Autumn P. Davidson and †George H. Stabenfeldt
Chapter 40 by Juan E. Romano and Steven P. Brinsko

CHAPTER 35
Control of Gonadal and Gamete Development

KEY POINTS

Development of the reproductive system
1. Organization of the gonads is under genetic control (genetic sexual differentiation).
2. Sexual organization of the genitalia and brain depends on the presence or absence of testosterone.

Hypothalamopituitary control of reproduction
1. The hypothalamus and anterior pituitary (adenohypophysis) secrete protein and peptide hormones, which control gonadal activity.
2. The adenohypophysis *(pars distalis)* produces follicle-stimulating hormone, luteinizing hormone, and prolactin, all of which control reproductive processes.

Modification of gonadotropin release
1. The pulsatile release of gonadotropin releasing hormone (GnRH) induces the critical pulsatile production of the gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH).
2. Gonadotropin release is then modulated by the process of negative feedback from estrogen and progesterone.

Ovarian follicle development
1. Gamete development occurs initially without gonadotropin support and subsequently with pulsatile gonadotropin secretion.
2. In the preantral follicle, gonadotropin receptors for luteinizing hormone develop on the theca, which results in androgen synthesis; follicle-stimulating hormone directs the granulosa to transform the androgens to estrogens.
3. Late in the ovarian follicular phase, luteinizing hormone receptors develop on the granulosa, which permits the preovulatory surge of luteinizing hormone to cause ovulation.

DEVELOPMENT OF THE REPRODUCTIVE SYSTEM

Organization of the Gonads Is Under Genetic Control (Genetic Sexual Differentiation)

The initial development of the embryonic ovary involves the migration of germ cells into the genital ridge from the yolk sac. These primordial germ cells populate sex cords that have formed in the cortical region of the embryonic gonad from the proliferation of cells from the *coelomic epithelium* (so-called germinal epithelium) of the genital ridge. The sex cords contribute cells, known initially as *follicle cells* and subsequently as *granulosa cells,* which immediately surround the oocyte. The mesenchyme of the genital ridge contributes cells that will become the theca. The entire structure is called a *follicle,* which includes oocyte, granulosa, and theca cells.

No direct connections are formed between the oocytes and the tubes destined to become the oviducts, which are derived from *müllerian ducts.* The final result is that oocytes are released through the surface of the ovary by rupture of tissue elements that surround the ovary; this process is called *ovulation.* A specialized end of the oviduct, the *fimbria,* develops to enable the oocyte to be removed efficiently from the surface of the ovary. In some animals, oocytes are funneled to the fimbria through the use of a bursa, which tends to encompass the ovary; oocytes are directed to a relatively small opening in the bursa.

The development of the embryonic testis is similar to that of the ovary: germ cells migrate into the genital ridge and populate sex cords that have formed from an invagination of the surface (coelomic) epithelium (Figure 35-1). *Sertoli cells* (male counterparts of granulosa cells) develop from the sex cords, and *Leydig cells* (male counterparts of thecal cells) develop from the mesenchyme of the genital ridge. One fundamental difference from ovarian development is that the invagination of the sex cords in the male continues into the medulla of the embryonic gonad, where connections are made with medullary cords from the mesonephros (primitive kidney). The duct of the mesonephros *(wolffian duct)* becomes the epididymis, vas deferens, and urethra, which has a direct connection to the seminiferous tubules. Thus, male germ cells pass to the exterior of the animal through a closed tubular system.

Sexual Orientation of the Genitalia and Brain Depends on the Presence or Absence of Testosterone

The development of the genital tubular system and the external genitalia *(genital sexual differentiation)* is under the control of the developing gonad. If the individual is female—that is, the developing gonad is an ovary—the müllerian duct develops into oviduct, uterus, cervix, and vagina, whereas the wolffian duct regresses; the absence of testosterone is important for both changes (Figure 35-2). If the individual is male, the rete testis produces müllerian-inhibiting factor, which causes regression of the müllerian ducts. The *wolffian duct* is maintained in the male because of the influence of androgens produced by the testis. To

