Chapter 10

The Endocrinology of Mammalian Reproduction

Reproduction is the process that perpetuates a species through evolutionary time. It includes the process of sex determination and sexual differentiation (conversion of the indifferent gonads into testes or ovaries), embryonic development and birth, sexual maturation or puberty, development of gametes, physiological and behavioral aspects of mating, fusion of gametes, and development of the resulting zygote. In addition, a period of complex parental care is intercalated between birth and sexual maturation and possibly extends longer, such as in the case of humans. Every step in this complicated reproductive process is controlled directly or is modified by bioregulators secreted within the body or by pheromones from other members of the species.

The "reproductive system" includes the complex **hypothalamus–pituitary–gonad (HPG)** axis as well as the targets of steroid hormones secreted by the gonads. Environmental factors (chemical, visual, photic, thermal, and tactile stimuli) operating through effects on neural and endocrine factors frequently determine the timing of many reproductive events. Regulators of other endocrine axes, such as the thyroid (HPT) and adrenal (HPA) axes, have important effects on reproductive events, too (see Chapters 6 and 8).

Reproductive mechanisms are of central importance to survival of a species and hence are under intense evolutionary selection. Consequently, the reproductive system has been highly responsive to selective forces throughout the long evolutionary history of vertebrates. Because the same selective forces in the environment act upon all animal species, it should not come as a surprise that similar mechanisms have evolved in diverse vertebrates to achieve reproductive success in the face of similar pressures. Progressive "improvements" in the endocrine mechanisms regulating reproduction may not show progressive "development" from fishes to mammals, but instead we see specific adaptations to solve common environmental problems appearing in diverse groups of vertebrates. A case in point would be the achievement of viviparity in all but two extant vertebrate classes, the jawless fishes (Agnatha) and birds (Aves), as specific adaptations that, when coupled with varying degrees of parental care, result in a greater percentage survival of a small number of offspring. Viviparity represents only one solution, however, to similar selective pressures that confront all species. In birds, extensive and complicated parental behavior serves a similar evolutionary role as viviparity does in mammals to ensure reproductive success. In spite of the problems of environmental adaptations that tend to confuse evolutionary relationships, there remain numerous conservative features in the regulatory mechanisms of reproductive biology, and it is these that are emphasized in this chapter and in Chapter 11, where reproduction of non-mammalian vertebrates is discussed.

I. GENERAL FEATURES OF MAMMALIAN REPRODUCTION

Mammals can be separated into three, distinct taxonomic groups: Prototheria (monotremes), Metatheria (marsupials), and Eutheria (placentals). All possess **mammary glands**, specialized skin glands that are employed in secretion of milk to feed their young. The egg-laying monotremes comprise the most ancient group of mammals of which only a handful of species are extant. The marsupials or pouched mammals (**marsupium** = pouch) are confined mostly to Australia with a few species in North, Central, and South America. The marsupials are evolutionarily intermediate between the prototherians and the placental mammals, and there are about 230 extant species of marsupials. Their survival in Australia is due largely to the late arrival of eutherian mammals to that continent, whereas competition with eutherians has severely limited them in North and South America. The placental mammals are the dominant group of living mammals in number of species, distribution over the Earth, and abundance. The **placenta** is a specialized structure that develops through interactions of zygote-derived extra-embryonic tissues and some maternal uterine tissues. It provides nutritional, respiratory, excretory, and endocrine support for the offspring developing within the uterus. Although a placenta is present in marsupials, it is very short-lived and the marsupial fetus soon exits the uterus via the vagina and finds its way into the pouch. Most development for this

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exteriorized fetus takes place within the pouch rather than *in utero*, with the mammary glands providing the nutrition for continued development.

Reproduction is influenced by environmental variables that are perceived and integrated by the **central nervous system**. Communication between the nervous system and the reproductive system is achieved through the HPG axis, which coordinates specific gonadal events through regulation by circulating **gonadotropins (GTHs)**. This gonadal axis is modified by other systems, especially the HPT axis (Chapter 6) and the HPA axis (Chapter 8). Factors influencing GTH release and hence gonadal functions were discussed in Chapter 4 and are summarized only briefly here (see also Table 10-1).

Reproductive events in mammals are controlled through the release of **luteinizing hormone (LH)** and **follicle-stimulating hormone (FSH)** from the adenohypophysis under the control of a single hypothalamic hormone, **gonadotropin-releasing hormone (GnRH)**. Pulsatile release is an innate feature of GnRH neurons. The **tonic center**, located in the hypothalamus of both males and females, maintains a relatively constant pattern of pulsatile release of GnRH and produces rather static circulating levels of both LH and FSH. The **surge center** found only in the brains of females is responsible for the midcycle **LH surge** observed in mature females in response to elevated estrogen. In some species, these neural centers are distinctly separated in females (e.g., the rat), and in others there is no obvious anatomical separation (e.g., human). Although the pulsatile rhythm of GnRH secretion is ultimately due to calcium cycling within GnRH neurons and autocrine feedback on GnRH neurons by GnRH (see Chapter 4), oscillations in GnRH secretion can be stimulated by catecholaminergic neurons such as **norepinephrine (NE)** and inhibited by **endogenous opioid peptides (EOPs)**. Positive feedback of estrogens on GnRH release from the surge center in females is mediated by **GABA (γ-amino butyric acid)** neurons. GnRH secretion also is modulated by **kisspeptin** acting via **GPR54** receptors located on GnRH neurons (see Chapter 4), whereas **gonadotropin inhibitory hormone (GnIH)** acts directly on gonadotropes to inhibit FSH and LH secretion (Chapter 4).

Gonadotropins stimulate gamete maturation in males and females as well as steroidogenesis and release of **estrogens**, **androgens**, and **progestogens** (= progestins) into the general circulation. **Gametogenesis** (**oogenesis** in females and **spermatogenesis** in males) is controlled primarily by FSH, whereas LH is mainly responsible for controlling androgen synthesis as well as release of gametes in both sexes. Estrogen synthesis is influenced by FSH in both males and females. In some species, **prolactin (PRL)** may play a role in regulating ovarian steroidogenesis. The details of these events are discussed later. GTHs may be responsible for synthesis by the gonads of a variety of paracrine or autocrine factors that play roles in steroidogenesis or gametogenesis.

Induction of ovulation and formation of corpora lutea from the remnants of the ovulated follicle in the ovaries are due to LH. Surges in both LH and FSH occur in response to elevated GnRH prior to ovulation, but the magnitude of the LH surge greatly exceeds the FSH surge. LH release is enhanced selectively by the neuropeptide **galanin**, a peptide that is co-released with GnRH just prior to the midcycle LH surge. Galanin has no effect on FSH release. Under the influence of FSH, the ovaries secrete a peptide called **inhibin** that selectively blocks FSH release from the pituitary and contributes to the reduced FSH surge. In **polyestrous** species (poly = many; i.e., a very short diestrous phase), the importance of the FSH surge may be related to initiation of follicle development for the next cycle.

The gonadal steroids, secreted as a result of the action of GTHs on special cells in the ovaries and testes, control differentiation and maintenance of many **primary sexual characters** (such as the uterus) and **secondary sexual characters** (such as muscle development and beard growth in men). These gonadal actions were recognized hundreds of years ago by the Chinese, who used gonadal (and placental) preparations routinely to treat conditions ranging from impotence in men to the inability of a woman to bear sons. The hypothalamic centers regulating GnRH release are sensitive to circulating steroids that generally produce negative feedback on GnRH release, the exception to this pattern being the positive feedback effect by estrogens on release of GnRH from the surge center and subsequent stimulation of LH release from the pituitary.

Several other hormones are involved in mammalian reproduction in addition to those of the HPG axis. The HPT and HPA axes as well as PRL have already been mentioned. In addition, nonapeptides from the pars nervosa influence reproductive events including courtship, birth, and parental behavior. The placenta of eutherian mammals assumes an endocrine role in pregnant females, producing steroids (primarily estrogens and progesterone) and polypeptide hormones (e.g., **chorionic gonadotropins, CG; chorionic somatomammotropin, CS; corticotropin-releasing hormone, CRH;** GnRH; PRL). In addition, the endocrine glands of the fetus may influence reproductive events—for example, contribution of the adrenal cortex to steroidogenesis by the placenta. The **pineal gland** may be a modulator of photoperiod and a source of antigonadotropic factors that influence gonadal function and prevent early onset of puberty (see Chapter 4). Finally, there are numerous reports of chemical agents termed **pheromones** that are produced by one sex to influence reproductive physiology, behavior, or both in the opposite sex.

TABLE 10-1 Summary of Generalized Hormone Actions in Mammalian Reproduction

	Action in:	
Hormone	**Females**	**Males**
Kisspeptin	Enhances GnRH secretion	Enhances GnRH secretion
GnIH	Inhibits LH/FSH secretion	Inhibits LH/FSH secretion
GnRH	Stimulates FSH and LH secretion	Stimulates FSH and LH secretion
FSH	Initiates follicle growth; conversion of androgen to estrogen; synthesis of inhibin, $P450_{aro}$	Initiates spermatogenesis; secretic of androgen-binding protein, STP, and inhibin by Sertoli cell conversion of androgen to estrogen by Sertoli cell
LH	Androgen synthesis; ovulation; formation of corpus luteum from granulosa; secretion of progesterone initiated in corpus luteum	Androgen secretion by interstitial cell (Leydig)
Prolactin	Synthesis of milk	Stimulates certain sex accessory structures (with androgen)
Oxytocin	Contraction of uterine smooth muscle; menstrual sloughing; birth; orgasm; milk ejection from mammary	Ejaculation of sperm; orgasm
Androgens	Precursors for estrogen synthesis; stimulates sexual behavior	Complete FSH-initiated spermatogenesis; stimulate prostate gland, other sex accessory structures; stimulate secondary sexual characters, such as beard growth in man
Estrogens	Stimulate proliferation of endometrium; induces LH surge; sensitize uterus to oxytocin; negative feedback on pituitary release; may be primate luteolytic factor (estrone); may induce PRL surge; maintain pregnancy; involved in birth	Converted from androgens; induces male hypothalamus; stimulates sexual behavior
Progesterone	Maintains secretory phase of uterus; inhibits release of gonadotropins from adenohypophysis; maintains pregnancy	Facilitates/inhibits sexual behaviors depending on physiological context; influences brain development
Prostaglandins	Causes corpus luteum to degenerate at end of luteal phase in some animals: may be involved in birth initiation (induction of labor)	Ejaculation
Relaxin	Softens pelvic ligaments and cervix; possible role in lactation	Enhances sperm motility (secreted by prostate)
Placental CRH	Stimulates fetal HPA axis	Stimulates fetal HPA axis
Chorionic gonadotropin	Stimulates corpus luteum to produce progesterone	Not present in males
Chorionic somatomammotropin	Stimulates mammary to synthesize milk during late pregnancy; growth hormone-like (somatotropin) actions on metabolism	Not present in males
Inhibin (Sertoli cell factor, folliculostatin)	Inhibits FSH secretion from pituitary	Inhibits FSH secretion from pituitary

A. Embryogenesis of Gonads and Their Accessory Ducts

Primary sexual characters include the vagina, uterus, and oviducts of the female and the penis, vasa deferentia, seminal vesicles, and prostate gland of the male. Secondary sexual characters are often dependent on gonadal hormones and usually enhance mating success but are not necessarily required for physically mating and producing offspring.

1. The Gonads

The paired gonadal primordia arise from the intermediate mesoderm of the mammalian embryo as a genital ridge on either side of the midline in close association with the transitory mesonephric kidney of the embryo. Numerous derivatives of the mesonephric kidney and its duct system are retained as functional portions of the adult reproductive system, although the bulk of the mesonephric kidney degenerates. A gonadal primordium consists of an outer **cortex** derived from peritoneum and an inner **medulla** (Figures 10-1 and 10-2). Germ cells do not arise within the gonadal primordium itself but migrate from their site of origin in the yolk sac endoderm to either cortex (female) or medulla (male) depending upon the genetic sex (Figure 10-2). The basic pattern of germ cell migration is evolutionarily conserved from fruit flies to humans and requires a complex interplay between (1) guidance signals and extracellular matrix attachment proteins that ensure directed migration of the germs cells to the genital ridge mesoderm, and (2) a host of chemical signals involved in alignment of the germ cells within the gonad and coalescence of the developing gonad. Some of the genes involved in regulating primordial germ cell differentiation, migration, and meiosis are listed in Table 10-2.

Initially, the medullary component in males and females differentiates into **primary sex cords**. Differentiation of the primary sex cords into **seminiferous cords** and regression of the cortex result in a testis. Each testis consists of seminiferous tubules derived from the primary sex cords. The germ cells migrate into the seminiferous tubules, give rise to spermatogonia, and eventually produce sperm. The **Sertoli** or **sustentacular cells** support sperm development. Steroidogenic **interstitial cells** or **Leydig cells** are located between the seminiferous tubules. These interstitial cells arise from medullary tissue surrounding the primary sex cords and become sources of androgens.

In females, the primary sex cords degenerate, and **secondary sex cords** differentiate from the cortical region. These secondary sex cords become the definitive ovary. In the ovary, the germ cells give rise to **oogonia**, which soon enter meiosis to form **primary oocytes**. The ovaries contain **follicles** that consist of one or more layers of **follicular cells** surrounding a primary oocyte.

2. Accessory Ducts

In males, the central portion of each differentiating testis forms a network of tubules, known as the **rete testis**, that do not contain seminiferous elements. The rete testis forms a connection between the seminiferous tubules and a surviving portion of the primitive mesonephric kidney duct called the **wolffian duct**, which, under the influence of testosterone, differentiates into the **vas deferens** and conducts sperm from the testis to the urethra. Most of the

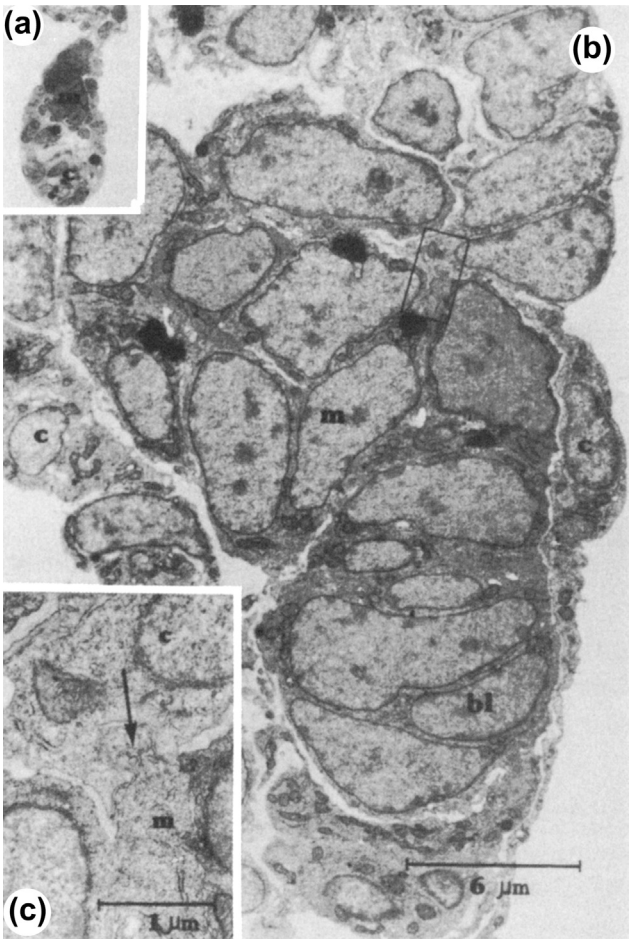


FIGURE 10-1 Undifferentiated gonad. Section of gonad from 25-mm tadpole of *Rana pipiens* showing cortical (c) and medullary (m) cells separated by a basal lamina (bl = basement membrane). (a) total gonad (upper left); (b) enlargement; (c) further enlargement showing contact between cortical and medullary cells (arrow). *(Reprinted with permission from Merchant-Larios, M., in "The Vertebrate Ovary" (R.E. Jones, Ed.), Plenum, New York, 1978, pp. 47–81.)*

mesonephric kidney in mammals degenerates, with the exception of some of the anterior mesonephric kidney tubules (see Box 10A). In the presence of testosterone, this tissue together with a portion of the wolffian duct forms two glandular structures, the **epididymis** and the **seminal vesicle** (Figures 10-2 and 10-3).

A second pair of longitudinal ducts develops in the embryo from the mesial wall of each wolffian duct and lie parallel to them. These structures are known as the **ducts**. In genetic females, the müllerian ducts develop into the oviducts, uterus and the upper part of the vagina (Figure 10-3), usually fusing together to form a common vagina and, in some species, a single uterus as well. The wolffian ducts degenerate in female mammals. In males, it is the müllerian ducts that are suppressed in favor of wolffian duct development.

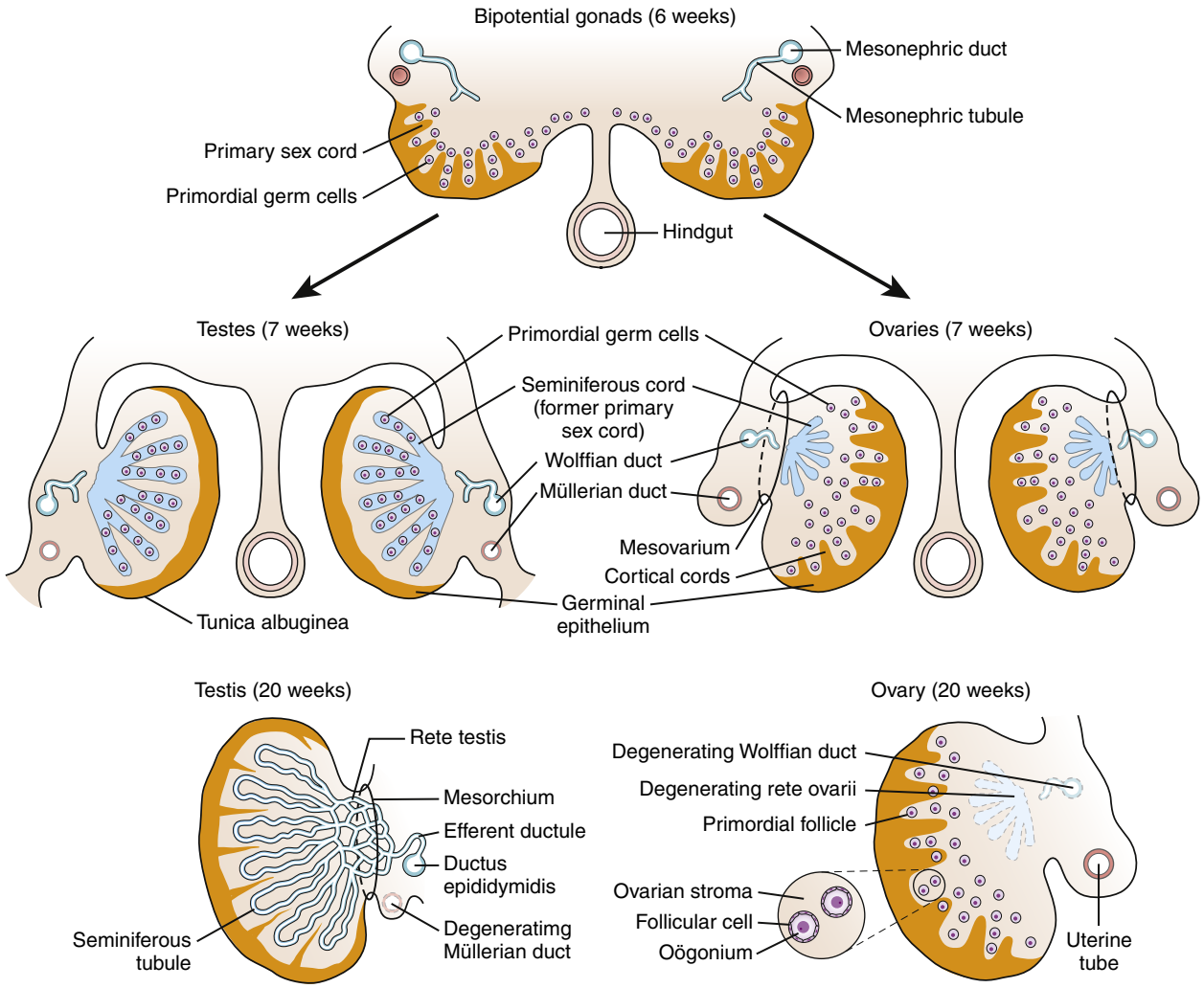


FIGURE 10-2 Development of testis and ovary in humans. Primordial germ cells migrate from the hindgut into the mesoderm of the bipotential gonad. In the male, the cortical tissue (orange) degenerates and the medullary tissue develops into the testis cords, which give rise to the seminiferous tubules including the Sertoli cells. Mesonephric tubules give rise to the intratesticular ducts such as the rete testis and the efferent ducts and vas deferens. In the female, the medullary cords degenerate, and the cortical cords (orange) give rise to an ovary. Some mesonephric elements remain in the female as well. The vasa deferentia are retained in amphibians but eventually they degenerate in reptiles, birds, and mammals in which the ureters develop to drain the metanephric kidneys (not found in anamniotes). *(Adapted with permission from Paxton, M., "Endocrinology Biological and Medical Perspectives," William C. Brown, Dubuque, IA, 1986.)*

Müllerian-inhibiting substance (MIS) was first proposed by Alfred Jost in the 1940s to explain the inhibitory effect of the testes on development of müllerian ducts in rabbit embryos. It also has been called the **anti-Müllerian hormone**, or AMH. AMH is a dimeric glycoprotein encoded by the *amh* gene that acts via a membrane serine/threonine kinase type-II receptor located in the gonads and in connective tissue near the müllerian ducts. Implantation of a testis into a female embryo results in sufficient AMH secretion to prevent development of the müllerian ducts. AMH not only blocks müllerian duct development but also is capable of inhibiting growth of tumors from ovaries and müllerian duct derivatives. It appears that AMH acts cooperatively with testosterone in producing these effects on the müllerian ducts. The ovary also makes AMH, but the müllerian ducts are protected by local estradiol secreted by the ovary.

Maleness in eutherian mammals is dependent upon secretion of androgens from the testis. In the absence of androgens or androgen receptors the male animal (genotype XY) will develop a female phenotype. Similarly, exposure of developing males to estrogens will result in female phenotype development to a degree proportional to the amount of estrogen and the timing of the exposure (see Table 10-3). Conversely, treatment of newborn females with androgens destroys the cyclical secretory pattern of the HPG axis and replaces it with a noncyclical or tonic pattern like that of males (see Box 10B). Becoming a male mammal, then, involves overcoming the basic tendency for mammalian embryos to develop as females. A gene

TABLE 10-2 Some Genes Involved in Primordial Germ Cell (PGC) Induction, Specification, Migration, and Meiosis

Gene	Name	Role
bmp 2/4/8	Bone morphogenic protein	Induction and competence of PGCs
prdm1 and *prdm14/blimp1* (mouse)	PR domain zinc finger protein 1	Required for PGC specification
pou5f1	POU domain class 5, transcription factor 1	PGC marker and specification
vasa; ddx4	DEAD box family of ATP-dependent RNA helicases	PGC marker and specification
nanos3	Nanos homolog 3	Migration, entry into mesoderm
dnd1	Dead end homolog 1	Migration, entry into mesoderm
kit	Mast/stem cell growth factor receptor (SCFR); proto-oncogene c-kit	Migration, entry into mesoderm
dazl	Deleted in azoospermia-like	Meiosis competency

BOX 10A Vertebrate Kidney Evolution

The first kidney in vertebrates was the **pronephros**. It appears in vertebrates only as a transitory structure during early development, and only remnants of the pronephros remain as the anteriormost part of the fish kidney that is known as the head kidney. This structure consists largely of lymphoid tissue as well as adrenocortical cells. The duct that drained the pronephros to the cloaca is retained. It is called the **pronephric** or **archinephric duct**. Posterior to the pronephros develops a second kidney, the **mesonephros**, which co-opts the pronephric duct as its conduit to drain urine to the cloaca. Developmentally, this **mesonephric duct** is called the wolffian duct in the embryo. The mesonephros becomes the definitive kidney of fishes and amphibians, where it is often designated as the **opistonephric kidney**. The wolffian duct is retained in both male and female fishes as a kidney duct and may also be used as a sperm duct in males of elasmobranchs as well as in amphibians. In amniote vertebrates, a third kidney that develops posterior to the opistonephros is called the **metanephric kidney**. A new urinary duct, the **ureter**, develops to connect the metanephric kidney to the urogenital sinus. The wolffian duct is retained as the epididymis and the vas deferens in males. A portion of the wolffian duct also gives rise to the seminal vesicles that retain a connection to the vas deferens. In addition, some of the mesonephric kidney tubules form the rete testis, which connects the seminiferous tubules of the testes to the epididymis. In female amniotes, the wolffian duct degenerates. Some mesonephric tubules are retained in females and become associated with the ovaries. In elasmobranchs, amphibians, and amniotes, a pair of müllerian ducts develops adjacent to the wolffian ducts. In females, these ducts give rise to the oviducts and uteri but usually degenerate in males. The utricle of the prostate gland in male mammals actually is a müllerian remnant. It is the stimulation of this female remnant by estrogens that is responsible for most prostate cancer.

seemingly responsible for male sex determination called ***sry*** (sex-determining region of Y chromosome) has been localized on the short arm of the Y chromosome that is characteristic of genetic males. In mice, the *sry* gene is activated in gonads of genetic males before they begin to differentiate into testes. Insertion of the *sry* gene into XX mice followed by its activation leads to formation of male-specific structures and regression of female ducts. The activated gonad secretes AMH, which causes regression of the müllerian ducts. The *sry* gene produces a factor called **testis determining factor (TDF)** (Figure 10-3) that activates the *amh* gene. Androgens secreted by the transformed gonad cause male-like differentiation of the external genitalia and the wolffian ducts as well as changes in the hypothalamus to suppress development of the surge center. This establishes the tonic secretory pattern for GnRH and GTHs that characterizes males. Studies with estrogen receptor knockout (ERKO) mice verify that defeminization of the male brain requires conversion of androgens to estradiol. Genetically male ERKO mice will exhibit female behavior, whereas wild-type males do not.

Although the female has been called the default sex in mammals, becoming a female is not just the absence of androgens. For example, studies have shown specific genes are required to be expressed in order for the ovary to form and that estrogens are necessary for development of the female difference in the corpus callosum of the brain.

Androgens and estrogens may alter basic traits through what are termed **organizational effects**. Stimulation of the development of male genitalia by androgens is an example of an organizational effect. Organizational effects are permanent and cannot be reversed later by exposure to other gonadal steroids. In contrast, **activational effects** can be induced by

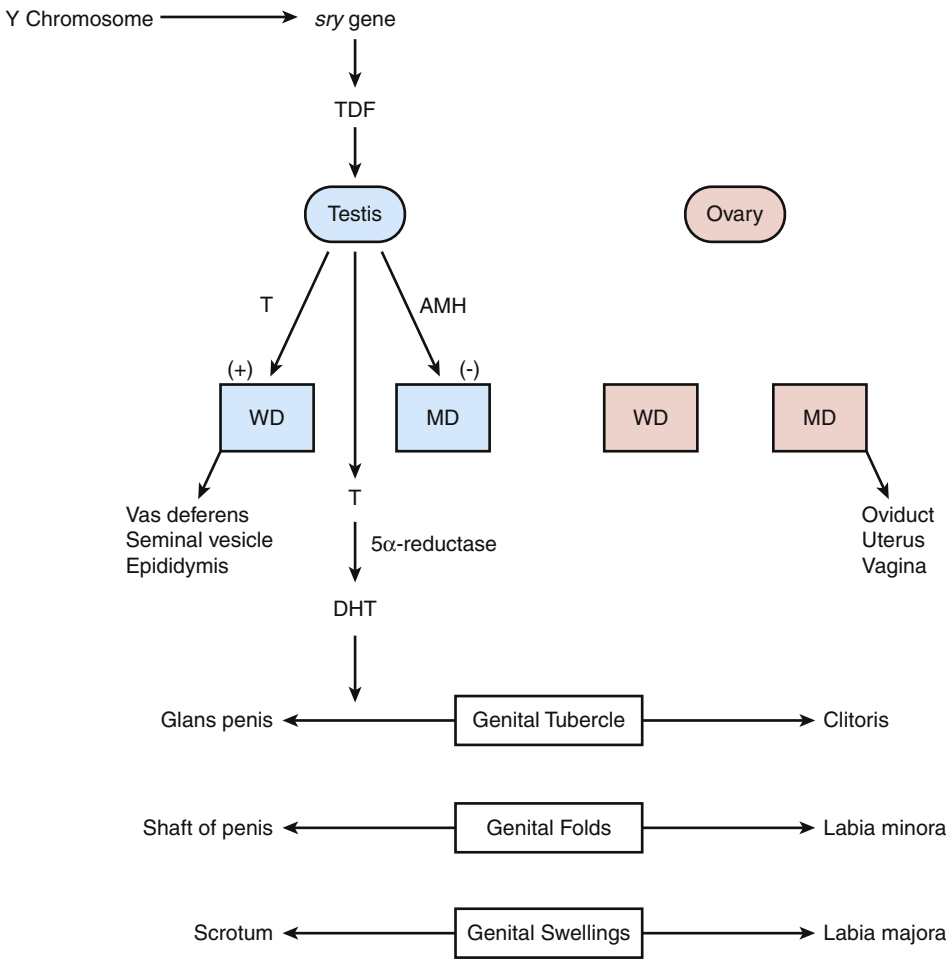


FIGURE 10-3 Patterns of development for ducts and genitalia. In males, the testis secretes testosterone (T), which stimulates differentiation of wolffian ducts, and müllerian-inhibitory substance (MIS), which causes regression of müllerian ducts. Dihydrotestosterone (DHT) is either produced by the testes or converted from T in the genital tubercle, genital folds, or genital swellings, causing them to differentiate in the male direction. Estradiol from the ovary prevents MIS (also secreted by ovary) from causing müllerian duct regression, and the absence of sufficient androgens determines the fate of the other structures. Abbreviations: *sry*, sex-determining region of the Y chromosome; TDF, testis determining factor.

TABLE 10-3 Critical Periods for Sexual Differentiation of the Brain in Mammals

Species	Gestation Period (days)	Critical Period (days)
Hamster	16	16–21
Laboratory rat	21–22	18–28
Laboratory mouse	18–22	20
Guinea pig	68	30–35
Human	270	84–126

gonadal steroids—for example, by inducing a specific behavior in adults. The type of behavior induced depends on the steroid applied, not on the genetic sex of the individual.

II. REPRODUCTION IN MONOTREMES AND MARSUPIALS

A. Monotremes

The monotremes have retained the reptilian feature of laying eggs but have mammary glands for feeding the young after they hatch. Unlike the eggs of reptiles and birds, monotreme eggs contain little yolk, and embryonic nutrition is supplied through uterine secretions that pass through the porous leathery shell as the egg passes through the female genital tract. Upon hatching, the new monotreme appears as a tiny, fetus-like creature with only a few well-developed features that enable it to attach itself to its mother's mammary gland and obtain nourishment. The mammary glands of monotremes lack external teats; consequently, milk is secreted onto a special common area, the **areola**. The newly hatched monotreme must attach

itself to its mother with its forelimbs and suck or lick milk from the areola. Development continues and the offspring, because of its primitive nature, is considered an exteriorized fetus until it has completed development.

