosylated; it is mainly synthesized and constitutively secreted by the décidua, which explains the different time course of PRL in the two fluid compartments. The lactotroph cell develops last among anterior pituitary hormones. The fetal pituitary starts secreting significant amounts of PRL only after the 25th week (now shown). (*Source:* Kletzky OA et al: Dynamics of human chorionic gonadotropin prolactin, and growth hormone in serum and amniotic fluid throughout normal human pregnancy. Am J Obstet Gynecol 151:876–884, 1985.)

PRL is widely expressed in tissues including the decidua, normal and tumorous mammary gland, endothelial cells, a specific set of neurons in the CNS, and T lymphocytes. At least the decidual expression is driven by a promoter distinct from that utilized in the pituitary gland. Extrapituitary PRL may be involved in local actions. PRL is present in several biologic fluids, including milk, CSF, and amniotic fluid (Fig. 13-32).

The PRL receptor (PRL-R) is a member of the cytokine receptor family; it is closely related to the GH receptor, and utilizes the Jak–STAT pathway. During evolution PRL acquired its classical target with the appearance of mammals. However, due to the widespread expression of PRL receptors, PRL exerts several biologic actions (Table 13-12).

Similar to GH, PRL is under a dual hypothalamic control (Fig. 13-33). However, PRL is unique among the anterior pituitary hormones in that it is primarily under an inhibitory control: *disruption of the pituitary stalk*

Table 13-12 Selected Biologic Actions of Prolactin

Category of action	Biologic effect(s)
Lactation	Increases milk yield.
	Promotes gene expression of milk proteins.
	Stimulates biosynthesis of lactose.
	Induces lipoprotein lipase in the breast.
	Stimulates reabsorption of sodium from milk.
Reproduction	Hyperprolactinemia inhibits gonadotropin secretion by hypothalamic actions.
	In nonlactating individuals: hyperprolactinemia induces galactorrhea amenorrhea syndrome in females, and impotence with or without galactorrhea in males.
	During lactation: PRL and nipple stimulation per se inhibit gonadotropin secretion; inhibits follicular growth and aromatase expression.
	In rodents: PRL is lutreotrophic (LH-like) in females and synergizes with androgens to stimulate male accessory gland function.
Water and electrolyte homeostasis	Milk production is a regulated loss of fluids and electrolytes.
	Electrolyte/volume homeostasis provides important feedback to PRL secretion.
	Stimulates intestinal absorption of sodium.
	Renal action of PRL on sodium reabsorption is minimal in humans.
	Stimulates renal 25(OH)vitamin D 1α-hydroxylase, thereby increasing intestinal Ca^{2+} and phosphate absorption (weak action).
Effects on the ectoderm and the skin	Mammary gland (a skin appendage): stimulates proliferation of epithelium during puberty and pregnancy, promotes maturation at the onset of lactation.
	Growth factor for keratinocytes (in culture).
	Regulates seasonal change of pelage in sheep.
Metabolism	Trophic factor for pancreatic islets.
	Stimulation of extrapancreatic PRL receptors may induce insulin resistance.
	Accumulation of breast adipose tissue especially after priming by estrogens.
	Modulates steroidogenesis in the adrenal cortex and the gonads.
Growth and morphogenesis	Depending on the signals from the extracellular matrix, stimulates either the growth or the differentiation of mammary epithelium.
	Inhibits morphogenesis of tadpoles (antagonizes T_4).
Immunoregulation	Promotes lymphocyte proliferation.
	May be involved in the mechanism of autoimmune arthritis.
Behavior/sleep	Stimulates REM sleep and decreases aggressive behavior.

Abbreviations: PRL, prolactin; LH, luteinizing hormone.

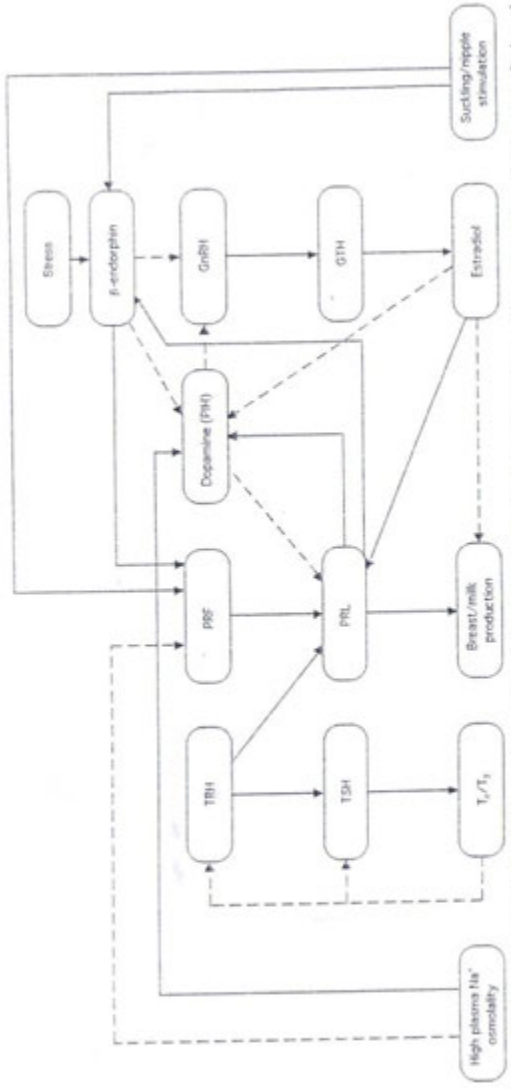


Figure 13-33. A model of the regulation of prolactin (PRL) secretion. Compare with Figs. 11-11 and 13-11. Several mechanisms are displayed which do not necessarily occur simultaneously. TRH, thyrotropin-releasing hormone; TSH, thyroid stimulating hormone; T_4/T_3, thyroid hormones; PRF, PRL-releasing factor(s); PIH, PRL-inhibiting hormone; GnRH, gonadotropin-releasing hormone; GTH, gonadotroph hormone(s). See text for details.

results in hyperprolactinemia. Note that this hyperprolactinemia is moderate, and usually does not reach the plasma PRL levels associated with PRL-secreting tumors of the pituitary gland. As opposed to most hypothalamic neurohormones, which are peptides, the *PRL-inhibiting factor* (PIF) is *dopamine*. Dopamine is produced by the *tuberoinfundibular* (TIDA) and in part by the *tuberohypophyseal* (THDA) dopaminergic neurons; the perikarya are located mainly in the arcuate nucleus of the hypothalamus. Dopamine acts on D_2 dopamine receptors (expressed from chromosome 11q22), which are coupled by $G\alpha_i$ to inhibit adenylyl cyclase activity. In addition, D_2 receptor stimulation activates K^+-channels and decreases intracellular concentration of Ca^{2+}. These effects inhibit both the release and (via the Pit-1 transcription factor) the synthesis of PRL. PRL stimulates dopaminergic activity of the TIDA, which constitutes a *short-loop negative feedback*. No hormone has been identified that would provide a long-loop negative feedback signal to PRL secretion in humans.

During the reproductive ages, PRL levels are higher in women than in men (Table 13-13); the difference is attributed to the difference in circulating estrogens. *Estrogens* stimulate PRL secretion mainly by three mechanisms:

- In concert with the Pit-1 transcription factor, estrogens directly stimulate PRL gene expression in the lactotroph.
- Estrogens inhibit the turnover of dopamine (i.e., inhibit the activity of TIDA) and decrease pituitary D_2 receptors which leads to disinhibition of PRL secretion.
- Estrogens induce lactotroph hyperplasia (chronic exposure to high levels of estrogens). Estrogens are trophic factors for PRL-secreting pituitary adenomas (*prolactinoma*; see Chap. 10). By this mechanism, oral contraceptives may lead to "postpill amenorrhea," usually manifesting as galactorrhea–amenorrhea syndrome. Development of hyperprolactinemia may sometimes present with symptoms while the individual continues her contraceptive pill regimen. In these cases unusually light menstrual flow (hypomenorrhea) may be observed during the days of steroid withdrawal and, in rare cases, mild galactorrhea may be continuously present.

Table 13-13 Normal Values of Serum Prolactin

Physiologic conditions	Concentration (ng/mL)
Adult male, postmenopausal female	3–15
Adult cycling female	5–25
Typical prolactinoma patient	>100
Pregnant female, third trimester	100–250
Newborn (1–7 d)	30–250
Prepuberal child	<10

Whereas *estrogens* are very important in the preparation of the breast for lactation and in inducing lactotroph hyperplasia/hyperprolactinemia during pregnancy, they *are not involved in the regulation of PRL secretion during lactation.*

Ample experimental evidence suggests the involvement of *PRL-releasing factor* (PRF) in the physiologic regulation of PRL secretion, including PRL release induced by nipple stimulation and stress. The chemical nature of the physiologic PRF(s), however, has remained elusive. A number of putative PRFs have been proposed to fulfill this function including the following selected examples:

- TRH is not a physiologic PRF during lactation. In *primary hypothyroidism*, TRH release from the median eminence is increased and stimulates both TSH and PRL secretion. The resulting hyperprolactinemia inhibits GnRH secretion and causes *galactorrhea-amenorrhea syndrome* in women, a condition similar to *lactational amenorrhea*. High levels of estrogens (such as those seen in pregnancy) may sensitize the lactotroph cells to the action of TRH by inhibiting their expression of the TRH-degrading ectoenzyme (see Fig. 11-11).
- VIP, a member of the secretin-GHRH family, produced by parvocellular neurons of the paraventricular nucleus and released into the pituitary portal circulation stimulates PRL secretion in vitro via activating adenylyl cyclase. At least in rodents, the expression of VIP is enhanced during lactation and after adrenalectomy, and bilateral lesion of the paraventricular nucleus inhibits suckling-induced release of PRL. VIP is also produced by the lactotroph cells and stimulates PRL secretion in an autocrine/paracrine manner.
- In contrast with the vasodilatory VIP, the vasoconstrictor *endothelin-1* (ET-1) acting on endothelin A receptors inhibits PRL secretion both in humans and rodents. It is currently unclear whether the ET-1-mediated inhibition is a physiologic regulator of PRL secretion. At least in rodents, ET-1 is produced by the lactotroph cells suggesting an autocrine function.
- *Oxytocin* is released both in the median eminence and in the posterior pituitary, and may stimulate PRL secretion. However, whereas audiovisual stimuli related to the infant (such as baby cry) stimulate oxytocin secretion and cause milk let-down, plasma PRL does not rise without nipple stimulation.
- *Angiotensin-II* (A-II) stimulates PRL secretion in vitro. A-II is secreted by pituitary gonadotrophs in response to GnRH. This explains why an acute GnRH challenge may increase plasma PRL. PRL is a weak regulator of renal Na^+ reabsorption, intestinal Na^+ absorption, and thus a regulator of plasma volume and osmolality. A-II primarily stimulates renal Na^+ reabsorption via aldosterone, but may cause Na^+ retention in part via PRL.

Various neurotransmitters influence PRL secretion via PRF and/or dopamine. Some of these neurotransmitter systems are targeted by commonly used drugs:

- *Histamine* has a dual role in PRL secretion. Via H_2 receptors, histamine inhibits PRL. Thus, H_2 receptor blockers (used for suppressing gastric acid production) stimulate PRL secretion and may cause gynecomastia in men. In contrast, H_1 receptor blockers (used for alleviating allergic symptoms) inhibit PRL secretion. This indicates a PRL-simulating role of histamine via H_1 receptors.
- *Serotonin* and *adrenaline* (produced by CNS neurons) stimulate PRL secretion. At least in rodents, the serotonin-induced release of PRL is mediated by PRF(s), mainly VIP.
- Morphine and its derivatives are strong stimuli of PRL secretion. These drugs utilize the physiologic mechanism of endogenous opioid peptides that are involved in nursing-induced PRL release.

The preeminent physiologic role of PRL is the regulation of lactation. In the presence of physiologic concentrations of insulin and cortisol, plasma PRL levels and the emptying of the mammary gland are the main determinants of milk yield (see The Composition of Milk and the Regulation of Milk Production). The three most important regulators of PRL secretion are nipple stimulation, hydration status, and stress.

- *Sucking the nipple* is the main regulator of PRL secretion during lactation. During any stage of lactation, the more the daily nipple stimulation, the higher the average plasma PRL level. Nocturnal breastfeeding is an important stimulus in maintaining high daily PRL output. When the mother is not nursing for several hours (a typical setting for working women), plasma PRL levels are very low and pulsatile secretion is suppressed. Upon nursing or using a breast pump, PRL secretion is rapidly stimulated. When spontaneous nursing activity (as dictated by the needs of the infant) is uninterrupted, PRL levels fluctuate in a pulsatile manner and follow a circadian pattern.
- *Hydration status* is an important regulator of PRL secretion, although no direct relationship between plasma osmolality and PRL levels has been demonstrated. *Milk production is a regulated fluid and electrolyte loss.* In concert with stimulation of mineralocorticoid receptors in the lactiferous ducts, PRL promotes sodium reabsorption from milk. Thus, milk secretion represents the loss of hyponatremic fluid, which could lead to hypovolemia and increased plasma osmolality. Under hyperosmotic conditions when ADH is increased to the level to yield a concentrated urine, PRL secretion is suppressed at least in part by an increased dopaminergic tone. Thus, to maintain milk yield, lactating women need to drink enough fluid to achieve *diluting* renal function. In essence, this calls for a physiologic primary polydipsia. Indeed, at a stage of lactation when fluid loss with milk is 500 mL/d (approximately half-maximal milk yield), lactating women drink about 2 L more water than nonlactating women. Angiotensin-II, which is elevated upon hypovolemia, stimulates thirst, and the secretion of ADH and PRL. This mechanism ameliorates the decrease in PRL and maintains milk production in cases of mild dehydration. It is noteworthy that this process is similar to that of salmonids: for spawning, salmonids swim from seawater to freshwater, a hyposmotic environment that induces

PRL secretion. In turn, PRL stimulates sodium reabsorption by acting on the gill and the skin. A role for PRL has been suggested in decreasing the volume of amniotic fluid by stimulating sodium reabsorption in humans.

- *Stress* has a complex effect on PRL secretion. In general, stress stimulates PRL secretion in both sexes. Unless the blood samples are collected under stress-free conditions, this mechanism may lead to a mistaken conclusion of hyperprolactinemia. On the other hand, stress inhibits nursing-induced PRL secretion in agreement with the everyday observation that stress inhibits lactation. Note, however, that stress-related inhibition of lactation includes additional components.

Increased secretion of PRL is *associated with non-REM sleep* in both sexes. PRL *stimulates REM sleep*. It has been suggested that PRL expressed by CNS neurons is the physiologic source of REM-stimulating PRL, but under conditions of hyperprolactinemia sufficient amount of PRL may cross the choroid plexus by a receptor-mediated transport to exert a somnogenic action.

Most women are amenorrheic during lactation. The mechanism of *lactational amenorrhea* is only partially understood. Immediately after parturition, the corpus luteum renders the pituitary gland unresponsive to GnRH. Hyperprolactinemia and the suckling stimulus per se inhibit GnRH secretion. The decreased pulse frequency of GnRH is a stronger inhibitor of LH than FSH secretion. In spite of the increase in plasma FSH, estrogen secretion remains low because of the ovarian actions of hyperprolactinemia (The Puerperium). Although in theory lactational amenorrhea could be utilized as a method of contraception, it is unreliable probably because todays' lifestyle does not permit sufficiently frequent (13 to 18 times per day) nursing. As a consequence, PRL is insufficiently elevated to antagonize follicular maturation induced by the normally increased FSH (see Fig. 13-28). If pregnancy occurs during the first ovulation, its discovery may be delayed because the continued amenorrhea is assumed to be related to lactation. In spite of breastfeeding at a frequency that leads to amenorrhea in most women (6 to 7 nursings per day), a minority of women experience menstrual periods during lactation. In some of these women, milk production ceases with the onset of the ovarian/endometrial cycle mainly because of the inhibitory action of estrogens on milk secretion. For reasons unknown, other women continue milk production in spite of the endogenous production of estrogens.