†Deceased

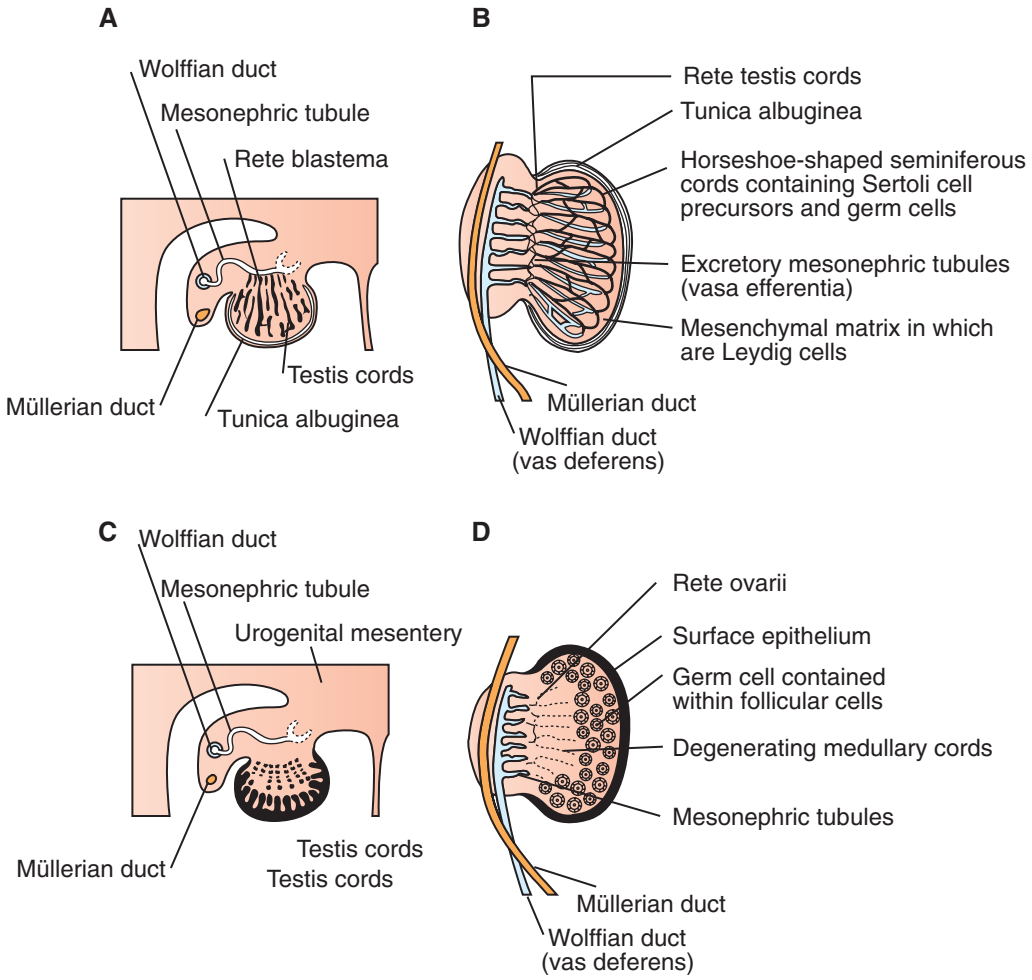

FIGURE 35-1 Testicular development during the eigth week **(A)** and the sixteenth to twentieth weeks **(B)** of human fetal life. **A,** The primitive sex cords proliferate in the medulla and establish contact with the rete testis. The tunica albuginea (fibrous connective tissue) separates the testis cords from the coelomic epithelium and eventually forms the capsule of the testis. **B,** Note the horseshoe shape of the seminiferous cords and their continuity with the rete testis cords. The vasa efferentia, derived from the excretory mesonephric tubules, connect the seminiferous cords with the wolffian duct (see text). Comparable diagrams of ovarian development around the seventh week **(C)** and the twentieth to twenty-fourth weeks **(D)** of development. **C,** Any primitive medullary sex cords degenerate and are replaced by the well-vascularized ovarian stroma. The cortex proliferates, and mesenchymal condensations later develop around the arriving primordial germ cells. **D,** In the absence of medullary cords and a true persistent rete ovarii, no communication is established with the mesonephric tubules. Therefore, in the adult, ova are shed from the surface of the ovary and are not transported by tubules to the oviduct. (From Johnson M, Everitt B, editors: *Essential reproduction,* ed 3, London, 1988, Blackwell Scientific.)

summarize, the müllerian ducts are permanent structures, and the wolffian ducts are temporary structures unless acted on by the presence of male hormones. The presence of an enzyme, *5α-reductase,* is important for the effect of the androgens because testosterone must be converted intracellularly into dihydrotestosterone for masculinization of the tissues to occur. The use of synthetic 5α-reductase inhibitors for the treatment of benign prostatic disease in humans is contraindicated without concurrent birth control measures, because drug levels in semen deposited in the female can lead to disorders of sexual development in male fetuses.

Development of the external genitalia follows the development and direction of the gonads. If the individual's genotype is female, folds of tissue called *labia* form the *vulva,* and a *clitoris* develops. If the individual is male, androgens from the testis direct formation of the *penis* (male counterpart of the clitoris) and the *scrotum* (male counterpart of the labia). Again, the absence or presence of androgens is an important factor influencing the formation of external genitalia.

The final organization of the individual with regard to gender comes with sexual differentiation of the hypothalamus. Exposure of the hypothalamus to androgens at about the time of birth causes the hypothalamus to be organized as male. A paradoxical finding is that conversion *(aromatization)* of androgens to estrogens is essential for maleness, mediated by enzymes in the neural tissue. In the absence of androgens, the *hypothalamus* is organized as female.

The fundamental concept of organization of the reproductive system with regard to genotype is that the female system is organized in the absence of testes. If the individual is to be male, there