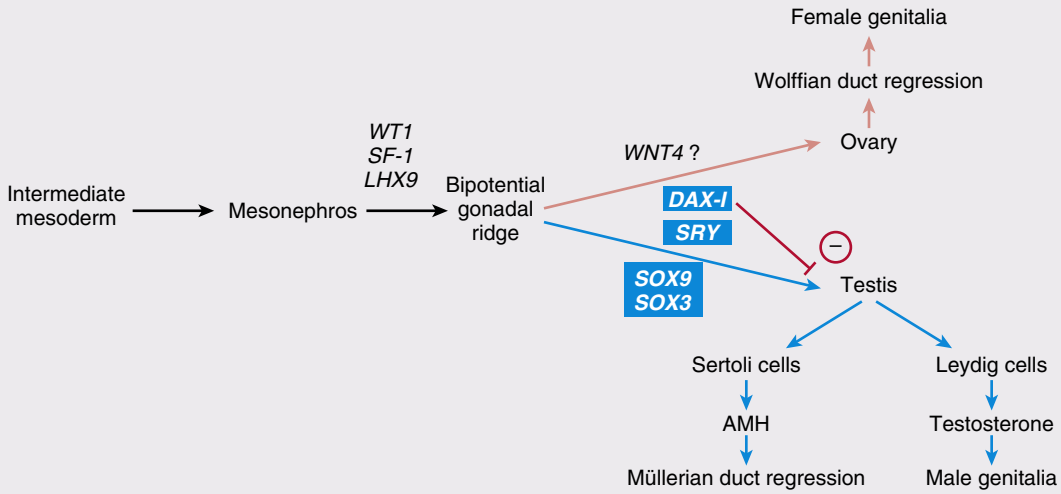
1. Monotreme Reproductive Patterns

Seasonally breeding monotremes are difficult to study because they are largely nocturnal animals and are carefully protected in nature. Furthermore, they do not breed readily in captivity. The duckbill platypus lays its eggs in a nest, but the female spiny echidna places her freshly laid eggs in a brood pouch that develops seasonally on her ventral surface. Formation of the pouch is probably dependent upon estrogens, although no experimental data are available to confirm this. The eggs develop and hatch within the pouch, and mom remains in her burrow while they develop. The brood pouch regresses after the breeding season. Echidnas enter a period of torpor and inactivity prior to the breeding season when they arouse to mate. Attempts to measure

BOX 10B Gene Regulation of Gonadal Development

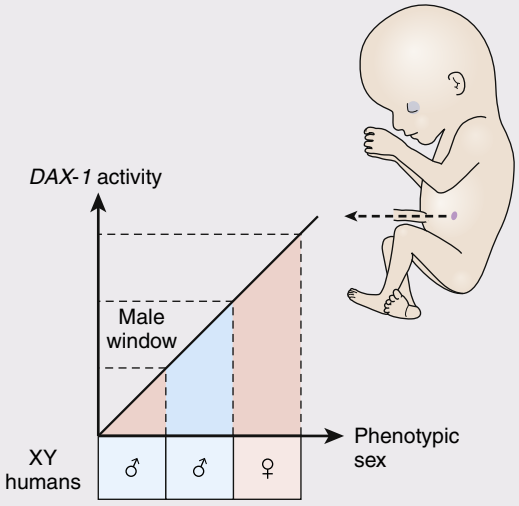
Gonadal phenotype in mammals ultimately is determined by the presence or absence of the Y chromosome and *sry* gene. However, even before the molecular switches governing testis formation are called into play there are cellular events that must occur for the bipotential gonad precursor to form from mesoderm. To simplify the seemingly complex process underlying sexual fate it is helpful to divide sexual development into the two major events that bracket gonad formation: **sexual determination**, the mechanisms involved in testis or ovary formation, and **sexual differentiation**, which are the events requiring normal gonadal hormone secretion resulting in the overall male or female phenotype (see Figure 10-3). In order for normal gonadal development to occur, many genes are believed to be involved in formation of the bipotential gonad, the testis, and the ovary. Identification of the genes regulating gonadal development is important not only for understanding subsequent problems with sexual differentiation but also for understanding the full spectrum of **disorders of sexual development** (**DSDs**) in which gonadal phenotype is atypical of the normal male or female structure. Understanding how these genes and their protein products interact and are modulated by endocrine-disrupting chemicals obviously will lead to a better understanding of the genetic and epigenetic basis for directing the development of the ovary or testis. A simplified scheme for gene regulation of gonadal development is shown in Box Figure 10B-1. Links between defects in these genes and known DSDs are summarized later in this chapter.

The fact that the absence of *sry* expression leads to ovary development may lead one to conclude that ovary development is the default *bauplan*. We now know this to be inaccurate, as both testis and ovary formation requires a cascade of gene expression. *dax-1*, a gene encoding an orphan member of the nuclear hormone receptor family, is a gene that was initially shown to be critical in ovary formation (Box Figure 10-B1) and at the same time function in XY cells. Its role in males as initially proposed is to antagonize *sry* function (Box Figure 10-B1). More recent evidence suggests a more complicated role for *dax-1* in gonad formation, with *dax-1* expression during critical windows of development (7 weeks gestation in humans). If *dax-1* expression exceeds or falls below normal levels during the critical period, problems in testis formation may occur (Box Figure 10B-2).



BOX FIGURE 10B-1 Genes regulating gonadal development in humans. Abbreviations: AMH, anti-müllerian hormone; *DAX-1*, dosage-sensitive sex reversal, adrenal hypoplasia critical region, on chromosome X, gene 1; *LHX9*, LIM homeobox 9; *SF-1*, steroidogenic factor 1; *SOX3*, sex-determining region Y box 3; *SOX9*, sex-determining region Y box 9; *SRY*, sex-determining region of Y chromosome; *WNT4*, wingless-type MMTV integration site family, member 4; *WT1*, Wilms tumor 1. *(Compiled from many sources.)*

BOX 10B Gene Regulation of Gonadal Development (Continued)



BOX FIGURE 10B-2 Testes formation requires *DAX-1* activity during a critical period of development. The *SRY* gene initiates testes development by the seventh week of pregnancy in humans. Full development of the testes requires a set level of *DAX-1* activity during this period (blue); *DAX-1* activity that is too high or too low will result in abnormal testes development in XY individuals. *(Reprinted with permission from Ludbrook, L.M. and Harley, V.R.,* Trends in Endocrinology & Metabolism*, **15**,116–121, 2004.)*

circulating estrogens by radioimmunoassay in platypus and echidnas have been unsuccessful as plasma levels are below the detectable level (<1 ng/mL). Examination of steroid metabolite levels in feces employed with chromatographic and mass spectrophotometry methods (see Chapter 2) would provide the sensitive noninvasive approach needed for these species. Measurable progesterone levels in female echidnas rise after mating as the pouch undergoes development and peak just prior to the appearance of an egg in the pouch (Figure 10-4). When males are in breeding groups with females, they exhibit elevated testosterone levels (Figure 10-4).

B. Marsupials

The marsupial placenta is rather primitive and apparently has no major endocrine function when compared to the eutherian placenta. The period of pregnancy or gestation is very short in marsupials (Table 10-4; Figure 10-5), and the young marsupial is born in an extremely immature condition. For example, among the macropodid marsupials (kangaroos, wallabies), the **joey** must find its way essentially unaided to the mother's pouch, where it permanently attaches to the nipple of a mammary gland. The attached joey, like the newly hatched monotreme, continues its development as an exteriorized fetus. After a long period of pouch development (about 200 days in the red kangaroo), the young marsupial disengages itself from the teat and ventures outside of the pouch, returning first at regular and later at irregular intervals for milk.

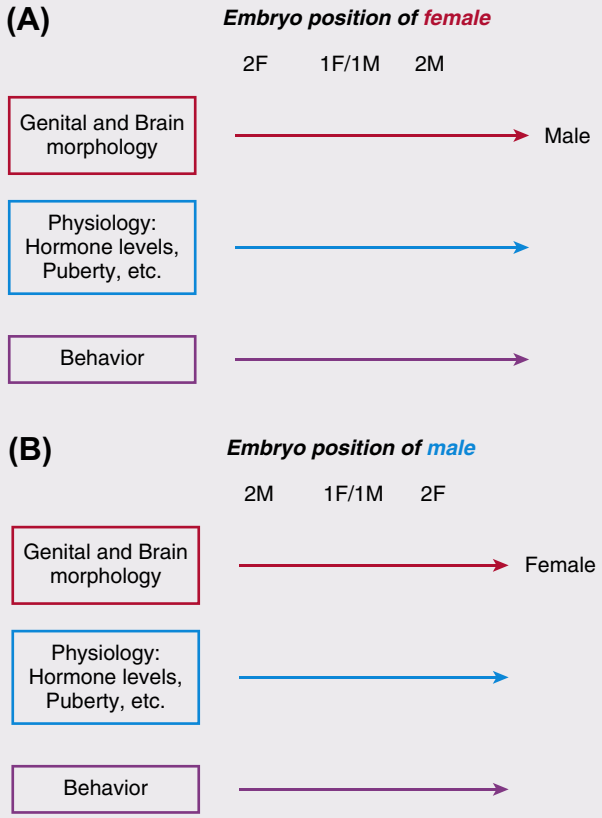
1. Marsupial Reproductive Patterns

Proestrus in females is characterized by follicular enlargement, estrogen-dependent uterine proliferation and enlargement of elements of the vaginal complex. Peak uterine and vaginal development coincides with estrus and copulation. Ovulation occurs spontaneously one to several days after the onset of estrus, and postovulatory follicles transform into corpora lutea that maintain a short secretory uterine condition. Progesterone continues the secretory uterine phase in castrates and is undoubtedly the hormone responsible for maintaining gestation as it is in eutherian mammals (Figure 10-5). In marsupials, estradiol is the main circulating estrogen and in males testosterone is the main circulating androgen (Table 10-5).

The luteal phase is the same in mated and unmated females, and no "pregnancy-recognition signal" is necessary. Pregnancy is very short (Table 10-4) and does not affect ovarian function. Both pregnant and unmated females return to proestrus at about the same time in most species. Equivalent mammary gland development occurs during post-estrus in both pregnant and non-pregnant females, and newborn foster young will develop normally if attached to virgin or non-lactating females at the equivalent post-estrous state in relation to the time of parturition or

BOX 10C Changes in Sexual Differentiation Caused by Exposure to Gonadal Steroids

Exposure of developing mammals to external (exogenous) sources of either androgens or estrogens can alter the sexual phenotype regardless of the genetic sex. The most elegant demonstration of the subtle effects of exposure to exogenous steroids was that of Frederick vom Saal, who observed that the position of the mouse embryo *in utero* could determine anatomical, physiological, and behavioral traits in the offspring (Box Figure 10-C1). Thus, a genetic female that developed between two males could be influenced by male hormones. When examined as newborns or adults, such females exhibited male traits (see Box Figure 10-C1A). Similarly, a male developing between two females will later exhibit some degree of feminization (see Box Figure 10-C1B). In cattle, when male and female twins share a common blood supply, the female will be masculinized to such a degree that it will be born as an intersex incapable of reproduction (called a *freemartin*). Similarly, recent studies of human dizygotic twins of opposite sexes provide evidence for masculinization of the hearing apparatus and behaviors in the female presumably as a result of *in utero* exposure to androgens.



BOX FIGURE 10-C1 The impact of position of embryos in utero on morphology, physiology and behavior. A female that develops between 2 females vs. between 1 female and 1 male vs. between 2 males shows a progressive tendency toward maleness as a newborn and an adult (Figure 10C-1A). Similarly, a male developing between 2 males vs. between 1 male and 1 female vs. between 2 females shows a progressive tendency in femaleness (Figure 10C-1B). *(After the work of Dr. Fredrick vom Saal and associates.)*

cycle cessation. Circulating progesterone and urinary pregnanediol levels are similar in pregnant, unmated, and luteal-phase females. That there are no endocrine differences between the pregnant and non-pregnant post-estrous phase supports the conclusion that the marsupial placenta is not an endocrine organ.

Birth in wallabies involves an increase in **relaxin**, an insulin-like hormone discovered in eutherian mammals, and a reduction in progesterone. Relaxin is thought to soften the cervix of the uterus to ease the birth process as it does in eutherians (see ahead). Increases in prostaglandins and upregulation of **mesotocin (MST)** and **oxytocin (OXY)** receptors in the uterus aid in birth. Uterine contractions are probably initiated by MST and/or OXY. Fetal glucocorticoids are also elevated at the end of gestation and may be involved in birth events as described for eutherian mammals (see ahead).

Lactation is somewhat unique in marsupials. Marsupials can simultaneously produce two kinds of milk. As in eutherian mammals, the first milk differs markedly in

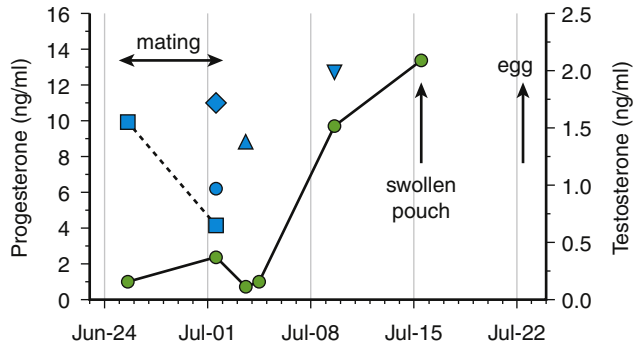


FIGURE 10-4 Progesterone and egg laying in the echidna. Progesterone levels (green circles) rise following mating and prior to the appearance of an egg. Testosterone levels in five attendant males are indicated by the blue symbols. *(Adapted with permission from Nicol, S. et al.,* General and Comparative Endocrinology, ***144****, 204–210, 2005.)*

composition from that produced later during lactation. However, macropodid marsupials, which may have both a newborn joey and one that has already detached itself from a teat, will produce early and late milk simultaneously in the respective glands. The developmental state of a particular mammary gland would seem to be independent of endocrine conditions and strongly influenced by external conditions—that is, the joey. Furthermore, some marsupials (i.e., kangaroos and wallabies) exhibit a **lactating diapause** that inhibits implantation of a developing embryo (see Box 10F).

A number of rodent-like marsupials have a reproductive cycle in which all of the males die after a single breeding. For example, in the brown antechinus (*Antechinus stuartii*), annual reproduction is controlled strictly by photoperiod. Mating is restricted to the same two-week period each year, with the males exhibiting extreme aggression to one another and mating vigorously and frequently with females. This mating frenzy is accompanied by reduced levels of **corticosteroid binding globulin (CBG)** (see Chapter 8) and elevated cortisol secretion in the males that leads to a spike in free or available cortisol and subsequent neural, gastrointestinal, and kidney degeneration and death. Interestingly, castration of the males prevents the reduction of CBG and increases survival. The females generally survive breeding and rear their young with no help from the departed males. In years of good environmental conditions, the females may live to breed a second year.

A major developmental difference between eutherians and marsupials has had a profound influence on reproductive patterns in the latter group. marsupials exhibit a primitive reptilian pattern of wolffian and müllerian duct origins. Instead of developing medially to the kidneys and ureters as in eutherians, these ducts develop laterally. Consequently, it is not possible for left and right Müllerian ducts of females to fuse in the midline without placing considerable strain on the ureters. It is believed that the short gestation period of marsupials (Figure 10-5) is

TABLE 10-4 Comparison of Length of Estrous Cycle, Gestation Period, and Ratio of Body Weight of Neonate to Body Weight of Mother in Metatherian and Eutherian Mammals

Species	Length of Estrous Cycle[a] (days)	Length of Gestation Period (days)	Neonate:Mother Body Weight Ratio
Eutheria			
Rat	4–5	22	—
Sheep	16	148	1:14
Metatheria			
Virginia opossum	29	12	1:8,300
Long-nosed bandicoot	26	12	1:4,250
Brush possum	26	17	1:7,250
Dama wallaby	30	29	1:10,000
Swamp wallaby	31	37	—
Red kangaroo	35	33	1:33,400
Western gray kangaroo	35	30	—

[a]*The estrous cycles are similar to the gestation period in metatherians and may not require a pregnancy-recognition mechanism.*
Adapted with permission from Sharman, G.B., in "Reproduction in Mammals, Vol. 6" (C.R. Austin and R.V. Short, Eds.), Cambridge University Press, Cambridge, U.K., 1976, pp. 32–70.

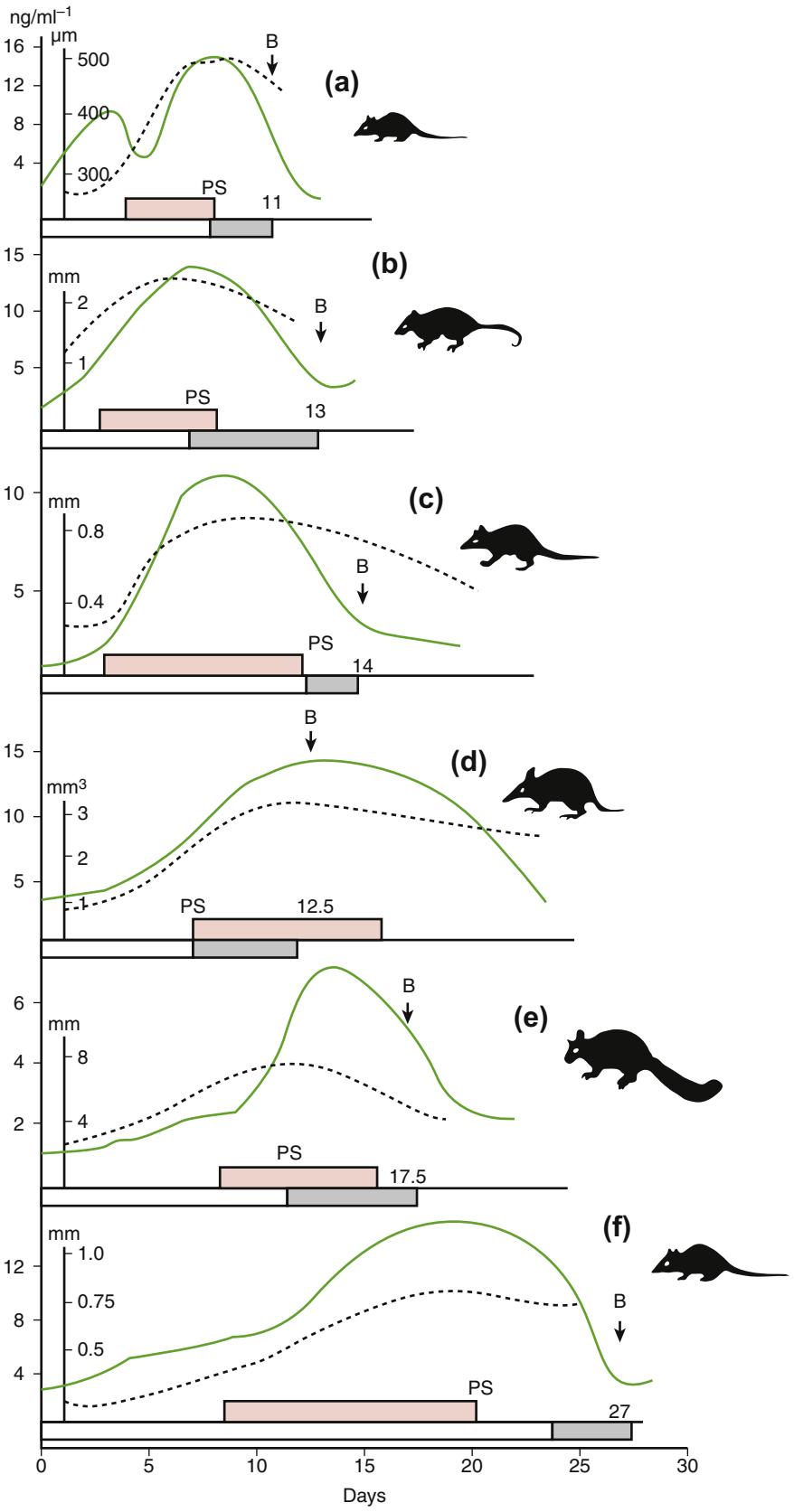


FIGURE 10-5 Reproductive cycles in six marsupial species show the relatively short gestation period before birth (B). Pink bar represents the luteal phase, and the dashed line represents the size of the corpus luteum with circulating progesterone levels shown in the green line. Pregnancy is shown by the bar at the bottom where the open bar represents the attached phase and the shaded bar represents the unattached phase. PS, time to primitive streak stage of development. (a) Stripe-faced dunnart (*Sminthopsis macroura*), (b) Virginia opossum (*Didelphis virginiana*), (c) eastern quoll (*Dasyurus viverrinus*), (d) northern brown bandicoot (*Isoodon macrourus*), (e) brushtail possum (*Trichosurus vulpecula*), (f) brown antechinus (*Antechinus stuartii*). *(Adapted with permission from Bradshaw, F.J. and Bradshaw, D.,* General and Comparative Endocrinology, ***170****, 18–40, 2011.)*

TABLE 10-5 Steroid Levels in the Tammar Wallaby (*Macropus eugenii*)

Tammar Wallaby	Reproductive Status	E_2 (pg/mL)	T (ng/mL)
Female	Day 5 of estrous cycle	15	N.D[a]
	Rest of cycle	5–10	N.D[a]
Male	Non-breeding	N.D	1–3
	Breeding	N.D	6

[a]*N.D., non-detect.*

a consequence of separate uteri and vaginas and limited space for uterine hypertrophy. A special birth canal must be formed so that parturition can occur, and in some species it forms anew each season. This development of separate vaginas has influenced evolution of the male reproductive system as well. Males of some species have a bifid penis, with left and right prongs apparently being inserted into the separate vaginas of the female during copulation.

III. REPRODUCTION IN EUTHERIAN MAMMALS

Reproductive events such as spermatogenesis and oogenesis and their hormonal control are similar in monotremes and marsupials to events described for eutherian mammals. Consequently, most of the events and associated terms described ahead for eutherians probably apply to monotremes and marsupials, although the latter groups have not been studied to the extent of eutherians. Only marked differences will be notes in the following accounts.

Eutherian mammals employ the placenta not only as an endocrine organ to maintain gestation but also as a replacement for the mammary glands to supply early nutrition to the fetus. Consequently, birth (**parturition**) is delayed considerably, and the newborn or **neonate** of most placental mammals is at a comparable stage of development to the young joey when it first ventures out of the pouch. Like the juvenile marsupial, the placental neonate relies at first on the mammary gland as the exclusive source of nourishment but gradually abandons it for other foods.

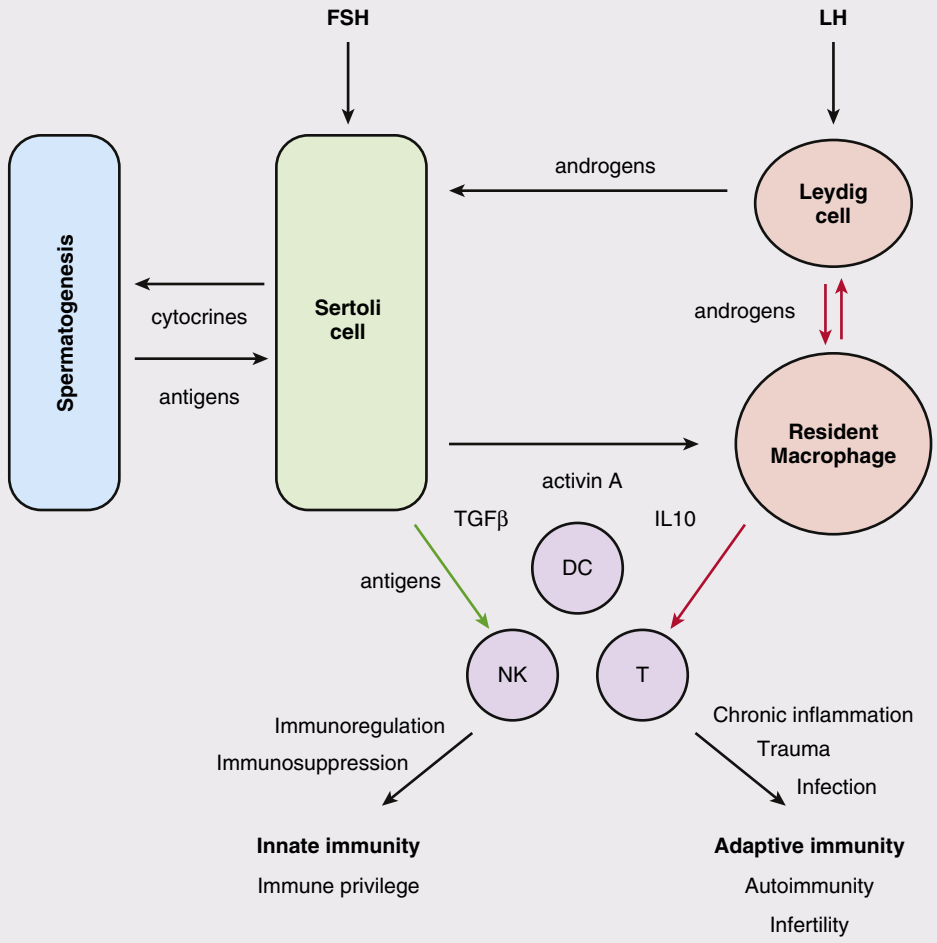
Three distinct reproductive patterns occur in sexually mature eutherians: one typical for all males and two among females. Males of some domesticated species and humans are characterized by continuous secretion of GTHs and occasionally continuous spermatogenesis, and these males are capable of siring offspring at any time of year. Most eutherian species, however, exhibit seasonal episodes of spermatogenesis, sexual activity, or both such as displayed by most non-mammalian vertebrates (see Chapter 11).

Although considered a continuous breeder, humans show some dramatic seasonal patterns of breeding activity correlated with photoperiod and latitude (based on the past 30 to 50 years of birth records). In Europe and Japan, there are more births in the early spring (March–April) with the amplitude of the birth peak increasing in a south-to-north gradient. In South Africa, Australia, India, and New Zealand, the birth peak is associated with the months of September through November. The amplitude of the birth peak decreases from north to south, which is opposite to the latitudinal change observed in the Northern Hemisphere. However, in North America, the greatest number of births for both white and non-white people (many having their origins as transplants from Europe and Africa, respectively, in the past 300 years) occurs from August to October, with a decrease in amplitude from warmer to colder latitudes as occurs in countries of the Southern Hemisphere of the Old World. It has been proposed that North American patterns are a response to environmental temperature superimposed over the Old World patterns that correlate more strongly with photoperiod. These variations with latitude are less obvious for the Northern Hemisphere in the Old World and are opposite to that observed in the New World and the Southern Hemisphere of the Old World. In spite of these differences, it is clear that humans show seasonal tendencies in reproductive activity, resembling those of other eutherians.

Females exhibit cyclic patterns of GTH secretion that can be traced to a basic rhythmicity probably residing within the hypothalamus. There are two types of female cycles: the ovarian-based estrous cycle and the uterine-based menstrual cycle. The **estrous cycle** is typical for mammals (except possibly humans) and consists of a series of precisely regulated endocrine events repeated in each cycle. **Proestrus** is characterized by hormonal changes that bring about follicular development and ovulation. **Estrus** is a short period when the female is receptive to the male and during which mating can occur. It immediately follows proestrus and coincides with ovulation. Estrus represents the time when fertilization is most likely to lead to pregnancy and successful birth of offspring. The interim between estrus and the onset of hormonal changes characteristic of proestrus in cases in which pregnancy did not result is termed **diestrus** and usually is associated with ovarian inactivity and uterine regression. Carnivores and some other mammals may be classified as **monestrous**. If mating does not occur in a monestrous species or if mating occurs but fertilization and implantation are unsuccessful, the female will not return to estrus until the next breeding season (mono = one). Hence, these species have a long diestrous phase. Many mammalian species are polyestrous,

BOX 10D Serftoli Cells and Organ Transplant

The testes are considered an "immune privileged" organ because they produce a number of chemical signals that protect cells of the spermatogenetic lineage from being recognized as being foreign by cells of the immune system. Sertoli cells modulate the activity of local white blood cells by secreting cytocrines that act in a paracrine fashion to influence resident macrophage cells in the testis (Box Figure 10D-1). The ability of Sertoli cells to modulate immune cells holds promise for organ transplant technology. For example, Sertoil cells protect pancreatic islet implants from being attacked by the hosts immune system. Co-implantation of Sertoli cells with pancreatic islets represents a promising treatment for type 1 diabetes.



BOX FIGURE 10D-1 Chemical signals involved in the immune network of the testes. The immune cell cohort of the testis includes macrophages, natural killer lymphocytes (NKs), T lymphocytes (T), and dendritic cells (DCs) that aid in antigen presentation to lymphocytes and macrophages. In the process of phagocytosing residual cellular material from developing sperm, Sertoli cells generate potential antigens to be recognized by the immune cells. In order to prevent the immune system from attacking developing sperm cells, adaptive immunity pathways mediated via T lymphocytes are suppressed, whereas innate immunity via NK cells is enhanced through signaling from transforming growth factor β (TGFβ), activin, and interleukin-10 (IL-10). Failure to keep adaptive immunity pathways in check will lead to T cells attacking spermatogenetic cells and infertility. *(Adapted with permission from Meinhardt, A. and Hedger, M.P.,* Molecular and Cellular Endocrinology, ***335,*** *60–68, 2011.)*

however, and will return immediately to proestrus if mating does not occur or if mating is unsuccessful. Eventually, the female will enter diestrus. In some mammals (e.g., certain rodents), mating without successful fertilization and implantation may result in a short period of simulated or false pregnancy, termed **pseudopregnancy**, after which the female re-enters proestrus without experiencing diestrus.

Some eutherian mammals exhibit a sequence of uterine events, known as a **menstrual cycle**, that is characterized by one phase in which rapid sloughing of the uterine epithelium (**menses**) occurs if fertilization does not lead to

pregnancy rather than the slow regression seen in diestrus. Often, these animals do not experience diestrus and may breed continuously, whereas others are seasonal breeders. Menstrual cycles are found in humans, monkeys, gibbons, the slender loris, marmosets, and several shrews and bats. The sloughing of the uterine epithelial lining often results in a vaginal discharge (**menstruation**) of uterine epithelial cells and trapped blood. The blood is trapped due to constriction of special spiral-shaped arteries that supply most of the blood flow to the uterine epithelium. The lack of blood flow to the outer portion of the epithelium results in cell death and hastens sloughing. A few of these mammals with menstrual cycles are known to exhibit **covert menstruation** where the sloughed tissues are resorbed and there is no uterine discharge. The onset of menses marks the end of one cycle and the beginning of the next. Although reproductive endocrinologists consistently focus upon the cyclic nature of estrous and menstrual cycles and their restarting with the failure of pregnancy to occur, it is important to remember that the normal sequel to ovulation is pregnancy. In nature, it is probably unusual for a female to enter estrus and not become pregnant.

Most primates exhibit estrous behavior to some degree at about the time of ovulation, including species characterized as having menstrual cycles, although a well-defined estrus is not observed in the human female. Exhibition of menstrual or estrous cycles should not be considered as alternative strategies, but that, with the possible exception of humans, the menstrual cycle is one variation within the estrous cycle.

Although there have been numerous hypotheses developed as to why menstrual cycles evolved, most are discounted because they cannot be generalized to all mammals exhibiting menses. Some, for example, relate menstruation to a placental type common to menstruating mammals, yet other species with this same type of placental anatomy do not have menstrual cycles. If it is simply a byproduct of endometrial function, then it is a costly one in terms of nutrient losses. One intriguing hypothesis suggests that menstruation evolved as an adaptation in mammals to neutralize and eliminate pathogens introduced during copulation and/or by sperm deposited in the vagina. However, this does not explain the scarcity of menstrual cycles among eutherian species. Perhaps the repeating menstrual cycle is simply another adaptation in favorable climates that allows for continuous breeding activity to ensure pregnancy will occur at some point but so that not all females in the population are pregnant at the same time.

Uterine bleeding and discharge occur at other times in the estrous cycles of some mammals; for example, the cow, domestic dog, and coyote discharge blood prior to ovulation and the onset of estrous behavior. This discharge is estrogen induced and does not involve degeneration of the uterine lining. Periovulatory bleeding also occurs either overtly or covertly in several primates, including humans, white-throated monkeys, vervet monkeys, and possibly in the cottontop tamarin.

A. Puberty

The achievement by the gonads of their full hormonal and gametogenetic capacity for reproduction is termed **puberty**. Remarkably, much remains to be learned regarding the mechanisms regulating the timing of puberty. Ultimately, puberty is the result of an increase in GnRH secretion (Figure 10-6), but the fact that GnRH is produced by one out of every 100 millions neurons in the human brain presents some technical difficulties in determining the precise mechanisms involved in the timing of GnRH secretion. Puberty may be gradual or rather sudden, depending on the species, and may be accompanied by a variety of morphological changes as well. In mammalian groups that have been the best studied such as rats, monkeys, and humans, puberty is associated with a marked increase in GnRH release from the hypothalamus resulting in elevated GTH secretion. In children, the frequency of these GTH pulses increases twofold at night and later during the day as puberty progresses. The amplitude of the GTH pulses also increases as the sensitivity of pituitary gonadotropes to GnRH is enhanced. Experimental studies suggest that the most critical factor in the induction of puberty is the increase in the frequency of GnRH pulses.

These changes in the HPG axis are paralleled in other endocrine systems. Nocturnal release of PRL also is elevated along with FSH and LH, although its role in puberty is uncertain. Adrenal androgens increase in boys and girls prior to the onset of puberty and continue to rise during puberty. This process is called **adrenarche** (see Chapter 8). These events are independent of the changes in the gonadal axis but are important contributions to puberty; for example, adrenal androgens stimulate the prepubertal growth spurt and the appearance of axillary and pubic hair.