Prolactinomas secrete PRL in an autonomous fashion. Although endogenous production of dopamine is insufficient to suppress PRL to the physiologic range, its inhibitory action can be demonstrated by administration of D_2 receptor antagonist drugs, such as *metoclopramide*. The functional evaluation of prolactinomas may also include provocation tests with bolus injections of TRH or VIP. Prolactinomas may be treated by the dopamine agonist *bromocriptine* (see Fig. 10-2). The shrinkage of the tumor during bromocriptine therapy indicates that cyclic AMP mediates important tro-

Table 13-14 Composition of Human Colostrum and Milk in Comparison with Cow's Milk

	Human colostrum	Mature human milk	Mature cow's milk
Caloric content (kcal/L)	671 (588–730)	747 (446–1192)	701 (587–876)
Fat (g/100 g milk)	2.9	4.5	3.8
Cholesterol (mg/L)	280	139	110
Lactose (g/100 mL)	5.3	7.3	4.8
Protein (g/100 mL)	2.7	1.0	3.3
Whey : casein ratio	—	70 : 30	22 : 78
Albumin (g/100 mL)	—	0.4	0.4
Globulin (g/100 mL)	1.5	0.2	0.2
Casein (g/100 mL)	1.2	0.4	2.7
Total ash (g/L)	3.08	2.02	7.15
	Selected minerals		
Na (mEq/L)	48	7.8	22
K (mEq/L)	15–19	13–14	36
Ca (mg/100 mL)	30	34	117
P (mg/100 mL)	15	15	92
Fe (mg/L)	0.1	0.3	0.5
I (mg/L)	0.045–0.450	0.044–0.093 (mean, 0.061)	0.036–1.05 (mean, 0.116)
	Selected vitamins		
Vitamin D (IU/L)	—	22	14
Vitamin K (μg/L)	—	15.0	60.0
Vitamin C (mg/L)	72	40	11
Carotenes (mg/L)	1.37	0.25	0.37

phic action in these cells. This finding is comparable with the somatotroph hyperplasia caused by the GHRH-cyclic AMP pathway.

The Composition of Milk and the Regulation of Milk Production

Milk Yield and the Composition of Milk Depend on the Stage of Lactation The composition of milk is summarized in Table 13-14. *Colostrum* (early milk) is produced during the first 2 to 4 days postpartum. Colostrum is yellowish due to its high β carotene content, and has a high protein concentration with a high whey : casein ratio. Colostrum is gradually replaced by transitional milk (days 6 to 10) and finally *mature milk*. Milk yield, which is mainly determined by the demand of the infant, rapidly increases during this period: from about 120 mL on the 2nd or 3rd day postpartum to 240 to 300 mL by the end of the 2nd week. The maximum milk yield is approximately 0.8 to 1.2 L/d, which is usually achieved during the 6th to 7th months of lactation, right before supplementation and/or weaning begins.

After an accumulation of milk in the breast during a 2- to 4-h period of nonsuckling, the composition and caloric value of split milk displays

marked differences: *foremilk* (milk ejected first) differs from *hindmilk* (milk ejected last). This difference must be taken into consideration when breastfeeding twins.

The Role of Colostrum Is to Provide Defense Factors for the Newborn The immune system of the newborn is not fully developed, and even if it were, it cannot rely on immunologic memory. Breast milk and in particular, colostrum, represents a significant protective mechanism. Breastfed infants are less likely to acquire infections than formula-fed babies. Defense factors in human milk include

- *antimicrobial agents*: secretory IgA (antigen-binder), lactoferrin (iron chelator), lysozyme (muramidase, degradation of bacterial wall), mucins and oligosaccharides (antirotavirus activity as a decoy receptor), and digestive products of milk lipids (disruption of viral envelope);
- *bifidus agent*: milk promotes colonization of the intestines by the harmless *lactobacillus bifidus*, thereby preventing colonization by pathogenic bacteria.
- *antiinflammatory factors* (antioxidants, epithelial growth factors, cellular protective agents, and enzymes that degrade mediators of inflammation);
- *immunomodulators*: IL-1 (activator of T cells), IL-6 (enhances IgA production), TNF-α (enhances secretory component production of IgA), TGF-β (induces isotype switching of antibodies in B cells), and antiidiotypic antibodies;
- *leukocytes* (neutrophils, macrophages, and lymphocytes).

Breast Milk Is a Liquid Diet that Simultaneously Satisfies Thirst and Hunger The *protein composition* of human and cow's milk is different. Casein is the mixture of cheese-generating proteins in milk (*case*, cheese) that precipitate on acidification to pH 4.0. The supernatant of this precipitate is known as *whey*, which contains albumins, immunoglobulins, and *whey acidic protein* in high concentrations. Cow's milk contains more casein than human milk does. Casein is not as rich in essential amino acids as albumin, and is more difficult to digest than albumin. Thus, the nutritional value of human milk proteins is higher.

The promoter region of most milk proteins has multiple cis-acting elements and requires the coordinated function of several *hormone-regulated transcription factors*, including STATs and steroid hormone receptors (see also C/EBPβ in Functional Development of the Breast). Via STAT5, PRL stimulates the expression of several milk proteins, including β-casein, whey acidic protein, UDP-galactosyl transferase, and α-lactalbumin. Stimulation of the glucocorticoid receptor by physiologic levels of cortisol is required for the expression of these proteins. The relatively high type II *11β-hydroxysteroid dehydrogenase* (11βHSD) *activity* in the fat pad and the epithelium of the mammary gland during pregnancy significantly declines after parturition thereby allowing normal glucocorticoid action in lactogenesis and galactopoiesis.

The main *carbohydrate* in milk is lactose, a disaccharide formed by galactose and glucose. About 42% of the caloric value of mature human milk is derived from lactose. Lactose synthesis requires two enzymes in the mammary epithelium: the ubiquitous *UDP-galactosyl transferase* and the mammary specific *α-lactalbumin*, which is a major whey protein. The latter is required for the *lactose synthase* activity; without it, UDP-galactosyl transferase may only glycosylate polypeptides. Immunoreactive α-lactalbumin has been demonstrated in about 70% of various breast cancers and can be used for the detection of micrometastases. Minor quantities of glucose, galactose and fucose are also present in milk; fucose may be part of the "bifidus factor."

On the average, about 50% of the caloric value of mature human milk is derived from lipids. PRL stimulates lipoprotein lipase activity in the breast, which aids synthesis of milk fat by the mammary epithelium. Unlike proteins, which are secreted by exocytosis of secretory vesicles (*merocrine secretion*), lipids enter milk as *fat globules* generated by pinching off the budding apical cytoplasm which contains large lipid droplets (*apocrine secretion*). Some lipid-laden epithelial cells become shed altogether and appear in milk as foam cells. Breast milk contains *fatty acids* that are important for myelinization and the maturation of brain. Infant formulas are deficient in the same fatty acids, which may contribute to a statistically significant difference in IQ test scores observed between 7- to 8-year-old children who were breastfed or formula-fed as infants.

Breastfed infants are less likely to develop *obesity* than formula-fed babies, in spite of the same caloric content of the two diets, and an essentially identical distribution of caloric content among the three main nutrients. The difference may be explained at least in part by the difference in work on behalf of the infant to obtain food. In spite of the oxytocin-mediated milk-ejection reflex, the infant has to suck hard to obtain sufficient volumes of milk. In contrast, sucking from the bottle requires minimal effort, and babies tend to overeat. The appetite-suppressing hormone leptin is present in human breast milk, and is absorbed from the GI tract of the newborn. Milk-derived leptin might be a physiologic regulator of appetite.

The *energy requirement* of the infant during the first year is approximately 100 kcal/kg body weight/24 h. The *fluid requirement* under normal circumstances is about 130 mL/kg/24 h. The caloric content of milk is approximately 70 kcal/100 mL. Thus, 100 kcal/kg energy is delivered by 143 mL breast milk. This means that the breastfed infant does not require fluid supplementation. However, infants may become rapidly dehydrated under conditions such as hot climate, diarrhea, or vomiting. Fluid supplementation with fruit juice (for water loss) or commercial pediatric electrolyte solutions (for electrolyte loss) is important under these circumstances.

Milk Is an Isosmotic Solution Containing Low Concentrations of Sodium Although milk is isosmotic, its *osmotically active constituents* are quantitatively different from those of plasma. Instead of sodium, which is the main determinant of plasma osmolality, lactose accounts for about 70% of the osmolality of milk. The low sodium concentration in milk is due to the actions of PRL and mineralocorticoid receptor stimulation (see below). The latter also increases K^+ concentration in milk, which is needed for the expanding ICF of the growing infant. The low sodium concentration in milk prevents hypernatremia in the infant. In term infants, the osmolality of maximally concentrated urine is 500 to 700 mOsm/kg water, i.e., about half of that in adults (1200 mOsm/kg). Thus, unlike adults who tolerate physiologic (0.15 *M*) saline as an intravenous fluid *maintenance*, newborns require a mixture of 1 volume physiologic saline: 3 volumes 5% glucose. This isosmotic fluid is designed to be closer to the sodium load infants receive with breast milk. Cow's milk contains enough sodium to induce hypernatremia in the newborn human infant.

The *mineralocorticoid receptors* are preferentially expressed in the duct system of the mammary gland. This arrangement is similar to that observed in salivary glands and eccrine sweat glands (see Chap. 12). As pointed out, 11βHSD is expressed in the nonlactating mammary epithelium. The function of this enzyme in the kidney is to assure that the mineralocorticoid receptor is not stimulated by glucocorticoids. The postpartum decline of 11βHSD suggests that sodium reabsorption and potassium secretion by the lactating mammary gland is regulated by cortisol. This mechanism may coordinate fuel and electrolyte composition of milk.

Lactation and Milk Play a Central Role in the Calcium Homeostasis of the Mother and the Infant Milk contains several essential components of *calcium homeostasis*, including magnesium, calcium, phosphorous and vitamin D. A significant portion of phosphorous is present in milk in the form of casein, a highly phosphorylated protein. The vitamin D content of milk is often insufficient; the supplementation of the infant is influenced by the seasonal changes of exposure to sunlight.

Milk contains several hormones (see second section on page 595), including *PTHrP*, a product of the mammary epithelium. PTHrP is an autocrine factor of the mammary gland that stimulates calcium transport into milk. PTHrP is expressed by breast cancer cells and, depending on the amount secreted, its PTH-like action may either cause *humoral hypercalcemia of malignancy* (systemic effect) or localized osteolytic metastatic bone lesions (see also in Chap. 8).

Maternal calcium homeostasis is different during pregnancy and lactation. Because placental 25(OH)vitamin D 1α-hydroxylase is absent after birth and estrogens are low, renal 1α-hydroxylase activity is maintained by two hormones: PRL and PTH. Because the effect of PRL is weak, *secondary hyperparathyroidism* normally develops during lactation and leads to a significant (6–7%) decrease of bone mineral content by the 6th month of lactation. Dietary calcium supplementation of lactating women is therefore exceedingly important. Maternal vitamin D stores decline during lactation as indicated by the decrease in plasma 25(OH)vitamin D levels. The decrease is due to losses of vitamin D with milk.

Breast Milk Is an Adequate Source of Trace Elements Such as Iron and Iodide The absorption of *iron* is facilitated from human milk by the presence of lactoferrin. Iron is absorbed together with intact lactoferrin from the GI tract of the newborn. Although the iron concentration of cow's milk is higher, it is less absorbed probably related to the very high concentration of casein, and the generation of ferrous phosphate.

Iodide is actively transported into milk by mechanisms similar to those involved in its transfer into the follicular lumen of the thyroid gland (see Figs. 11-3 and 11-10). Because the transfer of thyroxine with breast milk is minimal, the newborn relies on the iodide content of breast milk to support endogenous synthesis of thyroid hormones.

Milk also contains other trace elements such as *selenium*, *copper*, *zinc* and *fluoride*.

Protein and Peptide Hormones Are Present at High Concentrations in Breast Milk Several *hormones*, mainly peptides/proteins, have been demonstrated in milk, including leptin, PRL, releasing hormones (TRH, GnRH), growth factors (EGF, IGF-1), relaxin, PTHrP, and the lipophilic amino acid derivative melatonin. These hormones often reach concentrations in milk exceeding that in plasma. The GI tract of the newborn and especially the prematurely born infant is not mature; thus, these hormones may escape degradation and may be absorbed as intact, biologically active molecules. Some of these hormones, such as EGF, may act locally on the GI tract and promote the proliferation, maturation or function of the intestines. The impact of certain milk hormones in experimental animals has been demonstrated, but definitive human studies have not yet been reported.

Most of the PRL in breast milk is derived from the circulation by transcytosis. However, PRL is also expressed by the mammary epithelium. Because pituitary PRL is a mandatory requirement for lactation, mammary PRL presumably does not reach the basolateral surface of the epithelium where the PRL receptors are preferentially located. Together with appropriate attachment to the basal lamina, the epithelial polarity is lost in breast cancer. PRL has been suggested to function as an autocrine/paracrine growth factor under these circumstances.

Steroid hormones are also present in milk. Vitamin D is a prohormone steroid, and milk is a physiologic source of vitamin D for the newborn. Estrogens and progesterone are low during lactation. However, steroid compounds produced by the regressing corpus luteum are present in colostrum, and may contribute to the physiologic jaundice of the newborn by

competing with bilirubin for the limited glucuronyl transferase activity of the maturing liver.

Milk Production Is Mainly Regulated by Two Types of Humoral Signals: Systemic Hormones and Locally Acting Autocrine Factors Galactopoiesis is stimulated by *systemic hormones*. PRL, cortisol, and insulin stimulate synthesis and secretion of milk, oxytocin induces milk-ejection. The receptors of hydrophilic galactopoietic hormones are located in the basolateral membrane of the alveoli.

Humoral factors acting locally on receptors at the luminal surface of the alveoli inhibit milk secretion. A partially characterized protein hormone appears to mediate the *local autocrine inhibition of milk production*. Its function is to adapt milk production to the demand of the infant. If milk is completely emptied from the breast, the galactopoietic function becomes maximal. Milk retention decreases galactopoiesis even in the presence of appropriate circulating galactopoietic hormones. Preferential feeding from one breast may therefore result in different milk yields of the two breasts.

As mentioned, demand of the infant is the main determinant of milk production. The decreasing sucking activity of the infant during weaning results in the accumulation of the local inhibitor in the breast and the decrease of circulating PRL. Both factors are important components in the *cessation of lactation*. In several species, weaning, tooth eruption, and gastrointestinal function are coordinated during development. It is recommended that until tooth eruption (about 6 months of age), infants should receive no supplementation to breast milk. Supplementation of food may be initiated without weaning if it is given *after* breastfeeding. During the weaning process, supplemented food *precedes* breastfeeding, which satisfies appetite, decreases milk ingestion and nipple stimulation. Weaning in western cultures is usually completed by 1 year of age.

Breastfeeding is the best biologic support for the infant. In spite of advocacy by pediatricians, many healthy women *elect* formula feeding instead of breastfeeding: in the United States in 1993 less than 56% of women attempted breastfeeding, and less than 20% of women nursed their infants beyond 6 months postpartum. When required, *suppression of lactation* can be accomplished by several approaches:

- The chest/breasts are tightly wrapped with a cloth. Stimulation of the nipples is avoided and fluid intake is minimized. The initially accumulating milk inhibits further secretion by a local action. Nonsteroidal antiinflammatory drugs (NSAIDs) such as indomethacin or aspirin can be used to control the pain associated with the engorgement of the breasts. These drugs also inhibit the local synthesis of vasodilatory prostaglandins, and may decrease mammary blood flow and milk yield.
- Pharmacologic suppression of lactation is a controversial issue. The dopamine receptor agonist bromocriptine and (before the availability of bromocriptine) estrogens have been used for this purpose.