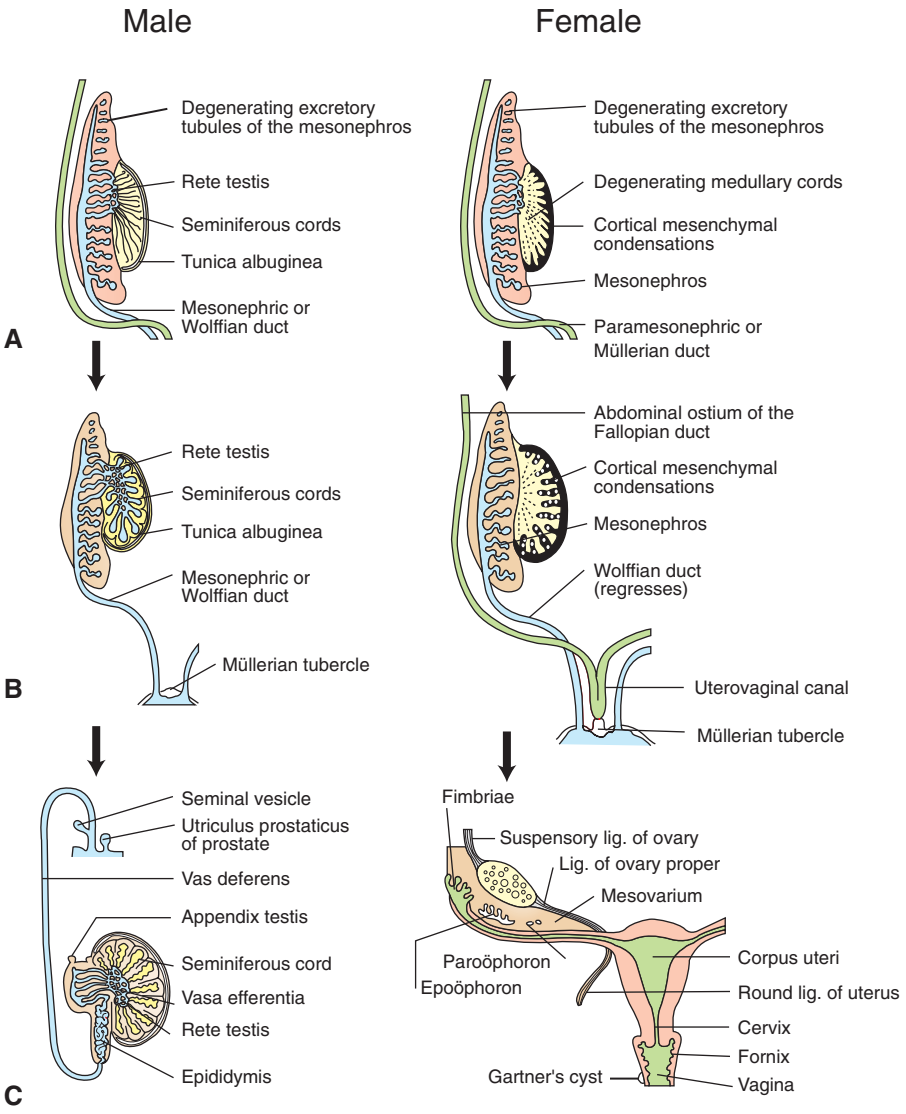

FIGURE 35-2 Differentiation of the internal genitalia in the human male and female at **A,** the sixth week of gestation, **B,** the fourth month of gestation, and **C,** the time of descent of testis and ovary. Note that the müllerian and wolffian ducts are present in both genders early on; the müllerian ducts eventually regress in the male and persist in the female, and the wolffian ducts regress in the female and persist in the male. The appendix testis and utriculus prostaticus in the male and epoöphoron, paroöphoron, and Gartner's cyst in the female are remnants of the degenerated müllerian and wolffian ducts, respectively. *Lig,* Ligament. (From Johnson M, Everitt B, editors: *Essential reproduction,* ed 3, London, 1988, Blackwell Scientific.)

must be active intervention by the testes through the production of androgens and appropriate tissue enzymes in two circumstances: (1) within the internal genitalia for conversion to more potent androgens, and (2) within the hypothalamus for conversion to estrogens.

HYPOTHALAMOPITUITARY CONTROL OF REPRODUCTION

The Hypothalamus and Anterior Pituitary (Adenohypophysis) Secrete Protein and Peptide Hormones, Which Control Gonadal Activity

Gonadal activity is under the control of both the *hypothalamus* and the *anterior pituitary gland* (Figure 35-3). The hypothalamus lies near the ventral midline of the diencephalon. It is divided into halves by the third ventricle and actually forms the ventral and lateral walls of the third ventricle. The hypothalamus has clusters of neurons, collectively called *nuclei,* which secrete peptide hormones important for controlling pituitary activity. As described in more detail later, these peptides move to the pituitary either directly by passage through the axons of neurons or by a vascular portal system. The pituitary responds to the hypothalamic peptides to produce hormones that are important for the control of the gonads.

The Adenohypophysis *(Pars Distalis)* Produces Follicle-Stimulating Hormone, Luteinizing Hormone, and Prolactin, All of Which Control Reproductive Processes

The pituitary gland is composed of three parts: an anterior lobe called the *adenohypophysis,* or *pars distalis;* an intermediate lobe called the *pars intermedia;* and a posterior lobe called the *neurohypophysis,* or *pars nervosa.* The lobes are of different embryological origins; the *pars distalis* is derived from the endoectoderm (derived in turn from a small diverticulum off the dorsal pharynx, called *Rathke's pouch*), and the *pars intermedia* and *pars nervosa* are derived from neuroectoderm. The adenohypophysis produces protein hormones that are important for the control of reproduction: two *gonadotropins* (*follicle-stimulating hormone* [FSH] and *luteinizing hormone* [LH]) and a third hormone called *prolactin;* other pituitary hormones include *growth hormone* (GH), *corticotropin* (adrenocorticotropic hormone, ACTH), and *thyroid-stimulating hormone* (TSH). FSH and LH are synergistic in *folliculogenesis* and ovulation in the ovary. FSH plays a more