There are several hypotheses concerning the mechanism(s) for the normal onset of puberty (Figure 10-6). The **gonadostat hypothesis** suggests that decreased feedback sensitivity to gonadal steroids develops in the hypothalamus and brings about increased release of GnRH. Accelerated GTH release from the pituitary activates gonadal steroidogenesis and gametogenesis or **gonadarche**. Gonadal responses also may involve receptor synthesis; for example, receptors for FSH are present in early ovarian follicles but not LH receptors. FSH stimulates production of LH receptors and increases the levels of **aromatase ($\mathbf{P450_{aro}}$)**. Now, the ovary can respond to both GTHs. The **missing link hypothesis** implies that some factor is missing and that it is the brain that is functionally incompetent prior to puberty. Data obtained from experimental and clinical observations that support these hypotheses also

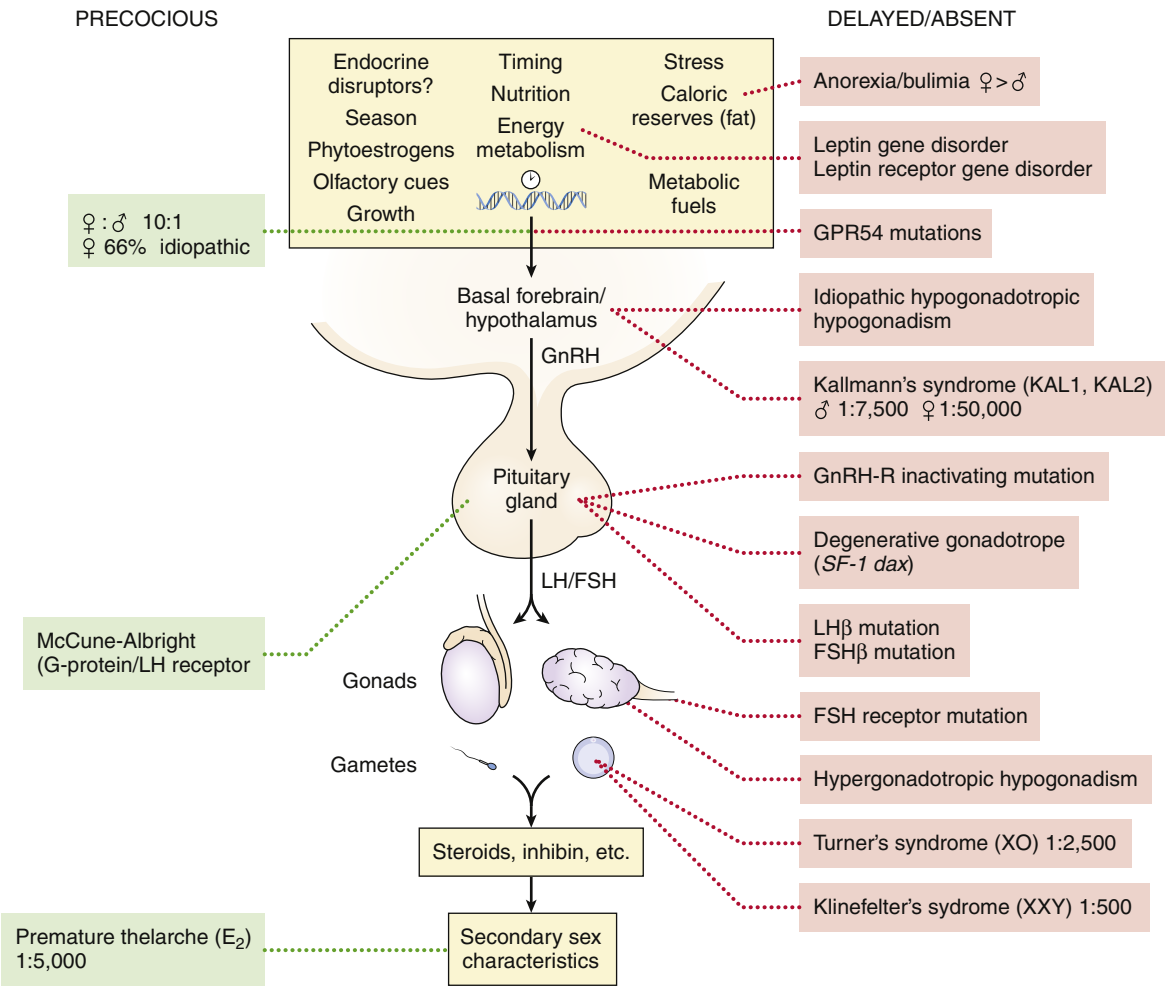


FIGURE 10-6 **Factors involved in the timing of puberty.** Puberty involves an increase in GnRH secretion from the hypothalamus. Many genetic and environmental factors come into play in determining the timing for the onset of GnRH secretion. Factors leading to precocious puberty are shown on the left. Factors that lead to delayed or absent puberty are shown on the right. Central nervous system mechanisms that may affect the onset of GnRH secretion are shown in the purple shaded box. *(Adapted with permission from Ebling, F.J.,* Reproduction, ***129****, 675–683, 2005.)*

support the **active inhibition hypothesis** that currently is in favor. This hypothesis states that puberty occurs because of a progressive decrease in physiological inhibition. The well-known actions of the pineal gland and melatonin on inhibiting reproductive function (see Chapter 4), and the observed reduction in melatonin secretion with precocial puberty offer strong support to the inhibitory hypothesis (see ahead). Early puberty events in humans also have been linked to exposures to endocrine-disrupting chemicals.

A separate hypothesis has been formulated to explain puberty and **menarche**, the onset of menstruation in girls. One fact supporting a separate mechanism in girls is that the likelihood of precocious puberty is ten times greater in girls than boys (Figure 10-6). This **lipostat hypothesis** or **critical weight hypothesis** implies that attainment of puberty is in part a function of fat storage in the body; consequently, girls who have more body fat reach menarche sooner than leaner girls. Furthermore, chronic strenuous exercise reduces body fat and may prevent young girls from reaching menarche just as it can block menstruation in women. Once the excessively lean girls are placed on a less severe regimen so that body fat increases, menarche usually is achieved. This mechanism probably is not related to puberty per se but rather may be an evolutionary mechanism to ensure that there is evidence of sufficient environmental resources (as evidenced in body fat) to support the energy demands of pregnancy and lactation for successful rearing of young. Recent studies of the peptide **leptin** that is secreted by adipose cells may influence GnRH release in the brain. Higher levels of leptin occur when lipid stores are greater, and leptin may provide a signal to the brain of the extent of fat storage in addition to suppressing appetite (Figure 10-6; also see Chapter 12). Excess kisspeptin secretion also may lead to precocious

puberty. It is worth mentioning that **McCune–Albright syndrome** also leads to precocious puberty and menstruation in girls (Figure 10-6), although the mechanism is linked to mutations in the *GNAS1* gene that encodes the α subunit of the G protein Gs. This condition also leads to gigantism and discoloration of the skin.

There are many potential causes for delayed or absent puberty (Figure 10-6). Mice lacking the GPR54 (kisspeptin) receptor fail to go through puberty, and many loss-of-function mutations in the *GPR54* gene have been described in patients with delayed or absent pubertal development (Figure 10-6). **Kallmann's syndrome** represents a wide spectrum of disorders associated with reduced GnRH secretion. Delayed puberty may manifest itself in individuals with eating disorders that lead to reduced caloric intake (Figure 10-6).

IV. ENDOCRINE REGULATION IN EUTHERIAN MALES

In some species, mature males are capable of copulating with a female whenever she is receptive. Secretion of GnRH and hence of GTHs is more or less continuous in these males but with daily and often seasonal fluctuations occurring in circulating levels of some GTHs. Daily secretory patterns for GTHs show considerable variation among different species. Hourly fluctuations of LH have been reported in bulls, and these variations in LH are correlated with following increases in circulating testosterone (Figure 10-7). In human males, FSH shows no cyclic variation in blood levels, although LH and testosterone exhibit obvious daily patterns with peak levels occurring during early morning hours and minimum values reported for the afternoon. Most wild mammals exhibit distinct seasonal breeding, and active spermatogenesis may be restricted to only a few months of the year or less.

A. Spermatogenesis

Each testis develops primarily from the medullary portion of an embryonic gonadal blastema as described previously (see Figure 10-2). Differentiation of the medullary portion with concomitant regression of the cortical components (progenitor of the ovary) appears to be controlled by local embryonic androgen secretion activated by the *sry* gene. The medullary region differentiates into seminiferous tubules and interspersed masses of interstitial cells. These interstitial cells are located between the seminiferous tubules and synthesize and release androgens into the general circulation.

The seminiferous tubules consist of large Sertoli cells, germ cells, spermatogonia, and cells derived from the spermatogonia (Figures 10-8, 10-9, and 10-10). Each tubule is surrounded by a thin layer of connective tissue. Under the influence of androgens, **peritubular myoid cells** develop in this connective tissue layer during puberty. They surround and provide support for the seminiferous tubules and are believed to be responsible for contractile activity of the tubules that propels sperm to the

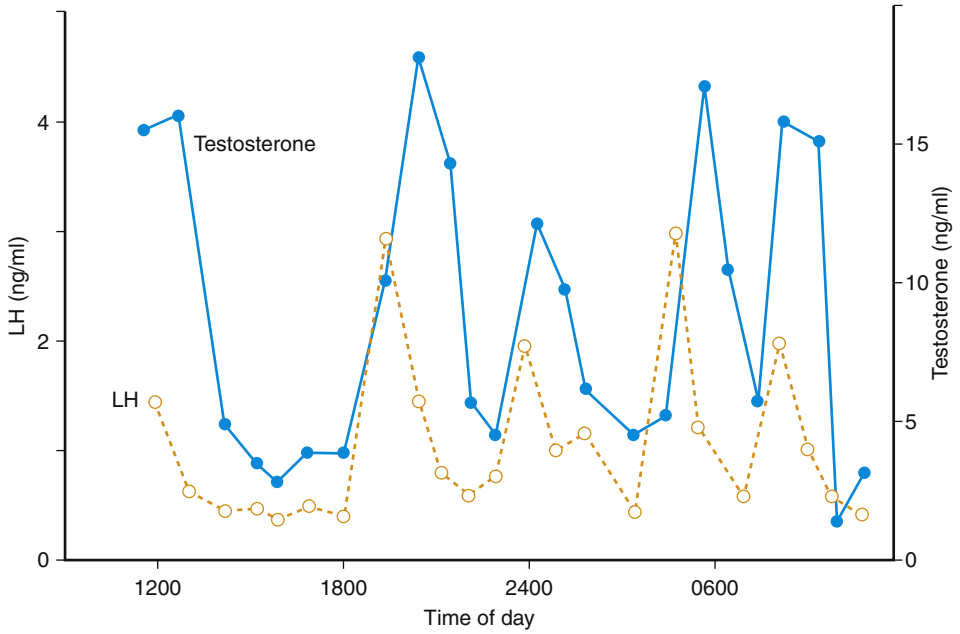


FIGURE 10-7 Male pattern of luteinizing hormone (LH) and testosterone secretion in a bull. Pulsatile release of GnRH (not shown) would precede each LH peak that precedes each testosterone peak. (*Adapted with permission from Short R.V.,* Reproduction in Mammals, Book 3, *Cambridge University Press, 1972.*)

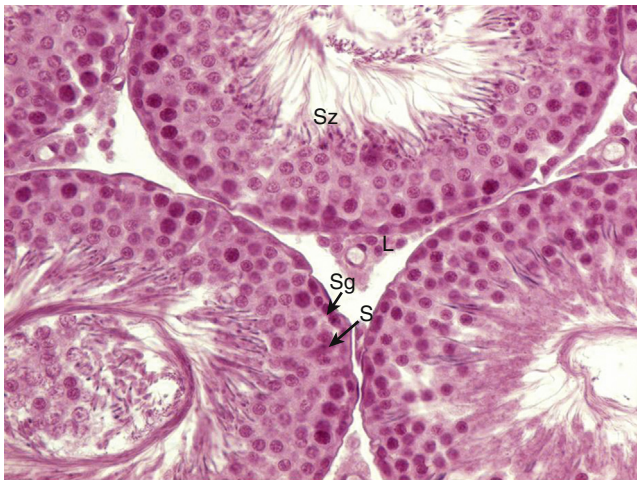


FIGURE 10-8 Spermatogenesis in rat testis. Section of rat testis showing edges of adjacent seminiferous tubules. Note the position of the Leydig cells adjacent to a capillary. Abbreviations: L, Leydig cells; S, nuclei of Sertoli cells; Sg, spermatogonia; Sz, sperm.

epididymis. The Sertoli cell has an extensive cytoplasm extending from the outer edge to the lumen of the tubule. The angular nucleus of the Sertoli cell is located at the outer edge of the tubule. Sertoli cells form tight junctions with adjacent Sertoli cells and together with the peritubular myoid cells they secrete the various collagen and laminin proteins that form the basement membrane. This basement membrane along with their **tight junctions** together form the blood/testis barrier, quite literally a cellular fence that isolates the seminiferous tubules into a 'backyard" (basal) compartment containing Sertoli cells and spermatogonia and a "frontyard" (adluminal) compartment containing the spermatogenetic cells (Figure 10-10). As a result of the blood testis barrier, nutrients and leukocytes cannot move between the Sertoli cells into the seminiferous tubules, thereby protecting developing spermatogenetic cells from exposure to cells and antibodies of the immune system. All chemicals must pass through the Sertoli cells in order to reach the spermatogenetic cells (Figure 10-10).

Germ cells are present along the outer margins of the seminiferous tubules and differentiate into **spermatogonia**. Spermatogonial cells proliferate mitotically under the influence of FSH to produce more spermatogonia. Eventually some of these spermatogonia will undergo differentiation characterized by nuclear enlargement and will become **primary spermatocytes** that are capable of entering spermatogenesis (Figure 10-11). Testicular androgens are somehow necessary for initiation of meiosis in primary spermatocytes that undergo the first meiotic division to give rise to two smaller **secondary spermatocytes**. These latter cells are infrequently observed in histological preparations because, once formed, they quickly enter the second meiotic division to yield four haploid **spermatids** which are transformed to sperm (spermatozoa) by concentrating the chromatin material into the sperm head and by elimination of the majority of the cytoplasm. The process of transformation of spermatids to sperm is termed **spermiogenesis**.

Spermatogenesis is a temperature-sensitive process, and high temperatures such as found within the body cavity of terrestrial eutherians can impair normal spermatogenesis and produce temporary sterility. Consequently, at some time prior to the attainment of sexual maturity or prior to the annual breeding season, the testes descend into the scrotum, where spermatogenesis can proceed at a slightly lower temperature. The failure of the testes to descend, a condition known as **cryptorchidism** (*crypto-*, hidden;

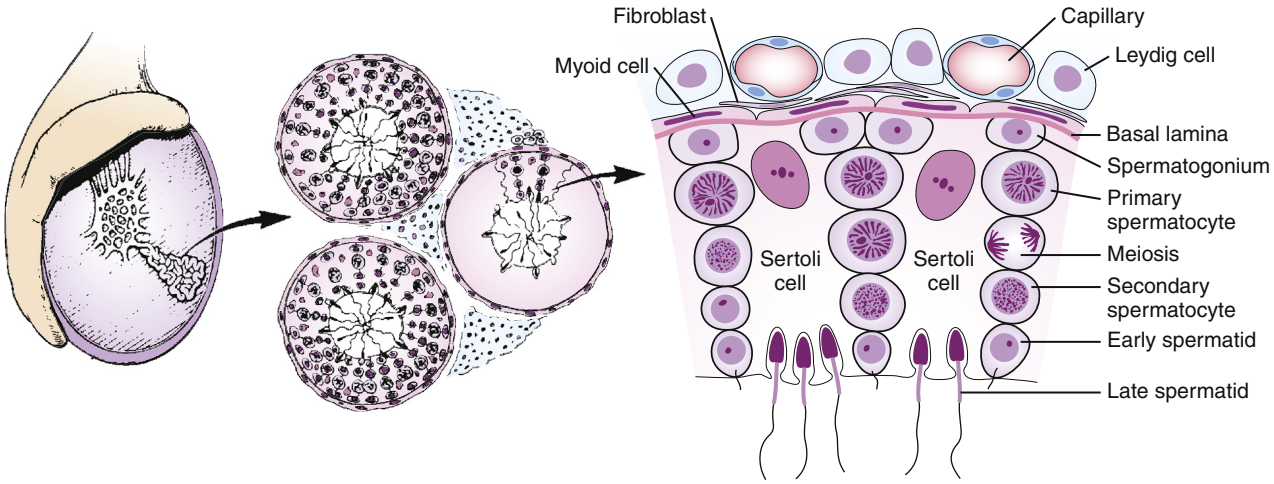


FIGURE 10-9 Organization of mammalian testis. Detail at right shows events of spermatogenesis and spermiogenesis in relation to Sertoli cells. The endocrine roles of interstitial (Leydig) cells, peritubular myoid cells, and Sertoli cells are described in the text. *(Adapted with permission from from Skinner, M.K.,* Endocrine Reviews*, **12**, 45–77, 1991; Junqueira, L.C. and Carneiro, J., "Basic Histology," 11th ed., McGraw-Hill, New York, 2005.)*

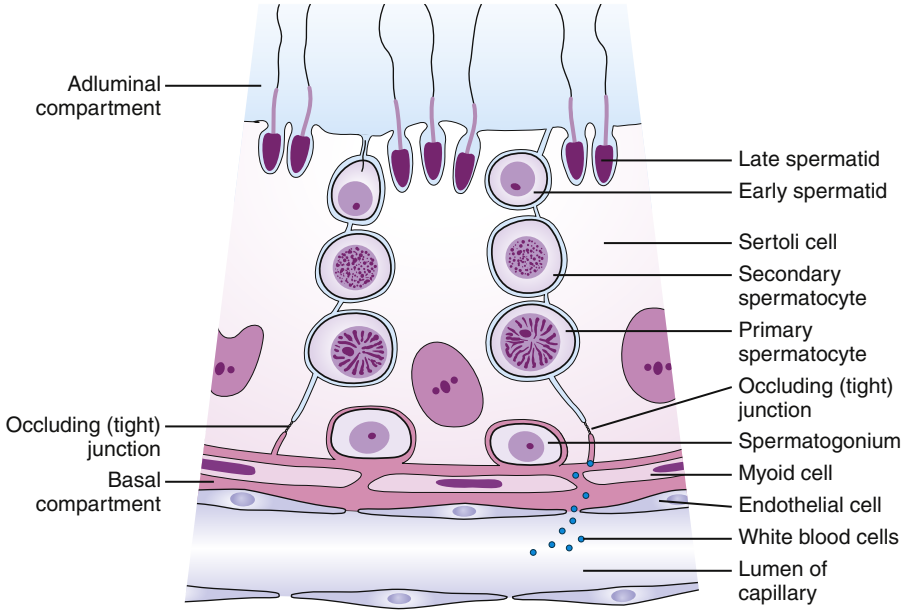


FIGURE 10-10 Occluding (tight) junctions between adjacent Sertoli cells form the blood testis barrier. The presence of the occluding junctions establishes an adluminal compartment isolating developing spermatocytes, spermatids, and spermatozoa from materials or white blood cells, leaving the capillaries in the basal compartment. Substances or leukocytes may diffuse or move by diapedesis through capillaries in the interstitium but cannot move past the occluding junctions linking the Sertoli cells. Spermatogonia lie basal to the occluding junctions in the basal compartment. *(Adapted with permission from Junqueira, L.C. and Carneiro, J., "Basic Histology," 11th ed., McGraw-Hill, New York, 2005.)*

orchi, testis), may cause irreparable damage to the seminiferous epithelium in most species. Some mammals lack a scrotum (e.g., elephants, whales, seals), and the testes are permanently located within the abdominal cavity. Male elephants, however, are capable of producing viable sperm and copulating with a female at any time of year. In such species, either spermatogenesis does not exhibit the same temperature sensitivity characteristic for scrotal species or these animals possess other mechanisms to reduce testicular temperature.

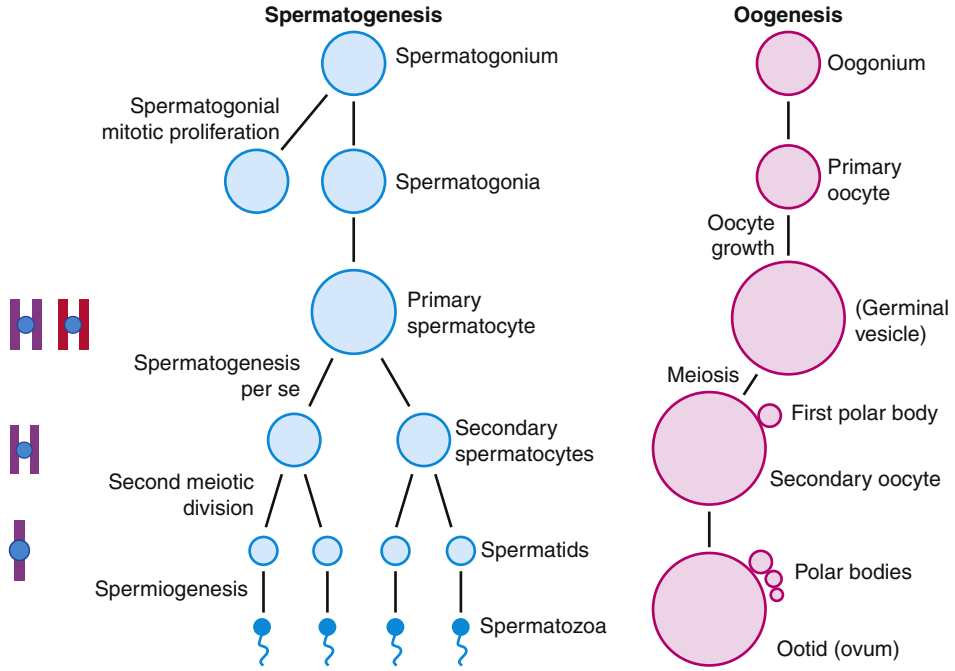


FIGURE 10-11 Comparison of spermatogenesis and oogenesis. Relative sets of chromosomes (ploidy) number is shown on the left. A major difference between males and females is the mitotic proliferation of gonial cells after birth in males, whereas the germ cells have all progressed to the primary oocyte stage in females and no new oocytes appear after birth. The primary spermatocyte undergoes meiosis to produce four spermatids, whereas meiotic division of the primary oocyte with unequal distribution of cytoplasm produces only one oocyte plus up to three polar bodies. The first polar body (a secondary oocyte with very little cytoplasm) often does not undergo the second division.

A given histological section of a seminiferous tubule may show varying numbers of spermatogonia, primary spermatocytes, possibly a few secondary spermatocytes, spermatids, and sperm in sequence from the outer margin (gonia) to the lumen (sperm). The tails of the sperm extend into the lumen, and the heads of the sperm typically are still surrounded by highly folded margins of Sertoli cells (Figure 10-9).

Millions of maturing sperm may be sloughed off into the lumina of the seminiferous tubules each day. This process is termed **spermiation** and is stimulated by LH. These sperm pass through the tubules that eventually coalesce into larger ducts of the rete testis that eventually join the epididymis associated with each testis. Vast numbers of maturing sperm are stored in the epididymis. Under the influence of androgens and PRL, the epididymis secretes materials into its lumen where the sperm are being held. Included in this secretion are protein-bound **sialic acids** (sialomucoproteins), **glycerylphosphoryl-choline**, and **carnitine**. These particular substances are involved directly in maturing and maintaining sperm in viable condition until ejaculation. Androgens and **androgen-binding protein (ABP)** produced by Sertoli cells in the seminiferous tubules are released along with sperm and travel to the epididymis. Androgen molecules freed from ABP in the lumen or androgen-ABP complexes or possibly both are absorbed by the epididymal cells. These androgens stimulate epididymal cells to secrete materials involved in maintenance of the sperm.

Contraction of the smooth muscles of the epididymis and vas deferens causes ejection of sperm (ejaculation). The mature sperm leave the epididymis, enter the vas deferens, travel to the urethra, traverse the length of the penis via the urethra, and are deposited in the female's vagina during coitus. **Seminal vesicles** and the **prostate gland** add their fluid secretions to the sperm and epididymal secretions to form a watery mixture of sperm and various organic and inorganic substances known as **semen**. The **bulbourethral gland** or **Cowper's gland**, which is homologous to the Bartholin's gland in females, produces a pre-ejaculate that cleanses and lubricates the urethra prior to the arrival of the semen. The entire ejaculatory event may be induced by the release of OXY from the pars nervosa in response to a neural reflex initiated by mechanical stimulation of the penis.

B. Endocrine Regulation of Testicular Functions

GTHs and a number of paracrine factors including testosterone (Table 10-6) control production of sperm. Many of the details of the endocrine regulation of these processes are not clear, but a generalized picture is emerging. Relatively separate roles have been defined for LH and FSH in males, although FSH and testosterone work cooperatively in some cases. Spermatogenesis is initiated indirectly by FSH through mitotic proliferation of spermatogonia and formation of primary spermatocytes. Spermatogonia lack FSH receptors, and the mechanism of spermatogonial activation is mediated by paracrine factors from the Sertoli cells that do have FSH receptors. One paracrine factor identified as having an important role in proliferation of spermatogonia is **glial cell line-derived neurotrophic factor (GDNF)**. The intermediary role of the Sertoli cell is supported by observations that FSH stimulates mitosis in Sertoli cells whose number in the adult testis is directly proportional to sperm abundance. Testosterone may initiate meiotic divisions of primary spermatocytes that differentiate from spermatogonia, resulting eventually in formation of spermatids. The production of ABP, cytoskeleton proteins (**actin, vinculin**), and $P450_{aro}$ by the Sertoli cell also is stimulated by FSH.

Testosterone is the major circulating androgen in mammals, although other androgens such as **androstenedione** or **5-α-dihydrotestosterone (5α-DHT)** may circulate in significant amounts (Table 10-7). Some 5α-DHT also is produced and stimulates red blood cell production in bone marrow and contributes to the higher hematocrit found in males as compared to females. Prior to attainment of puberty in bulls, androstenedione is the principal circulating androgen, but it is gradually replaced by testosterone at puberty. Testosterone within the testis, however, seems to be the most important androgen influencing sperm production.

Testosterone has several important paracrine effects on spermatogenesis and spermiogenesis. Testosterone concentrations within the testis are generally orders of magnitude greater than circulating testosterone levels (Table 10-8) and, for reasons still not understood, much higher than needed in order to maintain spermatogenesis. Perhaps testosterone is in part a precursor for testicular estrogen synthesis; for example, testicular estrogens reach dramatic levels in the stallion. Androgens are required for spermatogenesis to proceed normally, in part through the activation of a multitude of genes, one of which produces **gonadotropin-regulated testicular helicase (GRTH/DDX25)** in Leydig cells and germ cells. GRTH is a 56-kDa protein that is transported into the nucleus, where it binds to nuclear RNAs as a component of messenger ribonucleoprotein (mRNP) complexes. It regulates the activity and turnover of certain mRNAs involved in meiosis. GRTH-knockout mice are incapable of producing functional sperm. Androgen actions probably are indirect, as testosterone receptors appear to be absent or occur in very low numbers on germ cells. However, estrogen receptors (ERs) are present on germ

TABLE 10-6 Possible Local Actions for Gonadal Secretions

Factor	Source	Proposed Action
Activin	Granulosa cells Sertoli and interstitial cells	Unknown Specific receptors shown on germ cells
Epidermal growth factor (EGF)	Thecal/interstitial cells of ovary	Stimulates granulosa cell proliferation
Estradiol	Interstitial cells in male epididymis	Blocks androgen synthesis Stimulates fluid resorption
Fibroblast growth factor (FGF)	Granulosa cell	Causes epithelial proliferation in early follicular development and conversion of thecal cells to ovarian interstitial cells after ovulation
	Testicular germ cells	Binds to receptors on Sertoli cell; function unknown
Gonadotropin-releasing hormone (GnRH)	Ovary	Working through IP_3 second messenger, GnRH alters steroidogenesis by granulosa cells in certain follicular stages; may signal atresia
	Sertoli cell	Alters androgen synthesis by interstitial cells; increases local permeability of capillaries
Growth differentiation factor-9 (GDF-9)	Ovary	Maintains FSH receptors on oocyte
	Oocyte	Follicle growth and maturation (folliculogenesis)
Growth hormone-releasing hormone (GHRH)	Corpora lutea, oocyte	Promotes follicular development and ovulation
	Germ cells of tests	Stimulates sertoli cells to make stem cell factor
Insulin-like growth factor (IGF-I)	Granulosa cells	Increases number of LDL receptors on granulosa cells; stimulates cholesterol and inhibin synthesis
Interleukin 1(IL-1)	Sertoli cells	Decreases steroidogenesis in interstitial cells
PmodS protein	Peritubular myoid cells	Non-mitogenic factor that regulates differentiation and function of Sertoli cells
Interleukin 6 (IL-6)	Ovarian T cells	Suppresses response of granulosa cells to FSH,i.e., decreased progesterone synthesis; induces apoptosis and atresia of granulosa cells
KIT ligand	Oocyte	KIT ligand is a cytocrine that acts on a tyrosine kinase receptor (KIT) to promote folliculogenesis
Nerve growth factor (NGF)	Ovarian cells	Stimulates follicular formation and organization as well as differentiation of ovarian interstitial cells
Testosterone	Interstitial cell	Regulates functions of Seroli cells; stimulates meiosis in primary spermatocytes
Transforming growth factor (TGF-α)	Thecal cells	Facilitates proliferation of thecal and granulosa cells but slows their GTH-induced differentiation
	Sertoli and peritubular myoid cells	Causes EGF-like growth stimulation in interstitial cells; decreases steroidogenesis

cells that also possess aromatase activity. Furthermore, at least in rodents, the local metabolism of DHT to an androgen metabolite **5α-androstane-3β, 17β-diol (3β-diol)** (see Chapter 3; Table 10-8) has been reported in brain, prostate, and testes. 3β-Diol binds and activates ERs. Furthermore, aromatase knockout male mice exhibit disruption of spermatogenesis, supporting a local role for estrogen receptor-binding regulators.

TABLE 10-7 Plasma Levels of Reproductive Steroids in Mammals

Species	Testosterone (ng/mL)	Dihydrotestosterone (ng/mL)	Estradiol (pg/mL)	Progesterone (ng/mL)
Mustela ermina (stoat)				
Male (annual range)	4.5–26			
Ursus americanus (black bear)				
Female				
Nonpregnant			35–71	17.8
Pregnant			32,000–35,000	29.40
Elaphus maximus (Asian elephant)				
Male (annual range)	0.7–45			
Female				
Not pregnant (peak)			26	0.153–0.195
Pregnant (peak)			26	4
Macaca fuscata (Japanese monkey)				
Male (annual range)	0.2–19.8			
Female				
Follicular phase (peak)			150	2
Luteal phase (peak)			250	5.3
Homo sapiens				
Male (mean)	7.9	0.4	50	
Female				
Follicular phase (range)	0.6	0.3	60–600	0.3–1.5
Luteal phase (range)	0.6	0.3	200	3–20
Pregnant (range)			5,500–30,000	45–210

TABLE 10-8 Intratesticular Concentrations of Various Steroids in Humans

Steroid	Intratesticular Concentration(ng/mL)	Range
Testosterone[a]	572±102	103–1,085
DHT	13.4±1.8	4.9–26
3 β-diol	12.3±1.9	3.6–26
E_2	15.7±2.3	8–29

[a]*Plasma concentrations averaged 3.8 ng/mL.*
Abbreviations: 3 β-diol, 5α-androstane-3α, 17β-diol; DHT, dihydrotestosterone; E_2, estradiol.
Data from Jarow, J.P. and Zirkin, B.R., *Annals of the New York Academy of Sciences*, 1061, 208–220, 2005.

The attachment of Sertoli cells to spermatids involves cytoskeletal actin and vinculin interactions with the spermatids, as well as indirect effects of testosterone. Peritubular myoid cells (Figure 10-9) are stimulated by testosterone to release two proteins, **PModSA** and **PModSB**, that cause Sertoli cells to secrete additional paracrine regulators that may alter spermiogenesis (Table 10-6).

The Leydig cells of the testis also synthesize and release small quantities of estrogens. Locally, estradiol can block androgen synthesis by interstitial cells and can influence the responsiveness of these cells to GTHs. Estradiol also binds to receptors in the epididymis, where it regulates resorption of excess testicular fluid that was used to conduct sperm to the epididymis. The ratio of testosterone to estradiol in the general circulation may alter the ratios of FSH and LH being released from the pituitary through negative feedback. Finally, conversion of androgens to

estrogen occurs in certain brain cells, and circulating estrogens themselves may influence male sexual behavior.