Some women experience difficulty with breastfeeding. Total absence of lactation in postpartum women may suggest *Sheehan's syndrome*, a postpartum infarction of the pituitary gland (see Chap. 10). In other cases, insufficient milk production can be overcome by

- Appropriate hydration of the mother and stress-free environment.
- Complete emptying of the breast every 3 hours: after the infant stops sucking, the remaining milk is removed by a breast pump. This method eliminates the locally acting inhibitor of milk secretion, which otherwise accumulates in the lumen of the mammary gland because the initially weak sucking activity of the newborn.
- Pharmacologic approaches: dopamine antagonist drugs (such as metoclopramide) are in use when other attempts have failed. Recombinant human GH may also improve milk-yield; this expensive drug has only been used in limited clinical tests.

ONTOGENY OF THE REPRODUCTIVE SYSTEM

OBJECTIVES

1. Discuss the main checkpoints of sex determination: chromosomal sex, gonadal sex, genital sex, sexual/gender identity, and sexual orientation. Define the terms *hermaphroditism*, *pseudohermaphroditism*, and *ambiguous genitalia*.
2. Discuss the structure and main genes of the X and Y chromosomes. Describe the process of *lyonization* of the X chromosome, its role and physiologic consequences, particularly in sex determination. Discuss the significance of the *Barr body*.
3. Discuss intrauterine sexual development. Discuss the autosomal and sex chromosomal genes that are involved in the determination of testicular versus ovarian development from the *indifferent gonad*. Define the relationship between the development of the gonads and the adrenal glands. Contrast the timing of events in males versus females. Identify the onset of testicular hormone production (AMH and testosterone), and identify the critical timeframe of androgen exposure of the external genitalia in the process of masculinization. Describe the role of androgens in the development of Wolffian duct structures and the prostate, and identify dihydrotestosterone-dependent processes.
4. Discuss potential mechanisms of deranged intrauterine sexual development. Discuss chromosomal translocation of the *SRY* gene and the consequences of duplication and deletion of the *DSS* locus. Discuss deletions of *SF-1*, *WT1* and the *SOX9* genes. Discuss *androgen insensitivity syndrome* and enzyme defects (including the various types of *adrenogenital syndrome*, *type II 5α-reductase deficiency*, *aromatase deficiency*, and *17βHSD3 deficiency*), which may result in pseudohermaphroditism. Discuss the physiologic basis of the

potential intrauterine treatment of the classic form of 21-hydroxylase deficiency.

5. Discuss the four phases of increased pituitary gonadotropin secretion in females. Compare and contrast these phases with the three corresponding phases in males. Describe the intrauterine, postpartum, and pubertal activation of testosterone secretion. Identify the mechanisms resulting in pubertal development. Describe the physiologic changes associated with puberty. Be familiar with the *Tanner staging* of pubertal development and the timing of developmental milestones.
6. Discuss deranged pubertal development and its potential causes. Discuss delayed puberty, sexual infantilism (*absent puberty*), and *hypergonadotropic* and *hypogonadotropic hypogonadism*. Discuss *Klinefelter's* and *Turner's syndromes*. Discuss *precocious puberty* leading to either *isosexual* or *contrasexual* development. Compare and contrast *complete* (*true*) and *incomplete precocious puberty*.
7. Define the terms *menopause*, *andropause*, and *adrenopause*. Discuss the mechanism of perimenopause and the hormonal regulation in postmenopausal women. Distinguish early and late manifestations of menopause. Discuss the physiologic mechanism of *hot flashes* in early menopause, postpartum, and after orchiectomy. Review postmenopausal osteoporosis. Discuss postmenopausal alterations in plasma lipoproteins and the risk for cardiovascular disease. Discuss the benefits and risks associated with hormone replacement therapy in postmenopausal women.

Introduction

During embryonic development, the initially indifferent (bipotential) gonad differentiates into either an ovary or a testis determined by the sex chromosomes present. Human gonadal sex determination is synonymous with testis determination. The presence of the Y chromosome results in the development of a testis, which secretes AMH (the product of Sertoli cells) and *androgens* (the product of Leydig cells) during embryonic life.

- AMH is required for the regression of the Müllerian ducts, the primordia of the female genital tract (Fallopian tubes, uterus, and the upper third of the vagina).
- If the inherently female primordial external genitalia are exposed to sufficient androgen receptor stimulation within the critical period of embryonic development, scrotum and phallus develop. The androgens are also needed for the obliteration of the *urogenital sinus*, the development of the prostate, and the Wolffian duct-derived structures (vas deferens, accessory glands).

In the absence of the Y chromosome, ovaries develop that secrete neither AMH nor androgens during fetal life.

- In the absence of AMH, the female genital tract develops from the Müllerian ducts by default.

- In the absence of androgen exposure, the lower two-thirds of the vagina (from the urogenital sinus) and the vulva develop, also by default.

During the childhood years, psychological sexual identity develops, which is primarily based on the appearance of the external genitalia. During pubertal development, the hypothalamic–pituitary gonad axis is activated, and the gonad secretes the sex steroids appropriate for the sex of the individual, leading to either male or female secondary sexual characteristics, which reaffirm sexual identity, enhance interest in sexuality, and direct sexual orientation toward the opposite sex.

As we shall see, this simplified and seemingly deterministic developmental pattern is not always followed. However, it allows us to consider the main checkpoints of *sex determination*:

- *Chromosomal sex:* The presence of the Y chromosome determines male chromosomal sex.
- *Gonadal sex:* The presence of testis determines male gonadal sex. In cases of *pseudohermaphroditism*, when there is a mismatch between the gonad, the external genitalia, and secondary sexual characteristics, gender is designated by gonadal sex. In the unusual case when both testicular and ovarian tissue are present, the gonadal sex is assigned with the term *true hermaphroditism*.
- *Genital sex:* Genital sex is determined by the anatomy of the external genitalia irrespective of the internal genitalia (such as Wolffian and Müllerian duct structures). The term *ambiguous genitalia* refers to conditions that may reflect *intersex* developmental disorders. These include cryptorchidism, partial labioscrotal fusion, varying degrees of *hypospadias* (urethral opening on the ventral surface of the penis due to incomplete fusion of the urethral folds/labia minora), micropenis, clitoromegaly, a combination of these conditions, and other disorders.
- *Sexual/gender identity* is the psychological self-identification either as a male or a female.
- *Sexual orientation* is attraction/arousal felt toward the opposite sex (*heterosexual* orientation), the same sex (*homosexual* orientation), or both sexes (*bisexual* orientation).

An important feature of the developmental process that the structure and/or function has an *innate bisexual potency*, and these checkpoints decide whether the development proceeds toward male versus female direction. A consequence of this is a wide variety of mismatches between any of the above components of sex determination. These may include mismatches such as those between chromosomal sex and gonadal sex, gonadal sex and genital sex, genital sex and sexual identity, or sexual identity and sexual orientation.

In this section, we discuss the normal regulation of these developmental processes and some of the consequences of deranged development. The

subject matter has serious moral and ethical dimensions that are beyond the scope of this text.

Intrauterine Sexual Development

Except for Its Pseudoautosomal Regions, All But One Copy of the X Chromosome Is Permanently Inactivated in Each Cell Due to the fusion of haploid male and female pronuclei during fertilization, the zygote and all normal human somatic cells contain a diploid set of chromosomes consisting of 22 pairs of autosomes and a pair of sex chromosomes. The oocyte may contribute only an X sex chromosome; the spermatozoon may contribute either an X or a Y sex chromosome. Thus, the normal karyotype is 46,XX in females, and 46,XY in males. (By convention, the number reflects the *total* number of chromosomes, including the sex chromosomes.)

A unique feature of the sex chromosomes is that only one X chromosome may be active in each cell, whereas the other X chromosome is permanently inactivated, a mechanism originally proposed by Mary Lyon in 1961, and now termed *lyonization*. This constitutive heterochromatin appears in the cells as *Barr body* or *sex chromatin* (Box 13-12; see also Chap. 3). During the blastocyst stage of embryonic development, the maternal and

BOX 13-12 Barr Body

Demonstration of the Barr body (sex chromatin) can be used for the diagnosis of *chromosomal sex* by microscopic examination of thionine-stained buccal smear cells. The Barr body appears as a clump of chromatin associated with the nuclear envelope. In normal females, 20 to 80% of the cells are identified as sex chromatin positive. Up to 2% of the cells may be positive for Barr body-like chromatin clump in normal men. Although the buccal smear is not as accurate as karyotyping, it is fast, inexpensive and in case of unambiguous results, may be sufficient. The *drumstick configuration* of the chromatin structure of circulating polymorphonuclear neutrophilic granulocytes has the same significance as the Barr body; 1.5 to 15% of neutrophils display the drumstick configuration in normal females.

The number of Barr bodies equals the total number of X chromosomes minus one. The buccal mucosa of phenotypically female *Turner's syndrome* (45,X) patients is negative for Barr bodies, similar to normal males. Most *Klinefelter's syndrome* (47,XXY) patients are hypogonad males with a Barr body count normally observed in females (i.e., 20 to 80% of the cells displaying a *single* Barr body).

The *Y chromosome* can be detected in buccal smears by quinacrine staining and fluorescence microscopy: the Y chromosome appears as a highly fluorescent clump known as the *F body*.

paternal X chromosomes in the *inner cell mass* are assigned for inactivation in a random manner. However, only paternal X chromosomes are inactivated in the *trophoblast* cells (compare with Box 13-10). All daughter cells of the blastocyst inherit the same assignment of maternal and paternal X chromosomes. As a consequence, the adult female is a mosaic for the active X chromosome: in certain cell populations the paternal X chromosome is active; in others the maternal X chromosome is active. By chance, all cells may carry the same active parental (either maternal or paternal) X chromosome. This condition is known as *extreme lyonization*, which may result in the full manifestation of X-linked recessive genetic diseases typically encountered in males who are normally hemizygous for the X chromosome. The inactivation of an X chromosome in 46,XX females equalizes the active X chromosomal genes present in males and females, a phenomenon known as *gene dosage compensation*.

The telomeric regions of both arms of the X and Y chromosomes contain the same set of genes and are known as the *pseudoautosomal regions* (Figs. 13-34 and 13-35). *The pseudoautosomal region is not inactivated with the rest of the X chromosome, irrespective of the number of the X chromosomes present, and participates in meiotic crossing over between the X and Y chromosomes during spermatogenesis.* The rest of the X and Y chromosomes contain different loci and recombination between them outside the pseudoautosomal regions does not normally occur.

During meiosis, the homologous chromosomes that line up for meiotic crossing over occasionally fail to separate from each other. This phenomenon, known as *meiotic nondysjunction*, results in haploid gametes that either lack or gain a chromosome. Fertilization involving such gametes typically results in *aneuploidia*. As a rule of thumb, the consequences of autosomal meiotic nondysjunction are more severe than those of the sex chromosomes. In the simplest form of nondysjunction, gametes with 24,XX, 22,0 and 24,XY may be formed.

- Fertilization of a 24,XX egg with a 23,Y spermatozoon results in a zygote with a karyotype of 47,XXY (*Klinefelter's syndrome*).
- Fertilization of a 22,0 egg with a 23,X spermatozoon results in a zygote with a karyotype of 45,X (*Turner's syndrome*).
- For viability of the embryo, at least one X chromosome must be present; i.e., 45,Y is lethal.

Klinefelter's and Turner's syndromes are common conditions, which may serve as prototypes of deranged development and will be covered in this chapter.

The chance for meiotic nondysjunction increases with maternal age. Pregnant women above 35 year of age are screened by karyotyping of their fetus by amniocentesis for diseases due to meiotic nondysjunction, including Down's syndrome. Note that this maternal age coincides with the earliest

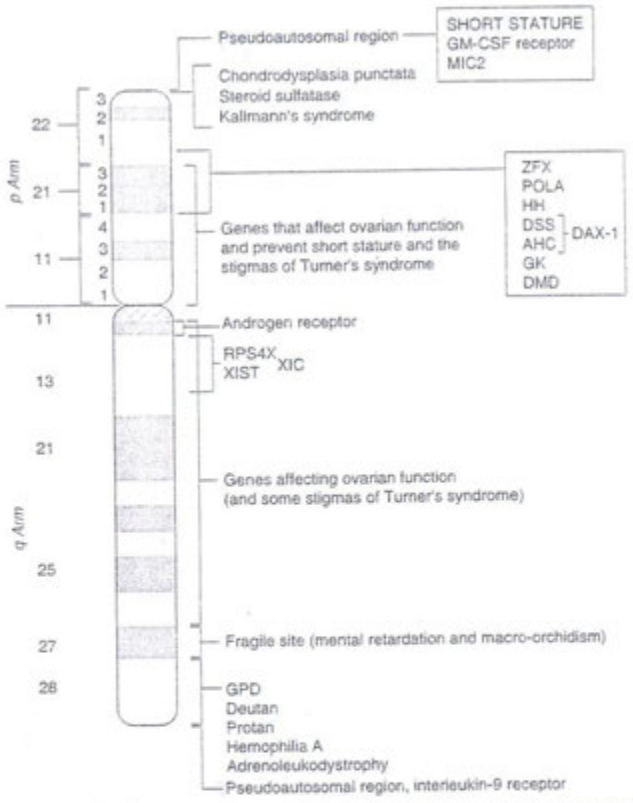


Figure 13-34. Diagrammatic presentation of the Giemsa- (G-) banded X-chromosome. Selected X-linked genes are shown. GM-CSF, granulocyte-macrophage colony-stimulating factor; MIC2, a cell surface antigen; ZFX, zinc finger X; POLA, RNA polymerase; HH, hypogonadotropic hypogonadism; DSS, dosage-sensitive sex reversal locus = AHC (adrenal hypoplasia congenita) = DAX-1 (DSS–AHC-critical region); GK, glycerol kinase; DMD, Duchenne muscular dystrophy; RPS4X, ribosomal protein S4; XIST, inactive X chromosome-specific transcripts; XIC, X inactivation center; GPD, glucose-6-phosphate dehydrogenase; Deutan & Protan, color blindness genes. Note that *XIST* is expressed only from *inactive* X-chromosomes. (*Source:* From Grumbach MM, Conte FA: Disorders of sex differentiation, in Wilson JD, Foster DW (eds): *Williams Textbook of Endocrinology*. 8th ed., Philadelphia, Saunders, 1992.)

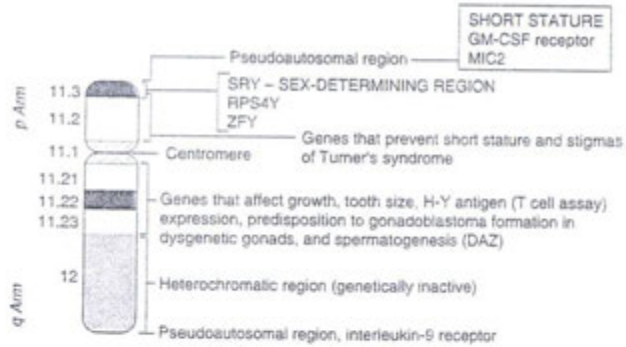


Figure 13-35. Diagrammatic presentation of the Giemsa- (G-) banded Y-chromosome. Selected Y-linked genes are shown. GM-CSF, granulocyte-macrophage colony-stimulating factor; MIC2, a cell surface antigen; RPS4Y, ribosomal protein S4; ZFY, zinc finger Y; DAZ, deleted in azoospermia. (*Source:* From Grumbach MM, Conte FA: Disorders of sex differentiation, in Wilson JD and Foster DW (eds): *Williams Textbook of Endocrinology*. 8th ed., Philadelphia, Saunders, 1992.)

perimenopausal symptoms and a depletion of ovarian follicles to 25,000, a "critical number" below which the fine-tuning of the hypothalamic–pituitary–ovarian axis deteriorates.