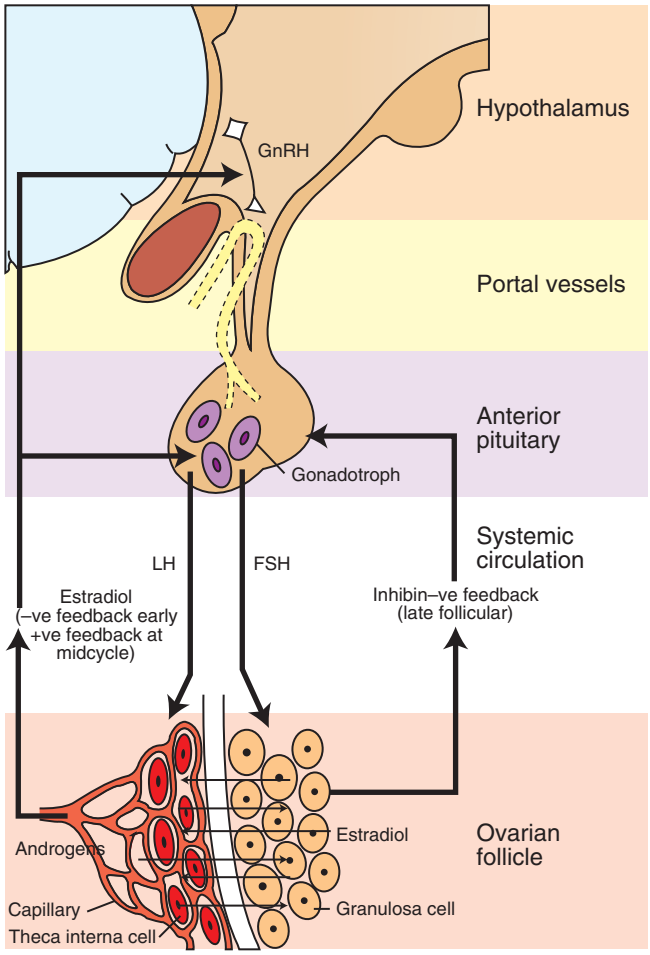

FIGURE 35-3 Summary of hypothalamic-pituitary-ovarian interactions during the follicular phase of the cycle. *FSH,* Follicle-stimulating hormone; *GnRH,* gonadotropin-releasing hormone; *LH,* luteinizing hormone; *-ve,* negative; *+ve,* positive. (From Johnson M, Everitt B, editors: *Essential reproduction,* ed 3, London, 1988, Blackwell Scientific.)

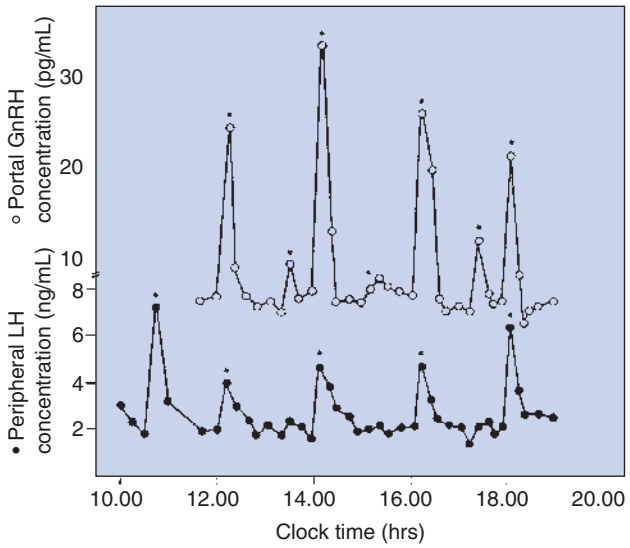

FIGURE 35-4 Concentrations of gonadotropin-releasing hormone *(GnRH)* in portal plasma *(open circles)* and luteinizing hormone *(LH)* in jugular venous plasma *(solid circles)* of four ovariectomized ewes. Asterisks indicate secretory episodes (pulses) of GnRH and LH. (From Johnson M, Everitt B, editors: *Essential reproduction,* ed 3, London, 1988, Blackwell Scientific.)

dominant role during the growth of follicles, and LH plays a more dominant role during the final stages of follicle maturation through ovulation. The *gonadotropins,* as well as TSH, are called *glycoproteins* because their molecules contain carbohydrate moieties that contribute to their function. *Oxytocin,* which is released by the neurohypophysis, is a hormone of importance in reproduction.

Besides being an important center for the control of reproduction, the *hypothalamus* regulates appetite and temperature and integrates the activity of the autonomic nervous system. Because of a common embryological origin, the *hypothalamus* has a direct connection to the neurohypophysis. This connection is through the neural stalk, which contains axons that originate from neuronal cell bodies located in the hypothalamus. Two sets of neurons within the hypothalamus, the *supraoptic* and *paraventricular* nuclei, are responsible for the synthesis of vasopressin and oxytocin, respectively. These small peptide hormones are coupled to larger peptide molecules, called *neurophysins,* and are transported from the site of synthesis in the hypothalamus (neuronal cell bodies) through axons to the site of storage and eventual release, the neurohypophysis.

The connection of the hypothalamus to the adenohypophysis does not involve the direct passage of axons through the *neural stalk.* A *venous portal system* connects the median eminence within the hypothalamus to the adenohypophysis. Hypothalamic substances that control the adenohypophysis are carried from the median eminence of the hypothalamus to the pituitary by a venous portal system. For example, *gonadotropin-releasing hormone* (GnRH), a peptide, is produced in the medial preoptic nucleus, and *dopamine,* an amino acid, is produced in the arcuate nucleus. Axons transport both substances from the hypothalamus to the *median eminence,* where they are released into the venous portal system. The synthesis of GnRH, as with oxytocin and vasopressin, involves the production of a larger precursor molecule, with a C-terminal region of 56 amino acids, called *GnRH-associated peptide* (GAP). Although GAP can stimulate the release of FSH and LH, GnRH is still thought to be the critical hormone for gonadotropin release. An even more important function of GAP may be its ability to inhibit prolactin secretion.

MODIFICATION OF GONADOTROPIN RELEASE

The Pulsatile Release of Gonadotropin Releasing Hormone (GnRH) Induces the Critical Pulsatile Production of the Gonadotropins, Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH)

The main secretory pattern of gonadotropins is *pulsatile;* the pattern is driven by pulsatile secretion of GnRH from the hypothalamus (Figure 35-4). The pulsatile release of gonadotropin-releasing hormone (GnRH) induces the critical pulsatile production of the gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH).