C. Actions and Metabolism of Androgens in Males

Circulating androgens influence development and maintenance of several glands and related structures associated with the male genital tract, such as the prostate gland and seminal vesicles, and induce development of certain secondary sexual characters such as growth of the beard and development of skeletal muscles in men. Androgens also exert a negative feedback effect upon the secretion of GTHs primarily through actions at the level of the hypothalamus (see Chapter 4).

The action of testosterone in some of its target cells involves its cytosolic conversion to DHT by the enzyme **5α-reductase**. DHT has a greater affinity for the androgen receptor than does testosterone and hence is more potent than testosterone. Timely development of prostate and bulbourethral glands, the penis, and scrotum are dependent upon conversion of testosterone to DHT by target cells in these structures. This conversion of testosterone to the stronger androgen DHT is necessary due to the low circulating level of testosterone at this time that is insufficient to activate these tissues. The testes generally do not synthesize DHT until puberty. Neurons in some brain areas express 5α-reductase, and some effects of testosterone on behavior may involve prior conversion to DHT.

Many androgenic responses, however, are not mediated by DHT, and this conversion is not necessary for testosterone to produce these effects. For example, development of wolffian duct derivatives (the epididymis, vas deferens, and seminal vesicles) is accomplished by a local level of testosterone that is sufficient to activate receptors without prior conversion to DHT. This effect may occur because local testosterone levels from the developing testes are sufficiently high to effectively activate androgen receptors in these structures. In the tammar wallaby, a marsupial, testosterone is converted to DHT in the wolffian duct, but this has not been shown to occur in a eutherian mammal.

In some target tissues, androgens have been shown to undergo conversion to estrogens through aromatization of the A-ring and removal of the C_{19} carbon atom (see Chapter 3). Conversion of androgens to estrogens also occurs in both Sertoli and Leydig cells. This process also occurs in the central nervous system, where aromatization may be essential to some androgen actions. Induction of some male behaviors in castrates requires aromatization and cannot be induced by essentially non-aromatizable androgens such as DHT, whereas others may be induced by either aromatizable androgens or by DHT. The Sertoli cells, under the influence of FSH, secrete two forms of inhibin that selectively block FSH release from the adenohypophysis. Inhibin activity also has been found in rete testis fluid, seminal plasma, testicular extracts, and ejaculate, suggesting local actions (see Table 10-6). Two forms of inhibin have been isolated: **inhibin A** and **inhibin B** (Figure 10-12). These molecules are glycoprotein heterodimers of 31 to 35 kDa that possess a common α-subunit combined with one of two β-subunits (A or B). Inhibins are believed to be the major factor responsible for negative feedback in the selective regulation of FSH release in both males and females. In addition, a β-subunit heterodimer called **activin** has been isolated from gonads. Activin is composed of two β-subunits (Figure 10-12) and is a potent releaser of FSH from the pituitary gland in laboratory experiments, although its physiological role is still undetermined. Activins bind to serine−threonine kinase type I and type II receptors on the plasma membrane (see Chapter 3), leading to phosphorylation of downstream signaling proteins (so-called **R-SMADs** for **receptor-regulated SMADs**, short for an isoform of the humorously named "mothers against decapentaplegic") and changes in gene transcription (Figure 10-12). Inhibins interfere with activin receptor interation by binding to activin type II receptors and blocking recruitment of type I receptors (Figure 10-12). Activin may be a local regulator, as activin levels within the testes are highly modulated during development (Figure 10-13) and activin receptors occur on spermatogenetic cells in the testis.

V. ENDOCRINE REGULATION IN EUTHERIAN FEMALES

Regardless of whether or not an animal exhibits a menstrual cycle in addition to an estrous cycle, there are numerous distinctive features that characterize reproduction in all female mammals. In addition to the roles of GTHs and gonadal steroids described below, numerous other regulators (including GnRH, inhibins, AMH, prostaglandins, and growth factors) are synthesized in the ovaries and are suspected of playing important paracrine roles in ovarian events. A partial listing and some demonstrated autocrine and paracrine ovarian regulators are provided in Table 10-6. One of those, **growth differentiation factor 9 (GDF-9)**, is produced by the oocyte and is required for proliferation of follicular cells and subsequent ovarian follicle development (Figure 10-14). Other candidate ovarian growth factors include **bone morphogenetic proteins 6 and 15 (BMP-6, 15)**, **fibroblast growth factor 8 (FGF-8)**, and **transforming growth factor β2 (TGF-β2)**. Developing follicles also produce AMH, which is responsible for recruitment of new follicles. Ovarian and uterine cyclical events during reproduction are similar. The ovarian events are discussed first followed by the uterine events that are linked to the ovarian cycle.

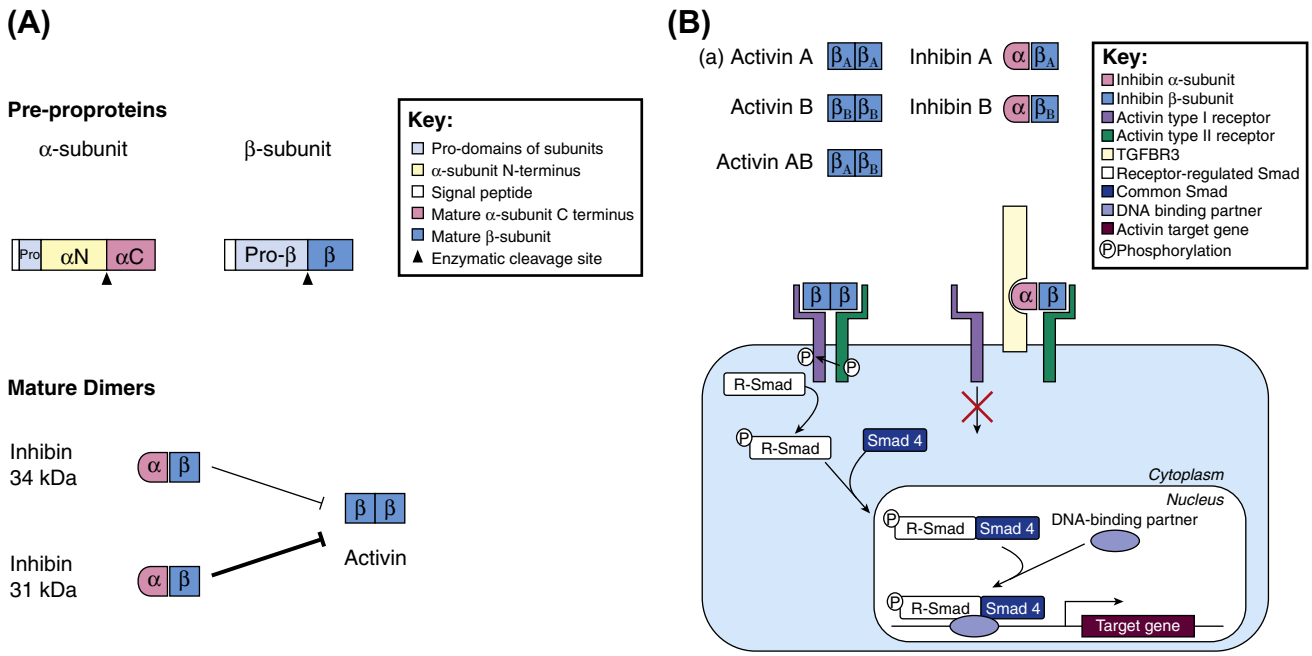


FIGURE 10-12 **Activin and its receptors.** (A) Activin and inhibins are formed by dimerization of two subunits, α and β, that are extensively modified by glycosylation and proteolysis after translation from either the α or β subunit mRNA. Activin is formed from two β subunits. Activins and inhibins act as natural inhibitors of each other's activity. (B) Activins bind to serine−threonine kinase type I and type II receptors on the plasma membrane. Binding to the type II receptor (green) leads to phosphorylation of the type I receptor (purple) and subsequent phosphorylation of downstream signaling proteins (R-SMADs). Inhibins interfere with activin receptor interaction by binding to activin type II receptors as well as TGFBR3 thereby blocking recruitment of type I receptors. Abbreviations: R-SMAD, receptor regulated mothers against decapentaplegic homolog 1; SMAD-4, SMAD family member 4; TGFBR3, transforming growth factor, beta receptor III. *(Adapted with permission from Stenvers, K.L. and Findlay, J.K.,* Trends in Endocrinology & Metabolism, ***21****, 174−180, 2010.)*

For sexually mature mammals in nature, fertilization and pregnancy are normal events, and coitus occurs frequently during estrus. The character of the ovarian cycle, with its rapid resumption in polyestrous species or following a short menses as in primates if fertilization and successful implantation do not occur, enhances the chances for successful reproduction. Either rapid reentry into estrus or rapid appearance of one or more new ova or both can occur, and a second opportunity to produce offspring is made possible during that season. In some cases, polyestrous animals (especially rodents) produce more than one litter in a single breeding season.

A. The Ovarian Cycle

The basis for the cyclical nature of female reproductive events resides in the hypothalamus and is a genetically determined female characteristic. The ovary undergoes cyclical development in response to GTHs. The duration of the ovarian cycle is characteristic for each species. During proestrous, the growth of one or more follicle occurs in the ovaries. This portion of the ovarian cycle is the **follicular phase**. The follicular phase results in development of one or more mature follicles, each containing one oocyte. Following ovulation, which ends the follicular phase, the remains of a ruptured follicle are transformed into a **corpus luteum**. Thus, ovulation also marks the onset of the **luteal phase** of the ovarian cycle**.** The luteal phase may last from a few days to weeks, depending upon the species. Some species, like humans, may begin another follicular phase during the latter portion of the luteal phase, whereas others may enter an inactive period (diestrus) that may last until the next breeding season when a new follicular phase is initiated.

1. The Follicular Phase of the Ovarian Cycle

During the follicular phase of the ovarian cycle (Figure 10-15), the tonic hypothalamic center releases small quantities of GnRH into the portal circulation and relatively low but rather constant circulating levels of FSH, LH, or both are maintained. Prior to puberty, which is characterized by increased GTH levels, the ovary contains **primordial follicles** consisting of **primary oocytes** invested with an additional layer of flattened follicle cells derived from the germinal epithelium that surrounds the ovary. In most mammals, it is assumed that there are no oogonia in the ovary because all of them entered meiosis and became primary oocytes prior to or shortly after birth. However, recent studies in mice have demonstrated new follicle development occurs after birth, and production of new oocytes has been described among certain primates as well.

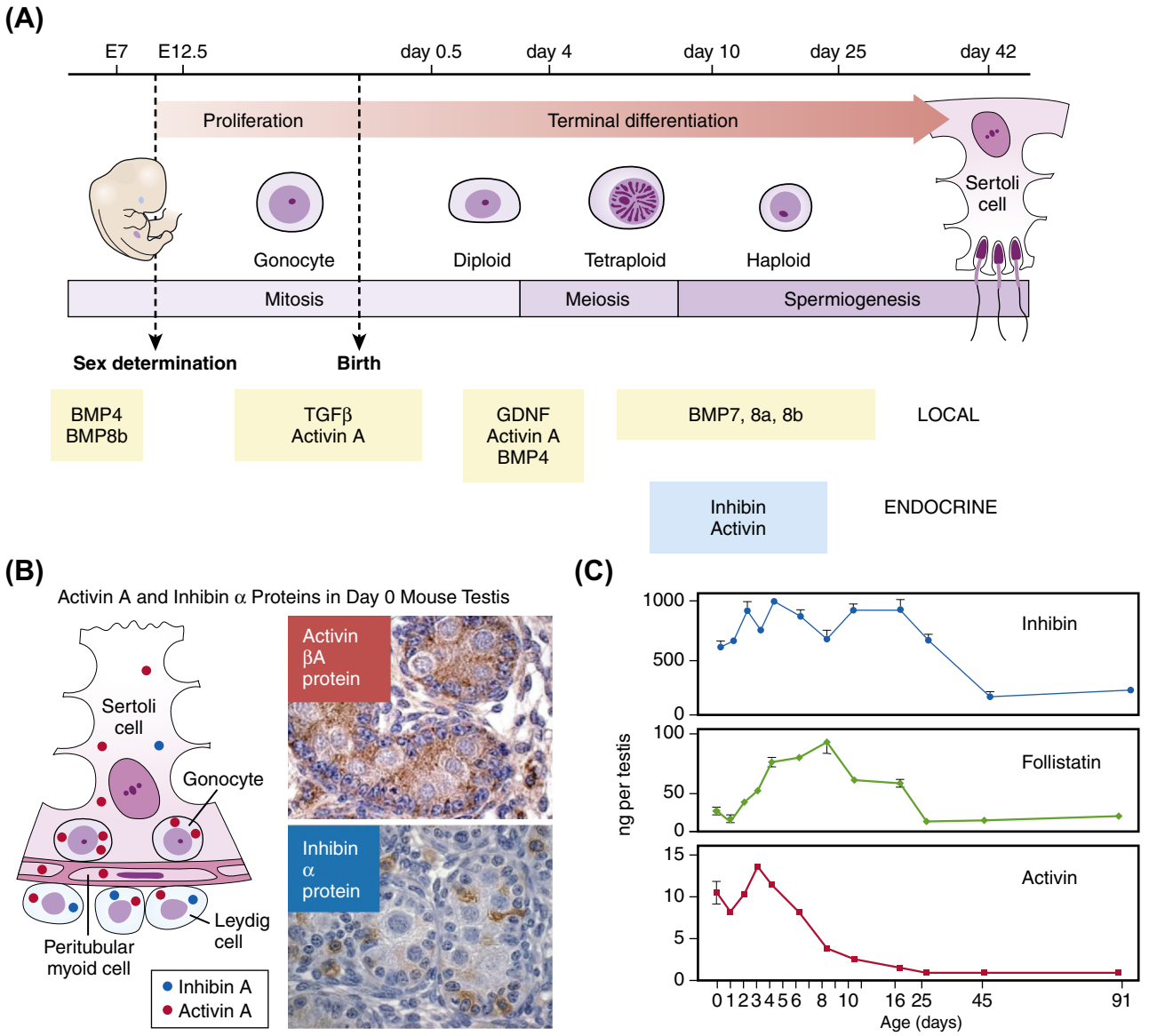


FIGURE 10-13 **Activin is secreted from the testis during embryonic development.** (A) Time course for expression of activin and other putative growth factors in testis during mouse development. Abbreviations: BMP, bone morphogenic protein; GDNF, glial cell-derived neurotrophic factor; TGFβ, transforming growth factor β. (B) Both inhibin A and activin A are highly expressed in the testis at birth. (C) Patterns in activin, inhibin, and follistatin production in mouse testis during postnatal development. See text for explanation of functions of these regulators. *(Adapted with permission from Barakat, B. et al.,* Molecular and Cellular Endocrinology, ***359****, 66–77, 2012.)*

The arrival of FSH at the ovary causes local release of AMH that stimulates a number of primordial follicles to begin to enlarge and differentiate into **primary follicles**. The follicle cells surrounding the growing oocyte develop into **granulosa cells**, which are in contact with the oocyte. The granulosa cells secrete a basement membrane along their outermost surfaces. A second layer of **thecal cells** derived from the ovarian stroma surrounds the granulosa outside of the basement membrane. Thecal cells further differentiate into inner and outer layers: the endocrine **theca interna** and the connective tissue-like **theca externa**. The rich supply of capillaries in the thecal layer do not cross the basement membrane and penetrate the granulosa layer. The presence of FSH receptors on the granulosa cells and their ability to proliferate mitotically is initiated by the oocyte through production of GDF-9 (Figure 10-14). In *Gdf9*-knockout mice, the ovary develops normally but follicle formation is blocked by the failure of follicle cells to respond to FSH.

As the follicle grows, the granulosa cells secrete the **liquor folliculi** or **antral fluid** that is primarily an ultrafiltrate of blood plasma. Increasing production of antral fluid

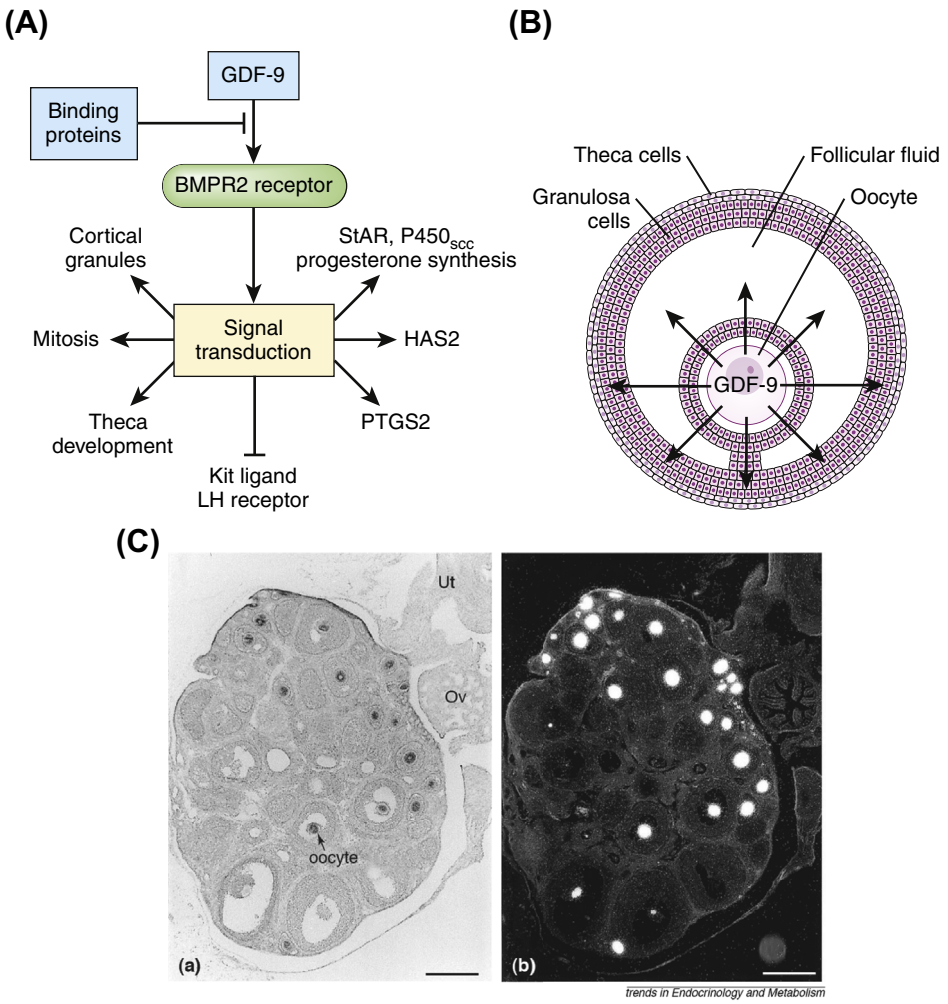


FIGURE 10-14 Growth differentiation factor-9 (GDF-9) is required for folliculogenesis in the mammalian ovary. (A) GDF-9 is produced by the oocyte and binds to surface membrane BMPR2 on follicle cells to initiate a wide variety of functions important for follicle growth including cholesterol transport and steroidogenesis (StAR, $P450_{scc}$), follicle growth (HAS2), prostaglandin synthesis (PTGS2), follicle recruitment (Kit ligand), cell division, theca growth, and the formation of cortical granules. (B) Gradient theory explaining the role of GDF-9 and other morphogens from the oocyte. (Adapted with permission from Erickson, G.F. and Shimasaki, S., *Trends in Endocrinology & Metabolism*, **11**, 193–198, 2000.) (C) Location of GDF-9 within oocytes. Mouse ovary showing follicle histology (a) and autoradiography showing location GDF-9 within oocytes only (b). Abbreviations: BMPR2, bone morphogenetic protein type II receptor; GDF-9, growth differentiation factor-9; HAS2, hyaluronan synthase 2; $P450_{scc}$, side-chain cleaving enzyme; StAR, steroid acute regulatory protein; Ut, uterus; Ov, oviduct; PTGS2, prostaglandin-endoperoxide synthase 2. *(Reprinted with permission from Erickson, G.F. and Shimasaki, S.,* Trends in Endocrinology & Metabolism*, **11**, 193–198, 2000. © Elsevier Science, Ltd.)*

results in formation and progressive enlargement of a fluid-filled cavity within the follicle, the **antrum**. The follicle is now called a **secondary follicle** or an **antral follicle**.

Under the influence of LH and FSH as well as a variety of paracrine factors (see Table 10-6), growing ovarian follicles synthesize and release estrogens, predominantly estradiol (= 17β-estradiol), into the general circulation. The synthesis of estrogens in the ovary appears to be a cooperative effort between cells of the theca interna and the granulosa (Figure 10-16). LH stimulates the thecal cells to produce androgens (principally androstenedione) that are aromatized by the granulosa cells to form estradiol (see Figure 3-25 for pathway). Conversion of androgens to estradiol by the granulosa cells is stimulated by FSH. In turn, FSH increases $P450_{aro}$ levels in these cells. In addition, FSH causes the granulosa cells to produce inhibins, which feed back on the pituitary to selectively inhibit FSH release as was described earlier for males. Inhibin also inhibits $P450_{aro}$ activity locally in granulosa cells, whereas the related peptide activin increases $P450_{aro}$ activity.

The final stage of follicle growth is the **tertiary** or **mature follicle** (also called a *graafian follicle*). This follicle has reached maximal size and often is characterized by a single large antrum surrounded by a relatively thin layer of granulosa cells with the oocyte relegated to and surrounded by a small mass of granulosa cells, the **cumulus oophorus**. The final meiotic maturation of the oocyte apparently is influenced by paracrine factors from cells of the cumulus oophorus following ovulation. The mature follicle is located just beneath the surface of the ovary.

Most (99%) of the follicles that begin to grow during a given ovarian cycle will exhibit apoptosis and degenerate, a process called **atresia** (Figure 10-17). These degenerating follicles are called **corpora atretica**. Atresia can occur at any stage of follicle development. Some of the steroidogenic cells from these atretic follicles will remain active and contribute to what has been called the **interstitial gland** of the ovary. Androstenedione produced in the interstitial gland by LH stimulation supplements thecal cell contributions for synthesis of estradiol by the granulosa cells.

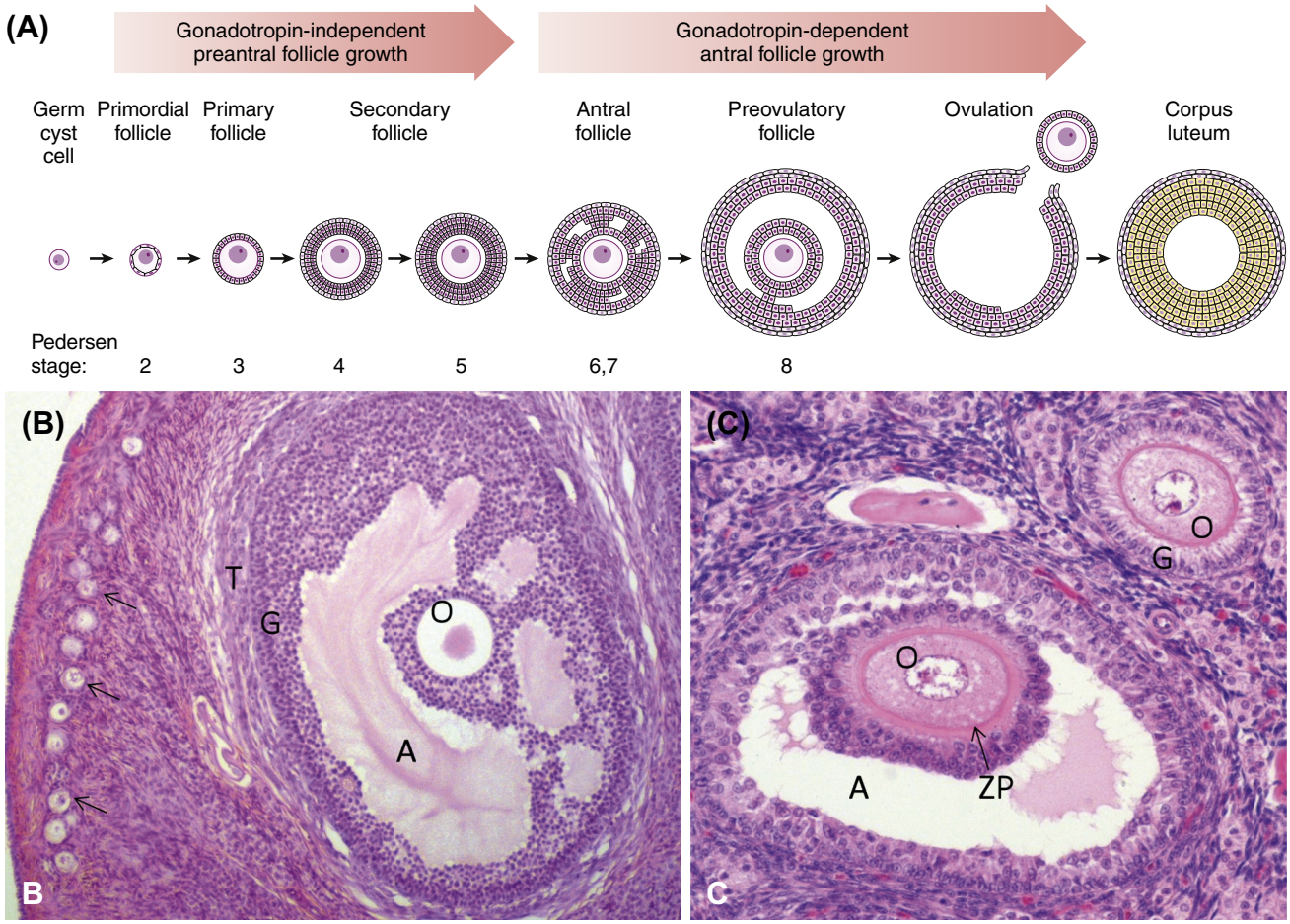


FIGURE 10-15 Ovarian follicle stages. (A) The appearance of the oocyte and follicle during oogenesis from germ cell to mature follicle and corpus luteum. (B, C) Sections of rat ovary showing stages of follicular development. (B) Mature or Graffian follicle with a large antrum (A) and clearly defined theca (T) and granulosa (G) cell layers. Primordial and primary follicles are indicated by arrows. (C) A secondary follicle and early antral follicle with a clearly defined zona pellucida (ZP). O, oocyte.

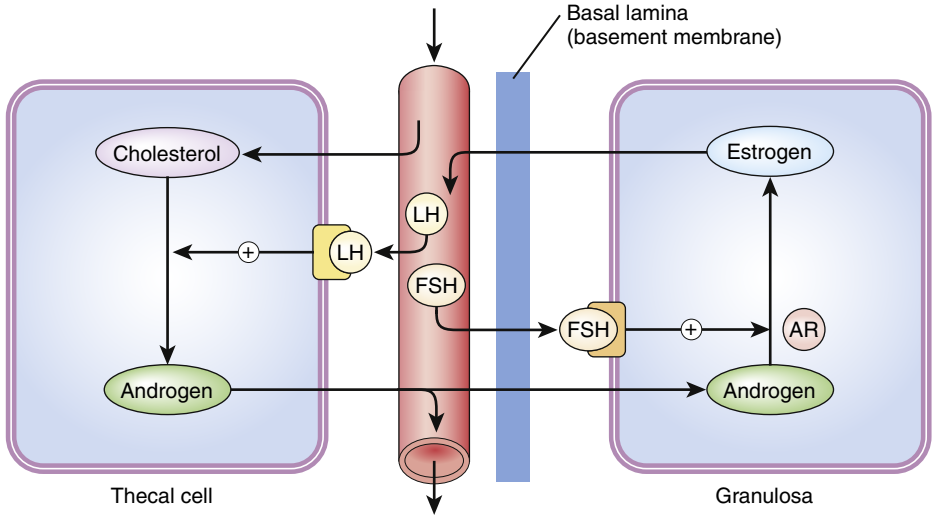


FIGURE 10-16 Two cell model for steroidogenesis. Binding of LH to receptors found only on thecal cells (or ovarian interstitial cells) stimulates androgen synthesis, most of which diffuses through the basal lamina (basement membrane) to the granulosa cell. FSH stimulates aromatase (AR) production, which transforms androgens into estrogens. A similar two-cell system is present in the testis but with both FSH and LH receptors associated with the interstitial cells (Leydig cells) and FSH receptors on the Sertoli cells. Androgens reach the Sertoli cell by diffusion through the basal lamina surrounding the seminiferous tubule.

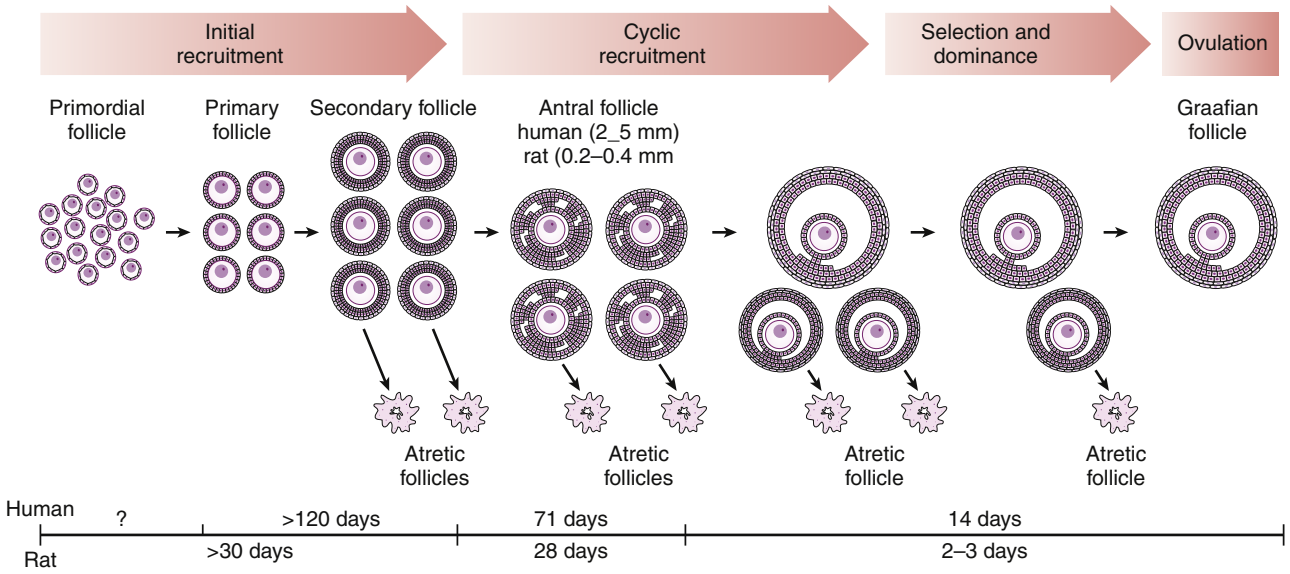


FIGURE 10-17 **Recruitment and death (atresia) of ovarian follicles.** Beginning at puberty a cohort of follicles selected from primordial follicles begins to grow. The majority of these follicles undergo programmed cell death, and one dominant follicle is selected for ovulation during each ovarian cycle from puberty to menopause. *(Adapted with permission from Norris, D.O. and Lopez, K.H., in "Hormones and Reproduction of Vertebrates. Vol. 5. Mammals" (D.O. Norris and K.H. Lopez, Eds.), Academic Press, San Diego, CA, 2011, pp. 59–72.)*

2. Ovulation

The process of ovulation involves the rupture of the mature follicle and release of the oocyte from the ovary into the body cavity. This event marks the end of the follicular phase and the beginning of the luteal phase in the ovary and is correlated with estrus. Ovulation occurs as a result of the progressive increase in circulating estradiol that occurs with the growth of the follicles. Increased estradiol also is responsible for estrous behavior in the female and the enhanced attractiveness of the female to the male at this time. A maximal or critical estrogen level in the blood in most cases activates the surge center in the hypothalamus, which releases a large pulse of GnRH as described in Chapter 4. The pulse of GnRH released results in the LH surge (Figure 10-18) that causes ovulation of one or more follicles within a matter of hours (usually 12 to 24 hours, regardless of the species). The LH surge results in a remarkable series of genetic switches being turned off (FSH gene expression program) and on (genes required for oocyte meiosis; expansion of the **cumulus cell oocyte complex**, or **COC**; and corpus luteum formation). The number of follicles that reach maturity and ovulate is species specific, varying from a norm of one in women to a dozen or more in the sow. The determining factors appear to be the amount of GTH available, and increased numbers of mature follicles are produced following supplementation with exogenous GTHs. The physical mechanism by which LH causes the mature follicle to rupture and release the mature oocyte is not understood completely but involves both an increase in growth of the COC and the production

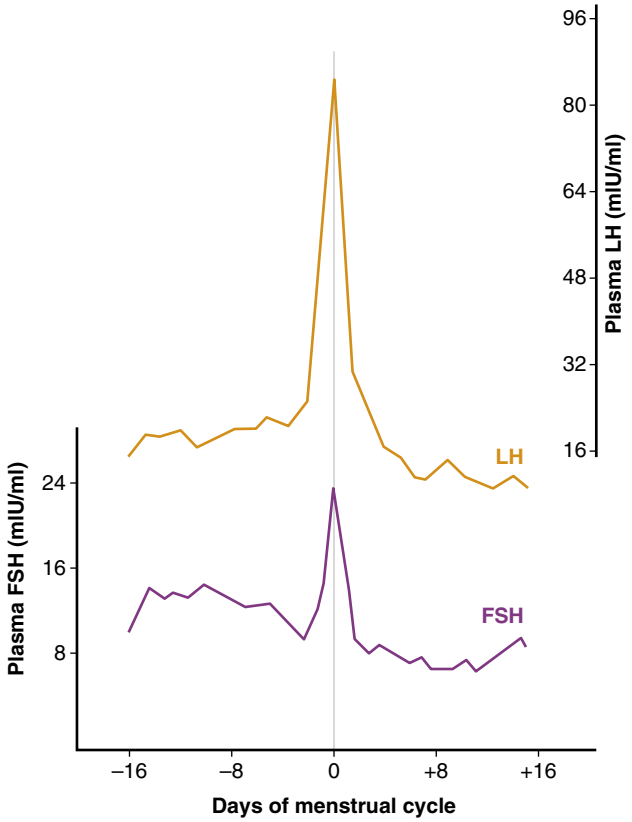


FIGURE 10-18 **Gonadotropin surge in normal women.** The greater release of LH is probably due to the presence of galanin released with GnRH at this time as well as negative feedback effects of inhibins on FSH release at the pituitary level. *Adapted with permission from McCann, S.M. (1974). Regulation of secretion of follicle-stimulating hormone and luteinizing hormone. Handbook of Physiology, Sec. 7, Endocrinology 4, 489–518.*

of **matrix metalloprotease (MMP)** enzymes that digest the collagen and elastic fiber components of the extracellular matrix (Box 10E).