Gonadal Sex Is Determined by the Temporal and Dosage-Dependent Expression of Several Genes Encoded by Autosomes and Sex Chromosomes, Including WT1, SF-1, SRY, DAX-1, and SOX9 The *urogenital ridge* develops by the 5th week of embryonic life adjacent to the developing adrenal gland at the medial aspect of the *mesonephros*. The *indifferent gonad* develops from the urogenital ridge during the 6th embryonic week (Fig. 13-36). The development of the indifferent gonad and the adrenal cortex requires the expression of the *WT-1* gene and the nuclear orphan receptor steroidogenic factor-1 (SF-1; Fig. 13-37).

- The *WT-1* gene, located on chromosome 11p13, encodes a zinc-finger transcription factor. Its loss-of-function mutation in a heterozygous state results in *Denys–Drash syndrome*, which is characterized in XY individuals as gonadal dysgenesis, persistent Müllerian structures, feminization of the external genitalia, nephropathy, and nephroblastoma (Wilms' tumor). Homozygous WT-1 knockout mice fail to develop kidneys and gonads.
- The *SF-1* gene is located on chromosome 9q33. Experiments with knockout mice indicate its role in inducing steroidogenic enzymes and development of the adrenal glands, gonads, and the ventromedial nucleus of the hypothalamus. SF-1 is involved in the development of both the indifferent gonad and in its differentiation into *testis*.

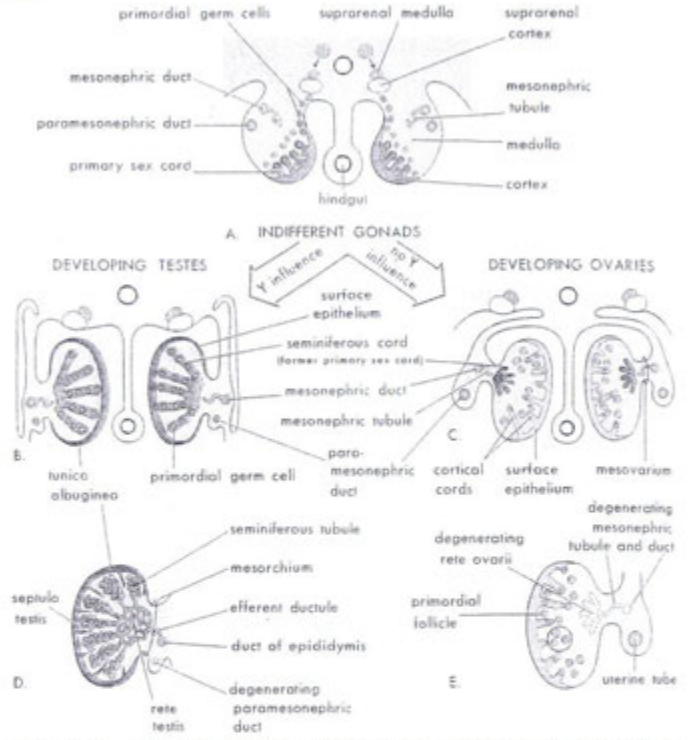


Figure 13-36. Schematic illustration of the morphologic differentiation of the indifferent gonads into testes and ovaries. Compare with regulatory steps shown in Fig. 13-37. Note the different timing of testicular and ovarian development. The primordial germ cells originate from the yolk sac. A. Sixth embryonic week (day 42): indifferent stage (biporential gonad). B. Seventh embryonic week (days 43 to 50): the primary sex cords develop into seminiferous sex cords which consist of Sertoli cells expressing anti-Müllerian hormone (AMH). AMH acts on ipsilateral structures reaching them by diffusion. Testosterone-producing Leydig cells appear by day 60 (not shown). C. Twelfth embryonic week (days 77 to 84): in the absence of testicular differentiation, ovarian differentiation commences. The cortical cords develop into the follicular epithelium of oogonia. D. Twentieth embryonic week: under the influence of AMH, the paramesonephric (Müllerian) duct degenerated (by the 9th week), and upon androgen exposure the mesonephric (Wolffian) duct developed (by the 12th week). E. Twentieth embryonic week: in the absence of AMH and androgens, the Müllerian duct developed, the Wolffian duct degenerated. (*Source:* Fig. 13-21, p 264 in Moore, KL: *The Developing Human. Clinically Oriented Embryology*. 4th ed. Philadelphia, Saunders, 1988.)

- The common embryologic origin explains the presence of *adrenal rest tissue* in the gonads, particularly in the testis. This ectopic adrenocortical tissue is typically negligible but may present as an ACTH-dependent testicular "tumor" in congenital adrenal hyperplasia, especially in the nonclassic form of p450c21 deficiency.

The development of the testis requires the mandatory expression of the sex determining region Y (*SRY*) gene encoding the *SRY transcription factor* also known as *testis determining factor* (TDF). The most likely function of SRY is the suppression of the developmentally expressed *dosage-sensitive sex reversal* (*DSS*) gene, which is probably identical with DAX-1. DAX-1 is a potent suppressor of SF-1. Thus, the effect of SRY is the disinhibition of SF-1 (see Fig. 13-37). In turn, SF-1 stimulates testicular differentiation via SOX9, which leads to the appearance of Sertoli cells. SF-1 also stimulates the production of AMH by Sertoli cells and the expression of steroidogenic enzymes by the Leydig cells that develop a few days later (see Regulation of the Gonadotropin–Gonad Axis in Postpubertal Males).

- SRY is a transcription factor. Its DNA-binding domain is an *HMG box*, i.e., it is homologous with the DNA-binding domain of the high mobility group (HMG) proteins. The *SRY* gene is closely linked with the pseudoautosomal region of the *short arm* of the Y chromosome, and may occasionally become *translocated* to the X chromosome during meiosis.
  - The presence of an X chromosome harboring the translocated *SRY* gene leads to the development of *46,XX males*, whose clinical presentation is similar to that of Klinefelter's syndrome patients.
  - The presence of a Y chromosome, whose *SRY* gene was translocated to an X chromosome during meiosis leads to the development of 46,XY females (*complete gonadal dysgenesis*). Note that this condition is distinct from 46,XY females whose condition is due to androgen insensitivity syndrome, or dosage-sensitive sex reversal.
- The SOX genes are related to SRY; they are termed for SRY HMG box. The *SOX9* gene is located on chromosome 17q24.1-25.1. This transcription factor is crucial in the development of cartilage and the testis. Its mutation results in *campomelic dysplasia* and partial male-to-female sex reversal as an autosomal dominant condition indicating that dosage of this factor is critical for its normal function (*haploinsufficiency*).
- DAX-1 has been localized to chromosome Xp21, the same location where the DSS locus had been mapped. Males and females have the same dosage of this gene because of X chromosome inactivation in females. The model shown in Fig. 13-37 explains clinical observations related to this gene:
  - Duplication of Xp21 (i.e., two copies of the gene on the *same* X chromosome) results in the development of 46,XY females because the double dose of DAX-1 cannot be sufficiently suppressed by SRY. This condition is referred to as *dosage-sensitive sex reversal* (DSS). Note that 47,XXY individuals do not express a

double dose of DAX-1 because of the inactivation of the extra X chromosome.

- Deletion of DAX-1 in 46,XY males results in normal sexual differentiation, which indicates that in males DAX-1 must be either suppressed (by SRY) or altogether absent for normal testicular development.

In females, SRY is absent, DAX-1 remains sustained, the SF-1/SOX9 pathway becomes suppressed, and ovaries develop. Note that *none of the classic hormones are required for the development of the ovaries and the testes.*

AMH and Testosterone Secreted by the Testis, Type II 5α-Reductase-Mediated Generation of Dihydrotestosterone by Target Cells, and Tissue Responsiveness to These Hormones Determine the Development of the Genital Tract, Accessory Sex Glands, and External Genitalia The Sertoli cell population is the first to develop in the testis and starts secreting AMH (chromosome 19p13.2-13.3) as early as days 43 to 50 of embryonic life (see Fig. 13-37). AMH, as a typical member of the TGF-β family (see Regulation of the Gonadotropin–Gonad Axis in Postpubertal Males), acts locally and causes the involution of the *ipsilateral* paramesonephric (Müllerian) duct by a paracrine mechanism. The cells of the Müllerian duct express the AMH-specific type II TGF-β receptor (chromosome 12q13). In the absence of AMH or AMH receptor, the Müllerian ducts develop irrespective of the sex steroid hormone environment, and give rise to the Fallopian tubes, uterus, and the upper third of the vagina. In females, this is the physiologic condition. In males, mutations of either AMH or AMH receptor cause *persistent Müllerian duct syndrome*, which is characterized by the coexistence of Müllerian and Wolffian structures, the prostate, and male external genitalia. Similar constellation may be seen in females who were exposed to high levels of androgens during the critical weeks (8th to 12th weeks) of embryonic life either from an exogenous source or by adrenocortical overproduction (see below).

The Leydig cells secrete testosterone from about embryonic day 60 (8th to 9th week). Because the pituitary hormones are first detectable at 12 weeks after conception and pituitary gonadotropins display a peak only during midgestation (see Fig. 13-41), testosterone production at this stage cannot be under the control of the hypothalamo–hypophyseal system. Instead, testosterone is stimulated by hCG, which is still rising to reach peak

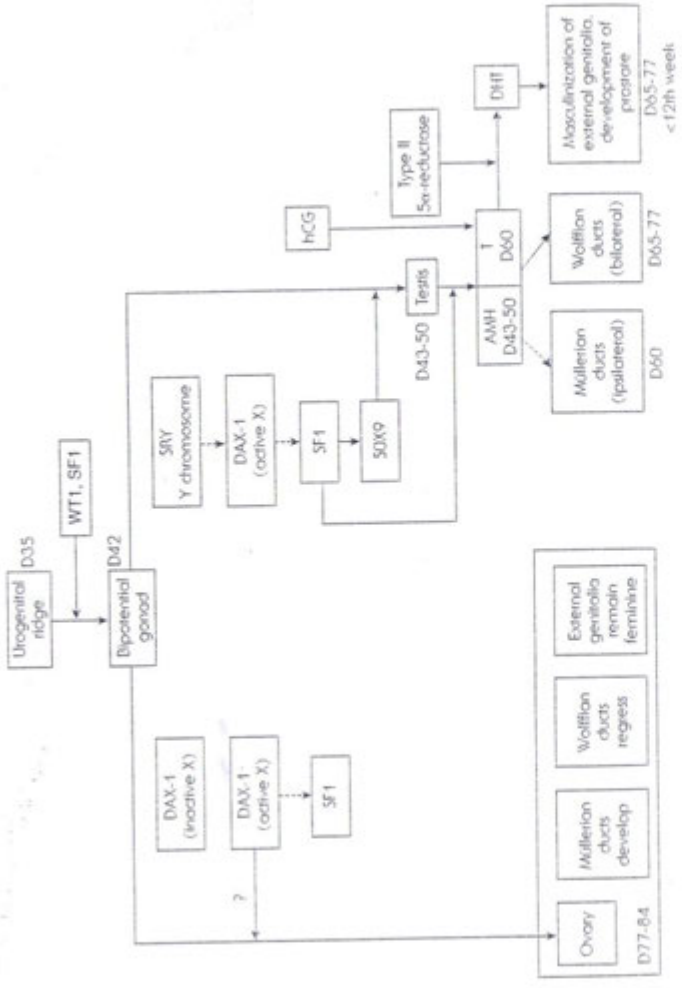


Figure 13-37. A model of the regulation of normal sexual differentiation. Solid arrows indicate stimulation, dashed arrows indicate inhibition, the letter D with a number indicates the day of embryonic life. The steps are dosage-sensitive and must occur within specified time windows. Transcription factors: WT1, Wilms' tumor 1; SF1, steroidogenic factor 1; SRY, sex-determining region Y; DAX-1, dosage-sensitive sex reversal/adrenal hypoplasia congenita critical region of chromosome X; SOX9, SRY high mobility box gene 9. Extracellular signals: AMH, anti-Müllerian hormone; hCG, human chorionic gonadotropin; T, testosterone; DHT, dihydrotestosterone.

levels during the latter part of the first trimester (11th to 12th embryonic week, Fig. 13-23). This peak activity of hCG is crucial in the masculinization of the male fetus. Unlike hCG, which declines after the 12th week, testosterone remains elevated throughout midgestation because of pituitary LH secretion (see Fig. 13-42). The pituitary-stimulated testicular androgen secretion is important in the descent of the testicles into the scrotum, a process completed by the 32nd week. The *absence of functional LH receptors* result in androgen deficiency throughout life: pseudohermaphroditism in newborn males is followed by hypergonadotropic hypogonadism and sexual infantilism (lack of pubertal development). Like the testis, the developing ovary is also exposed to hCG; however, it does not produce steroid hormones.

The testosterone secreted under the influence of hCG has the following roles (see Table 13-4):

- Testosterone stimulates the development of Wolffian duct structures: epididymis, vas deferens, and seminal vesicle.
- Androgens may achieve complete masculinization of the external genitalia only if they are present before the 12th week of embryonic life (Fig. 13-38). After the 12th week, exposure to androgens may only achieve clitoromegaly/phallic enlargement.
- Masculinization of the external genitalia and the proper development of the prostate require conversion of testosterone into dihydrotestosterone (DHT). If the type II 5α-reductase is absent, male pseudohermaphroditism develops (see Box 13-1) in spite of retaining the Wolffian structures. The 5α-reductase deficiency is also known as "*penis at twelve syndrome*" because pubertal testosterone production causes clitoromegaly that usually proceeds to the development of a functional penis. Pubic hair develops without a male escutcheon. Although these individuals are usually raised as females, they change gender identity at the time of phallic development and develop sexual orientation toward women. Their phallus enables them to have intercourse and the seminal vesicle produces a near-normal volume of seminal fluid.

The androgenic action requires the presence of functional androgen receptors. *Androgen insensitivity syndrome* (*Morris syndrome*), a form of male pseudohermaphroditism, is due to the loss of function mutation of the androgen receptors (Fig. 13-39). Because of its psychological impact, it has been recommended to avoid using the term *testicular feminization* when relating this condition to patients. Because the gene of the androgen receptor is located on the X chromosome, *the X chromosome is a determinant of male sexual development*. Mutations of the androgen receptor are without negative consequences in heterozygous females (carriers). If the male infant of a carrier female inherits the X chromosome with the nonfunctional androgen receptor, a phenotypically female cryptorchid XY individual develops.

- *During embryonic development*, androgens are produced by the testis but fail to masculinize the external genitalia, to induce prostate devel-

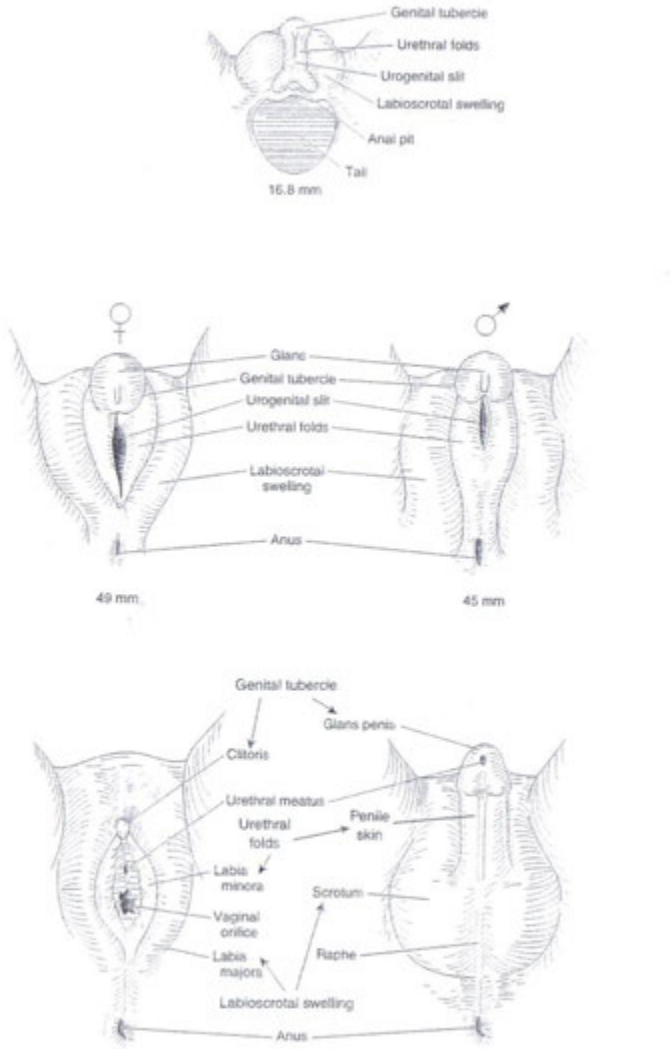


Figure 13-38. Differentiation of the male and female external genitalia from bipotential primordia. (From Fig. 14-9, p 495 in Conte FA, Grumbach MM: Abnormalities of sexual differentiation. Chapter 14 in Greenspan FS, Strewler GJ (eds): *Basic & Clinical Endocrinology*, 5th ed., Stamford, CT, Appleton & Lange, 1997.)