Gonadotropin Release Is then Modulated by the Process of Negative Feedback from Estrogen and Progesterone

Gonadotropin release is then modulated by the process of negative feedback from estrogen and progesterone. The importance of this mode of delivery is shown by the fact that if GnRH is

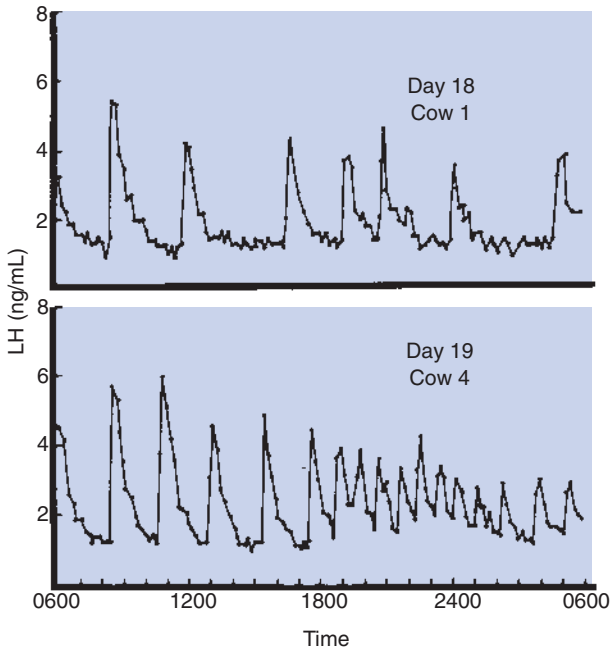

FIGURE 35-5 Pattern of plasma luteinizing hormone (LH) concentration on day 18 or 19 of the estrous cycle in two cows. (From Rahe CH, Owens RE, Fleeger JL, et al: Pattern of plasma luteinizing hormone in the cyclic cow: dependence upon the period of the cycle, *Endocrinology* 107(2):498–503, 1980.)

administered in a continuous (pharmacological) manner, the system can be downregulated. Continual occupancy of GnRH receptors on gonadotrophs by GnRH interrupts the intracellular signal for the synthesis and release of gonadotropins. Successful induction of a fertile estrus in bitches can be performed by administering canine analogue GnRH; however, the dose must be diminished as ovulation approaches, or downregulation will occur.

In general, *the pulse generator system* for gonadotropin secretion is increased in the *follicular phase* and decreased in the *luteal phase* of the estrous cycle (Figure 35-5). Estrogen decreases the pulse amplitude, and progesterone decreases the pulse frequency of *gonadotropin* secretion. This means that during the follicular phase, pulse frequency increases because of the absence of progesterone, and pulse amplitude decreases because of the presence of estrogen. This combination of increased pulse frequency and decreased pulse amplitude is important for nurturing the final growth phase of the developing antral follicle.

The hypothalamus and adenohypophysis are capable of responding to a sustained increase in estrogen secretion by increased secretion of gonadotropins, a relationship that is termed *positive feedback*. The sudden sustained increase in estrogen levels, which occurs over one to several days during final antral follicle development, causes an increase in gonadotropin secretion by increasing the frequency of pulsatile release of GnRH and, as a result, gonadotropin secretion. In essence, the frequency of pulsatile release of gonadotropins overcomes the metabolic clearance rate. The purpose of the gonadotropin surge is to induce changes within the follicle that lead to its rupture (ovulation). The duration of the gonadotropin surge is relatively short, usually 12 to 24 hours, possibly because the main factor driving the response, estrogen, declines in concentration as the follicles respond to the preovulatory gonadotropin surge. This particular physiological mechanism for initiating the onset of ovulation is effective because the follicle is able to signal its stage of maturity to the hypothalamus and adenohypophysis by a product (estrogen) that is produced in increasing amounts with increasing follicle maturity.

The secretion of gonadotropins is modified by the ovarian steroid hormones *estrogen* and *progesterone*. With time, the effect of these hormones is suppressive for gonadotropin secretion. Estrogens, in particular, cause *negative-feedback inhibition* of gonadotropin secretion, which is characterized by its sensitivity (effective at low concentrations) and its rapid onset (within a few hours). The substantial increase in gonadotropin concentrations that occurs after ovariectomy is caused largely by the removal of estrogens.

Because progesterone affects gonadotropin pulse frequency, it is thought that its modulatory effect is at the level of the hypothalamus. Estrogens are thought to affect gonadotropin secretion through an effect on both the pituitary gland and the hypothalamus. Although there are differences in the site of action among species, it appears that the hypothalamic site for negative-feedback inhibition of gonadotropins by both progesterone and estrogen is in an area immediately above the median eminence, known as the *arcuate nucleus*. The hypothalamic site for positive-feedback stimulation of gonadotropin release by estrogen is probably further anterior, that is, in the preoptic anterior hypothalamic region.

The secretion of gonadotropins can be modified by peptide and protein hormones produced by both the hypothalamus and the ovary. β-Endorphin, an opioid peptide produced from the hypothalamic precursor molecule pro-opiomelanocortin, can inhibit LH secretion when pharmacologically administered systemically. Its role in the physiological modulation of gonadotropin secretion, however, remains to be identified. Another hormone, inhibin, a protein produced by the granulosa cells of the developing follicle, also inhibits gonadotropin secretion, particularly FSH, during the final stages of follicle development. As described in the section on *folliculogenesis,* this depression of FSH secretion may be important to the animal for controlling the number of follicles that are brought to final maturation.

Control of gonadotropin secretion in the male is similar to that in the female; pulses of GnRH, arising in the hypothalamus, affect pulsatile secretion of the gonadotropins. This in turn causes the secretion of testosterone, also in pulsatile form, from the testes. One major difference between the genders is that the need for positive-feedback release of gonadotropins in males does not exist; gametes are produced and released on a continuous basis within a tubular system that opens to the exterior. This negates any need for a surge release of gonadotropins, as is required in the female to rupture the ovarian surface for the release of oocytes.