In some species, meiosis in the oocyte that began early in life is not completed until after fertilization. Prior to fertilization, the ovulated cell is an arrested oocyte. If meiosis were completed prior to ovulation, this cell would be termed an ovum. The situation in mammals apparently varies from ovulation of oocytes to ova, but in the following discussions the ovulated cell in every case will be referred to as an ovum to simplify terminology.

Some mammals ovulate following coitus and are termed **induced ovulators**. Several carnivores (e.g., ferret, mink, raccoon, cat), rodents (e.g., *Microtus californicus*), lagomorphs (e.g., cottontail and domestic rabbits), at least one bat (lump-nosed bat), and several insectivores (e.g., hedgehog, common shrew) are confirmed induced ovulators. In these species, coitus is immediately followed by an LH surge that induces ovulation (Figure 10-19). Some other species are suspected to be induced ovulators, including the elephant seal, nutria, and long-nosed kangaroo rat (a marsupial). Most mammals are believed to be **spontaneous ovulators** in that the LH surge and ovulation are independent of coitus; however, even some spontaneous ovulators can be induced to ovulate following copulation under special conditions. Evidence from humans suggests that ovulation may be induced in rape cases especially if the female is very young.

3. The Luteal Phase of the Ovarian Cycle

Ovulation marks the onset of the **luteal phase** of the ovarian cycle as well as the end of the follicular phase. In addition to causing ovulation, the LH surge induces granulosa cells as well as some theca interna cells to differentiate into the corpus luteum (Box Figure 10E-1). This process, known as **luteinization**, results in the corpus luteum, which functions as an endocrine gland, secreting both estrogens and progesterone into the general circulation. One corpus luteum will form from each ovulated follicle. In addition, other developing follicles may undergo premature luteinization and function as **accessory corpora lutea** during pregnancy. The corpus luteum begins secreting large quantities of progesterone, along with lesser amounts of estradiol as well as other estrogens and progestogens. Circulating progesterone and estrogens inhibit both the tonic and cyclic hypothalamic GnRH centers during the luteal phase so that additional follicular development is arrested and a second ovulatory episode is prevented. All developing follicles that do not ovulate undergo atresia or form accessory corpora lutea. Some of the follicular cells from the atretic follicles will persist as part of the ovarian interstitial gland that is responsive to LH and synthesizes androstenedione that can be used as a substrate by the corpus luteum to form estrone and then estradiol (see Figure 3-25).

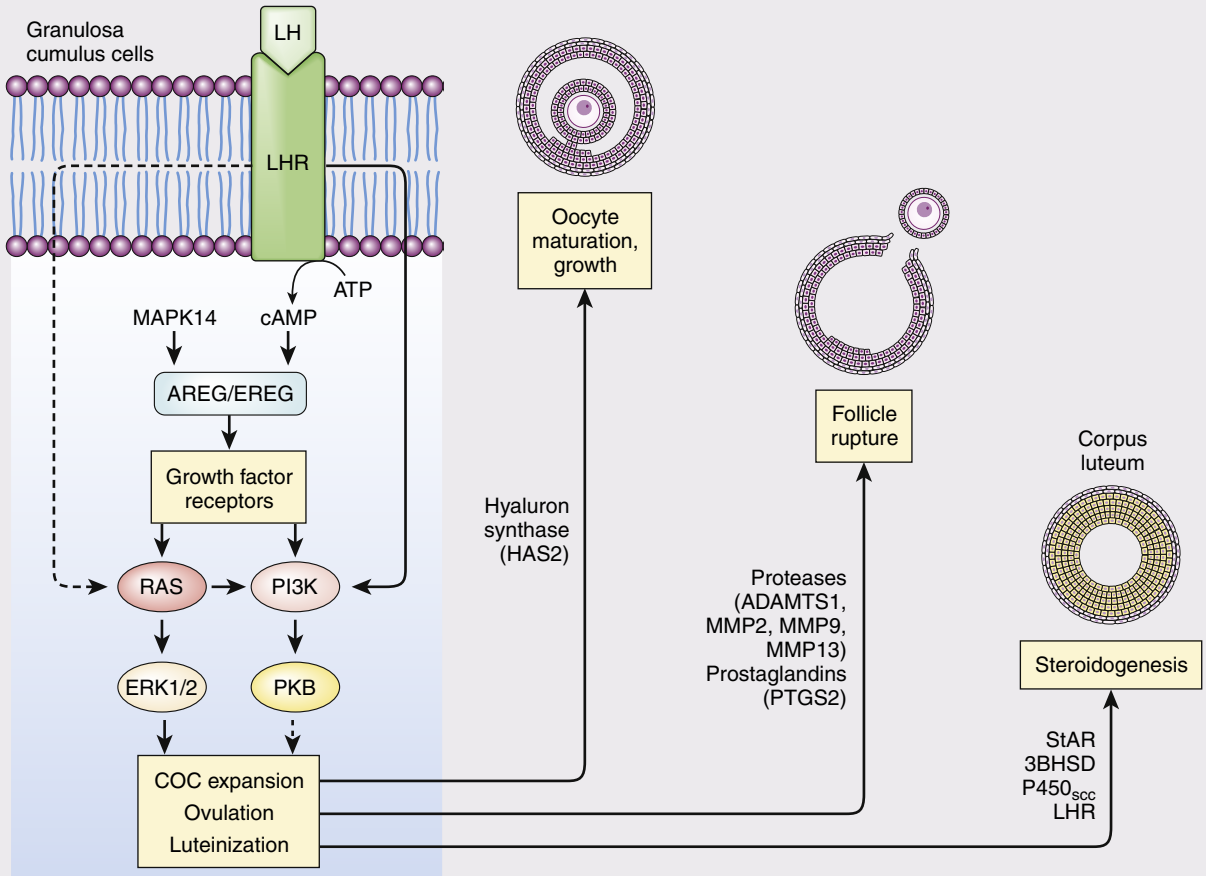
Depending on the species, regulation of corpora lutea function may require LH or be independent of LH once it has formed. In sheep, PRL together with LH apparently stimulates steroid secretion by the corpus luteum; however, only PRL is necessary to maintain the activity of the rat corpus luteum. Preovulatory estradiol can produce a surge of PRL release in several species and might be related to corpora lutea function. These actions of PRL on the corpus luteum were the basis for the older name of luteotropic hormone for this molecule; however, PRL has no role in corpus luteum functions in primates and most other mammals, and the older name should not be used.

The corpus luteum secretes steroids for only a relatively short period in many species (5 to 8 days in humans) after which it begins to degenerate if mating and fertilization were not successful. As the corpus luteum undergoes degeneration, steroidogenesis declines, and the uterus enters a regressive phase. In some species, the corpus luteum is relatively long-lived, especially in carnivorous species like the dog. Corpora lutea in the bitch are active for about 63 days after ovulation, which is equivalent to the normal gestation period, regardless of whether fertilization and pregnancy occurred. If unmated, the bitch will not reenter estrus until the next breeding season.

The predetermined life span for the functional corpus luteum has provided one of the most intriguing mysteries of the ovarian cycle. Apparently, the corpus luteum sows the seeds of its own destruction (Figure 10-20). In female rats, mice, hamsters, rabbits, guinea pigs, and ewes, breakdown of the corpus lutem (luteolysis) requires the production of the prostaglandin **$PGF_{2\alpha}$** from the uterine lining. Production of luteolytic pulses of $PGF_{2\alpha}$ requires the coordinated action of estradiol, OXY, and progesterone on their respective receptors in uterine epithelial cells (Figure 10-20). First, estradiol secretion from mature follicles increases the expression of **progesterone receptors (PRs)**, **OXY receptors (OXTRs)**, and **estrogen receptors (ERs)** in uterine epithelial cells. Progesterone action on the PR causes uterine cells to build up phospholipid in order to generate arachidonic acid, the precursor for prostaglandin synthesis. (see Chapter 3). Progesterone action then causes a down-regulation or block of ERs and OXTRs during this period of phospholipid buildup followed by an upregulation or release of ERs and OXTRs due to suppression of PR. Pulses of OXY secreted from the posterior pituitary and corpus luteum act on uterine OXTRs to stimulate $PGF_{2\alpha}$ synthesis and luteolysis. The exact mechanism of luteolytic activity caused by $PGF_{2\alpha}$ is not clear, although it may relate to reducing blood flow to the corpus luteum via an interaction locally with **angiotensin II** and **endothelin 1**. In primates, the destruction of the corpus luteum toward the end of the luteal phase is not influenced by the uterus but appears to be

BOX 10E Mechanism of Ovulation

Ovulation occurs as a consequence of a series of events (Box Figure 10-E1) that take place in the follicular wall at an avascular site called the **stigma**. LH action on its receptor during ovulation triggers not only the **protein kinase A (PKA)** signaling pathway (see Chapter 3) but also the **rat sarcoma signaling (RAS)** cascade. Stimulation of the PKA pathway by LH results in the production of a series of epidermal growth factor-like signaling molecules, including **amphiregulin (AREG)**, **betacellulin** and **epiregulin (EREG)**. These **epidermal growth factor (EGF)**-like messengers act on their respective receptors on granulosa cells and cells in the cumulus oophorous to stimulate RAS and **extracellular signal-regulated kinases (ERK1/2)** that have several important effects. First, activation of ERK1/2 turns off FSH signaling and FSH-induced gene expression. Second, ERK1/2 activation increases the transcription of a number of genes involved in (1) **follicle rupture**, including chemokines (IL-6, for example), protease enzymes, and **prostaglandin-endoperoxide synthase 2 (PTGS2)**; (2) **luteinization**, including genes regulating steroidogenesis; and (3) **COC complex formation**, including **hyaluronan synthase 2 (HAS2)**. HAS2 plays an important role just prior to ovulation in making a hyaluronan-rich matrix that envelops the oocyte. The activity of a number of protease enzymes acts to weaken the follicular wall. Enzymatic breakdown products bring about an inflammatory response and the release of **prostaglandin (PGE_2)** that causes local blood vessels to constrict, leading to local ischemia and cell death. These events weaken the follicular wall further. Pressure builds up in the antral cavity and causes the follicular wall to rupture at its weakest point, the stigma. The ovum and surrounding cells of the cumulus oophorus (now called the **corona radiata**) are expelled along with the antral fluid. It is speculated that the smooth-muscle-like cells located in the follicular wall may be responsible for producing the increased antral pressure that triggers ovulation. Another hypothesis suggests that the increased pressure results from water influx into the antral fluid.



BOX FIGURE 10E-1 Signaling events triggered by LH during ovulation. How does the binding of a single hormone, LH, to its receptor trigger such a diverse array of cellular changes? In part because the hormone's signal is **amplified** by activation of multiple signaling pathways within the granulosa cells. The LH receptor triggers changes in the cAMP, RAS, and PI3K/AKT signaling pathways that lead to the production of proteins involved in oocyte maturation and cell growth, follicle rupture, and steroidogenesis required for ovulation and corpus luteum formation. 3βHSD, 3β-hydroxysteroid dehydrogenase; ADAMTS1; A disintegrin and metalloprotease (ADAM) with thrombospondin type 1 motif, 1; AKT, protein kinase B; AREG, amphiregulin; COC, cumulus cell oocyte complex; EREG, epiregulin; ERK, extracellular-signal-regulated kinases; LHR, LH receptor; MMP2, matrix metalloproteinase-2, 72 kDa type IV collagenase; MMP9, matrix metalloproteinase-9, 92 kDa type IV collagenase; MMP13, matrix metalloproteinase-13, collagenase 3; $P450_{SCC}$, P450 side-chain cleaving enzyme; PI3K, phosphatidylinositol 3-kinases; PKB, protein kinase B; RAS, rat sarcoma; StAR, Steroid acute regulatory protein. *(Adapted in part from Fan, H.Y., Liu, Z., Mullany, L.K., Richards, J.S., Consequences of RAS and MAPK activation in the ovary: the good, the bad and the ugly.* Molecular and Cellular Endocrinology, ***356(1–2)***, *74–79, 2012).*

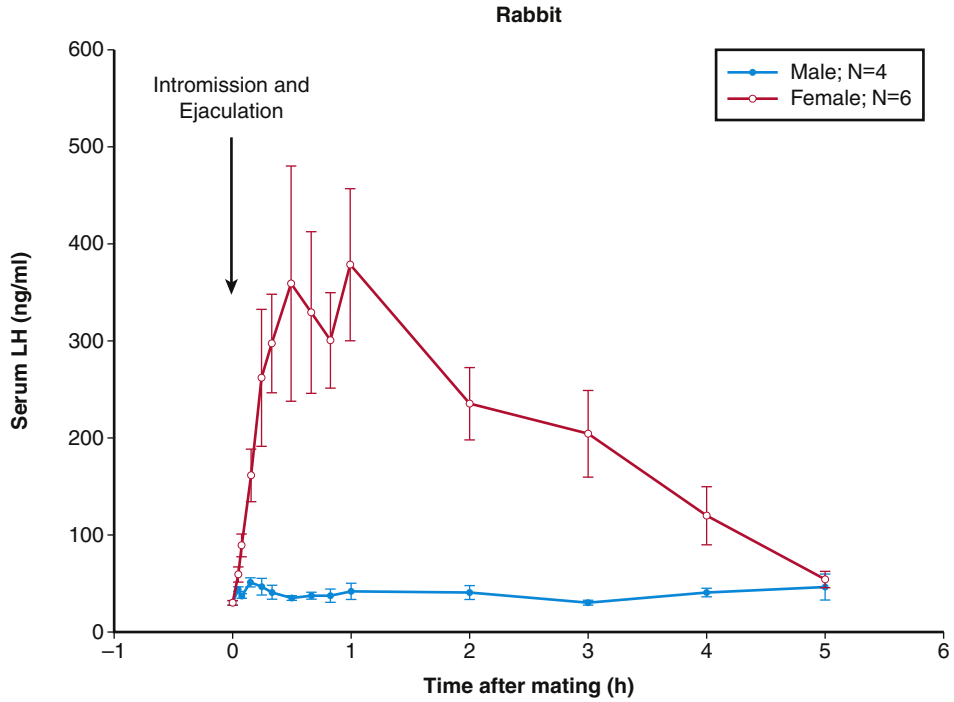


FIGURE 10-19 Coitus induced LH secretion in a rabbit. *(Adapted with permission from Jones, E.F. et al.,* Fertilization and Sterilization*, 27, 848–852, 1976.)*

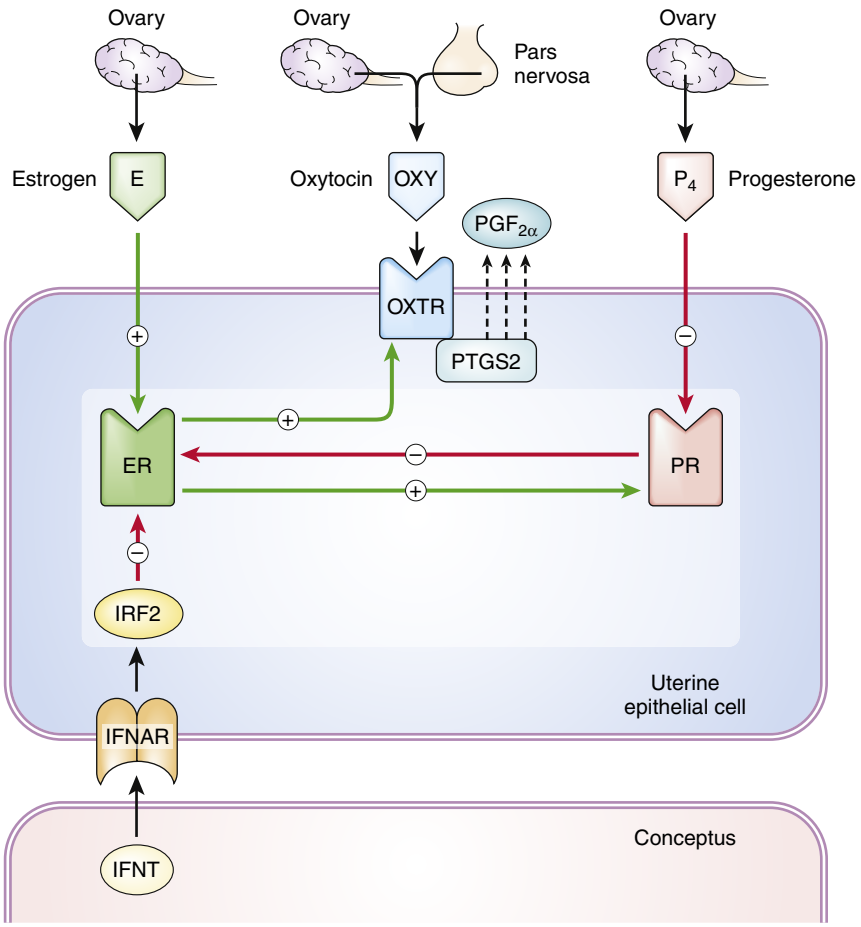


FIGURE 10-20 Mechanism of luteolysis. Estradiol action on uterine epithelial cells leads to increased expression of estrogen receptor alpha (ER), progesterone receptor (PR), and oxytocin receptor (OXTR). Increased PR availability leads to an increase in arachidonic acid, the substrate for prostaglandin PGF_2 synthesis. Increased progesterone secretion downregulates PR, leading to even greater expression of ER and OXTR. Oxytocin secretion from the pars nervosa leads to pulses of PGF_2 secretion, causing luteolysis. Signals (interferon tau, IFNT) from the developing embryo and associated tissues (conceptus) block this pathway, prolonging the life of the corpus luteum. Abbreviations: IFNAR, type 1 interferon receptor; IRF2, interferon regulatory factor 2; PTGS2, prostaglandin-endoperoxide synthase 2. *(Adapted from Bazer, F.W. and Spencer, T.E., in "Hormones and Reproduction of Vertebrates. Vol. 5. Mammals" (D.O. Norris and K.H. Lopez, Eds.), Academic Press, San Diego, CA, 2011, pp. 73–94.)*

caused locally by a luteolytic factor (e.g., estrone), produced by the corpus luteum itself. Once fertilization has taken place, there are several mechanisms by which corpus luteum degeneration may be prevented and the life of the corpus luteum prolonged (see ahead).

Degeneration of corpora lutea frees the hypothalamic GnRH centers from the inhibitory influence of estrogens and progesterone, resulting in a moderate increase in circulating GTHs and consequent renewal of follicular development. In fact, increased FSH release occurs in many species during the later stages of the luteal phase so that follicular growth may resume even before regressive uterine events become obvious.

The importance of the corpus luteum in maintaining pregnancy varies considerably as does the role of pituitary hormones in stimulating corpus luteum function. For example, in rats, PRL is necessary for maintaining the first half of gestation through actions on the corpus luteum. However, in pigs, the corpora lutea secrete progesterone to maintain the uterine secretory phase during the early portion of the gestation period without aid of any pituitary hormones. In ewes, both LH and PRL are necessary for maintaining corpus luteum function during the first third of pregnancy, but maintenance of pregnancy actually resides in the ability of the conceptus to neutralize the uterine luteolytic factor $PGF_{2\alpha}$. Estrogens of placental origin apparently are responsible for prolonging the life span of corpora lutea in rabbits as well as promoting progesterone synthesis. If the estrogen-secreting placental cells are damaged (for example, by x-rays), pregnancy is abruptly terminated.

B. The Uterine Cycle

The uterine cycle can be separated into a **proliferative phase** corresponding to the ovarian follicular phase, a **secretory phase** corresponding roughly to the ovarian luteal phase, and a **post-luteal phase** (Figure 10-21). The proliferative phase is separated from the secretory phase by the occurrence of ovulation. The end of the luteal phase marks the entrance into the post-luteal phase, which is a quiescent period of slow regression of the uterine epithelium in most mammals and is termed the **menses** in mammals that exhibit menstrual cycles. The wall of the uterus consists of an outermost connective tissue covering, a thick intermediate layer of smooth muscle called the **myometrium**, and an innermost epithelium, the **endometrium**, that contacts the uterine lumen. The endometrium can be further separated into an outer **basal layer** that proliferates during each uterine cycle to produce an inner **functional layer** that later regresses.

1. The Proliferative Phase of the Uterine Cycle

Estradiol produced during the follicular phase by the ovary stimulates differentiation and proliferation of the endometrium in preparation for implantation of the **blastocyst**, an embryonic stage formed from the first series of cellular divisions following fertilization. The blastocyst consists of an outer extraembryonic layer of cells, the **trophoblast**, which will form the fetal component of the placenta, and an **inner cell mass**, which will become the embryo proper. The proliferative phase of the uterine cycle is characterized by hyperplasia of the basal layer of the endometrium to form the functional layer in response to estrogens secreted by the growing ovarian follicles. In addition, there is a marked increase in the vasculature (hyperemia) of the functional layer as a result of estradiol stimulation. In higher primates, this hyperemic response includes the development of special **spiral arteries** that play an important role in the menstrual cycle of these mammals. There may be two independent targets for estradiol in the uterus. One target is the basal epithelial cell that responds to estrogens with new protein synthesis and mitosis. This results in an increase in the functional layer as well as in the development of tubular uterine exocrine glands. A second target for estrogens is the uterine eosinophil, which is a white blood cell that has infiltrated the uterine lining. These eosinophils possess specific receptors for estradiol and appear to be responsible for the rapid uptake of water and release of histamine that causes local hyperemia.

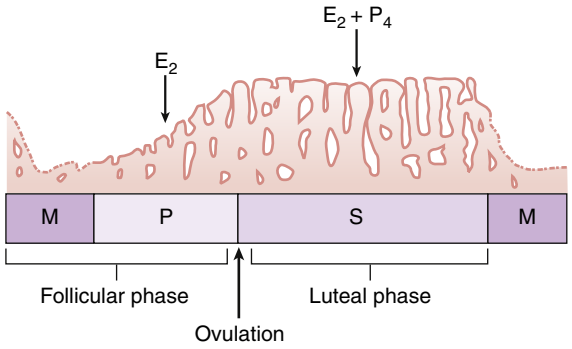


FIGURE 10-21 The uterine cycle. The menses (M) occupies the first 5 days of the cycle. The endometrium is stimulated by estradiol (E_2) during the proliferative stage (P). The follicular phase in the ovary corresponds to M + P. Following ovulation the corpus luteum secretes E_2 and progesterone (P_4) during the luteal phase which maintain the vascularity of the endometrium as well as secretion by exocrine glands during the secretory phase (S). Following the death of the corpus luteum, the uterine lining degenerates and the uterus reenters menses.

2. The Secretory Phase of the Uterine Cycle

Progesterone and estradiol secreted by corpora lutea in the ovary are the hormones controlling this phase, which is characterized by secretion of the exocrine glands in the functional layer of the endometrium. During the secretory phase, estradiol maintains the proliferated uterine endometrium and increased hyperemia that were initiated

during the proliferative phase. Progesterone stimulates the uterine glands to secrete a fluid called **uterine milk** or **embryotroph**. Uterine milk is believed to be a source of nourishment for unimplanted blastocysts. The endometrium of both eutherian and marsupial mammals produces uterine milk. Progesterone and estradiol also maintain the highly vascularized state of the uterus necessary for implantation and early development of the embryo. Uterine muscle becomes desensitized by progesterone, reducing the chance that rhythmic contractions of uterine smooth muscle might dislodge an implanting or recently implanted blastocyst.

3. The Post-Luteal Phase of the Uterine Cycle

If fertilization is successful and implantation occurs, the secretory phase will continue throughout pregnancy. Should implantation not occur, the corpus luteum of many eutherian mammals will rapidly degenerate, resulting in a marked decrease in circulating levels of progesterone and estrogens. This decrease in circulating ovarian steroids causes regressive changes in the endometrium following steroid withdrawal. The endometrium becomes less vascular, and secretion by the uterine glands is reduced. Thus, the uterus becomes less capable of supporting implantation of a blastocyst. In monestrous species such as carnivores, the uterus would enter the quiescent post-luteal phase called diestrus during which the functional layer would be slowly resorbed. In polyestrous species, however, the female may quickly re-enter proestrus, resulting in the resumption of endocrine secretions that would prevent uterine regression.

Instead of a quiescent post-luteal phase, animals with a menstrual cycle exhibit a rapid regression and actual sloughing of the outer portion of the endometrium during the menses if implantation is not successful. In higher primates, the spiral blood vessels constrict, preventing flow of blood to the functional layer of the endometrium and causing extensive cell death. The degenerating tissue and trapped blood are sloughed into the uterine lumen, where they are resorbed or discharged as menstrual flow. Following the menses, considerable rebuilding of the endometrium (another proliferative phase) must occur during the next follicular ovarian phase to prepare for implantation of blastocysts resulting from the next ovulation.

C. The Pregnancy Cycle

Estrus usually occurs just prior to ovulation and normally leads to mating. The recently ovulated ovum still surrounded by some of the granulosa cells, the **corona radiata**, enters the open upper end of the fluid-filled oviduct and is propelled toward the uterus by the action of cilia lining the oviduct and the muscles of the oviduct wall. Contractility of the oviductal smooth muscle is controlled by adrenergic nerves, steroid hormones, nitric oxide, and prostaglandins. Sperm deposited in the vagina by the copulating male during estrus are transported at least in part by peristalsis through the uterus and ascend into an oviduct in which recently ovulated ova are descending. Fertilization leading to successful implantation typically occurs in the upper third of the oviduct. Cleavage begins soon after fertilization, and the **zygote** or fertilized egg rapidly becomes a minute, multicellular blastocyst. The trophoblast produces enzymes that enable the blastocyst to implant (i.e., erode the highly vascularized, secretory uterine endometrium and settle in for development). Following implantation, the outer layer of the blastocyst, the trophoplast, will give rise to the extraembryonic membrane called the **chorion**. Implantation marks the beginning of gestation or pregnancy. In some species, the blastocyst may not implant immediately into the endometrium but may remain in the uterine lumen for a period of time before implanting. This **delayed implantation** (see Box 10F) allows species with a short developmental period to prolong the time before birth will take place after mating. The gestation period is specific for each species and may be as short as 12 days in the opossum (a marsupial) or as long as 22 months in an elephant (eutherian).

In carnivores, such as the domestic dog, the corpora lutea normally function throughout gestation since the length of the normal luteal phase is equal to the gestation period (see before). In others, the corpora lutea would degenerate much earlier with respect to the time required for gestation if fertilization and implantation were not successful. A central question puzzled reproductive physiologists for many years: How did mammals "know" they were pregnant and how did they prolong corpora luteal function and prevent premature regression or sloughing of the endometrium? It turns out there are several mechanisms.

The signal for prolongation of corpora luteal function in some species is the synthesis of an LH-like **chorionic gonadotropin** (CG) by the blastocyst even before implantation. Placental GTHs are structurally very similar to pituitary GTHs and generally produce LH-like effects (see Chapter 4). Their synthesis and release, however, are not influenced in a negative way by steroids in the manner of the steroidal feedback on pituitary GTHs. The trophoblast of the developing human blastocyst begins to secrete hCG prior to implantation. hCG appears in maternal blood within a few days of fertilization and soon after appears in sufficient quantities in urine to be detected with antibody-based pregnancy kits. Later, the trophoblast will contribute to the placenta following implantation and will continue to secrete hCG throughout pregnancy.

In the mare, only fertilized ova ever reach the uterus, implying some sort of early chemical recognition that

BOX 10F Delayed Implantation

Several eutherian mammals, such as mink, bats, and skunks, have evolved a mechanism known as **delayed implantation** whereby development of the blastocyst is arrested and the unimplanted blastocyst remains in the oviduct or uterus for an extended period prior to implantation. Among some eutherian mammals, delayed implantation appears to be an adaptation allowing copulation to occur at a particular time that is especially advantageous to the parent while ensuring that the young are born at the most favorable time for their survival. Neither the basis for causing the blastocyst to remain in a healthy, arrested state nor the stimulus to bring about implantation is known.

Macropodid marsupials have developed a form of delayed implantation called **embryonic diapause**. Embryonic diapause has been reported for 14 macropodid species but does not occur in at least one species, the western gray kangaroo. One major difference from delayed implantation occurring in eutherian mammals is the condition of the resting blastocyst. The macropodid blastocyst consists of about 70 to 100 cells of a uniform type termed **protoderm**. The macropodid blastocyst is surrounded by a shell membrane and an albumen layer. It has not yet differentiated into embryonic (inner cell mass) and extraembryonic (trophoblast) regions like that of the eutherians.

Presence of a joey suckling on a teat presumably evokes release of OXY from the pars nervosa. Oxytocin is believed to arrest corpus luteum functions while allowing lactation to occur. Removal of the suckling joey will allow the resting blastocyst to implant. Ovariectomy following ovulation induces diapause, but if ovariectomy is performed during diapause there is no effect on the duration of diapause. Progesterone administered to either intact or ovariectomized females stimulates cessation of diapause and reinstates blastocyst development. Estrogen is also effective, but continued embryonic development is not as successful as following progesterone treatment.

In the red kangaroo, embryonic diapause may be an adaptation to renew pregnancy immediately following the death of the joey living in the pouch. The gestation period for the red kangaroo is 33 days. After birth the newborn must find its way to the pouch virtually unaided. When it reaches the pouch, the joey attaches itself permanently to a teat and continues development as an exteriorized fetus. Soon after parturition, the mother kangaroo enters estrus again and mates. The presence of one joey in the pouch inhibits implantation of the new blastocyst resulting from the second mating. The new blastocyst remains in a suspended state of development for up to 200 days, at which time the first joey normally disengages itself from the teat and ventures into the outside world as a juvenile kangaroo. The newly liberated kangaroo will return at intervals to the teat to which it was formerly attached for nourishment. Meanwhile, the detachment of the first joey from the teat either releases an inhibition to implantation or provides an endocrine stimulus for implantation of the waiting blastocyst.

In about 4 weeks, the gestation period terminates in birth of the second joey. The new joey enters the pouch and attaches to a teat. The mother kangaroo again enters estrus and mates, and another blastocyst enters embryonic diapause. Thus, a female red kangaroo may have a young juvenile that requires occasional nourishment, a joey attached to a teat, and a blastocyst "waiting in the wings." Secretion of PRL stimulates milk secretion and prevents progesterone synthesis by the corpus luteum that is necessary to sustain an implantation. During extensive periods of drought, the older joey could be denied milk and allowed to die. The implantation of the waiting blastocyst soon provides another joey whose demands upon the mother's nutritional reserves and water supply would be very small in comparison to the demands of the larger joey. Next, the mother would reenter estrus, produce another diapausing blastocyst, and be ready to continue reproducing should conditions improve.

fertilization has occurred. The equine chorionic gonadotropin is called **pregnant mare serum gonadotropin (PMSG)** and appears in large amounts in the urine of pregnant horses.