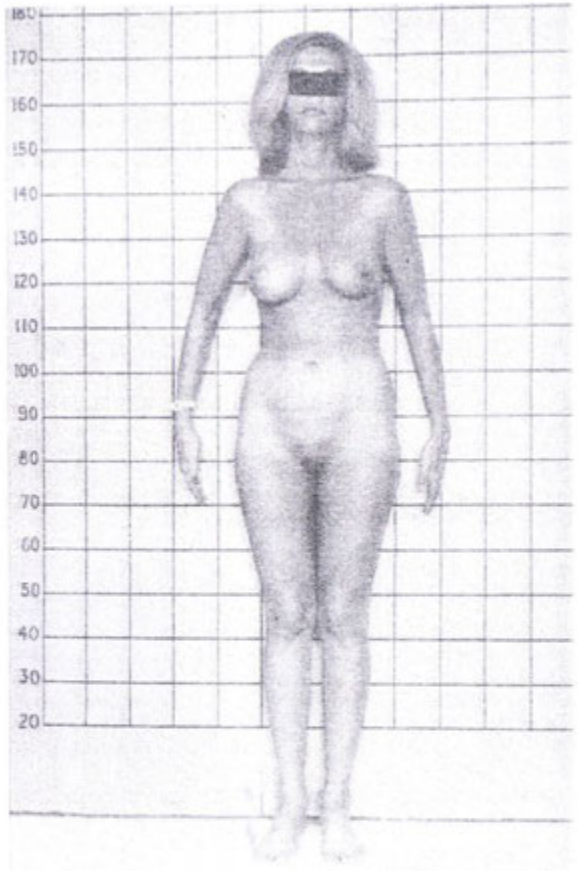


Figure 13-39. Complete androgen insensitivity syndrome (male pseudohermaphroditism). The karyotype is 46,XY. Note the perfectly female phenotype, including the external genitalia, breasts, hips and acne-free complexion. The pubic and underarm hair is absent (or scanty), which is characteristic for the condition. The patients are usually taller than average phenotypic females with somewhat larger than average hands and feet. The condition is usually inherited from the mother, and follows an X-linked recessive pattern because the androgen receptor is encoded by the X chromosome. The vagina ends blindly; Müllerian duct-derived structures are absent due to the intrauterine action of antiMüllerian hormone (AMH) secreted by Sertoli cells. (*Source*: Fig. 5-20, p 121 in Strenchever MA, Jones Jr HW: Genetic disorders and sex chromosome abnormalities, in DeCherney AH, Pernoll ML: *Current Obstetric and Gynecologic Diagnosis and Treatment*, 8th ed., Norwalk, CT: Appleton & Lange, 1994.)

opment and to sustain the Wolffian duct structures. However, AMH action leads to the involution of the Müllerian ducts, including the upper third of the vagina. The lower two-thirds of the vagina develops from the urogenital sinus just as in normal females; thus, the vagina ends blindly. In cases of complete androgen resistance, the male infant is born with female external genitalia and the condition may remain unsuspected until puberty. The condition in the newborn is the *mirror image* of the persistent Müllerian duct syndrome of males and the fetal masculinization of females.

- *Puberty* is delayed for a female, and about normal for a male, which results in a relatively tall stature with normal epiphyseal closure mediated by estrogen receptors. With puberty, testosterone production commences but no androgenic effects are exerted. In the absence of androgenic effects, AMH production by Sertoli cells remains high (see also Regulation of the Gonadotropin–Gonad Axis in Postpubertal Males). The lost *androgenic feedback* to the hypothalamus usually results in an increased LH output, and increased Leydig cell aromatase activity. Testosterone is converted into estradiol mainly within the testis, exerts its actions unopposed by testosterone or progesterone and results in feminization: female contours and breasts develop, but no menstruation occurs (primary amenorrhea). Although the vagina is shorter than average, it is distensible, and is appropriate for normal intercourse. Androgen-dependent hair growth is missing or scanty (this the condition is also known as "*hairless woman syndrome*"), neither male-type balding nor acne develops. The psychosexual development is typically normal female. The phenotypically female individual presents with cryptorchidism, a condition predisposing for testicular malignancy. For this reason, after pubertal development is complete, removal of the testes is recommended. After orchiectomy, *female* hormone substitution is indicated to maintain secondary sexual characteristics and to prevent osteoporosis. Inguinal hernias in preadolescent/adolescent females may direct attention to this condition.

Certain mutations of the androgen receptor result in *incomplete androgen resistance (Reifenstein's syndrome)*. Typical features include the presence of testes, regression of Müllerian ducts, postpubertally elevated estrogen production (compared to normal male levels), and resultant gynecomastia. The development of androgen-dependent features is highly variable.

Dysfunction of the Enzymes of the Steroidogenic Pathway May Interfere with Sexual Development by Altered Gonadal and/or by Altered Adrenocortical Hormone Secretion Disturbances in the core steroidogenic pathway may have two consequences:

- Defects that result in *decreased production of androgens* result in *male pseudohermaphroditism*, but cause no sexual abnormalities in females.
- Defects that result in *overproduction of androgens* cause varying degree of masculinization/virilization in females (*female pseudohermaphroditism*), and may present as a type of precocious puberty in males.

The defects may involve enzymes specific for either the corticosteroid or the sex steroid synthetic pathway, and the enzymes shared by these two pathways (see Fig. 12-8). The types of congenital adrenal hyperplasia affecting genital development are also referred to as *adrenogenital syndrome*. The common feature of congenital adrenal hyperplasia is the defective production of cortisol, which leads to increased ACTH production (see Box 12-6). Table 13-15 summarizes the various types of congenital adrenal hyperplasia. Note that whereas the effects of these enzyme defects on sexual development may be influenced by gonadal sex, the effects on adrenal function, electrolyte homeostasis, and blood pressure are independent of gonadal sex.

The phenotypes in Table 13-15 can be explained with the aid of Fig. 12-8 as follows:

- *StAR deficiency* precludes steroid hormone biosynthesis in both the adrenals and the gonads. The absence of androgen does not alter

Table 13-15 Enzyme Defects Causing Congenital Adrenal Hyperplasia (Adrenogenital Syndrome)

Enzyme	Gonad	External genitalia	Postnatal sexual development	Salt-loss/ Addisonian crises	Hypertension, hypokalemic alkalosis
StAR	Male	Female	Sexual infantilism	Yes	No
	Female	Female			
3βHSD type II	Male	Ambiguous	Moderately decreased secondary sex characteristics	Variable	No
	Female	Ambiguous	Moderate virilization		
p450c17	Male	Female	Sexual infantilism	No	Yes
	Female	Female			
p450c21	Male	Male	Precocious "puberty" (adrenal virilization)	Variable	No
	Female	Ambiguous/ female	Virilization		
p450c11	Male	Male	Precocious "puberty" (adrenal virilization)	No	Yes
	Female	Ambiguous/ female	Virilization		

the external genital development of females, but prevents the normal masculinization in males. During puberty neither androgens nor estrogens are produced, leading to sexual infantilism. Corticosteroid deficiency presents as Addisonian crises mainly because of the absent mineralocorticoids.

- The deficiency of type II 3βHSD prevents the synthesis of all corticosteroids, the synthesis of potent androgens (androstenedione, testosterone) and their aromatized estrogenic derivatives (estrone, estradiol). However, DHEA (a weak androgen) is overproduced due to the increase in ACTH (absent feedback by cortisol). Thus, in the absence of testosterone the male is insufficiently androgenized by the increased DHEA both in utero and during postnatal development. The female is exposed to unusually high androgenic stimulation both in utero and during postnatal/pubertal development. During puberty, appropriate estrogen production is missing. Depending on the degree of enzyme deficiency, the presentation is variable both in terms of sexual function and in Addisonian crises.
- The deficiency of p450c17 does not affect the zona glomerulosa where its absence is physiologic. Thus, mineralocorticoid production is not prevented and Addisonian crises do not occur. However, the *zona fasciculata* produces overt quantities of DOC, a potent mineralocorticoid, which leads to hypertension. In the absence of the 17,20-lyase activity of p450c17, neither androgens nor estrogens may be produced. Thus, masculinization of the external genitalia does not occur in males, and no sexual development may occur in either sex during puberty. Rare cases have been reported with an isolated defect of the 17,20-lyase activity of p450c17. In these cases neither congenital adrenal hyperplasia nor mineralocorticoid excess occurs, but the impact on sexual development remains.
- The deficiencies of p450c21 (Fig. 12-9) and p450c11 have variable manifestation. The classic forms are associated with severe virilization of the female fetus and severe manifestations of the corticosteroid defect. In terms of the corticosteroid defect, p450c21 and p450c11 deficiencies present with opposite clinical pictures explained by the overproduction of DOC in p450c11-deficiency (see Chap. 12). The nonclassic and cryptic forms manifest only postnatally and the external genitalia of females is normal at birth. The classic forms may be treated by prenatal exposure to glucocorticoids. Dexamethasone administered to the mother crosses the placenta and prevents masculinization and virilization of the external genitalia if the treatment is started in early gestation (preferably before the 7th to 10th week of gestation LMP). The success of this treatment implies that *the fetal pituitary already secretes physiologically relevant amounts of ACTH by the 8th to 12th fetal week, and this ACTH secretion is subject to feedback regulation by glucocorticoids*. Indeed, the corticotroph lineage appears to diverge the earliest during development from the rest of the anterior pituitary hormone-producing cells.

It is noteworthy that the masculinization of the external genitalia is rarely complete in females presenting with any of the virilizing types of

congenital adrenal hyperplasia. Although under the influence of ACTH the fetal zone of the adrenal cortex already produces substantial amounts of DHEA and DHEAS during the critical period of external genital development (8th to 12th fetal weeks) as evidenced by placental estriol production (see Fig. 13-23), the androgenic activity of DHEA is insufficient to effect *complete* masculinization even at drastically elevated concentrations. As noted, masculinization depends on the presence of 5α-reductase activity and DHT. In the rare cases of complete masculinization, enhanced peripheral conversion of DHEA by 3βHSD and 17βHSD is assumed to provide testosterone for DHT production in its targets.

The deficiencies of sex steroid specific enzymes (see Fig. 12-8) include *aromatase*, 17βHSD3, and type II 5α-reductase.

- Absence of p450arom (*CYP19*) results in *female pseudohermaphroditism*, which is usually characterized by complete masculinization of the external genitalia, and transient *virilization of the pregnant mother*, which resolves after parturition. This indicates that in the absence of placental aromatase, the fetal adrenal and maternal androgens may reach both the fetus and the mother in high concentrations. Undiagnosed female patients grow up as boys and their Müllerian duct structures persist. During puberty, the ovaries are unable to aromatize androgens. Because the negative feedback to gonadotropin secretion is mainly performed by estrogens, pituitary gonadotropins become elevated. The stimulated ovarian androgen production results in multicystic ovaries and mild virilization. The lack of estrogens explains the absence of breast development, the severely retarded bone age, the delayed epiphyseal closure leading to tall stature, and the early development of osteoporosis. Aromatase deficiency *in males* does not prevent male sexual development. The affected male presents with elevated plasma androgens and *macroorchidism* due to the lost estrogenic feedback suppression of gonadotropins. The delayed epiphyseal closure leads to tall stature with eunuchoid proportions (low upper-to-lower segment ratio), severely retarded bone age, and osteoporosis. The high levels of androgens decrease HDL, and increase plasma LDL, total cholesterol, and triglycerides. The associated hyperinsulinemia is probably the consequence of insulin resistance secondary to increased plasma FFA.
- Type 3 17β-hydroxysteroid dehydrogenase (17βHSD3) is a testis-specific isoenzyme that converts androstenedione into testosterone (see The Biosynthesis, Mechanism of Action and Metabolism of Sexual Steroids). In its congenital absence, male pseudohermaphroditism develops. During puberty, the testes may descend into the labioscrotal folds and produce large amounts of androstenedione, which is converted into testosterone by extratesticular 17βHSD isoenzymes. This leads to phallic development and virilization (Fig. 13-40). When these pubertal changes take place, most affected individuals request sex reassignment to male and develop sexual orientation toward females. This pattern is similar to that observed in cases of 5α-reductase deficiency.

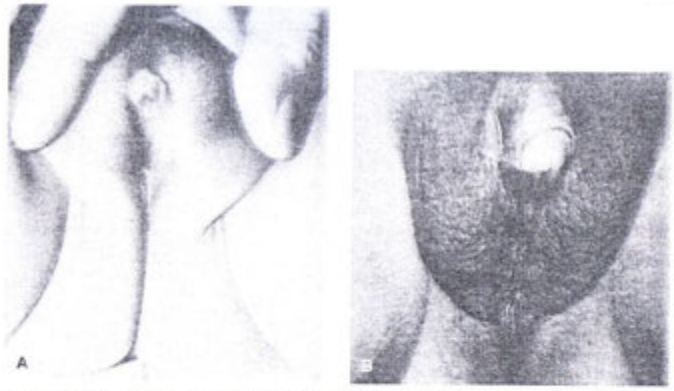


Figure 13-40. Deficiency of type 3 17β-hydroxysteroid dehydrogenase. Affected males are born with almost completely female external genitalia (A) but marked virilization occurs during puberty (B) which typically leads to a change in gender identity. (*Source:* Fig. 109-6, p 1918 in Forest MG: Diagnosis and treatment of disorders of sexual development. Chapter 109 in DeGroot LJ (ed): *Endocrinology*, 3rd ed., Philadelphia, Saunders, 1995.)

Masculinization of the fetus is sometimes caused by exogenous steroids. A potential exogenous source of androgens for the fetus is the mother. In rare cases of *mildly virilizing adrenocortical adenomas of the pregnant mother* which obviously did not cause infertility, the androgen output of the adenoma is increased during pregnancy along with cortisol, and may overwhelm both the SHBG and the capacity of placental aromatase. The consequence on the female fetus is comparable with aromatase deficiency, except that the perfectly masculinized female who is raised as a boy undergoes a quasinormal female puberty. Another exogenous source of steroids are the *synthetic progestins* that possess androgenic activity such as medroxyprogesterone. Before their masculinizing side effect was recognized, these drugs were administered to pregnant women to maintain pregnancy.

Fusions of the labia majora due to the intrauterine masculinizing effect of overt androgen exposure should not be confused with *labial adhesions*. Labial adhesions are characterized by the adherence of labia minora immediately under the clitoris. This condition arises only postpartum on the basis of the hypoestrogenic state of the infant, and may involve an inflammatory mechanism. The condition responds to topically applied estrogen ointments.