Prolactin is the third adenohypophysis-produced hormone that is important in the reproductive process, mainly because of its effect on the mammary gland and lactation in mammals. Although the secretion of prolactin is pulsatile, the control of secretion has more emphasis on inhibition than does stimulation of secretion. This concept is supported by the finding that prolactin secretion increases if the pituitary gland is disconnected from the hypothalamus by either cutting the pituitary stalk or transplanting the pituitary gland to another site (e.g., kidney capsule). Thus, most attention has focused on factors that inhibit prolactin secretion. The catecholamine dopamine, which is produced by neurons in the ventral hypothalamus (arcuate nucleus), is a potent inhibitor of prolactin secretion (Figure 35-6). Other

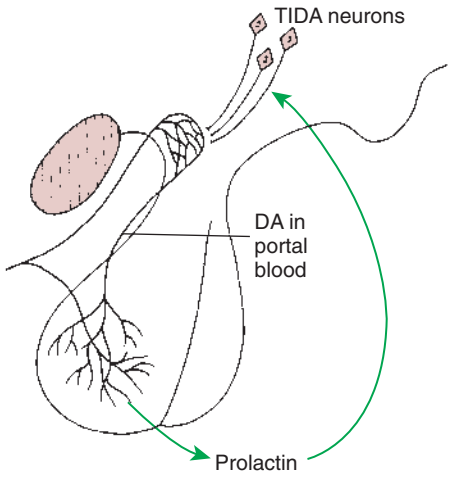

FIGURE 35-6 Diagrammatic summary of the proposed negative-feedback relationship between prolactin and dopamine *(DA).* Prolactin is believed to accelerate dopamine turnover in the arcuate nucleus neurons (tuberoinfundibular dopamine *[TIDA]* neurons), and the amine is then released into the portal capillaries to gain access to the lactotropes. Hyperprolactinemia could be caused by either a failure of prolactin inhibitory factor activity at the dopamine receptor level in the anterior pituitary or a reduction of TIDA neuron activity in the hypothalamus. (From Johnson M, Everitt B, editors: *Essential reproduction,* ed 3, London, 1988, Blackwell Scientific.)

factors that inhibit prolactin secretion are γ-aminobutyric acid (GABA) and GAP. Dopamine agonists, such as the ergot-type compounds *bromocriptine* and *cabergoline,* can be used to suppress prolactin secretion in cases of hyperprolactinemia. Cabergoline, a potent prolactin inhibitor, can be used to shorten interestrous intervals in female dogs and promote luteolysis in female dogs and female cats during the latter half of pregnancy (prolactin is a luteotropin). The negative-feedback control of prolactin is shown in Figure 35-6.

One of the first known prolactin-releasing factors was *thyrotropin-releasing hormone* (TRH). The physiological relevance of TRH in prolactin secretion is still unknown despite that receptors for TRH have been identified on lactotropes within the adenohypophysis. *Vasoactive intestinal peptide* (VIP), a potent stimulator of prolactin secretion, may play a physiological role in prolactin secretion through inhibition of dopamine synthesis within the hypothalamus. Estrogens can increase prolactin secretion by lactotropes by decreasing lactotrope sensitivity to dopamine and increasing the number of TRH receptors. Interestingly, female dogs undergoing ovariohysterectomy with a cesarean section usually maintain the ability to lactate effectively afterward, but this should always be evaluated before ovariohysterectomy or ovariectomy is performed. Removal of the ovary, the primary source of estrogen, could be detrimental if lactation is marginal.

OVARIAN FOLLICLE DEVELOPMENT

Gamete Development Occurs Initially Without Gonadotropin Support and Subsequently with Pulsatile Gonadotropin Secretion

Oocyte proliferation, which occurs by *mitotic division* during fetal development, ends at about the time of birth in most mammalian species. Oocytes begin the process of reduction of chromosome numbers to the haploid state by *meiosis* shortly after birth under the influence of *meiosis-initiating factor,* thought to be produced by the *rete ovarii.* The process is soon interrupted at the *diplotene,* or *dictyate,* stage of meiosis I by the meiosis-inhibiting factor, which is probably produced by the developing follicle cells. Oocytes remain in this stage until the follicle begins its final development, an interval that can be as long as 50 years or more in humans. The *follicle,* at this point, is delineated by an outer basement membrane *(membrana propria),* which is secreted by the follicle cells.

The initial development of the follicle involves growth of the oocyte. This growth is accompanied by intense synthetic activity; a large amount of ribonucleic acid (RNA) is synthesized. At the same time, follicle cells begin to divide and form a *granulosa* that is several cells thick. The granulosa cells then secrete another boundary substance, the *zona pellucida,* that lies within the granulosa and that immediately surrounds the oocyte. Granulosa cells maintain contact with the oocyte through the zona pellucida by means of the development of cytoplasmic processes. Interaction among granulosa cells is facilitated by the development of gap junctions. This form of communication is important because the granulosa has no blood supply; blood vessels are excluded at the level of the membrana propria. The *thecal layer* of the follicle forms around the membrana propria to complete the layers of the follicle. Follicles at this stage are called *primary,* or *preantral, follicles.*

Factors that control initial follicle growth are not known. External factors, such as gonadotropins, are not required because preantral follicles can develop in hypophysectomized animals. In species such as cattle and horses (perhaps sheep and goats as well), in which several dominant follicles develop during the estrous cycle, it is likely that a few follicles begin to develop each day. In animals in which a cohort of follicles develops synchronously (pigs, cats, dogs), there appears to be less tendency to have competing follicle growth waves during the luteal phase (pig) and a tendency to have only one cohort of follicles during the preovulatory period (cat and dog). Thus the development of a cohort of follicles may limit follicle development from the primordial state, at least during the period of active follicle development leading to ovulation. Initial follicle growth is under genetic control, and the pattern reflects the needs of the particular species.

In the Preantral Follicle, Gonadotropin Receptors for Luteinizing Hormone Develop on the Theca, Which Results in Androgen Synthesis; Follicle-Stimulating Hormone Directs the Granulosa to Transform the Androgens to Estrogens

For follicles to progress beyond the preantral stage, the granulosa and theca need to develop *receptors* for gonadotropins. FSH and LH receptors develop on the granulosa and theca, respectively. The onset of the antral follicle is marked by the appearance of fluid that begins to divide the granulosa. The follicular fluid, a secretory product of the granulosa, coalesces to form an increasingly larger fluid cavity *(antrum)* within the granulosa. In later development of the antral follicle, the oocyte remains surrounded by a layer of granulosa cells called the *cumulus oophorus,* which are attached to the wall of the follicle by a small stalk of granulosa cells.