LH-like GTHs can prolong the life of the corpus luteum that continues to secrete progesterone and estradiol, thus maintaining the secretory phase of the uterus. Secretion of ovarian steroids by the corpus luteum inhibits hypothalamic centers controlling pituitary GTH release so that follicular development and subsequent ovulation are blocked in pregnant animals. Suppression of subsequent follicular development and ovulation prevents having several embryos in the uterus at different stages of development. This would create the problem of expulsion of the younger embryos and fetuses during parturition of the oldest one(s).

While the HPG system is more or less shut down during pregnancy, the placenta begins to function as a composite HPG in the female. Thus, we observe secretion of hypothalamic peptides (e.g., GnRH, TRH, CRH), tropic-like hormones (e.g., ACTH, CG), and gonadal steroids (estrogens, progesterone). During the last third of pregnancy, another pituitary-like hormone, **chorionic somatomammotropin (CS)**, is secreted by the placenta in a number of species (primates, mice, rats, voles, guinea pigs, sheep, chinchillas, and hamsters but not bitches or rabbits). This placental hormone has both growth hormone (GH)-like and PRL-like activities. Antibodies to CS will cross-react with both GH and PRL in at least some of these species. The major roles for CS appear to be effects on metabolism (GH-like) and stimulation of the mammary gland to begin milk synthesis during the later stages of pregnancy. In humans, hCS formerly was called **human placental lactogen (hPL)**. The human placenta also secretes PRL that is identical to pituitary PRL. Placental

PRL accumulates in the amniotic fluid during pregnancy where it is thought to regulate volume and ionic composition of amniotic fluid. Levels of amniotic PRL are not affected by drugs that block maternal pituitary PRL release or even by hypophysectomy of the mother.

D. Birth (Parturition)

The birth process requires coordinated hormonal changes that culminate in the expulsion of the fetus and the associated placenta. Birth can be related to levels of estrogens, progesterone, OXY, prostaglandins, relaxin, corticosteroids, and CRH. The pattern varies in different mammals as to what hormones are involved and the patterns of their secretions. In many mammalian species, the end of gestation is marked by a dramatic decrease in circulating progesterone levels (Figure 10-22). However, in humans and guinea pigs, progesterone levels do not decline before birth and parturition (Figure 10-23).

In sheep, there is a marked reduction in circulating progesterone levels just prior to birth that, presumably, sensitizes the uterus to OXY. The contractions of the uterus initiated by OXY result in expulsion of the fetus as well as of the **afterbirth** (the detached placenta). Experimental studies in sheep show that the fetal adrenal axis plays an essential role in the initiation of the birth process (Figure 10-24). Factors that interfere with adrenal function at any level retard the normal onset of labor, and premature birth can be induced by addition of ACTH or corticosteroids. In sheep, the timing of parturition appears to depend on maturation of the fetal hypothalamus and CRH (Figure 10-24).

In humans, there is no drop in progesterone to trigger birth, although there is a relative increase in estrogen production as compared with progesterone (see Figure 10-23). Not only is the fetal adrenal essential for maintaining pregnancy, but it also is involved in the events associated with birth (Figure 10-25). Recent studies demonstrate that CRH from the placenta acts upon the fetal adrenal to produce DHEA, which is converted to estriol and estradiol, thereby altering the blood ratio of estrogens to progesterone (Figure 10-25). Estrogens increase synthesis

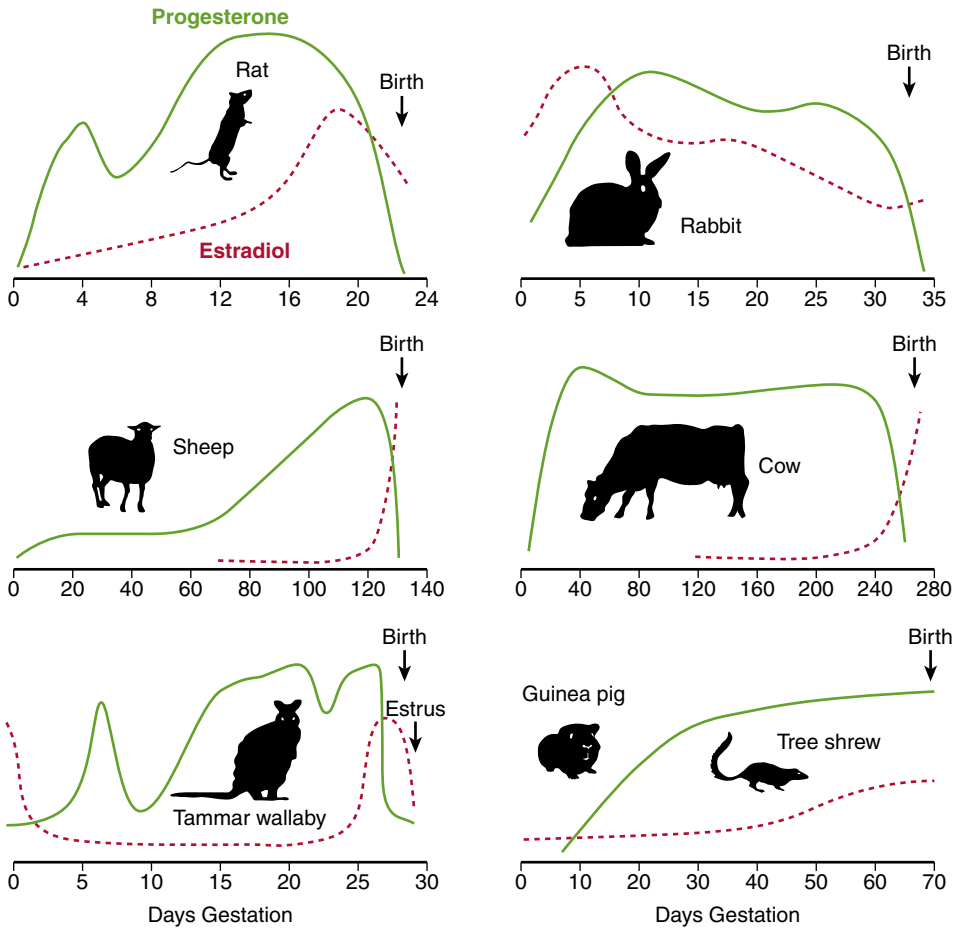


FIGURE 10-22 Patterns of circulating progesterone in various mammalian species. *(Adapted with permission from Young, I.R. et al., in "Hormones and Reproduction of Vertebrates. Vol. 5. Mammals" (D.O. Norris and K.H. Lopez, Eds.), Academic Press, San Diego, CA, 2011, pp. 95–116.)*

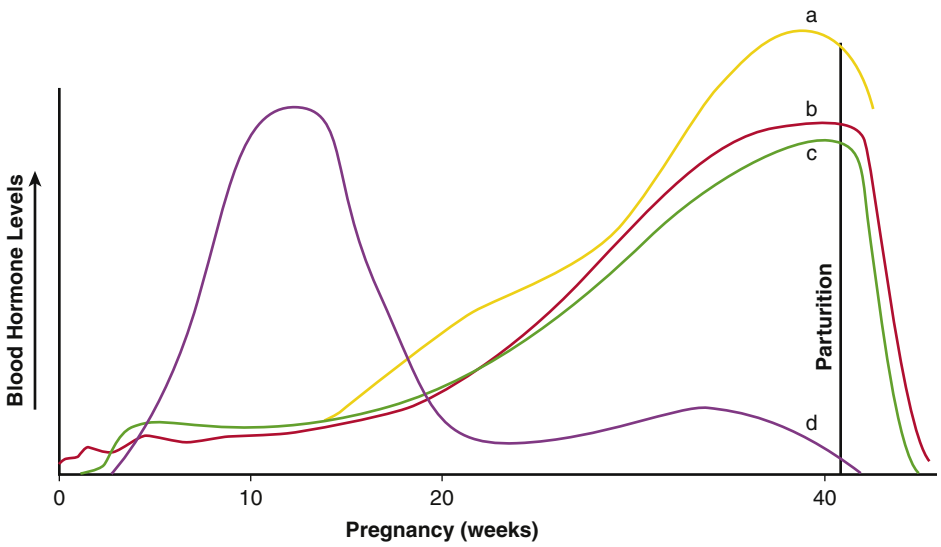


FIGURE 10-23 Pattern of hormone secretion during human pregnancy. Note that maternal progesterone levels do not decrease until detachment of the placenta. (a) hCS; (b) estrogens; (c) progesterone; (d) hCG. *(Adapted with permission from Bolander, F.F.* Molecular Endocrinology, *Academic Press, 1989.)*

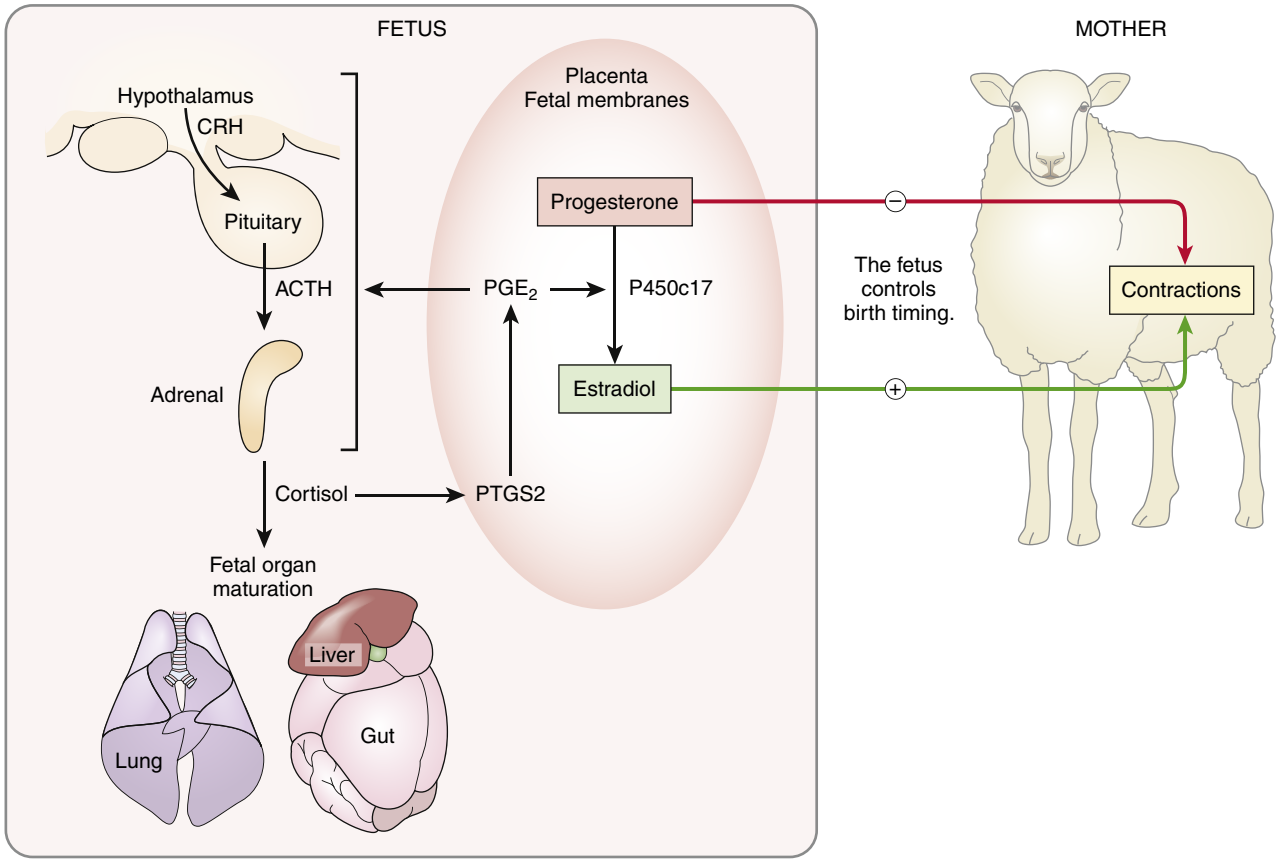


FIGURE 10-24 Timing of birth in sheep. In sheep, the timing of birth is determined by maturation of the fetal hypothalamus–pituitary–adrenal axis. Abbreviations: P450c17, 17α-hydroxylase/17,20 lyase; PGE_2, prostaglandin E_2; PTGS2, prostaglandin synthase 2. *(Adapted with permission from Young, I.R. et al., in "Hormones and Reproduction of Vertebrates. Vol. 5. Mammals" (D.O. Norris and K.H. Lopez, Eds.), Academic Press, San Diego, CA, 2011, pp. 95–116.)*

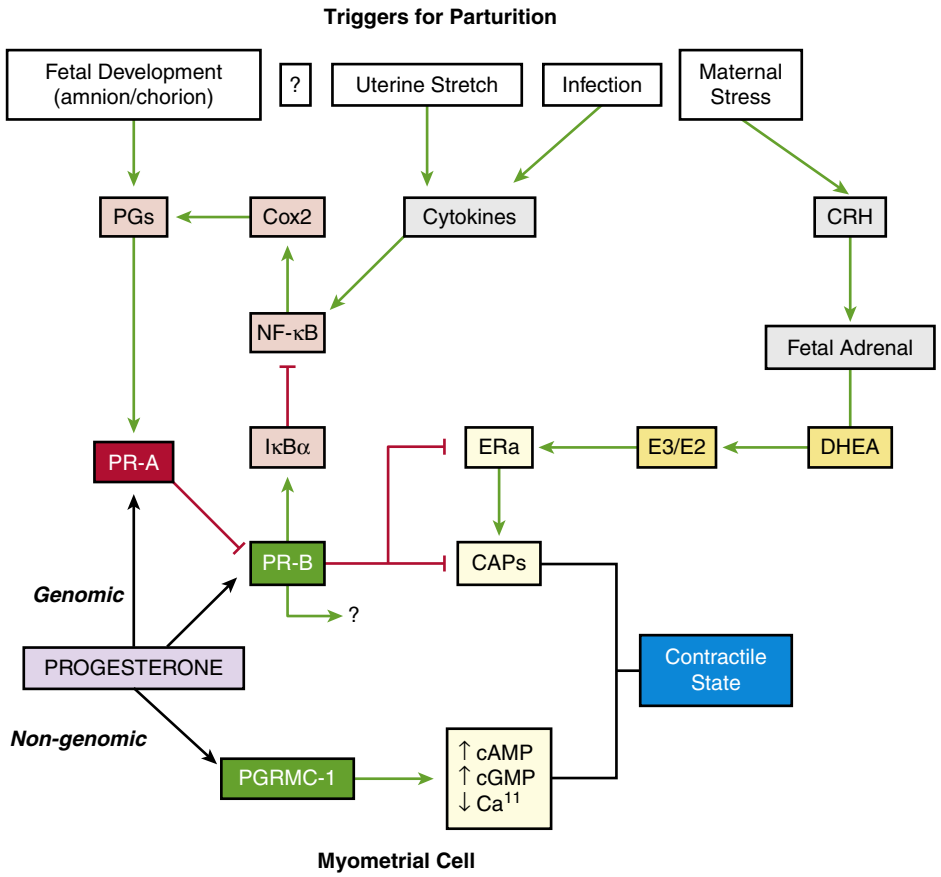


FIGURE 10-25 Timing of birth in humans. Contraction of the uterus is stimulated by placental CRH stimulating estrogen synthesis by the placenta and by blocking the PR-B form of the progesterone receptor, which promotes uterine relaxation. Both cytoplasmic (PR-A, PR-B) and surface membrane receptors for progesterone are involved in the control of uterine muscle contraction. The blockade of PR-B receptors is initiated by NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells), a transcriptional regulatory protein found in virtually all cells. NF-κB promotes the synthesis of PR-A, which represses the expression of PR-B, thereby allowing for stronger uterine contractions. Abbreviations: CAPs, contraction-associated proteins; COX2, cyclooxygenase isoform 2 (also known as prostaglandin-endoperoxide synthase 2, PGS2); DHEA, dehydroepiandrosterone; E_3, estriol; ER, estrogen receptor; Iκβα, nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha; P_4, progesterone; PG, prostaglandin; PGRMC-1, progesterone receptor membrane component 1. *(Adapted with permission from Young, I.R. et al., in "Hormones and Reproduction of Vertebrates. Vol. 5. Mammals" (D.O. Norris and K.H. Lopez, Eds.), Academic Press, San Diego, CA, 2011, pp. 95–116.)*

of prostaglandins and of OXY receptors in the uterine myometrium, facilitating contractions. Cortisol also increases CRH production by the placenta at this time, setting up a positive feedback on the fetal adrenal via placental CRH as well as increasing the effects of CRH on the myometrium. Women with higher levels of CRH early in pregnancy are more likely to exhibit higher levels later on and give birth prematurely (Figure 10-26). Cortisol from the fetus near term also induces the lungs to begin production of surfactants that reduce surface tension on the lung surface and will be essential for the switch to breathing air that occurs after birth.

Mechanical stimulation of the vagina, cervix, or uterus can release OXY in humans and induce a fetal ejection reflex. Administration of prostaglandins also can induce uterine contractions, and OXY may stimulate prostaglandin synthesis in the uterus. Synthetic OXY is normally used to induce labor in women and frequently is given to reduce postpartum bleeding following detachment of the placenta. OXY is preferred over prostaglandins for clinical uses even though both are involved in normal births because administration of prostaglandins tends to produce strong contractile effects on non-reproductive smooth muscle as well (i.e., gastrointestinal smooth muscle).

Relaxin causes relaxation and softening of estrogen-primed pelvic ligaments, allowing the pelvis to stretch and expand (relax) during birth. This expansion allows the relatively large head of the eutherian fetus to pass through the pelvis during parturition. Relaxin reaches peak levels prior to birth and rapidly disappears from the maternal circulation afterward. Spontaneous motility of the uterus may be inhibited by relaxin in some mammals, thereby reducing the risk of premature birth. Relaxin working with estrogens, progesterone, and prostaglandins actually can alter the structural collagen of the uterine cervix, increasing its distensibility at parturition. There are data supporting an action of relaxin in combination with steroids and PRL on the mammary gland and the onset of lactation following birth.

The corpus luteum is the major source of relaxin in species where the corpus luteum is retained throughout gestation (pig, rat, carnivores). Relaxin is produced by the human corpus luteum during early gestation and to some extent by the placenta. Only a little relaxin is found in placentas of sheep, rats, cows, and rabbits, but in horses the placenta is the major source of relaxin. In humans, the

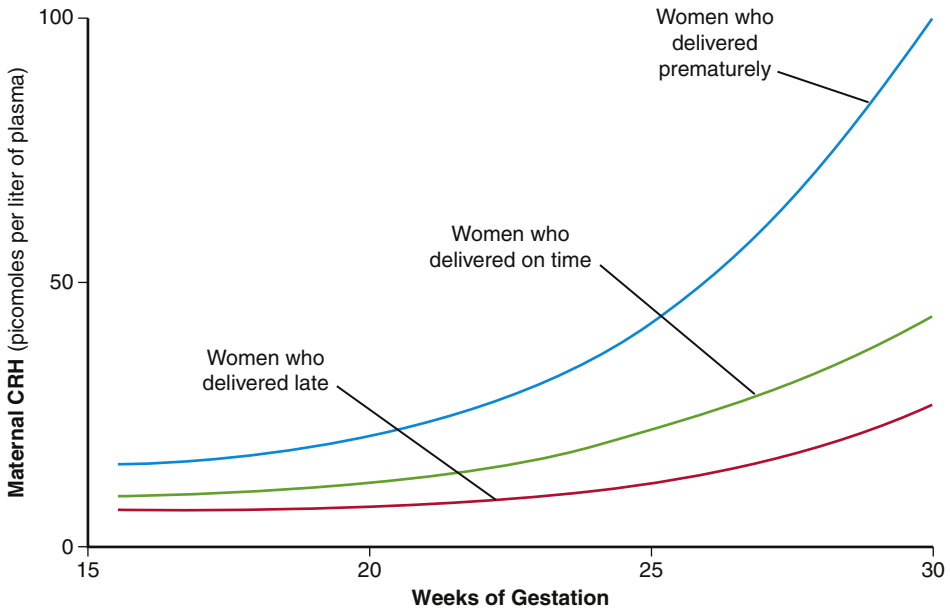


FIGURE 10-26 Relationship between circulating corticotropin-releasing hormone (CRH) and timing of parturition. As early as 15 weeks of pregnancy, it is possible to predict which women are at risk for premature birth by monitoring maternal blood levels of CRH. Low CRH delays birth, whereas high CRH levels indicate premature delivery is very likely. *(Adapted with permission from Young, I.R. et al., in "Hormones and Reproduction of Vertebrates. Vol. 5. Mammals" (D.O. Norris and K.H. Lopez, Eds.), Academic Press, San Diego, CA, 2011, pp. 95–116.)*

ovarian interstitial cells continue to be the major site for relaxin synthesis during pregnancy even after death of the corpus luteum.

Relaxin is chemically similar to insulin and insulin-like growth factors (IGFs) and consists of two short A-chains (22 to 24 amino acids) and a longer B-chain (26 to 35 amino acids) joined together by disulfide bonds (see Chapter 3, Figure 3-4). The positioning of the disulfide bonds is the same as for insulin and the IGFs, although there are many differences in amino acid sequences. It has been suggested that the relaxin gene arose by duplication from the insulin gene. Subsequent to this duplication, there has been considerable divergence in the relaxin genes among mammals as evidenced by considerable variation in amino acid sequences of mammalian relaxins. Relaxin acts on two G-protein-coupled receptors called the **relaxin/insulin-like family peptide 1 and 2 receptors (RXFP1 and 2)** to carry out its actions on the reproductive tract. Both receptors stimulate the cAMP/PKA signal transduction pathway (Figure 10-27). There also is evidence that relaxin/RFXP interaction can transactivate receptor tyrosine kinase and that relaxin can interact directly with glucocorticoid receptors (Figure 10-27). Relaxin receptors are part of a family of G-protein-coupled receptors called the **leucine-rich repeat-containing G-protein-coupled receptors (LGRs)**. Other family members include the receptors for the glycoprotein hormones FSH, LH, and TSH. Relaxin receptors were first identified as members 7 and 8 (LGR-7, LGR-8) of this receptor family.

E. Lactation

The development of mammary glands, their synthesis of milk, and the ejection of milk to the suckling offspring are all regulated by hormones. Mammary glands in eutherian mammals usually occur as paired structures, from 2 to 18, and may be located on the thorax (human, elephant, bat), along the entire ventral thorax and abdomen (sow, rabbit), in the inguinal region (horse, ruminants), along the abdomen (whale), or even dorsally (nutria, a South American rodent). These glands are apparently modified sweat glands, glands that are also unique to mammals. The internal structure is rather uniform and includes supporting stromal cells and a glandular epithelium that is organized into clusters of minute, sac-like structures called **alveoli**. It is this glandular epithelium that is responsible for the synthesis of milk. The alveoli are continuous with ducts and various duct-derived enlargements for storing milk. In addition, there are modified epithelial cells that contain muscle-like myofilaments parallel to the long axis of the cells. These cells are termed **myoepithelial cells** and are capable of contracting and causing ejection of milk from the alveoli into the duct system and out of the gland in the region of the nipple.

Information obtained from the mouse and rat indicates that differentiation of mammary glands from ectoderm involves specific induction by a particular underlying mesenchyme. These glands in both mother and fetus normally undergo hyperplasia and hypertrophy with the aid of estrogens during the last third of the gestational period. The

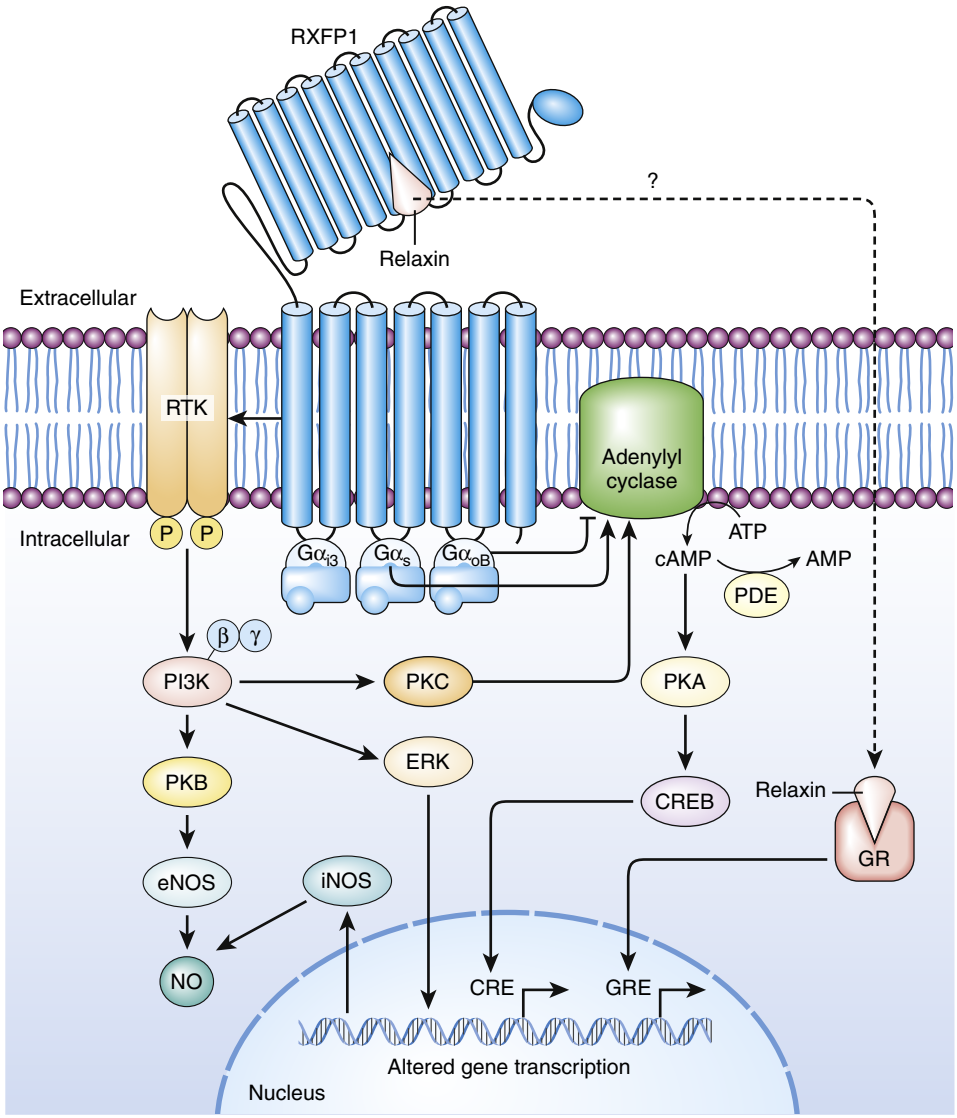


FIGURE 10-27 Relaxin signal transduction and control of transcription. Relaxin binds to RFXP1 receptors to stimulate cAMP production, triggering the PKA pathway and leading to phosphorylation and activation of cAMP response element-binding (CREB) protein which acts as a transcription factor. Relaxin bound to RFXP1 may transactivate receptor tyrosine kinase (RTK) pathways. Relaxin also may interact directly with the glucocorticoid receptor to modulate transcription, although the mechanism is unknown. Abbreviations: cAMP, cyclic AMP; CRE, cAMP response element; eNOS, endothelial nitric oxide synthase; ERK, extracellular signal-regulated kinase; GR, glucocorticoid receptor; GRE, glucocorticoid response element; iNOS, inducible nitric oxide synthase; NO, nitric oxide; PDE, phosphodiesterase; PKA, protein kinase A; PKB, protein kinase B; PKC, protein kinase C; PI3K, phosphoinositide 3-kinase; RXFP1, relaxin family peptide receptor 1. *(Adapted with permission from Du, X.-J. et al.,* Nature Reviews Cardiology, *7, 48–58, 2010.)*

placenta is the source of these estrogens. Androgens partially suppress mammary gland development and are presumably responsible for the lack of stimulation seen in the male fetus.

Postnatal mammary development involves hormones from the pituitary, ovaries, and adrenal cortex, at least in mice and rats. Growth of mammary ducts requires estrogens, GH, and corticosterone working in concert. However, expansion of the alveoli (called lobuloalveolar growth) is dependent upon the direct interactions of estrogens, progesterone, PRL, GH, relaxin, and corticosteroids.

Lactation can be separated into two basic processes or phases under separate endocrine control mechanisms. The first phase is milk secretion or **lactogenesis**. This process primarily is controlled by pituitary PRL (or placental CS), growth factors, and glucocorticoids. In primates, lactogenesis also is stimulated by GH. Lactogenesis involves synthesis of milk fat, milk protein, and milk sugar, typically lactose. The synthesis of lactose ultimately depends upon

TABLE 10-9 Some Bioregulators Found in Milk[a]

Regulator Type	Examples	Regulator Type	Examples
Adenohypophysial hormones	PRL	Gastrointestinal peptides	VIP
	GH		CCK
	TSH		Gastrin
	FSH		GIP
	LH		Substance P
	ACTH		Neurotensin
Growth factors	IGF-I	Steroid hormones	Estradiol
	NGF		Progesterone
	EGF		Testosterone
	TGF-α		Corticosterone
	PDGF		Vitamin D
Neurohormones	TRH	Other regulators	Prostaglandins (PGE, $PGF_2\alpha$)
	GnRH		cAMP
	SS		Delta sleep-inducing peptide
	GHRH		Relaxin
	Oxytocin		Thyroid hormones (T_3, T_4)
			Calcitonin
			Parathyroid hormone

[a]*See Appendix A for abbreviations.*

protein synthesis; that is, the enzyme responsible for lactose synthesis, lactose synthetase, must be induced. Lactose synthetase is composed of two protein units, one of which is lactalbumin, which also is found in milk. Lactose, fat, and milk protein (largely casein) are secreted into the lumen of the alveolus. Water and numerous water-soluble substances enter the lumen by osmosis and result in a watery liquid known as milk. Many hormones are present in milk, including hypothalamic peptides, pituitary hormones, growth factors, steroids, gastrointestinal peptides, and others (see Table 10-9). In addition, the mammary route may conduct lipid-soluble pollutants such as PCBs and pesticides accumulated by the mother to the offspring.

The composition of milk produced by the mammary gland associated with suckling the young is very different at birth from what it will be shortly thereafter. This first milk, known as **colostrum**, is characterized by having a greater concentration of protein and less carbohydrate than does later milk. Colostrum contains antibodies and other substances that serve to protect the neonate against allergies and diseases while its own immune response system is developing.

The second phase of lactation is **milk ejection**, a simple reflex mechanism controlled by OXY from the pars nervosa. Mechanical stimulation of the nipple (suckling) evokes release of OXY from the pars nervosa via a spinohypothalamic neuronal pathway. Release of PRL also occurs when milk is ejected and stimulates further milk synthesis. OXY stimulates contraction of myoepithelial cells which causes milk to be ejected from the alveoli into the ducts and storage channels of the mammary gland. Suckling by the young animal strips this milk from the gland by expressing it between the tongue and hard palate.

The milk ejection neurohormonal reflex exhibits classical conditioning responses as evidenced by the stimulation of milk flow in the cow by sight and sounds of the milking parlor or in women by the cries of their hungry infant. This reflex can be influenced by other neural or chemical inputs to the hypothalamus; for example, stress or physical discomfort can inhibit ejection of milk in the presence of the stimulus that would normally elicit release of OXY.

F. Menopause

In nature, few animals live beyond their peak of reproductive activity due to predation, disease, or other environmentally related phenomenon. In contrast, life after reproductive age is a common occurrence in human females. Whereas men may produce viable sperm most of their lives, the ovary becomes refractory to GTHs, usually during the mid to late 40s. This transitional stage is called **menopause**. Cycles of these women become irregular and eventually they cease to ovulate and menstruate. This is accompanied by a marked reduction in circulating levels of gonadal steroids as well as of adrenal androgens and by an elevation in GTH levels. The transition from **premenopausal** (actively reproductive) to **postmenopausal** (nonreproductive) usually is gradual over several years and may be accompanied by additional symptoms, including vaginal atrophy, hot flashes or flushes, reduced libido, and accelerated bone resorption leading to calcium deficiency syndromes such as osteopenia and osteoporosis (see Chapter 14). Many studies have shown that heart disease and other cardiovascular disorders increase exponentially in postmenopausal women and deaths due to cardiac disease are several-fold greater than for uterine and breast cancer combined.