Psychosexual Development Is Primarily Determined by the Appearance of the External Genitalia and the Secondary Sex Characteristics Studies in rodents conclusively demonstrated the role of prenatal exposure to sex steroids in the development of sexual behavior on reaching maturity. Such a mechanism does not seem to dominate human psychosexual development. *Gender identity* is usually firmly established by the age of 18 to 30 months. Gender identity is usually based on self-observation of unambigu-

ous external genitalia, comparison of the genitalia with those of the siblings and parents, verbal reinforcement of the gender identity by the family members, and unambiguous rearing. As we have seen, in cases of severe pubertal virilization of male pseudohermaphrodites (17βHSD3 and 5α-reductase deficiency), gender identity may change. It has been suggested that androgens are responsible for the development of *sexual orientation* toward female sexual partners. However, the development of sexual orientation involves complex psychological mechanisms and environmental influences.

Puberty

Pituitary Gonadotropin Secretion Displays Four Phases of Increased Activity Corresponding with Midgestational Fetal Life, the Neonatal Period, the Reproductive Period, and, in Women, the Postmenopausal Years

We have discussed the regulation of the hypothalamic–pituitary–gonad axis in postpubertal males and females. This pattern of regulation is attained by a cascade of events that starts with the intrauterine development of the structures involved. The adult regulation dramatically changes in women with menopause; the decline of testicular function is more subtle. Throughout the lifespan of an individual, four phases of increased *pituitary* gondotroph function can be identified; the first three are present in both sexes (Fig. 13-41):

- Midgestational fetal life.
- Neonatal period.
- Adult reproductive period attained during pubertal development.
- Postmenopausal rise of gonadotropin secretion.

In females, *the ovaries respond to the high levels of gonadotropins with follicular maturation and sex steroid production only during the third phase, i.e., starting with puberty and ending with menopause*. The ovary remains quiescent during fetal and neonatal life, and does not respond to placental hCG or pituitary gonadotropins. In contrast, *the testis responds to each of the three phases of increased gonadotropin exposure with testosterone secretion* (Fig. 13-42).

The *fetal increase* of gonadotropin secretion is attributed to the immaturity of the negative feedback system and, in females, to the absence of gonadal sex steroid production. Some degree of negative feedback function during the midgestational peak is indicated by the higher gonadotropin levels in female than in male fetuses. Gonadotropin levels decrease in both sexes and remain low during the third trimester. This decrease may be explained by the development of the negative feedback regulatory system of

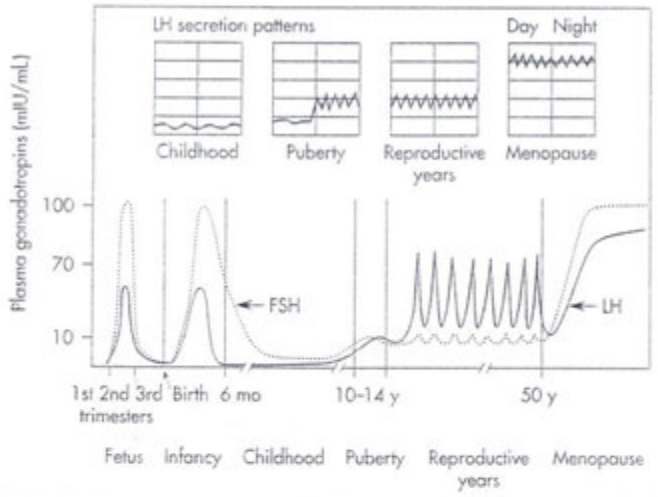


Figure 13-41. The four phases of increased pituitary gonadotropin secretion during the human lifespan. The figure shows the pattern seen in women. Men follow a similar pattern except for (1) the cyclic secretion (seen only in women during their reproductive years), (2) the average age at the onset of puberty, and (3) the absence of menopause. The inset shows the changes in pulsatile secretory pattern and the nyctohemeral rhythm of LH secretion. Note that the ovaries secrete sex steroids in response to gonadotropins only during the reproductive years. (*Source:* Fig. 9-15 in Porterfield SP: *Endocrine Physiology*. St. Louis, Mosby 1997; the figure originally appeared in Braunwald E et al: *Harrison's Principles of Internal Medicine*. 4th ed., New York, McGraw-Hill, 1987.)

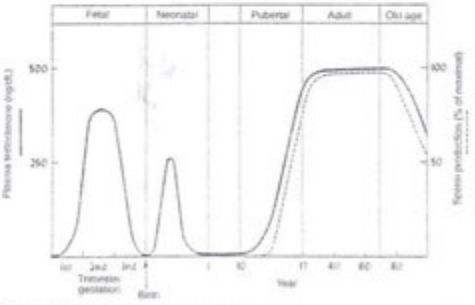


Figure 13-42. The phases of increased testosterone secretion during the lifespan of men. Compare with Fig. 13-41. Note that testosterone increases before the increase in pituitary gonadotropin secretion during fetal life. The early rise of testosterone is in response to hCG. (*Source:* Fig. 10-17, p 271 in Griffin JE, Wilson JD: Disorders of the testes and male reproductive tract, Chapter 10 in Wilson JD, Foster DW (eds): *Williams' Textbook of Endocrinology*. 7th ed., Philadelphia, Saunders, 1985.)

hypothalamic GnRH, and the high concentrations of placentally produced estrogens and progesterone.

The *neonatal increase* of gonadotropins is the consequence of the elimination of placentally provided negative feedback. A curious feature of this period is that gonadotropin secretion remains high for several months even in males, who respond to the increased gonadotropin exposure with up to midpubertal levels of androgen secretion. This androgen output by the neonatal testes is not the cause of the decrease of gonadotropin secretion to childhood levels.

During the *childhood years*, the circulating levels of sex steroids and gonadotropins are low (*juvenile pause of gonadotropin secretion*). The neuroendocrine mechanisms of this period are still enigmatic. The negative feedback regulation responds to 10 to 25% of the concentration of sex steroids required for comparable suppression of gonadotropins in adults. This exquisite sensitivity is undoubtedly a component of the *low setpoint of the negative feedback regulation* as evidenced by the supranormal gonadotropin levels of Turner's syndrome patients (Fig. 13-43). Turner's syndrome patients have streak gonads, which are devoid of follicles and do not secrete sex steroids. Although their gonadotropin levels are always higher than those of age-matched controls, they still display a characteristic decrease between the neonatal period and puberty, suggesting that mechanisms other than sex steroid feedback are primarily responsible for the juvenile pause of gonadotropin secretion. There is some evidence that the low levels of gonadotropins are related to the action of a specific group of GABAergic neurons that tonically inhibit GnRH secretion, and that NMDA receptors are also involved in the mechanism. The involvement of pineal melatonin secretion has also been suggested as a mechanism of the juvenile pause of gonadotropin secretion (see Chap. 14).

The *prepubertal/pubertal increase of gonadotropin secretion* is the result of poorly understood maturational changes in the CNS. The tonic inhibition responsible for the juvenile pause is relinquished. Leptin plays a gatekeeper role in this process to assure that adequate energy resources are available to support the energy expenditure required by the reproductive system (see Regulation of the Gonadotropin–Gonad Axis in Postpubertal Males). Both circulating gonadotropins and gonadal hormones increase during puberty indicating that the setpoint of negative feedback is gradually elevated to attain a new equilibrium characteristic of adults. The increased plasma concentration of sex steroids brings about the physiologic changes of puberty (see below). The onset of puberty is heralded by the reawakening of a partially quiescent GnRH pulse generator.

• Even though plasma gonadotropin levels are very low during the juvenile pause, a low-amplitude pulsatile secretion can be demonstrated. The GnRH/LH *pulse frequency* displays an approximately twofold increase from midchildhood (about 6 years of age) to the clinical onset of puberty,

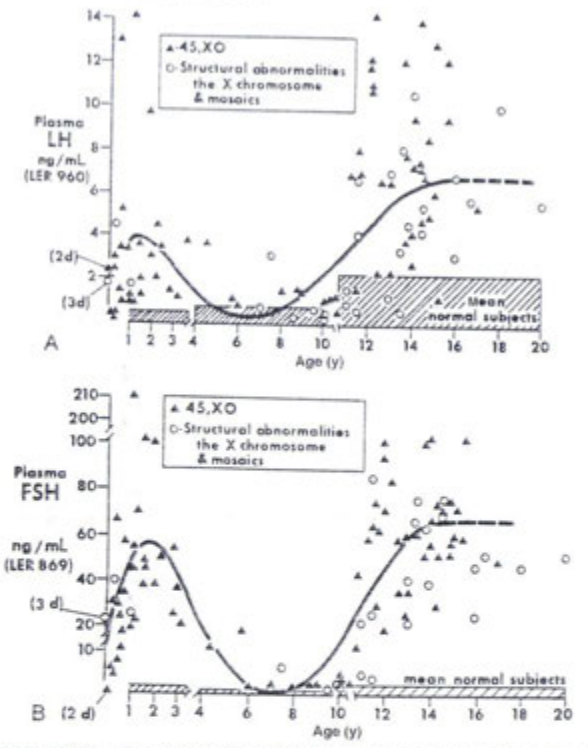


Figure 13-43. The ontogeny of plasma LH (upper panel) and FSH (lower panel) in normal subjects and Turner's syndrome patients. The ontogenetic pattern in the two groups is practically identical, except that it is accentuated in Turner's syndrome patients due to the absence of gonadal feedback-suppression of pituitary gonadotropins. The neonatal peak is followed by a juvenile pause of gonadotropin secretion. Note that Turner's syndrome patients who present with the absence of pubertal development due to gonadal dysgenesis, still display the pubertal onset of gonadotropin secretion. (*Source:* Redrawn from Conte FA et al: A diphasic pattern in gonadotropin secretion in patients with the syndrome of gonadal dysgenesis. J Clin Endocrinol Metab 40:670–675, 1975.)

with no subsequent changes during the continuing development toward adulthood. The twofold increase in GnRH pulse frequency *enhances the GnRH responsiveness of the pituitary* to increase the mass of LH produced to a standardized bolus of exogenous (or a pulse of endogenous) GnRH.

• From midchildhood to sexual maturity, LH production rate increases about fortyfold. Approximately 90% of this increment is accounted for by an increase in the *amplitude* of a preexisting pulsatile secretion. It

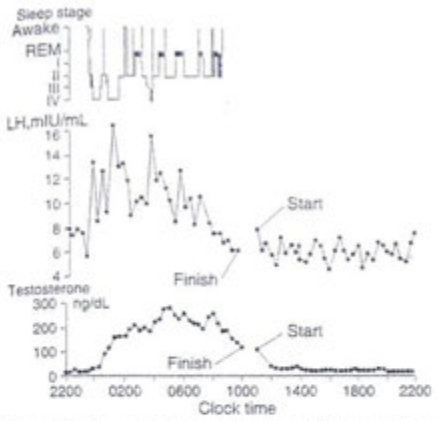


Figure 13-44. Nyctohemeral rhythm and sleep-entrained secretion of LH in a 14-year-old boy in pubertal stage 2. The top panel shows the sleep stages as evaluated by electroencephalogram. Peak LH levels mostly coincide with non-REM sleep. The circadian rhythm of plasma testosterone is shown at the bottom panel. (*Source:* Fig. 15-5 in Styne D: Puberty, Chapter 15 in Greenspan FS, Strewler GJ (eds): *Basic & Clinical Endocrinology*, 5th ed., Stamford, CT, Appleton & Lange, 1997; originally appeared in Boyar RM et al: Simultaneous augmented secretion of luteinizing hormone and testosterone during sleep. *J Clin Invest* 54:609, 1974.)

has been suggested that the enhanced amplitude of LH is mainly a result of the increased pituitary responsiveness to GnRH.

• GnRH/LH pulses coincide with non-REM sleep. This *sleep-entrained GnRH/LH secretion* is already present in midchildhood, but no circadian changes are seen in the amplitude of the LH pulses or the average plasma concentrations of LH. During the pubertal activation of the GnRH—gonadotropin axis, the appearance of a *nyctohemeral LH rhythm* is the first event (Figs. 13-41 and 13-44). *The amplification of sleep-entrained GnRH/LH secretion occurs about 2 years before the clinical onset of puberty.* The nyctohemeral rhythm of GnRH/LH secretion disappears during young adulthood, when the fairly regular LH pulses occur throughout the entire 24-h day and result in a continuously elevated concentration of plasma LH (see Fig. 13-41). This ontogenetic pattern is useful in the evaluation of pubertal development: low levels of plasma LH in blood samples collected during the daytime are far less informative than those collected during normal sleep.

Adrenarche (the increased production of adrenal androgens; see Chap. 12) normally precedes pubertal development by about 2 years, but there is no clear causal relationship between adrenarche and puberty. Neither Addison's disease nor premature (but otherwise normal) adrenarche has a clear influence on the age of the onset of puberty. In contrast, exposure to high levels of androgens such as in virilizing adrenogenital syndrome

may result in *precocious puberty* even if treatment of androgen production is instituted in the meantime. Although adrenarche contributes to physiologic changes at puberty, pubertal maturation is primarily due to the activation of the gonads (*gonadarche*).

The *postmenopausal increase* of gonadotropins reflects the exhaustion of the nonrenewable pool of ovarian follicles: the decline of ovarian hormone production (sex steroids and inhibin) release gonadotropin secretion from the negative feedback suppression. A similar pattern of gonadotropin secretion is seen in cases of testicular failure and after castration (see Figs 13-12 and 13-41).

Pubertal Development Is a Sex Steroid-Regulated Process that Begins Earlier and Does Not Last as Long in Females as in Males Puberty is characterized by the attainment of secondary sexual characteristics and the pubertal growth spurt, which is normally terminated by epiphyseal closure. Pubertal development is assessed by *Tanner staging* of the breasts and pubic hair development in females (Figs 13-30 and 13-45), the genital (penis, testis, scrotum) and pubic hair development in males (Fig. 13-46). The typical time-course of developmental stages is shown in Fig. 13-47.

The *first sign* of puberty in males is the *increase in testicular volume* (from 1 mL to >3 mL). This occurs between the ages of 9 and 14 years, and reflects the combined effects of FSH and LH-driven testosterone on the seminiferous tubules. In contrast, the first sign of puberty in females is related to the effect of increased estrogen production. (As mentioned, the first menstrual cycles are usually anovulatory; thus progesterone is not involved in the first manifestations of female puberty.) Breast development (*thelarche*) is usually noticed first between the ages of 8 to 13 years, although the onset of pubertal growth spurt usually precedes thelarche. Similar to the testis, the ovary grows in size, and follicles appear with diameters of ≥9 mm. These changes in the ovary can be evaluated by ultrasonography.

Underarm and *pubic hair* development (*pubarche*) requires androgenic action. Although the adrenal cortex is a significant source of androgens in females, normal ambisexual hair development also requires ovarian testosterone production. Thus, in Turner's syndrome patients whose streak ovaries do not secrete steroid hormones, pubic hair growth is either absent or markedly decreased as a part of sexual infantilism (Fig. 13-48), in spite of normal adrenarche. A major difference between mature female and male pubic hair distribution is the *escutcheon* (the spreading of pubic hair to the umbilicus), the development of which depends on a normal production rate of testosterone plus local conversion into DHT. Thus, patients with type II 5α-reductase deficiency or Klinefelter's syndrome (a condition with decreased testosterone secretion, Fig. 13-49) present with mature *female* type of pubic hair. In males, the growth of mustache and beard is initiated with some delay after pubarche. The appearance of androgen-dependent hair usually coincides with the activation of other (type I 5α-reductase-

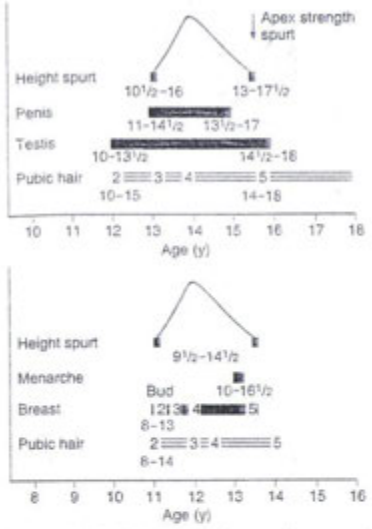


Figure 13-47. Sequence of secondary sexual development in British males (top) and females (bottom). (*Source*: From Marshall WA, Tanner JM: Variations in the pattern of pubertal changes in boys. Arch Dis Child 45:13, 1970.)

dependent) cutaneous structures leading to the development of *body odor* (and pheromonal signaling of sexual maturity) by secretions of *apocrine* sweat glands and *acne* due to overproduction of sebum.