The proximity of the granulosa and theca cells allows cooperative estrogen synthesis. The theca produces *androgens* (testosterone and androstenedione) under the influence of LH, which diffuses across the membrana propria into the granulosa, where

the androgens are transformed into *estrogen* (estradiol-17). At this time of development, the granulosa is incapable of forming androgens, the precursors of estrogen biosynthesis, and the theca has limited capacity for producing estrogens. This concept of cooperative effort, called the *two-cell mechanism* for estrogen secretion, is generally accepted as being the way most follicular estrogen is produced. These estrogens have a positive-feedback effect on the granulosa; they stimulate the cells to undergo mitotic division, and thus the follicle grows in size as the granulosa proliferates in response to its own secretory product (estrogen).

One effect of estrogen is the formation of additional receptors for FSH as follicle development proceeds. In this situation the antral follicle becomes increasingly sensitive to FSH as it develops and is able to grow under a relatively steady state of FSH secretion.

Late in the Ovarian Follicular Phase, Luteinizing Hormone Receptors Develop on the Granulosa, Which Permits the Preovulatory Surge of Luteinizing Hormone to Cause Ovulation

Late in antral follicle development, FSH and estrogens initiate the formation of LH receptors on the granulosa, whereas FSH receptors begin to diminish. Increasing secretion of estrogen by the antral follicle finally results in the initiation of the *preovulatory surge of gonadotropins*. Thus, in the last stages of development, the follicle falls progressively under the control of LH as it makes its last growth spurt to the point of ovulation.

CLINICAL CORRELATIONS

ANDROGEN INSENSITIVITY

History. You are called to examine a mare that has recently been brought to a broodmare farm after a successful racing career. It is late spring, but the mare has shown estrous behavior only on an intermittent basis.

Clinical Examination. As you approach the mare, you notice that she is large. The genital examination reveals a normal vulva, but when you introduce the speculum, it can be inserted only about 5 to 6 inches. Digital examination of the genital tract through the vulva results in a finding of complete blockage at the level of the vestibulovaginal conjunction, with no evidence of the external os of the cervix. On examination per rectum, you find the vagina, cervix, uterus, and oviducts to be absent; the gonads are symmetric in shape without the usual indentation caused by the ovulation fossa that is characteristic of equine ovaries.

Comment. You tell the shocked owner that you are suspicious that the animal is not really a mare, but a male masquerading as a female. One of the easiest ways to confirm the diagnosis is to have a testosterone analysis done on plasma. If the gonads are testes, they still retain the ability to secrete significant amounts of testosterone, even though they are retained (cryptorchid, in a sense) within the abdominal cavity. You could also have a chromosomal analysis to verify that the animal has an XY sex chromosome complement. In this case, it is likely that the testes were able to secrete the müllerian-inhibiting factor, which resulted in regression of the tubular system of the genital tract that forms the female system (oviducts, uterus, cervix, vagina). But why, asks the owner, did the external genitalia not turn out to be male? There is evidence, in cases such as this, that the tissues of the external genitalia lacked critical receptors for androgens; thus the external genitalia were female in type. The rule of sexual development is that the female state develops in the absence of testicular input, the latter including müllerian-inhibiting factor and testosterone. In this case the lack of sexual differentiation also appeared to involve the hypothalamus, because the "mare" did not exhibit male behavior despite relatively high testosterone concentrations.

Treatment. There is obviously no treatment for this syndrome. It would be unethical to take her back to the track and race her again as a female, when the owner knows that "she" is a male. The horse could be used as a performance horse (i.e., hunter/jumper, dressage, eventing) or for pleasure riding.

PRACTICE QUESTIONS

1. Which of the following statements is true?
   a. Müllerian ducts develop in the female because of the presence of estrogen.
   b. Müllerian ducts develop in the female because of a müllerian-stimulating factor.
   c. Wolffian ducts develop in the male because of a wolffian-stimulating factor.
   d. Wolffian ducts develop in the male because of the presence of androgen.

2. The most potent factor involved in the organization of the internal and external parts of the genital tract is:
   a. Müllerian-inhibiting factor.
   b. Müllerian-stimulating factor.
   c. Estrogen.
   d. Androgen.

3. Which of the following groups of hormones is transported to the anterior pituitary by the hypothalamohypophyseal portal system?
   a. Oxytocin, GnRH, and dopamine
   b. GnRH, dopamine, and vasopressin
   c. Dopamine, vasopressin, and oxytocin
   d. Dopamine and GnRH

4. Which of the following groups of hormones controls the synthesis and release of hypophyseal hormones involved in reproductive processes?
   a. Oxytocin, GnRH, VIP, and dopamine
   b. GnRH, dopamine, VIP, and vasopressin
   c. Dopamine, vasopressin, VIP, and oxytocin
   d. GAP, dopamine, VIP, and GnRH
   e. GAP, GnRH, VIP, and oxytocin

5. Which of the following factors is responsible for causing oocytes to remain in a diplotene or dictyate state?
   a. Müllerian-inhibiting factor
   b. Müllerian-stimulating factor
   c. Meiosis-inhibiting factor
   d. Meiosis-stimulating factor
   e. Wolffian-inhibiting factor
   f. Wolffian-stimulating factor

BIBLIOGRAPHY

Austin CR, Short RV, editors: *Reproduction in mammals*, vols 1-6, Cambridge, UK, 1986, Cambridge University Press.