Estrogen replacement therapy, usually in combination with a progestogen, alleviates many of the symptoms of menopausal and postmenopausal women. Estrogen therapies also have been associated with improvement in cognitive skills due to their stimulatory actions on neural development and learning tasks. When taken with calcium supplements and a regimen of weight-bearing exercise, steroid therapy also can prevent bone resorption. Studies report that estrogens reduce the risk of heart disease by as much as 50% in postmenopausal women as well as slow skeletal calcium losses. Apparently, estrogens or estrogens plus progestogens elevate high-density lipoproteins (HDLs) (see Chapter 12) which are associated with reduced cardiovascular risk. Potential benefits of estrogen therapy need to be considered in the light of other evidence linking estrogen replacement therapy to breast cancer. The decision for a woman to elect estrogen therapy involves many complicating factors that must be weighed. For example, is osteoporosis, heart disease, or breast cancer a serious problem in her family? How severe are the symptoms of menopause and/or osteoporosis and how do they affect her family life, her job? Exposure to other estrogenic chemicals through food, water, cosmetics, and other sources (e.g., phytoestrogens, bisphenol A, nonylphenols, ethinylestradiol, phthalates, certain pesticides) should also be considered as they do contribute to the total estrogen exposure. Careful scientific studies have verified that mixtures of estrogenic chemicals at levels unlikely for each to produce estrogenic effects are additive when they all work through the same mechanism (e.g., binding to and activating the estrogen receptor).

Men also experience reproductive decline with age, although this "male menopause" or **andropause** occurs more gradually and is not so evident as female menopause. Testosterone levels begin to decline after about age 30 and can lead to clinical signs in the 50s and 60s. Symptoms of andropause include reduced libido (sex drive), depression, loss of skeletal muscle mass, increased body fat, declines in cognitive ability, and osteoporosis. Metabolic changes may be responsible for the correlation of low testosterone with increased risk for cardiovascular disease. Although testosterone therapies are readily available, the known relationships between excess androgens and cancer induction should be considered before embarking on either a preventative or restorative course.

VI. REPRODUCTIVE CYCLES IN SELECTED EUTHERIAN FEMALES

In this section, four reproductive cycles are presented as being representative of eutherian mammals: four-day cycling rats, ewes, women, and elephants. These four examples emphasize both the features described previously that are characteristic of eutherian mammals and some of the differences seen among different species. The cycles of these species are among the best known, but not necessarily representative of all mammals. Ewes and rats are polyestrous species with distinct periods of estrus, whereas women have no seasonal estrous behavior and exhibit a menstrual cycle. Reproductive cycle length varies from 4 or 5 days in rats to 16 days in sheep, 28 days in humans, and 16 weeks in elephants. Cows and pigs have cycles that are essentially like the ewe cycle although they differ somewhat in timing of the various events. Both rats and women are continuous breeders, but ewes, like elephants, are distinctly seasonal breeders. Rats have a short gestation period lasting only 22 days, whereas elephants at the other extreme have a 22-month gestation period. None of these species exhibits delayed implantation, and all are believed to be spontaneous ovulators except under special conditions for the rat and possibly the human.

A. The Four-Day Cycling Rat

The laboratory rat cycle (Figure 10-28) is separable into **proestrus** (1 day), **estrus** (1 day), and **diestrus** (2 days in the four-day rat and 3 days in the five-day rat) and is cued closely to environmental events. Typically, several follicles develop and ovulate during each cycle, resulting in multiple corpora lutea in the postovulatory ovary.

GTHs from the pituitary stimulate ovarian follicle development and steroidogenesis. On the morning of the day prior to estrus (that is, during proestrus), the levels of

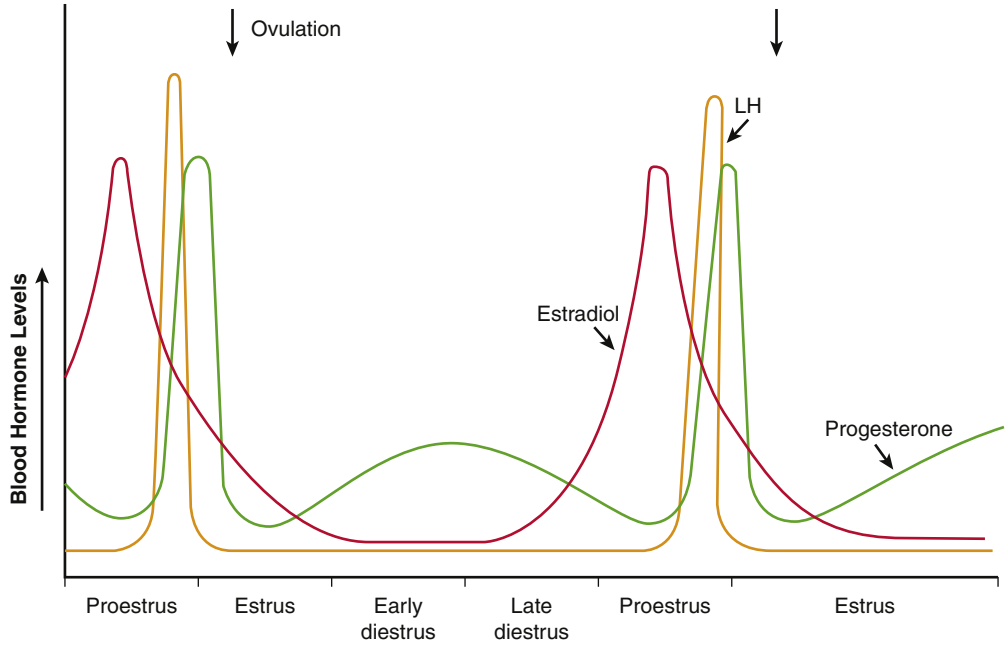


FIGURE 10-28 Ovulatory cycle of 4-day rat. The progesterone surge following the LH surge and prior to ovulation (arrow) is responsible for increased receptivity in the female for the male. There is a secondary, slow increase and decline in progesterone secretion from the corpus luteum that occurs during diestrous in the unmated female. *(Adapted with permission from Short R.V.,* Reproduction in Mammals, Book 3, *Cambridge University Press, 1972.)*

estrogen in the plasma reach a peak that stimulates an LH surge accompanied by a small surge in FSH. The GTH surge occurs on the afternoon of proestrus and is followed rapidly by a marked surge of progesterone from several short-lived corpora lutea. Ovulation occurs a few hours after midnight on the day of estrus. Several follicles usually mature simultaneously, and multiple ovulations commonly occur. Estrus lasts about 9 to 15 hours, during which time the female is highly receptive to the male. Ovulation occurs during estrus. Cornified cells, which were produced by the actions of estrogens during proestrus, appear in the superficial layers of the vagina, and their presence in vaginal smears characterizes estrus (Figure 10-29). In fact, this action was used as a bioassay for many years to define whether any compound had estrogenic activity (see Appendix D). Today, we have substituted more molecular techniques to assess estrogen activity (see Chapter 2 and ahead in Section VII of this chapter).

The third and fourth day of the cycle are termed **diestrus I** and **diestrus II**. Vaginal smears prepared during diestrus are characterized by the absence of cornified cells and a pre-dominance of leukocytes in the smear. There is limited secretory function by the corpora lutea, as evidenced by a slight increase in plasma progesterone. Much of this progesterone is produced by ovarian interstitial cells, which constitute what has been called a permanent corpus luteum in the rat ovary. This interstitial tissue responds to LH by secreting both progesterone and **20α-dihydroprogesterone**.

Many researchers recognize a short transitional period between estrus and diestrus in rats termed **metestrus**. The female in metestrus is no longer receptive to the male, but some cornified cells still appear in smears prepared from the vaginal mucosa.

Mating stimulates the GnRH surge followed by ovulation and consequently the corpora lutea form and begin to secrete significant amounts of progestogens. This increased production of progestogens will inhibit hypothalamus—pituitary function and delay the onset of the next cycle. If fertilization and implantation do not result from mating, the rat will not return immediately to proestrus but will delay resumption of proestrus for a few days. This period of copulation-induced **pseudopregnancy** often occurs in laboratory rodents and sometimes in other domestic mammals. The condition of pseudopregnancy is often accompanied by PRL-like effects on lactation and behavior presumably due to pituitary release of PRL.

If implantation does occur, the corpora lutea continue to secrete progestogens under the influence of placental CG and begin to secrete relaxin. The rat placenta also produces CS, which contributes to stimulation of mammary gland development and lactogenesis prior to birth.

Pheromones play central roles in mating and successful pregnancy in rodents. Most of the scientific studies have been done with mice rather than rats or wild rodents but may apply to many rodent species. Crowded female mice become anaestrus when no males are present (called the Lee—Boot effect). However, simply the odor from a male

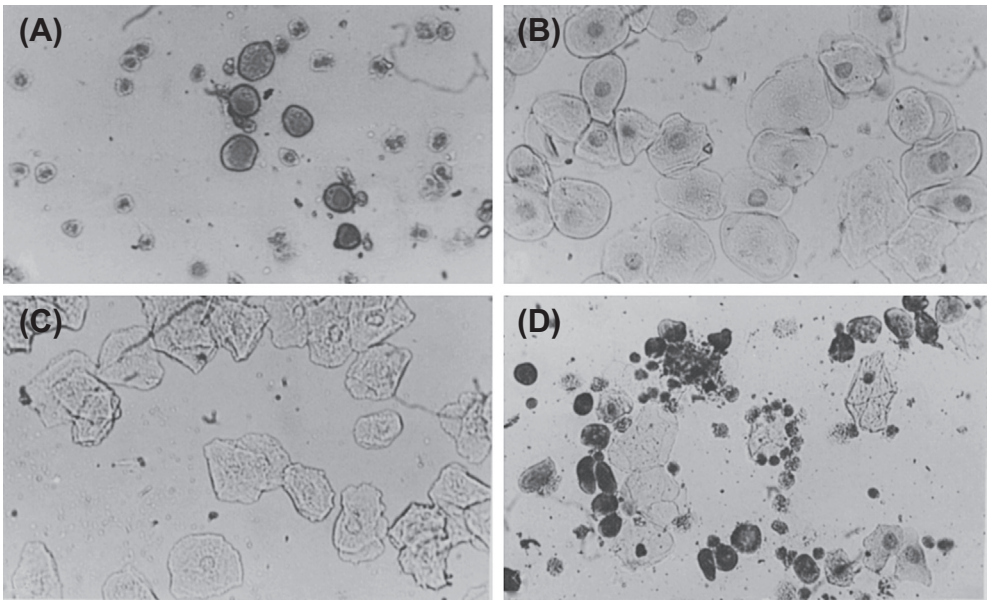


FIGURE 10-29 Vaginal smears from rats during estrous cycle. (A) *Diestrus:* absence of cornified (keratinized) cells and presence of small leukocytes. (B) *Proestrus:* many live epithelial cells with smooth margins; leukocytes absent. (C) *Estrus:* large cornified cells with irregular margins. (D) *Metestrus:* leukocytes have infiltrated among the cornified cells; this stage is transitional between estrus and diestrus. (*Adapted with permission from Short R.V.,* Reproduction in Mammals, Book 3, *Cambridge University Press, 1972.)*

mouse can cause them to synchronously ovulate and enter estrus (Whitten effect). The endocrinological basis for these effects is suggested by observations that pheromones from female mice suppress pituitary release of FSH, whereas male pheromone stimulates GTH release that is followed in normal sequence by an LH surge and ovulation. A newly mated female mouse may abort if placed with a "strange" male (not the previous mate), and the likelihood of spontaneous abortion increases with the genetic dissimilarity of the strange male to the male with whom she originally was mated (this is called the Bruce effect). If offspring result, they are always from the "strange" male.

The sexual pheromones involved in the Lee–Boot and Bruce effects are probably modified steroids (steroid metabolites) and are transmitted via the urine of the male to the olfactory apparatus of the female. Male mouse urine induces and accelerates estrous cycles of females (Whitten effect), and the effect is most pronounced on Lee–Boot groups of females. The time of vaginal closing in females is also influenced by male urine. Anosmic females (animals whose nostrils have been blocked or whose olfactory bulbs have been removed surgically) do not respond to male urine.

Pregnant and lactating rats produce pheromones that influence other females. Odors from a lactating female with pups lengthens estrous cycles of non-pregnant females. Thus a socially dominant, lactating female may suppress fertility of other females until she is again in estrus herself.

Males also may be influenced by female pheromones. Pairing of a previously paired male mouse with a strange female results in elevation of plasma testosterone in the male, indicating that endocrine responses of both males and females may be influenced through bisexual encounters.

B. The Ewe

Sheep estrous cycles occur seasonally, and the duration of one complete cycle is 16 days. The ewe may return to proestrus at least once if fertilization does not occur (Figure 10-30). Reproductive cycles can be blocked in ewes by **genistein**, a phytoestrogen found in certain clovers (genistein is also present in some plant products consumed by humans such as soy). The structure of genistein is provided in Chapter 3, Figure 3-20. Grazing of sheep in pastures containing this clover can have contraceptive effects.

During the follicular phase (= proestrus), there is a marked increase in estrogen and androgen levels in ewes, a peak being reached about 24 hours after the onset of proestrus. About 12 hours later, a surge of plasma LH occurs caused by the action of estradiol on the cyclic hypothalamic neurosecretory center. A high level of circulating androstenedione actually may be responsible for inducing estrous behavior by being a substrate for estradiol synthesis. Usually, a single ovulation follows the LH surge by about 24 hours, and a corpus luteum forms from the ruptured follicle under the influence of LH. Low levels of LH following ovulation and the estrogen-induced surge of PRL stimulate the corpus luteum to secrete progesterone. Under the influence of progesterone, the

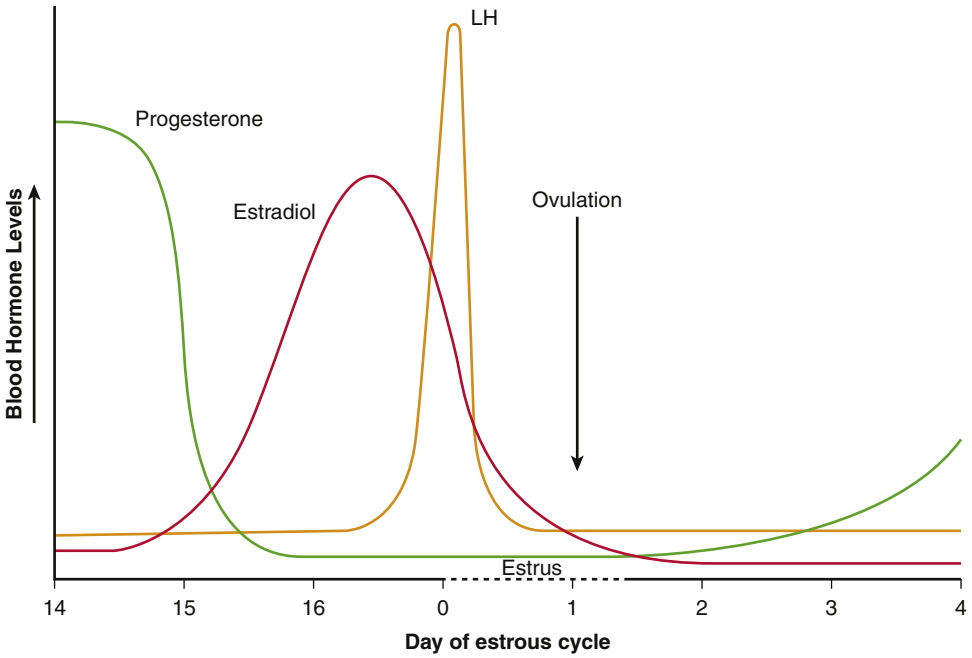


FIGURE 10-30 Ovulatory cycle of sheep (ewe). Note that ovulation occurs during the later part of estrus. *(Adapted with permission from Short R.V.,* Reproduction in Mammals, Book 3*, Cambridge University Press, 1972.)*

uterine endometrium synthesizes a luteolytic prostaglandin ($PGF_{2\alpha}$) that causes degeneration of the corpus luteum and resumption of proestrus. It appears that LH alone can cause follicle growth, ovulation, and luteinization, although several studies have shown that FSH can stimulate follicle growth, too; however, both postovulatory LH and PRL are necessary to induce progesterone synthesis by the sheep corpus luteum.

Fertilization followed by implantation delays degeneration of the corpus luteum. Although oCG is produced, the conceptus somehow neutralizes the PGFs synthesized under the influence of progesterone so that the corpus luteum may continue to secrete progesterone until the placenta is capable of producing sufficient steroids to maintain gestation. The placenta also secretes oCS throughout gestation. Birth seems to be triggered by a marked reduction in progesterone production near term (birth) (see Figure 10-24) and an increase in the activity of the HPA axis in the fetus.

C. Women

The human female exhibits continuous reproductive cycling with a mean non-pregnancy cycle length of 28 days for most reproductively active women (Figure 10-31). Timing of reproductive events is related to the uterine menstrual cycle. The rhesus monkey also has a menstrual cycle of 28 days, and there are many parallels in the menstrual cycles of these two primates. Thus, studies of the rhesus monkey have provided valuable insight into factors

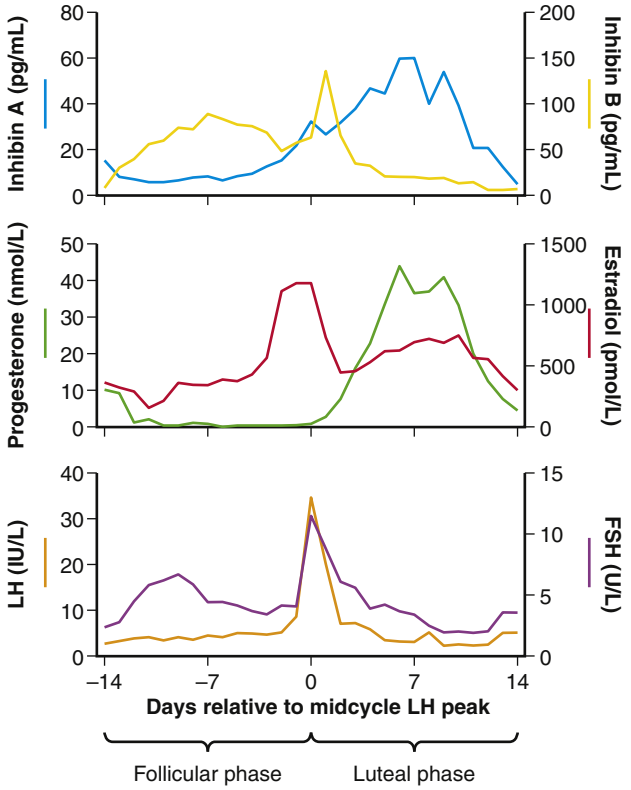


FIGURE 10-31 Ovulatory cycle of woman. Changes in blood levels of inhibin A and B (top), progesterone and estradiol (middle), and LH and FSH (bottom). See Appendix E for explanation of LH and FSH units. *(Adapted with permission from Burger, H.G. et al.,* Recent Progress in Hormone Research*, **57,** 257–275, 2002.)*

regulating the human menstrual cycle. Although this menstrual cycle is sometimes referred to as a lunar cycle because its periodicity is equivalent to approximately one lunar month, the human menstrual cycle is not correlated with any particular phase of the lunar month and should not be termed lunar. It could be that at one time the menstrual cycle was correlated more closely with moon phases but it has become highly modified by numerous environmental and internal factors.

The duration of menstrual cycles of women can vary from as short as 14 days to as long as 360 days, depending upon both endocrine and psychological factors. Furthermore, considerable variation can occur in cycle length in a given woman at different times in her life history. In general, short cycles and irregular cycles are associated with the onset of puberty and with the end of the reproductive life prior to menopause when the ovaries become refractory to pituitary GTHs and both estrogen synthesis and ovulation cease. The reduction or absence of circulating estrogens and progesterone results in elevated levels of circulating GTHs in menopausal and postmenopausal women through the normal negative feedback mechanism.

By convention, the menstrual cycle begins at the onset of the menses, which occupies the first 5 days of the cycle (Figure 10-31). However, as soon as the corpus luteum of a previous cycle begins to regress prior to the onset of menses, there is a depression in plasma gonadal steroids and a resultant moderate elevation in plasma FSH that initiates growth of new follicles. Typically, only one follicle (dominant follicle) in one of the ovaries will reach maturity in a given cycle, with ovulation often occurring in the alternate ovary during the following cycle (Figure 10-17). The largest follicle in the other ovary will ovulate the next month; hence, the true ovarian cycle is at least twice as long as the menstrual cycle (56 days, and some estimates suggest as long as 98 days). At least two periods of follicular growth are correlated with the proliferative phase of two successive uterine cycles.

The thecal cells of the growing follicles in both ovaries begin to secrete estrogens, progestogens, and inhibin B (Figure 10-31). Secretion of estrogens and progestogens peaks on about day 14 of the normal menstrual cycle. Steroidogenesis is regulated by LH and FSH operating on the thecal and granulosal cells, respectively. The peak in follicular phase estrogen level stimulates accelerated pulsatile GnRH release from the cyclic center, causing a large surge of LH accompanied by a lesser surge of FSH. Galanin released with GnRH enhances the LH response, whereas inhibin B secreted by the ovary under the stimulation of FSH actually reduces the release of FSH in response to the GnRH surge. Ovulation of typically only the largest follicle follows the LH surge by about 24 hours, and the LH-stimulated granulosa cells and some thecal cells from the ruptured follicle undergo luteinization to form a corpus luteum. The resulting corpus luteum is independent of pituitary hormones and begins production of progesterone and estradiol and inhibin A. The hypothalamic centers governing LH and FSH release are inhibited by the high levels of these circulating steroids and inhibins so that neither follicular growth nor ovulation can occur during the early portion of the luteal phase of the cycle. However, the corpus luteum functions for only a few days if the ovum is not fertilized. Unlike the case for sheep, the human uterus plays no active role in causing degeneration of the corpus luteum. Surgical removal of the uterus (hysterectomy) does not affect the duration of the luteal phase. Production of estrone by the corpus luteum of the rhesus monkey increases markedly prior to the onset of luteal degeneration. A similar mechanism employing estrone as a luteolytic factor may be operating in women. As the production of steroids by the corpus luteum decreases, the pituitary is released from steroid-induced inhibition, and a new cycle of follicular growth begins during the latter portion of the luteal phase. The outer portion of the uterine lining begins to slough off following the decline in progesterone levels, and the menses begins.

The limiting factor for follicle growth appears to be the availablility of GTH. Treatment with fertility drugs that enhance GnRH secretion and hence GTH levels often can cause development and ovulation of multiple follicles resulting in multiple births. Hence, women may transition from being non-reproductive to producing litters.

Should fertilization occur at the appropriate time during the menstrual cycle, the trophoblast of the blastocyst begins to secrete hCG prior to implantation, and the production of hCG continues at an accelerated rate during early pregnancy (Figure 10-30). Under the influence of hCG, the corpus luteum continues to secrete both progesterone and relaxin for about 60 days, after which it degenerates even in the presence of exogenous hCG. However, the normal fetal adrenal—placenta unit by this time produces sufficient estrogens and progesterone to continue the inhibition of pituitary GTH release and to maintain the secretory and hyperemic condition of the uterus. The placenta can synthesize progesterone but lacks the necessary enzymes to synthesize androgens. The fetal adrenal provides weak androgens (androstenedione, DHEA, DHEA-S) so that the placenta using $P450_{aro}$ can convert them into estrogens. Failure of the fetal adrenal to produce adequate androgens before the corpus luteum degenerates invariably results in a miscarriage at about 2 to 3 months following implantation.

At one time, a synthetic estrogen **diethylstilbestrol (DES)**, discovered in 1938, was prescribed for thousands of women who were unable to sustain sufficient estrogen levels to maintain pregnancy. Many women were treated with DES as a precautionary therapy even without a history of miscarriage. Although this treatment was claimed to be

effective in preventing miscarriages, it has not been proven, and DES was found to increase the incidence of cancer in both the treated women and their offspring. For these reasons, this practice was discontinued in the early 1970s. Similar observations had been made in animals years before, but the medical community was slow to recognize the danger and the Federal Drug Administration (FDA) approved its use. There are recent published reports of similar problems in the grandchildren of the DES-treated women.

Pheromones have been documented as important agents for coordinating events of sexual reproduction among many mammals as discussed earlier for rodents, and may even play important roles in human reproduction as inferred from studies of monkeys. Female rhesus monkeys produce a mixture of fatty acids of low molecular weight. The compounds appear in vaginal secretions and stimulate sexual interest of males as well as mounting behavior and ejaculation. The major volatile agents are fatty acids, including acetic acid, butanoic acid, propanoic acid, methylbutanoic acid, and methylpropanoic acid. Synthetic mixtures of these fatty acids in appropriate ratios stimulate male interest in females. Estrogens stimulate fatty acid secretions, and progesterone is inhibitory, observations that correlate well with levels of fatty acids observed in vaginal secretions throughout the menstrual cycle. Human vaginal discharges exhibit a similar cyclical variation in fatty acid composition (Figure 10-32), although human females produce a much greater percentage of acetic acid than do rhesus monkeys. The administration of oral contraceptives to these females effectively obliterates the preovulatory increase in volatile fatty acids, suggesting either an inhibition caused by high levels of estrogen or that the specific pheromonal agents are GTH dependent (recall that negative feedback by the contraceptive steroids blocks GTH release and hence prevents follicle development and ovulation).

Although studies of pheromones in humans are complicated by a number of psychological and social considerations, some evidence exists for production of pheromones and their roles in reproduction. A "dormitory effect" of menstrual synchrony has been described by McClintock for all-female living groups. Even though it is generally accepted that the olfactory sense in humans is limited as compared to most mammals, several studies have shown definite sensitive olfactory discriminations, including sexually based differences in the abilities to perceive certain odors. Trained perfumers can apparently distinguish between different skin and hair types, and some psychiatrists claim to be able to smell schizophrenics because of abnormal production and elimination of *trans*-3-methylhexanoic acid. The ability to detect some odors is sex dependent, such as the greater sensitivity of women to "boar taint" associated with spoiled pork. Some odors (e.g., licorice, lavender, doughnuts, pumpkin pie) are claimed to induce sexual arousal. Clearly, controlled experimental studies are needed to establish what roles pheromones have in controlling human behavior and reproductive functions.

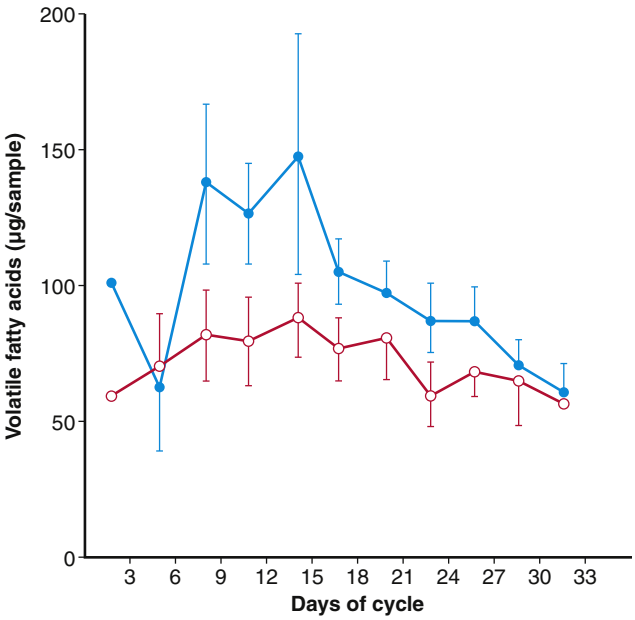


FIGURE 10-32 Effect of oral contraceptives on vaginal secretions in humans. Lipid composition of human vaginal secretions collected at 3-day intervals during the menstrual cycle (blue). Treatment with oral contraceptives (red) reduced the normal midcycle rise observed in the volatile fatty acid content of vaginal secretions in 47 women (blue line). *(Data originally reported in Michael, R.P. et al.,* Science, ***186****, 1217–1219, 1974.)*

D. Elephants

Asian (*Elephas maximus*) and African (*Loxodonta africana*) elephants have similar seasonal reproductive cycles with a 4- to 5-year birth interval in females due to the relatively long gestation period and extended lactation. The following account merges information from both species. Females are most likely to mate with older males that exhibit **musth**, a period of increased activity, increased association with females, and elevated aggressiveness toward other males. A female may signal her receptiveness to a male by calling or through release of pheromones. In the Asian elephant, a forthcoming ovulation is advertised by the excretion of **(Z)-7-dodecen-1-yl acetate** in urine that arouses interest by males. When a male is in musth, he secretes more fluid from his temporal glands and dribbles strong urine about that is attractive to females during the follicular phase of their ovarian cycles.

The ovarian cycle appears to be 16 weeks in duration with ovulation and a one-week period of estrus occurring near the middle of the cycle (Figure 10-33). There appear

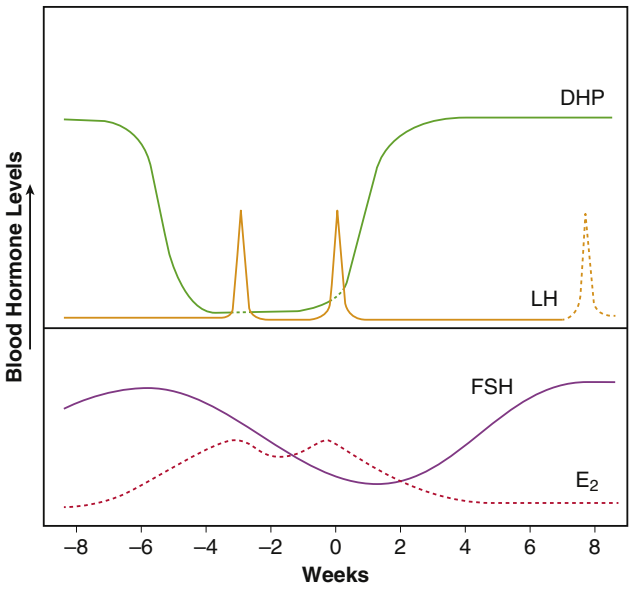


FIGURE 10-33 Ovulatory cycle of the elephant. Two LH surges are observed but it is believed that only the second results in ovulation and corpora lutea formation as evidenced by the levels of progestogens (DHP). *(Adapted with permission from Hodges, J.K.,* Animal Reproduction Science*, **53**, 3–18, 1998. © Elsevier Science, Inc.)*

to be be two successive LH surges about 2 to 3 weeks apart and both are related to elevated estradiol levels. The first surge is believed to stimulate development of accessory corpora lutea and the second LH surge stimulates ovulation. The single follicle that usually ovulates following the second LH surge forms a corpus luteum that together with the other corpora lutea are responsible for the observed rise in circulating progestins characterizing the luteal phase. Typically, only one follicle probably ovulates as twins are rare among elephants and usually only a single offspring is produced following 22 months of gestation. Progestogen levels rise during the luteal phase and remain elevated in the pregnant elephant until about 30 weeks prior to birth, reaching prepregnancy levels about 2 to 5 days before parturition. Relaxin appears in the blood about 20 weeks after the onset of pregnancy and also shows a decline with the progestogens; however, relaxin rises again during the last 8 weeks of pregnancy. Nothing is known about the factors responsible for initiation of birth in elephants.

Elephants are unique in producing very low amounts of progesterone but instead produce large amounts of the progestogens **5α-dihydroxyprogesterone (DHP)** and **5-pregnan-3-ol-20-one**. DHP has the strongest affinity for the progesterone receptor and may be the physiologically important progestogen in elephants. Following birth of the offspring, the female elephant enters a prolonged period of lactation during which GTH release is suppressed and the ovary remains quiescent.

VII. ENDOCRINE DISRUPTORS AND MAMMALIAN REPRODUCTION

In July 2012, the U.S. Food and Drug Administration banned the use of a plasticizer named **bisphenol A** (BPA) in baby bottles. Concerns regarding the health effects of BPA arose from several lines of evidence. First, over 6 billion pounds of BPA are produced worldwide every year and used for manufacuring the plastic lining of metal cans, the polycarbonate plastic in many household items and drinking bottles, and in dental sealant products. BPA has been measured in human tissues at concentrations higher than those found in the environment and has been measured in umbilical cord serum and amnionic fluid. Decades of research indicate that BPA is an estradiol mimic on estrogen receptors, and new data support a role for non-genomic actions of BPA by acting on membrane estrogen receptors. While BPA in many ways is a model for understanding how environmental estrogen mimics can adversely impact reproduction, there are many endocrine-disrupting chemicals (EDCs) that have been proposed to impact reproduction ranging from natural estrogen mimics such as genistein, a phytoestrogen found in soy, to industrial contaminants such as PCBs that may disrupt both androgen and estrogen actions. The structures of some selected EDCs are shown in Figure 10-34.