The onset of menses (*menarche*) is an important developmental milestone indicating that estrogen production is sufficient to stimulate endometrial proliferation. In the United States, the average age at menarche is 12.8 years; African American girls experience menarche about 4 months earlier than Caucasians. Menarche is expected to occur within 5 years after thelarche. The absence of menarche beyond 16 years of age is termed *primary amenorrhea*, which may indicate deranged development of the gonadal axis. The first menstrual cycles are usually anovulatory bleedings. The relative number of ovulatory cycles gradually increases to over 80% within 4 to 5 years after menarche. Under the influence of estrogens, the uterus grows to reach adult size. However, this growth can be evaluated only by ultrasonography. Bimanual examination is not only imprecise but is also a traumatic experience during childhood that should be avoided.

In males, the developmental milestone comparable with menarche is termed *spermarche*, which is associated with the appearance of spermatozoa in early morning urine samples, usually in the absence of previous ejaculation. This developmental milestone, however, is not apparent unless urine sediment is microscopically evaluated. The average age at spermarche is 13.4 years at gonadal stage 3 to 4. The ejaculation-independent appearance of spermatozoa subsides later in development. Stage 5 pubertal development is expected within 4.5 years after the first sign of puberty.

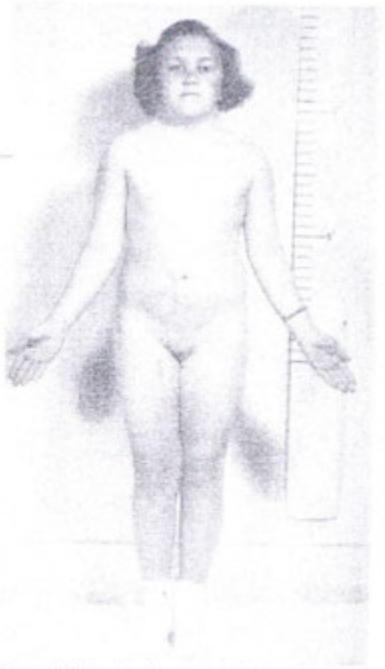


Figure 13-48. A 14-year-old 45,X female with characteristic manifestations of Turner's syndrome. Note the short stature, the absence of pubic hair or breast development, broad chest with widely spaced nipples, webbed neck, increased "carrying angle" of the elbow, and the old-looking face. (*Source*: Photograph by Earl Plunkett; courtesy of G.H. Valentine; Fig. 4, Chapter 8 in Mange AP, Mange EJ: *Genetics: Human Aspects*, 2nd ed., Sinauer Associates, 1990.)

The pubertal *growth spurt* is related to an increase in GH pulse *amplitude* and average concentration of IGF-1, which indicates resetting of the negative feedback exerted by IGF-1 on GH at a higher IGF-1 level. This is gradually achieved by the increasing concentrations of testosterone and estradiol. Sexual steroids are unable to effect pubertal growth spurt in Laron dwarves (GH receptor deficiency). In males, the growth spurt starts later, growth velocity is higher, and epiphyseal closure occurs later than in

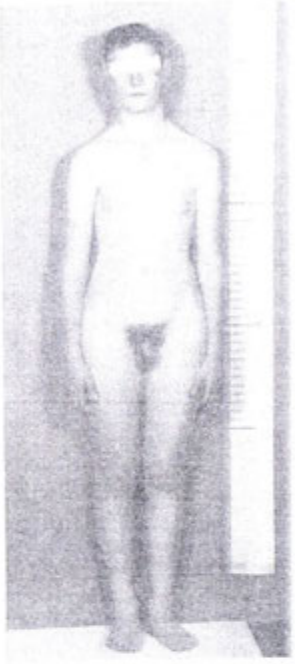


Figure 13-49. A 47,XXY male with Klinefelter's syndrome. The condition is associated with hypogonadism with increased of estrogen : androgen ratio. Note the relatively tall stature with lower than normal upper-to-lower segment ratio (eunuchoid body proportion) and long arms. The breasts are Tanner stage 3 for a female (gynecomastia) and pubic hair displays female distribution. The testes are firm, the testicular volume and the stretched length of the penis are below average. (*Source*: Photograph by Earl Plunkett; courtesy of G.H. Valentine: Fig. 4, Chapter 8 in Mange AP, Mange EJ: *Genetics, Human Aspects*, 2nd ed., Sinauer Associates, 1990.)

females (Fig. 13-50). Thus, males attain a 9-cm taller stature *before* the onset of pubertal growth spurt, and they add 3 cm more to their height *during* the growth spurt (28 cm in men versus 25 cm in women). These two factors together explain that on the average males are about 12 cm taller than females.

The sexual differences in *body contour and composition* develop during puberty. In males, androgen production increases lean body mass: muscle mass increases and fat stores may become decreased. The shoulders become prominent, the hip is narrow. The same presentation in females is a component of virilization. Females are normally under the predominant influence of estrogens (and progesterone), depositing more body fat and less lean muscle mass than males. The hips become more prominent than the shoulders and adipose tissue accumulates in the developing breasts, buttocks, and thighs.

A number of biochemical differences emerge during puberty, including a higher hematocrit, plasma LDL, and lower plasma HDL in males than in females.

The Main Forms of Deranged Pubertal Development Include Delayed Puberty, Sexual Infantilism (Absent Puberty), and Precocious Puberty Leading to Either Isosexual or Contrasexual Development Deranged pubertal development is often indicated by the abnormal time-course of puberty.

- *Delayed puberty* is considered either in a male ≥14 years or in a female ≥13 years of age if no sign of pubertal of pubertal development is present. Because this definition is based on ±2.5 standard deviations around the mean onset of puberty, in 0.6% of the population the delayed puberty does not indicate a disease but *constitutional delay*. In certain conditions, such as Kallman's syndrome or Turner's syndrome, pubertal development is absent permanently, i.e., does not occur without treatment.
- *Precocious puberty* is defined by the commencement of secondary sexual development either in males <9 years, or in females <8 years of age.
  - *Complete* (true) *precocious puberty* involves the activation of the hypothalamic–pituitary–gonad axis, indicating that the normal mechanism of puberty is activated prematurely. In most males presenting with complete precocious puberty, the underlying cause is a hypothalamic tumor such as that of the pineal gland, which involves the posterior hypothalamus. These tumors destroy the neural structures responsible for the juvenile pause of gonadotropin secretion and involute during puberty in healthy individuals. In most females with complete precocious puberty, no pathology is found and, in the absence of familial predisposition to precocious puberty (which is known as *constitutional precocious puberty*), the diagnosis of *idiopathic complete precocious puberty* is made.
  - *Incomplete precocious* puberty indicates that sex steroid production is not regulated by the hypothalamo–hypophyseal system.

Pubertal development may be isosexual or contrasexual.

- *Isosexual pubertal development* indicates that the secondary sexual characteristics developing during puberty are appropriate for the presumed gonadal sex of the individual.
- *Contrasexual pubertal development* indicates that the secondary sexual characteristics developing during puberty are opposite to the presumed gonadal sex of the individual. Examples of contrasexual pubertal development include
  - conditions leading to virilization/masculinization in *presumed* females, such as certain types of *male* pseudohermaphroditism (type II 5α-hydroxylase deficiency, deficiency in 17β-HSD3) and the nonclassic form of p450c21 deficiency.
  - conditions leading to feminization in *presumed* males, such as certain types of *female* pseudohermaphroditism (fetal masculinization by exogenous androgens).
  - In cases such as androgen insensitivity syndrome, pubertal

otropins. This is the case in conditions such as *Kallmann's syndrome* (see Regulation of the Gonadotropin–Gonad Axis in Postpubertal Males), defective GnRH gene, pituitary gonadotropin deficiency (which may either be isolated or part of combined pituitary hormone deficiency or *panhypopituitarism*), CNS tumors disrupting the normal adult-type regulation of the pituitary gland (such as craniopharyngioma), anorexia nervosa, hypothalamic amenorrhea (involving increased opioid-mediated inhibition of the GnRH pulse generator), low body weight (leptin mechanism), and genetic defects (such as Prader-Willi, Laurence-Moon and Bardet-Biedl syndromes).

Hypergonadotropic hypogonadism indicates gonadal failure and the absent negative feedback suppression of gonadotropin secretion. The condition may be due to cryptorchidism and various forms of *gonadal dysgeneses* (see Intrauterine Sexual Development).

Klinefelter's and Turner's Syndromes Are the Most Prevalent Forms of Hypergonadotropic Hypogonadism in Males and Females, Respectively *Klinefelter's syndrome* (*seminiferous tubule dysgenesis;* 47,XXY) and its variants (e.g., 48,XXYY) are due to meiotic nondysjunction and are the leading cause of infertility in males (see Fig. 13-49). The gonocytes are present at birth but disappear before the age of 2 years, which results in azoospermia in adults. The timing of puberty is usually normal, but the pubertal development reflects hypogonadism. The impaired Sertoli cell function leads to the *gonadotropin-dependent* hyalinization and fibrosis of the tubules; thus, the testes remain small and become firm to the touch during puberty. The associated destruction of the Sertoli cells leads to a marked decrease in the circulating levels of inhibin B. The primary cause of Leydig cell dysfunction is unknown. Although pseudoadenomatous clusters of Leydig cells give the histologic impression of hyperplasia, the *total number* of Leydig cells is decreased. The *total volume* of Leydig cells is normal, which indicates hypertrophy of the cells.The hypertrophic cells often display abnormal ultrastructure. The subnormal testosterone production increases the pulsatile release of GnRH. The decreased inhibin and androgen feedback to the pituitary together with a longer plasma half-life of FSH result in a disproportionately higher increase in FSH than in LH. The existing Leydig cells are overstimulated by LH and increase their aromatase activity. Because the condition develops in a gonadotropin-dependent manner, the decline in the production of testosterone is progressive. The progressive nature is also indicated by normal fetal masculinization of the genitalia and the typically normal descent of the testes in utero. The progressively subnormal pubertal production of testosterone is associated with normal to supranormal production of estradiol *compared to normal males*. The estrogen production is *subnormal for a female*. The consequences include *hypogonadism* (the *total androgen and estrogen levels are low*) and an elevated *estrogen:androgen ratio*, which leads to varying degrees of *feminization*. The hypogonadism results in delayed epiphyseal closure,

which manifests in low upper-to-lower body segment ratio, long upper limbs, and relatively tall stature. The decreased levels of androgens lead to varying degrees of *eunuchoid habitus* including the decrease in the pubertal growth of the penis, female-type pubic hair distribution, decreased facial hair growth, absence of hair line recession, relatively high-pitched voice, decreased libido, and impaired erectile function. The high estrogen:androgen ratio often causes *gynecomastia*; the stimulated ductal growth increases the risk of breast cancer twentyfold over normal males. The condition often remains undiagnosed before puberty. In childhood, behavioral problems and/or mild mental retardation may be present, and the lower limbs may be disproportionately longer than the trunk. The latter indicates a genetic component of the low upper-to-lower body segment ratio independent of delayed epiphyseal closure. Note that in the *Sertoli cell only syndrome* (also known as *germinal cell aplasia;* Spermatogenesis) only the FSH levels are increased (due to impaired inhibin secretion), but testosterone production and secondary sexual characteristics are normal.

Turner's syndrome (45,X), a condition due to meiotic nondysjunction, is the female prototype of gonadal dysgenesis and hypergonadotropic hypogonadism (Fig. 13-48). Similar to Klinefelter's syndrome, the germinal cells rapidly disappear from the gonad. The follicular epithelial cells, the female developmental equivalents of the Sertoli cells, also involute, and in their absence the ovarian stroma is unable to differentiate into a steroidogenic tissue. The result is an inert connective tissue termed *streak gonad*. At the time of puberty, gonadotropin secretion increases to postovariectomy levels (see Fig. 13-43) indicating normal hypothalamic and pituitary mechanisms of both puberty and gonadotropin regulation. As opposed to Klinefelter's syndrome, Turner's syndrome invariably results in *short stature*. The short stature is related to decreased gene dosage involving the pseudoautosomal region of the short arm of chromosome X (see Fig. 13-34). Growth hormone secretion is normal for a prepubertal female. Nevertheless, the affected children fail to show pubertal growth spurt and their growth can be stimulated by exogenous GH. The epiphyseal closure is finally achieved by adrenocortical androgens after their conversion to estrogens. This indicates normal adrenarche and adrenocortical function, which may also cause the appearance of *scanty* pubic hair. The hypogonadism manifests in *primary amenorrhea, absent breast development*, and decreased growth of ambisexual hair. The manifestations of physical stigmata (such as webbing of the neck, short neck with low hairline, broad chest, coarctation of the aorta, cubitus valgus, and [rarely] mild mental retardation) are variable and in their absence the diagnosis may be delayed until puberty.

Most Forms of Incomplete Precocious Puberty Are Associated with Low Levels of Pituitary Gonadotropin Secretion Apart from rare cases of pituitary gonadotroph tumors, the secretion of pituitary gonadotropins is suppressed by feedback mechanisms in the incomplete forms of precocious

puberty. In the absence of the appropriate endogenous pulsatile pattern of GnRH, the gonadotropin response to exogenous GnRH is decreased.

In males, due to the absence of increased FSH levels, incomplete precocious puberty is characterized by the absence of an increase in testicular volume. Testosterone production can be stimulated by extrapituitary gonadotropin-secreting tumors, most notably *hCG-producing tumors* of the testis. The hyperstimulation of Leydig cells increases aromatization just like in Klinefelter's syndrome, increases the estrogen:androgen ratio, and often results in gynecomastia. However, unlike Klinefelter's syndrome, the condition is not hypogonadism. Testosterone may be hypersecreted in an autonomous manner in *male-limited familial precocious puberty* (*testotoxicosis*), which is due to an activating mutation of the LH receptor (see Regulation of the Gonadotropin–Gonad Axis in Postpubertal Males). A similar condition may develop if a temperature-sensitive mutation of the $G\alpha_s$ subunit of the trimeric G-protein complex occurs; the mutation functions as a gain-of-function mutation at low temperature (scrotum) but as a loss-of-function mutation at core body temperature (see also Chap. 8). Virilizing forms of *congenital adrenal hyperplasia* (CAH) may present as isosexual incomplete precocious puberty in males. The ACTH-dependent growth of "adrenal rest tissue" usually appears as a bilateral testicular mass rather than the mostly unilaterally presenting testicular tumors. Congenital adrenal hyperplasia may present as contrasexual incomplete precocious puberty in females, who enter pubertal growth spurt, develop muscular male habitus, body odor, pubic, and axillary hair, but these quasipubertal changes are unaccompanied by breast development and menarche (see Fig. 12-9).

McCune-Albright syndrome is an activating somatic mutation of the $G\alpha_s$ subunit of the trimeric G-protein complex that occurs during early embryonic life (Fig. 13-51). Because the mutant protein is used by several hormone receptors, the mutation has variable consequences depending on the contribution of the mutant cell population in the development of hormone target cells (see Chap. 8). The prolonged exposure of the hypothalamus to the sexual steroids may advance pubertal development and the initially incomplete precocious puberty may be converted into the complete form.