A. Estrogen Receptor (ER) Agonists and Antagonists

A seemingly endless number of manmade and natural chemicals have the ability to bind to ERs and produce estrogen-like effects or inhibit ER functions by acting as an antagonist. Considerable effort has been directed at developing so-called **high throughput assays** to rapidly screen hundreds and thousands of chemicals for ER action. **Molecular docking** and **three-dimensional quantitative structure–activity relationships (3D-QSARs)** also have been developed to perform so-called *in silico* or desktop identification of a chemical's potential interaction with ERs. Whereas high-throughput assays are certainly needed to test the sheer number of contaminants released into the environment and to reduce the number of animals required for testing, whole animal studies are also needed, especially given the number of chemicals such as dioxins and PCBs that may indirectly interfere with ERs (and in fact androgen receptor) signaling by activating the **aryl hydrocarbon receptor (Ah receptor)**. The U.S. Environmental Protection Agency Endocrine Disruptor Screening Program (EDSP), tasked with reviewing pesticides and environmental contaminants for endocrine disruption, currently employs five assays for ER action in its initial (Tier 1) battery of tests (Table 10-10). Two of these assays are high-throughput cell-culture-based assays (ER binding assay,

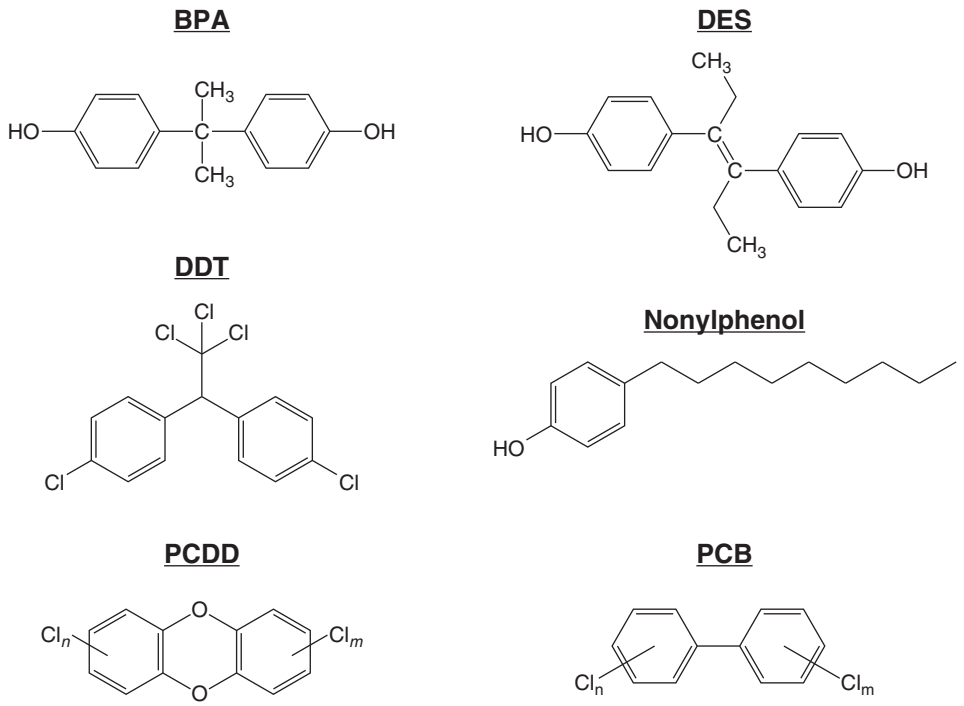


FIGURE 10-34 Chemical structures of some reproductive endocrine disruptors. Abbreviations: BPA, bisphenol A; DDT, dichlorodiphenyltrichloroethane; DES, diethylstibestrol; PCB, polychlorinated biphenyl; PCDDs, polychlorinated dibenzodioxins.

TABLE 10-10 Screening Assays Approved for the U.S. Environmental Protection Agency Endocrine Disruptor Screening Program

	Mode of Action						
	Receptor Binding				Steroidogenesis		
Assay	**ER agonist**	**Anti-ER**	**AR Agonist**	**Anti-AR**	**Estradiol**	**Androgen**	**HPG Axis**
Cell based							
ER binding	X	X					
ERα transcptional activity	X						
AR binding			X	X			
Steroidogenesis					X	X	
Aromatase					X		
Whole animal							
Uterotrophic	X						
Hershberger			X	X		X	
Pubertal male			X	X		X	X
Pubertal female	X	X			X		X
Fish reproduction	X	X	X	X	X	X	

ER, estrogen receptor; AR, androgen receptor.
Data from U.S. EPA Endocrine Disruptor Screening Program (EDSP), Harlan Laboratories, Indianapolis, IN.

ER transcriptional activation assay) and three are whole animal assays (uterine growth in rats, female puberty in rats, and fish reproduction). Chemicals known to interfere with reproduction by binding to ERs include pesticides (aldrin, DDT, dieldrin), phthalates, dioxins, nonylphenols, and PCBs. There is even evidence that cadmium, a heavy metal contaminating water supplies as a result of mining activity, can bind to ERs. Undoubtedly, as the U.S. EPA proceeds with the enormous task of reviewing the thousands of chemicals released into the environment for endocrine activity the list of chemicals known to bind to ERs will grow.

B. Androgen Receptor (AR) Agonists and Antagonists

As is the case with ER disruptors, there are both cell culture and whole animal assays for testing the ability of contaminants to activate or inhibit ARs including the **Hershberger assay**, which measures the weight of secondary sex organs such as the seminal vesicles and prostate gland which are androgen sensitive. In the Hershberger assay, rats are castrated and potential AR agonist effects are tested by administering the contaminants and examining the effect on organ weight. Potential AR antagonist effects are tested by administering the contaminants along with testosterone. Contaminants known to act on the AR include pesticides (*p*,*p*′-DDE, a DDT metabolite; fenitrothion; lindane; linuron; permethrin; and vinclozolin), phthalates, benzopyrene, and PCBs, all of which are AR antagonists. Very few data exist on contaminants that may activate the AR.

C. EDCs That Impact Steroidogenesis and Aromatase Activity

Steroidogenesis can be tested using a number of cell lines or primary culture with gonadal tissue, but the most widely used assay utilizes a human adrenocortical cell line called **H295R**. Activity of aromatase, the enzyme converting testosterone to estradiol, is generally measured for EDSP purposes in cells engineered to express the aromatase (*cyp19*) gene, although any tissue expressing the aromatase enzyme could be used in theory. Flavones, a type of phytoestrogen, are potent inhibitors of aromatase activity, possibly by competing with the natural substrates for the aromatase enzyme. Atrazine, a pesticide that is widely used in the United States as a pre-emergent herbicide on corn and sugarcane, increases aromatase activity in cell lines and has been implicated in altering steroidogenesis in the male pubertal development assay at high doses, although the mechanism may involve indirect action on GnRH neurons in the hypothalamus. BPA and the anti-AR pesticide vinclozolin also are suspected of disrupting aromatase activity. Many fungicides alter aromatase activity because of the similarity of this enzyme to the CYP proteins targeted by chemically engineered fungicides. Fadrozol, an aromatase inhibitor widely used by researchers and as a therapy for breast cancer, belongs to a class of chemicals called **azoles** that includes many fungicides. **Organotins** are organic metal hybrid chemicals known to interfere with aromatase activity. Tributyltin (TBT), widely used as an antifungal and wood preservative and as an anti-fouling agent in marine vessels, is a potent aromatase inhibitor. The organochlorine pesticide methoxychlor interferes with the expression of a wide number of genes involved in steroidogenesis.

D. EDCs with Epigenetic Effects on Reproduction

In 2005, Michael Skinner's laboratory at Washington State University reported in the journal *Science* that exposing pregnant rats to vinclozolin and methoxychlor led to aberrant spermatogenesis in the initial offspring and all other generations after that. This was the first published evidence of EDCs acting to disrupt reproduction through epigenetic mechanisms (i.e., altering gene expression and phenotype in offspring without altering the maternal DNA sequence). As we discussed in Chapter 1, EDCs may affect offspring phenotype without altering maternal DNA by altering DNA methylation and histone proteins. Epigenetic effects may also explain why short-term exposure of neonates to EDCs may have life-long consequences for reproduction. A diverse array of EDCs have been reported to cause epigenetic changes. BPA treatment of neonatal rats alters DNA methylation in the testis, possibly explaining the adverse effects of BPA exposure on spermatogenesis and fertility. Fetal exposure to methoxychlor also leads to hypermethylation of DNA in the ovaries of female offspring, leading to alterations in the expression of ERβ but not ERα genes. The epigenetic effects of methoxychlor extend to effects on the preoptic region of the hypothalamus which controls gonadotropin secretion. Embryonic rats exposed to methoxychlor had altered methylation of the ERα gene that led to earlier reproductive aging and altered estrous cycles. Genistein, a phyto-estrogen from soy, and phthalates, used as plasticizers, has also been implicated in epigenetic disruption of reproduction. Some epigenetic effects of EDCs are listed in Table 10-11.

VIII. MAJOR HUMAN ENDOCRINE DISORDERS RELATED TO REPRODUCTION

Reproductive disorders have been studied extensively in order to prevent their occurrence as well as to correct

TABLE 10-11 Examples of Endocrine-Disrupting Chemicals (EDCs) That Disrupt Reproduction Through Epigenetic Mechanisms

EDC	Exposure Period	Gene Targeted	Tissue	Epigenetic Effect	Phenotype	Reference
Bisphenyl SA	Neonate	*Esr1, Esr2* (estrogen receptors 1 and 2; estrogen receptors α and β)	Testis		Altered spermatogenesis, fertility	[1]
Diethylstilbestrol	Neonate	*FOS*	Uterus	Demethylation	More tumors	[2]
Methoxychlor	Embryonic	*Esr1*	Hypothalamus	Hypermethylation of *Esr1* promotor	Early reproductive aging; altered estrous cyclicity in females	[3]
Methoxychlor	Embryonic	*Esr2*	Ovary	Hypermethylation of *Esr2* promotor	Increase in the number of preantral and early antral follicles and a reduced number of corpora lutea	[4]

[1] *Doshi, T. et al.,* Toxicology, ***289***, *74–82, 2011.*
[2] *Li, S. et al.,* Molecular Carcinogenesis, ***38***, *78–84, 2003.*
[3] *Gore, A.C. et al.,* Molecular Endocrinology, ***25***, *2157–2168, 2011.*
[4] *Zama, A.M. and Uzumcu, M.,* Endrocinology, ***150***, *4681–4691, 2009.*

defects and increase reproductive capacities of both men and women. Some of the more common reproductive disorders are described here. Additional disorders related to reproduction include congenital adrenal hyperplasia (see Chapter 8), osteoporosis (see Chapter 14) and a host of **disorders of sex development (DSDs)** (see Box 10B), or **genetic disorders of gonadal development**, which are summarized in Table 10-12. The discussion of major endocrine disorders is separated into three major categories. The first two categories consider factors that influence the timing of puberty. The third deals with major genetically based disorders many of which can result in ambiguous sexual determination.

A. Precocious Puberty

Puberty is a delayed period of development focusing upon activation of the HPG axis and the functional integrity of sex accessory structures that may lead to successful sexual reproduction. **Precocity** is defined as the appearance of any one indicator of puberty at an age earlier than 2.5 to 3 standard deviations below the mean age at which the indicator normally appears in that population (see Table 10-13). The sequence and mean age for appearance of these indicators should be considered only as a guide. Implied precocity may not be evidence of an endocrine disorder, and some variation in the sequence of these events is normal. Major deviations in a number of indicators may signal precocial endocrine activity of a pathological nature (Figure 10-6). **Isosexual precocity** involves early appearance of the genetically determined sex. It is termed **heterosexual precocity** if male features develop precocially in a female or if female features appear precocially in a male.

It should be noted that there has been a shortening of the mean prepubertal period in the last several decades resulting in earlier puberty. Evidence from studies of endocrine disruption (see Section VII) through accidental exposures to estrogenic compounds such as phthalates suggest environmental causes for this accelerated timing of puberty. Exposure to artificial lighting that extends daylight and perhaps to television and computer monitors also has been hypothesized to influence pineal function (see ahead) and contribute generally to precocity.

1. Precocity with Normal Endocrinology

Idiopathic (of unknown cause) precocity may be familial. Sexual development and body growth appear normal but are accelerated. Reproduction may be possible at an early age. For example, the youngest mother on record was 5 years 8 months of age at delivery.

2. Precocity and Pineal Tumors

Pineal tumors are not common but occur most frequently in young males. Precocious sexual maturation occurs in about one third of these cases. Pineal tumors may impair release of melatonin from the pineal and allow sexual maturation to occur prematurely (see Chapter 4). In contrast, some pineal

TABLE 10-12 Some Genes Involved in Diseases of Sexual Development (DSDs) in Humans

Gene	Protein	DSD Phenotype
Bipotential gonad formation		
NR5A1	Steroidogenic factor 1 (SF-1)	Loss leads to XY gonad failing to form properly (gonadal dysgenesis)
M33/CBX2	Chromobox protein homolog 2, required for *SRY* expression	Loss results in male-to-female sex reversal, XY develops ovary, uterus, full female phenotype due to lack of *SRY* expression and function
WT1	Wilms tumor protein, transcription factor	Loss results in Denys-Drash syndrome, gonadal dysgenesis, pseudo-hermaphroditism
Testes formation		
DMRT1	Doublesex and mab-3 related transcription factor 1	Loss leads to XY gonadal dysgenesis, failure of Sertoli cells to develop
SOX9	*SRY* (sex-determining region Y)-box 9 protein, transcription factor	Loss leads to XY ovary and female phenotype; mutations upstream of *SOX9* leads to female-to-male sex reversal
SRY	Sex-determining region of Y chromosome encodes testis-determining factor (TDF), transcription factor	Loss leads to male-to-female sex reversal, XY develops ovary
Ovary formation		
FOX12	Forkhead transcription factor 12	Loss leads to premature ovary failure (POF)
RSPO1	R-spondin-1, possibly ligand for surface membrane protein	Loss leads to female-to-male sex reversal, ovotestis
WNT4	Wingless-type MMTV integration site family, member 4, ligand surface membrane receptor	Loss leads to müllerian duct agenesis

From Eggers, S., Sinclair, A. (2012). Mammalian sex determination—insights from humans and mice. Chromosome Res. **20,** 215–238.

tumors have been related to delayed puberty in a few cases. These tumors possibly secrete more melatonin than does a normal gland and hence delay puberty.

3. Precocity from Ectopic Gonadotropins or Gonadal Steroids

Rarely, pituitary tumors secrete excessive amounts of gonadotropins, and sometimes a non-pituitary tumor secretes chorionic gonadotropin, causing premature gonadal maturation. Likewise, certain ovarian or testicular tumors can produce sufficient steroids to cause external evidence of puberty although the gonads themselves are still quiescent with respect to gamete production. Heterosexual precocity can occur from feminizing tumors in testes or androgen-secreting tumors of the female adrenals or ovaries. (See also the discussion of compensatory adrenal hyperplasia in Chapter 8.)

4. Precocity from Endocrine Disrupting Chemicals

Exposure of animals to compounds such as phthalates and BPA associated with plastics have been shown to accelerate puberty in laboratory animals. Young girls exposed to phthalates exhibit early and sometimes excessive breast development (thelache).

B. Delayed Puberty

Numerous examples of delayed puberty are known. The causes and characteristics of delayed puberty are unique for males and females. However, mice lacking the G-protein-coupled receptor **GPR54** fail to undergo puberty (Figure 10-6). The natural ligand for GPR54 is kisspeptin, a peptide of 54 amino acids that is produced by the *KISS-1* gene. Kisspeptin was originally named metastin for its ability to suppress the metastatic potential of melanoma

TABLE 10-13 Mean Age for Normal Attainment of Certain Indicators of Puberty in Humans

	Mean Age (in years ± SD)
Female	
Budding of breasts	11.2 ± 1.1
Sparse pubic hair	11.7 ± 1.2
Peak vertical growth rate	12.1 ± 1.0
Menarche[a]	
United Kingdom	13.5 ± 1.0
United States	12.9 ± 1.2
Male	
Enlargement of testes and scrotum	11.6 ± 1.1
Lengthening of penis	12.8 ± 1.0
Sparse pubic hair	13.4 ± 2.2
Peak vertical growth rate	14.1 ± 0.9
Adult genital size and shape	14.9 ± 1.1

[a] *Ninety-five percent reach menarche between ages 11 and 15.*

and breast carcinoma cells. Knockout mice for GPR54 (the so-called Harry Potter strain) fail to undergo puberty. (See Chapter 4 for a review of kisspeptin's actions on the HPG axis.) Recent clinical studies have observed mutant GPR54 genes in patients with idiopathic hypogonadotropic hypogonadism.

1. Causes of Delayed Puberty in Males

Prevalence of undescended testes or cryptorchidism is common at birth (10%) but is reduced to only 1% of males by one year of age. Only about 0.3% of adult males exhibit cryptorchidism, and the case of only one undescended testis is much more common than the bilateral condition. Testicular descent normally is initiated by local effects of DHT. Because of higher temperatures experienced by an undescended testis, the spermatogenetic tissue degenerates at about the time when spermatogenesis would normally begin (at about age 10). Androgen production usually is normal but may be reduced in some cases. The external testis of a unilateral cryptorchid develops normally, and these males usually are fertile. There are several other causes for **hypogonadism** in males including insufficient levels of LH and FSH due to hypothalamic or pituitary dysfunction. In some very rare cases, the interstitial cells may be unresponsive to GTHs. Genetic causes for hypogonadism and gonadal dysgenesis are summarized in Table 10-12. Abuse of anabolic steroids for performance-enhancing effects can cause hypogonadism by reducing FSH and LH secretion via negative feedback.

2. Causes of Delayed Puberty in Females

Primary amenorrhea is the failure for menarche to occur at the normal time (Table 10-13). This condition can be related to many different causes, including disorders of the hypothalamus, pituitary, and ovaries. Poor nutrition, stress, or rigorous athletic training programs can delay puberty through inhibitory actions on the HPG axis. For example, levels of LH and FSH are depressed in women suffering from **anorexia nervosa**, a disorder in which food intake is greatly reduced. Simple weight loss can depress FSH levels for a time but does not inhibit LH secretion.

Secondary amenorrhea occurs after menarche and can result from many endocrine disorders including thyrotoxicosis, drug therapy, and premature menopause as well as from a variety of hypothalamic, pituitary, and gonadal disorders. Two of the more common gonadal disorders associated with secondary amenorrhea are **polycystic ovarian syndrome (PCOS)** and **luteinization of atretic follicles (LAF)**.

Approximately 10% of women are affected by PCOS. The name for PCOS is derived from the general thickening and simultaneous luteinization of several ovarian follicles resulting in formation of numerous cysts in the ovaries. These cysts develop from thecal cells, and there is a corresponding decrease of granulosa cells in these follicles. Current research indicates that excessive AMH production decreases the sensitivity of ovarian follicles to FSH but at the same time increases the recruitment of new follicles (Figure 10-35). Progesterone and estrogen production are diminished and gonadotropin secretion consequently is elevated. This resultant elevation of gonadotropins stimulates excessive production of androgenic steroids (DHEA, androstenedione) that cannot be aromatized to estrogens in the absence of granulosa cells. These androgens are responsible for the masculinization that accompanies PCOS. Uterine abnormalities and infertility result as well as obesity, hirsuitism, and occasional balding from the androgens. An association has been recognized between diabetes mellitus and the tendency to develop PCOS and it appears to be linked to a persistent metabolic disturbance.

The LAF syndrome results from premature luteinization of ovarian follicles prior to formation of the cumulus oophorus. GTH levels are elevated, but the masculinization described for PCOS usually does not happen. Numerous small ovarian cysts may be present, but these are easily distinguished from the large cysts that characterize PCOS.

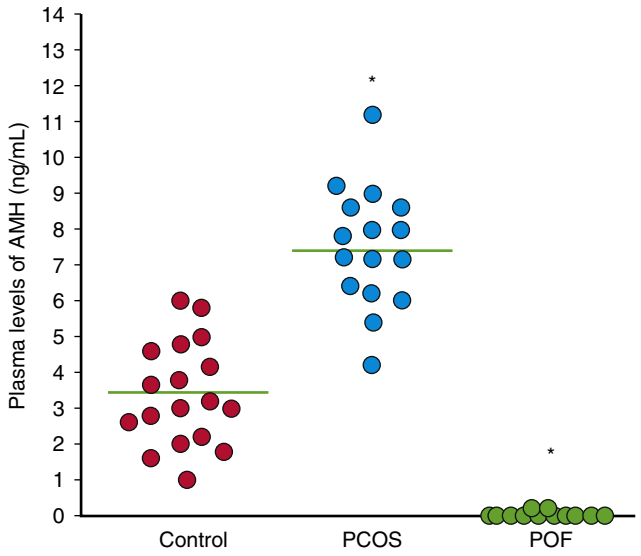


FIGURE 10-35 Anti-müllerian hormone (AMH) and polycystic ovarian syndrome (PCOS). AMH is responsible for stimulated growth of new follicles. Elevated levels of AMH cause additional follicle recruitment but prevents follicles from reaching the antral stage. The arrested follicles produce large amounts of the androgens dehydroepiandrosterone and androstenedione, causing the associated masculinization of the woman. In premature ovarian failure (POF) no AMH is produced and masculinization is rare. *(Adapted with permission from Broekmans, F.J. et al.,* Trends in Endocrinology & Metabolism, ***19****, 340–347, 2008.)*

C. Hereditary Disorders

Mutations in key genes regulating gonadal development and chromosomal rearrangements are common causes for deviations in sexual determination and/or expression (Table 10-12). A common disorder is the consequences of meiotic **nondisjunction** associated with the sex chromosomes; that is, the paired sex chromosomes fail to separate during the first meiotic division, resulting in gametes with either two or no sex chromosomes. Zygotes produced by such gametes would have only one sex chromosome or would have three sex chromosomes. Thus, one can obtain XO and XXY or XXX individuals following fertilization of these zygotes. YY zygotes are not viable.

Occasionally, people are born with a combination of ovarian and testicular tissue and sometimes are termed **hermaphrodites**. These are rarely functional in mammals but are often functional among non-mammalian vertebrates (see Chapter 11). This term comes from Greek mythology, combining the names of Hermes, a god who sometimes played tricks on lovers, and Aphrodite, the goddess of love. Most hermaphrodites possess an ovotestis on one or both sides of the body and are infertile or exhibit reduced fertility. These conditions are commonly termed **intersexes**. Rarely, **gynandromorphs** are discovered; these are individuals with an ovary and attendant müllerian derivatives on one side and a testis with its wolffian duct derivatives on the other. **Pseudohermaphrodites** have gonads of the genetic sex but externally resemble the opposite sex.

1. Klinefelter's Syndrome (XXY)

Persons with **Klinefelter's syndrome** are born with an abnormal number of sex chromosomes due to nondisjunction: 47,XXY (total number of chromosomes, sex chromosomes). This familial disorder occurs at fertilization and is present in about 0.2 to 0.3% of males. Klinefelter's syndrome can exist without obvious somatic abnormalities, although these persons are infertile and exhibit differing degrees of mental retardation. Similar syndromes have been described with additional sex chromosomes (48,XXYY, 48,XXXY, etc.). Severity of the symptoms increases with the number of X chromosomes present.

2. Turner's Syndrome (XO)

Another disorder arising at fertilization is **Turner's syndrome**, in which there is a loss of one sex chromosome so that the resulting genotype is 45,XO (one X chromosome and no Y or no second X chromosome). Sometimes this condition occurs when a twin is found to exhibit Klinefelter's syndrome. Individuals with Turner's syndrome have a female phenotype but are infertile. They also exhibit a number of anatomical defects as well as cardiovascular and kidney disorders. These patients do exhibit H-Y antigen that is characteristic of males but at a lower concentration, suggesting some activation of an H-Y antigen gene on the remaining X chromosome. Thus, the presence of H-Y antigen in blood cells is not indicative of the presence of a Y chromosome.

3. Galactorrhea

Secretion of a lactescent (milky) fluid from the breasts of either sex is called **galactorrhea**. It is usually caused by excessive secretion of PRL. Breast enlargement is not a prerequisite for its appearance. Galactorrhea frequently occurs in severe hypothyroidism characterized by elevated circulating levels of TSH and TRH. Prolactin release may be evoked by the high TRH levels.

4. Complete Androgen Insensitivity Syndrome (CAIS)

A person with CAIS is a genetic male (46,XY) that lacks androgen receptors. The testes are normal and secrete testosterone but do not descend from the body cavity. The testes may be removed, generally after puberty, to prevent testicular cancer in the undescended testes. Müllerian duct derivatives are absent because the embryonic testes also secreted AMH. However, the external appearance is that of a woman because of the congenital absence of androgen

receptors in the tissues. This is an example of male pseudohermaphroditism.

5. 5α-Reductase Deficiency

A most unusual example of pseudohermaphroditism is the apparent shift of sex at puberty. This condition was first reported from several small villages of the Dominican Republic. This disorder is the result of a genetic deficiency for the ability to synthesize the enzyme 5α-reductase. Males with this defect are born with undescended testes that synthesize testosterone like normal testes. However, these males lacking 5α-reductase cannot convert testosterone to DHT, and early development of male external genitalia does not take place. Although testosterone also can bind to the receptors in these tissues, DHT has a much greater affinity for them. Levels of testosterone in normal prepubertal males are too low to activate these receptors sufficiently, hence the need for 5α-reductase to convert testosterone to the more effective androgen, DHT. Apparently, in men with 5α-reductase deficiency, there is sufficient testosterone to stimulate other androgen-dependent structures that develop normally (such as the vasa deferentia and epididymi) but the normally DHT-dependent structures such as the prostate gland, penis, and scrotum do not develop. These males understandably are raised as girls until these latter structures appear at puberty when testicular testosterone levels are elevated suitably to stimulate the appropriate tissues. The marked increase in circulating testosterone at puberty allows the penis and other structures to enlarge and causes facial hair to appear. Although these men usually change gender roles after puberty, they are infertile.

IX. SUMMARY

The reproductive system includes the HPG axis and sex accessory structures. Primary control resides in pulsatile production of GnRH, which controls pituitary production of FSH and LH, which in turn cause gamete formation, gonadal steroid secretion, and ultimately the regulation of sexual characters and reproductive behaviors. FSH is involved primarily with gamete production whereas LH is responsible for initiating steroid secretion and release of mature gametes (ovulation and spermiation). FSH and testosterone work cooperatively to produce mature sperm. Androgens, progestogens, and estrogens stimulate other primary reproductive structures and secondary sex characters as well as mating behavior. Numerous paracrine regulators stimulated by GTHs or gonadal steroids contribute to reproductive events. Pheromones may play important roles in reproductive behavior and in the timing and success of reproduction.

In mammals, the male sex must be determined by the *sry* gene, which is responsible for production of AMH and androgens by the testis. AMH causes regression of some potential female structures (e.g., oviducts, uterus), and androgen production allows development of male sex accessory structures and reprograms hypothalamic reproductive centers in the brain to the male secretory pattern. Brain neurosteroids may also play a role in development of male brain differences. The default sex in mammals is female although a number of transcription factors and nuclear receptor proteins are required for ovary development and estrogens are essential for completely normal female development.

Mammalian reproductive cycles show great variations among the major taxonomic groups. Monotremes are egg-laying mammals that nourish their hatchlings with milk from their mammary glands. Marsupials allow their young to develop in a pouch after a short gestation period involving a nonendocrine placenta, relying on the mammary glands to support continued development of an exteriorized fetus. Eutherian mammals have a prolonged period of intrauterine fetal development supported by an endocrine placenta. Mammary glands are used for extended nutritional support after birth in all mammals.

Most female mammals exhibit an ovarian-based estrous cycle characterized by a period of enhanced receptivity of the female to the male called estrus. In some species (e.g., primates, including humans and the rhesus monkey), a special phase of the uterine cycle occurs, the menses, which involves sloughing and discharge of a portion of the endometrium and trapped blood. Because of this special uterine cycle, their reproductive cycles are typically called menstrual cycles and most may (e.g., rhesus monkey) or may not (human) exhibit a distinct period of estrus. The proliferative phase of the uterine cycle corresponds closely to the follicular phase of the ovarian cycle, and the secretory phase of the uterine cycle that follows ovulation coincides exactly with the luteral phase of the ovarian cycle. The uterine secretory phase is followed by a quiescent phase called diestrus in most mammals or by the menses in species having a uterine menstrual cycle. The menses corresponds to the first few days of the next follicular phase in the ovary.

Species may exhibit one (monestrous), two (diestrous), or many reproductive cycles (polyestrous) during the breeding season should fertilization or pregnancy not occur. In eutherians, the cycle can be separated into a follicular phase during which one or more ova develop in follicles and a luteal phase that prepares the uterus for implantation of the blastocyst. The luteal phase is named for one or more corpora lutea that develop from ruptured follicles and possibly some atretic follicles that continue to secrete estrogens and progestogens. These phases are clearly separated by ovulation.

During pregnancy, the eutherian placenta in cooperation with the fetal adrenal functions as an endocrine gland to maintain pregnancy, initiate birth, and prepare the mammary glands for postnatal functions. The corpus luteum performs various roles in pregnancy depending on the species. The placenta also functions in gaseous, nutrient, and metabolic waste exchanges. Numerous modifications of the placenta have evolved in different eutherian groups. OXY, prostaglandins, fetal adrenal steroids, and placental CRH, CG, CS, etc. play important roles in pregnancy and in the birth process. Postnatal functions of the mammary gland are controlled by PRL and OXY from the pituitary gland.

Gonadal secretory activities involve two special cell types responsive to FSH and LH, respectively. Ovarian granulosa cells and testicular Leydig cells are responsive primarily to LH and synthesize androgens. Ovarian thecal cells and testicular Sertoli cells as well as Leydig cells respond to FSH with conversion of androgens into estrogens ($P450_{aro}$ activity). FSH also stimulates Sertoli cells to synthesize inhibin, activin, and other local bioregulatory factors. Gonadal cells make many other local regulators (e.g., GnRH, IGFs, AMH, OXY, GDF-9, prostaglandins) in response to GTHs or independently, and these regulators may have autocrine and/or paracrine actions that control local events in the gonads.

STUDY QUESTIONS

1. Identify key steps in germ cell migration to the undifferentiated gonad. How would an endocrinologist identify primordial germ cells before they have reached the undifferentiated gonad?
2. What embryonic tissue type gives rise to the undifferentiated gonad?
3. Outline the major steps involved in differentiation of the bipotential gonad into a testes and ovary.
4. What information is coded in the mammalian Y chromosome that leads to the formation of a testis from a bipotential gonad?
5. Identify genes involved in determining whether the bipotential gonad differentiates into a testis or an ovary.
6. Identify how the differentiated gonad controls secondary sexual development.
7. As a researcher you are interested in performing tests to determine whether androgen receptors are involved in sexual libido. You have the choice of administering either testosterone or dihydrotestosterone to the test animals. Which androgen would you choose to unequivocally demonstrate an action at androgen receptors and why?
8. Explain general differences in reproduction between monotremes and eutherian mammals.
9. Outline the major steps involved in the process of spermatogenesis.
10. Identify which cells of the spermatogenetic lineage possess 4*N* DNA and 2*N* chromosomes.
11. Which cells of the spermatogenetic lineage are haploid?
12. Identify factors that can lead to precocious or delayed puberty.
13. Explain the role of the Sertoli cell tight junctions in forming the blood–testis barrier.
14. Which parts of the seminiferous tubules reside in the adluminal and basal tissue compartments and why is this segregation important?
15. Describe the regulation of testosterone synthesis by Leydig cells.
16. Identify secretory products of the Sertoli cells.
17. Differentiate between the roles of FSH and LH in testes regulation.
18. Describe how inhibin and activin peptides are synthesized and their mode of action.
19. Describe how Sertoli and Leydig cells modulate the activity of macrophage, T lymphocyte, and natural killer cells in the testes. Describe why the testes are considered to be an immune privileged organ.
20. Describe the roles of AMH and growth differentiation factor-9 in folliculogenesis.
21. Identify the major features of oogenesis.
22. How does the recruitment of new germ cells differ in the ovary and testis?
23. What is follicular atresia and how is it essential for normal reproduction?
24. Outline the major steps involved in corpus luteum lysis in non-primate mammals. Identify specifically the roles of the pituitary gland and uterus in this process.
25. Discuss factors involved in the timing of birth in mammals.
26. Describe the "two cell, two hormone" theory of estrogen synthesis in the ovarian follicle and compare to gonadal steroidogenesis in males.
27. Describe how the single event of LH binding to its receptor on ovarian follicle cells can lead to the transcription of multiple genes involved in such diverse processes as steroidogenesis and follicle rupture during ovulation.
28. Identify modes of action for endocrine disruptors targeting the reproductive system.
29. Identify assays used to test chemicals that are released into the environment for potential endocrine-disrupting activity.
30. Compare the reproductive cycles of the rat, woman, ewe, and elephant.

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