Menopause, Andropause, and Adrenopause

Menopause, Andropause, and Adrenopause Are Characterized by the Decreased Production of Sexual Steroids by the Ovaries, Testicles, and Adrenal Cortex, Respectively *Menopause* is defined as the permanent cessation of the menstrual cycle secondary to the cessation of the ovarian cycle. The diagnosis of menopause is based on the absence of menstrual bleedings for at least 12 months. Thus, the diagnosis of menopause and its

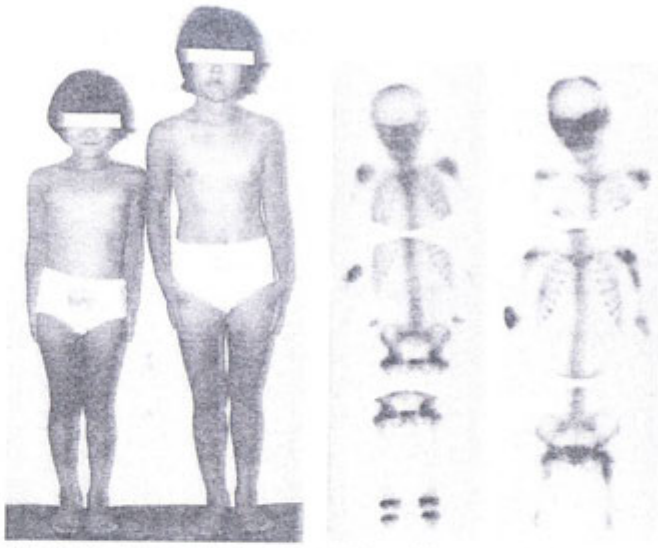


Figure 13-51. *McCune–Albright syndrome in 4-year-old monozygotic twins discordant for presenting with precocious puberty and polyostotic fibrous dysplasia. The discordance indicates that a somatic mutation occurred in one of the two sisters only after the separation of the two embryonic cell masses. A, Twin 1 (right) has entered the pubertal growth spurt and has reached Tanner stage 3 breast development. B, ^{99m}Tc-MDP scintigram of the bone indicating bone lesions in twin 1 (right) in the frontal bones, the base of the skull and the left humerus. The high density in the right arm is the site of injection of the radioisotope. (Source: Figs. 1 and 2 in Endo M et al: Monozygotic twins discordant for the major signs of McCune–Albright syndrome. Am J Med Gen 41:216–220, 1991.)*

timing is always retrospective. On average, last menstrual bleeding occurs at the age of 51.4 years (ranging between 42 and 60 years). Whereas life expectancy has increased over the past 100 years, the timing of menopause has remained the same. Thus, postmenopausal life has increased dramatically, and today's women are postmenopausal for approximately one-third of their entire lifespan. The absence of menstrual bleeding indicates that the endometrium is no longer exposed to sufficient cyclic stimulation by estrogens and progesterone. The average time elapsing between menarche and menopause (i.e., the reproductive phase) is approximately 38 to 39 years. A prolonged reproductive phase due to early menarche and/or late menopause indicates a prolonged exposure of the breasts to estrogens and progesterone, which is associated with an increased risk for breast cancer.

Andropause is defined as the age-dependent decrease of free testosterone in plasma below the low end of the normal range of young (30- to 35-year-old) adult men. The condition is due to a decreased number of Leydig

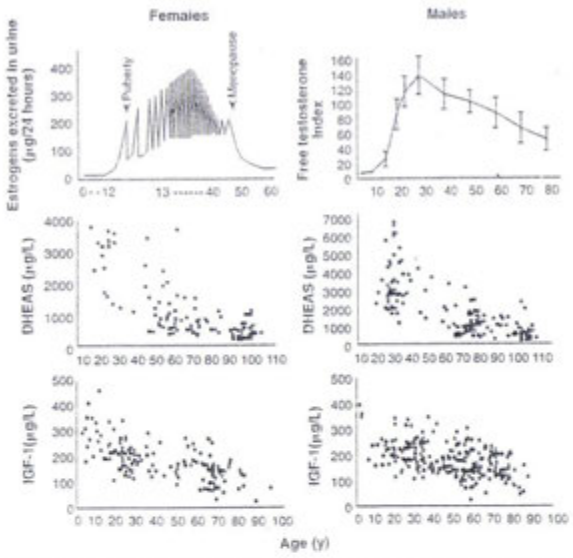


Figure 13-52. Age-related changes in various hormones in females (left) and males (right). Note the modified bell-shaped curves of sexual steroid parameters, the different scales for dehydroepiandrosterone sulfate (DHEAS) for females vs. males, the exponential decrease of DHEAS after the age of 30 years, and the approximately linear decrease of IGF-1 after its pubertal peak. Elderly men, who normally maintain higher production of sexual steroids (including DHEAS) than elderly women, also maintain higher levels of IGF-1. (*Source:* Fig. 3, p 421 in Lamberts SWJ et al: The endocrinology of aging. Science 278:419–424, 1997.)

cells. More than 60% of healthy men over the age of 65 years fulfill the laboratory diagnostic definition of andropause (Fig. 13-52). Nevertheless, significant amounts of testosterone continue to be secreted into old age, and the only unequivocal andropause is castration (*surgical andropause*).

Adrenopause is defined similar to andropause, and it is related to the age-dependent decrease in the production of DHEA and DHEAS by the zonae reticularis and fasciculata of the adrenal cortex.

The Mechanism of the Perimenopausal Process Involves the Decrease of Ovarian Follicles Below a Critical Number and Possibly the Age-Related Derangement of the Hypothalamic Regulation of Circadian Rhythms

Puberty is due to the maturation of the hypothalamus, and menarche is a developmental milestone that signals an underlying process that began years earlier. *Menopause is explained by a combination of the decline/exhaustion of the nonrenewable pool of ovarian follicles and probably the aging of the hypothalamus.* Similar to menarche, menopause is also the result

of a process that began years earlier. This process is termed *perimenopause*, which commences at about the age of 35 to 40 years. During perimenopause, fertility is decreased and the rate of meiotic nondysjunction progressively increases.

Perimenopausal symptoms usually begin with irregular menstrual cycles at the age of 35 to 40 years, when the depletion of the ovarian follicles reaches a *critical number*, when the total number of remaining follicles in the two ovaries is <25,000. In age-matched women, who still display regular menstrual cycles, the number of ovarian follicles is higher. Unilateral oophorectomy accelerates the onset of irregular cyclicity by decreasing the total follicular pool.

The exponential decrease of ovarian follicles implies that the number of follicles entering follicular development and becoming available for FSH-mediated rescue also decreases. The decreased follicular numbers translate into decreased granulosa cell mass and weaker inhibition of FSH secretion. As a consequence, the first signs of menopause are the irregular cycles (see below), *increased levels of plasma FSH, and decreased levels of inhibin and estradiol during the early follicular phase in cycling women.* The derangement is progressive (see Fig. 13-52), and is often associated with *hot flashes* and sleep disturbances. At the early stages, no change in the average plasma LH is observed, but the pulse frequency of LH decreases and the pulse duration increases during the follicular phase. In postmenopausal women, both FSH and LH are elevated, similar to those in castrated men (see Figs. 13-12 and 13-41).

The circadian rhythmicity of several hormones and neurotransmitters is regulated by the hypothalamic suprachiasmatic nucleus (see Chap. 14). As a part of the aging process, the biologic clock deteriorates. The consequence is the progressive derangement of the temporal organization of several functions, including the regulation of gonadotropin secretion. It has been proposed that the perimenopausal endocrine changes are at least in part secondary to hypothalamic aging. Although this notion has gained recognition, there is overwhelming experimental evidence supporting the role of the declining ovarian function as the primary cause of menopause.

The *irregularity* of the menstrual cycle (menometrorrhagia) is due to the unpredictable combination of several factors:

- Depending on the FSH responsiveness of the dominant follicle (which was selected from a very limited pool of developing early antral follicles), the follicular phase may either be shortened or prolonged, which leads to varying overall length of the menstrual cycle. A rapidly growing dominant follicle may achieve plasma estradiol *concentration* that results in positive feedback and ovulation earlier, but the total *amount* of estrogen secreted during the follicular phase is diminished.
- The decreased production of estrogens during the follicular phase may result in a diminished proliferation of the endometrium. Luteal progesterone prevents further proliferation as expected. After the involution of

the corpus luteum, the endometrial shedding is decreased: the menstrual bleeding is shorter and lighter.

- Estradiol secretion during the follicular phase may become insufficient to result in positive feedback, ovulation, and formation of the corpus luteum. In the absence of corpus luteum and progesterone, breakthrough bleeding may occur at irregular intervals.

Surgical menopause is the consequence of bilateral oophorectomy. Its manifestations are usually more severe than those due to natural menopause because the gradual perimenopausal endocrine changes are replaced by the abrupt elimination of ovarian hormones. However, even in the absence of hypothalamic aging and in the presence of relatively high adrenal production of androgens, the main manifestations and gonadotropin secretory patterns are essentially identical.

Postmenopausal Physiologic Changes Are Attributed to the Decrease of All Ovarian Steroid Hormones, Including Estrogens, Progesterone, and Androgens Menopausal changes are usually classified as early and late manifestations (Table 13-16). A leading early manifestation of menopause is a thermoregulatory vasomotor imbalance known as "hot flashes" (Fig. 13-53). *Hot flashes develop because of the sudden, transient decrease in the temperature setpoint of the hypothalamic "thermostat."* It appears that the same mechanism that results in pulsatile release of GnRH is also responsible for the phasic resetting of the hypothalamic thermostat (Fig. 13-54). The new setpoint results in the sensation of a hyperthermia, which activates countermeasures by the "cooling center" (see Box 11-3). Hot flashes are defined as recurrent, transient periods of sensation of heat, sweating, and (sometimes) flushing, which are accompanied by increased heart rate, the sensation of palpitations and anxiety, and are followed by chills. The cutaneous vasodilatation and sweating result in an acute heat dissipation which leads to a decrease of core body temperature ranging between 0.1 to 0.9°C. Hot flashes affect 24 to 93% of postmenopausal women in western countries. They occur with the highest frequency during the first 2 years after menopause, followed by gradual dissipation of the symptoms.

Table 13-16 Manifestations of Menopause

Early	Late
Perimenopausal irregularity of the menstrual cycles	Coronary heart disease
Hot flashes	Osteoporosis and periodontal disease
Atrophy of estrogen-dependent tissues: breast, genitourinary system (vaginal atrophy, urinary incontinence)	Cutaneous changes: loss of elastic fibers, decreased dermal water content and turgor, loss of ambisexual hair
Decreased sexual activity	
Depression	

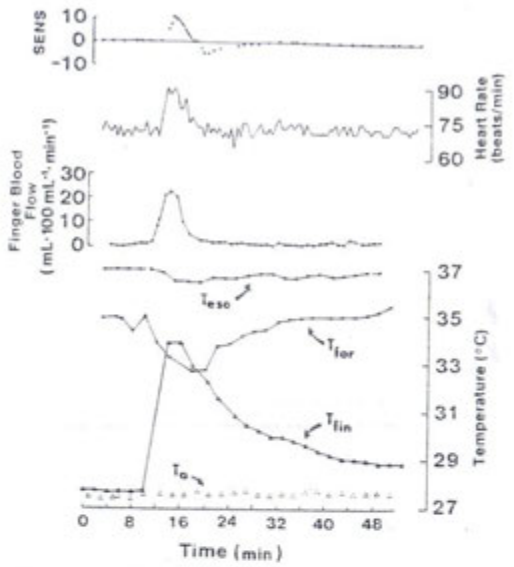


Figure 13-53. Characteristic physiologic changes during a hot flash. SENS, sensation; T, temperature; T_a, ambient, T_{fin}, finger, T_{eso}, esophagus; T_{for}, forehead. The cutaneous/peripheral vasodilatation increases finger temperature, but such an effect is offset by sweating-related heat-loss of the forehead. Note the slight decrease of core body temperature (T_{eso}). (*Source:* Fig. 1 in Kronenberg F, Downey JA: Thermoregulatory physiology of menopausal hot flashes: a review. Can J Physiol Pharmacol 65:1312–24, 1987.)

The ultimate cause of the deranged hypothalamic temperature regulatory mechanism is the decrease of the sex steroids, including progesterone. Thus, hot flashes are not specific for menopause: they occur during the puerperium (postpartum drop of placental steroids), and in men upon orchiectomy. Replacement of these hormones alleviates hot flashes; progesterone appears to be more effective than estrogens administered alone. In women experiencing surgical menopause secondary to salpingoophorectomy combined with hysterectomy, a combined regimen of estrogens and *androgens* instituted immediately after oophorectomy has been found most effective in eliminating or decreasing these symptoms. The sexual steroids have two sites of action:

- feedback regulation to neurotransmitters that directly regulate the GnRH pulse generator and the hypothalamic thermoregulatory centers; and
- direct action on the thermoregulatory centers to decrease their sensitivity to the feedback-regulated neurotransmitters.

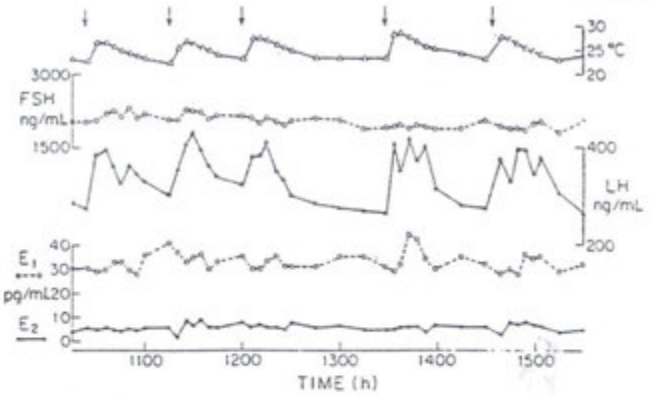


Figure 13-54. Serial measurements of finger temperature and luteinizing hormone (LH) indicate a close association between the pulsatility of gonadotropin releasing hormone (GnRH)/LH and hot flashes. The onset of hot flashes is indicated by the arrows. The association is due to (probably central adrenergic) mechanisms that simultaneously increase GnRH output and decrease the setpoint of temperature regulation. (*Source:* From Meldrum DR, et al: Gonadotropins, estrogens, and adrenal steroids during the menopausal hot flash. J Clin Endocrinol Metab 50:685–689, 1980.)

Chronic absence of the steroid hormones results in adaptive changes in the heat center responsiveness, which explains why pulsatile LH secretion continues after hot flashes have disappeared.

Postmenopausal *osteoporosis* is a severe consequence of estrogen deficiency. It is discussed in detail in Chap. 8.

During the reproductive years, women enjoy a decreased risk for *coronary heart disease* and *stroke* in comparison with men. The risk for these cardiovascular diseases increases after menopause and equals that of men of the same age. The main determinant of cardiovascular risk is the plasma lipoprotein profile. Estrogens decrease LDL and increase high density lipoprotein (HDL) levels is plasma. The effect on LDL is related to the estrogen-induced expression of LDL receptors that are crucial for the plasma clearance of LDL by binding its apolipoprotein B-100 (see Box 9-5).

Hormonal Therapy Alleviates the Symptoms of Menopause but Might Increase the Risk for Breast Cancer Various combinations and regimens of hormone therapy are in use, which are adjusted to the risk factors and preferences of the individual. Most postmenopausal complaints can be alleviated by estrogens. The early start of estrogen replacement is crucial for maintaining bone structure because estrogens can only prevent but cannot reverse osteoporosis. Estrogen replacement decreases the risk and severity of Alzheimer's disease, and may be beneficial in the treatment

of postmenopausal urinary incontinence. Estrogens increase the risk for endometrial cancer if their action is unopposed by progestins. Thus, in nonhysterectomized women estrogens are never administered without progestins. The estrogen/progesterone regimens may be tailored to yield cyclic bleeding or (by continuous administration) to avoid cyclic bleeding. Progestins, however, attenuate the beneficial effects of estrogens on plasma lipid profile. Prolongation of estrogen exposure of the breast might increase the risk for breast cancer, and this effect may not be antagonized by progestins. Coadministration of low-dose androgens with estrogens (in hysterectomized patients) does not deteriorate the plasma lipid profile compared to treatment with estrogen alone, but is more effective in controlling hot flashes, and in increasing libido and sexual activity.