Cain JL, Lasley BL, Cain GR, et al: Induction of ovulation in bitches with pulsatile or continuous infusion of GnRH, *J Reprod Fertil Suppl* 39:143–147, 1989.

Concannon PW, Morton DB, Weir BJ, editors: Dog and cat reproduction, contraception and artificial insemination, *J Reprod Fertil Suppl* 39, 1989.

Cupps PT, editor: *Reproduction in domestic animals*, ed 4, New York, 1991, Academic Press.

Feldman EC, Nelson RW, editors: *Canine and feline endocrinology and reproduction*, ed 4, Philadelphia, 2009, Saunders.

Hafez ESE, Hafez B, editors: *Reproduction in farm animals*, ed 7, Baltimore, 2000, Lippincott Williams & Wilkins.

Jöchle W, Arbeiter K, Post K, et al: Effects on pseudopregnancy, pregnancy and interoestrous intervals of pharmacological suppression of prolactin secretion in female dogs and cats, *J Reprod Fertil Suppl* 39:199–207, 1989.

Johnson MH, Everitt BJ, editors: *Essential reproduction*, ed 5, London, 2000, Blackwell Scientific.

Neill JD, editor: *Knobil and Neill's physiology of reproduction*, vols 1 and 2, ed 3, Philadelphia, 2005, Elsevier.

Pineda MH, Dooley MP, editors: *McDonald's veterinary endocrinology and reproduction*, ed 5, Ames, 2003, Iowa State University Press.

Romagnoli S, Schlafer DH: Disorders of sexual differentiation in puppies and kittens: a diagnostic and clinical approach, *Vet Clin North Am Small Anim Pract* 36(3):573–606, 2006.

CHAPTER 36
Control of Ovulation and the Corpus Luteum

KEY POINTS

Ovulation
1. Ovulatory follicles are selected at the onset of luteolysis (in large domestic animals).
2. Ovulation is caused by an estrogen-induced preovulatory surge of gonadotropins.

Corpus luteum
1. The corpus luteum secretes progesterone, which is essential for pregnancy.
2. Luteinizing hormone is important for the maintenance of the corpus luteum.
3. Regression of the corpus luteum in nonpregnant large domestic animals is controlled by uterine secretion of prostaglandin $F_{2\alpha}$.
4. Changes in luteal life span in large domestic animals occur because of changes in prostaglandin $F_{2\alpha}$ synthesis by the uterus.

Ovarian cycles
1. In spontaneously ovulating animals, ovarian cycles have two phases: follicular and luteal; animals that require copulation for ovulation can have only a follicular phase.
2. The luteal phase is modified by copulation in some species.

OVULATION

Ovulatory Follicles Are Selected at the Onset of Luteolysis (in Large Domestic Animals)

Until the advent of ultrasonography, it was difficult to identify growth patterns of follicles in domestic animals, especially those of follicles that develop during the luteal phase of the cycle. The concept that follicles do develop during the luteal phase was emphasized by the earlier work of Rajakowski, who described the midcycle follicle in the cow. With ultrasonography, it has been possible to define follicular growth and regression during the luteal phase of the cycle in the cow and mare. In cattle the predominant pattern is for several dominant (large) antral follicles to develop sequentially during the cycle (Figure 36-1). The follicular cycles are distinct to the extent that follicle regression usually begins (as indicated by follicle size) before the onset of the growth of the next follicle. The first dominant follicle regresses about midluteal phase, with a second dominant follicle beginning growth immediately. Whether the second dominant follicle is the ovulatory follicle, or whether a third develops, depends on the stage of the follicle at the time of regression of the *corpus luteum* (CL). If the second dominant follicle has begun to regress at the time of CL regression, a third follicle develops. Thus the selected ovulatory follicle is, by chance, the dominant follicle that is still in a developmental stage at the time that regression of the CL is initiated. The duration required for the development of the antral follicle to the point of ovulation has been estimated by various techniques to be about 10 days in domestic animals, perhaps slightly longer in some primates.

From ultrasonographical and endocrinological studies, two different phases in final antral follicle development apparently occur in large domestic animals: a relatively slow phase that lasts 4 to 5 days, followed by a second phase of accelerated growth, again lasting 4 to 5 days, that terminates in ovulation (Figure 36-2). Because the final growth phase of follicle development can be initiated during the luteal phase, the initiation of this phase can occur under the influence of a relatively slow pulse rate of gonadotropin release that occurs during the luteal phase. The rapidly growing follicle requires exposure to a faster gonadotropin pulse rate by the third, or fourth, day in order for the follicle(s) to complete the normal growth pattern through ovulation. This situation usually occurs in conjunction with the onset of CL regression, which passively allows an increase in pulsatile rate of gonadotropin secretion (see Figure 35-4).

One of the ways the *dominant follicle* maintains its status is to produce substances that inhibit the development of other antral follicles. One of the substances is *inhibin,* a peptide hormone produced by the granulosa, which inhibits the secretion of follicle-stimulating hormone (FSH). The dominant follicle is able to compensate for the lower FSH concentrations and continue to grow because of the numbers of FSH receptors it has compared with competitor follicles. Follicle development is dynamic once the rapid growth phase is achieved; the follicle(s) must be acted on through proper gonadotropin stimulation within a few days, or the result is death of the follicle. If the rapidly growing antral follicle is not exposed to the proper gonadotropin environment, *atresia* (regression) of the follicle begins almost immediately. Follicles that regress are invaded by inflammatory cells, and the area previously occupied by the antral follicle is eventually filled by connective tissue; that is, the follicle is replaced by an ovarian scar.

Ovulation Is Caused by an Estrogen-Induced Preovulatory Surge of Gonadotropins

The preovulatory surge of luteinizing hormone (LH), which begins about 24 hours before ovulation in most domestic species, including the cow, dog, goat, pig, and sheep, initiates the critical changes in the follicle that affect its endocrine organ status and

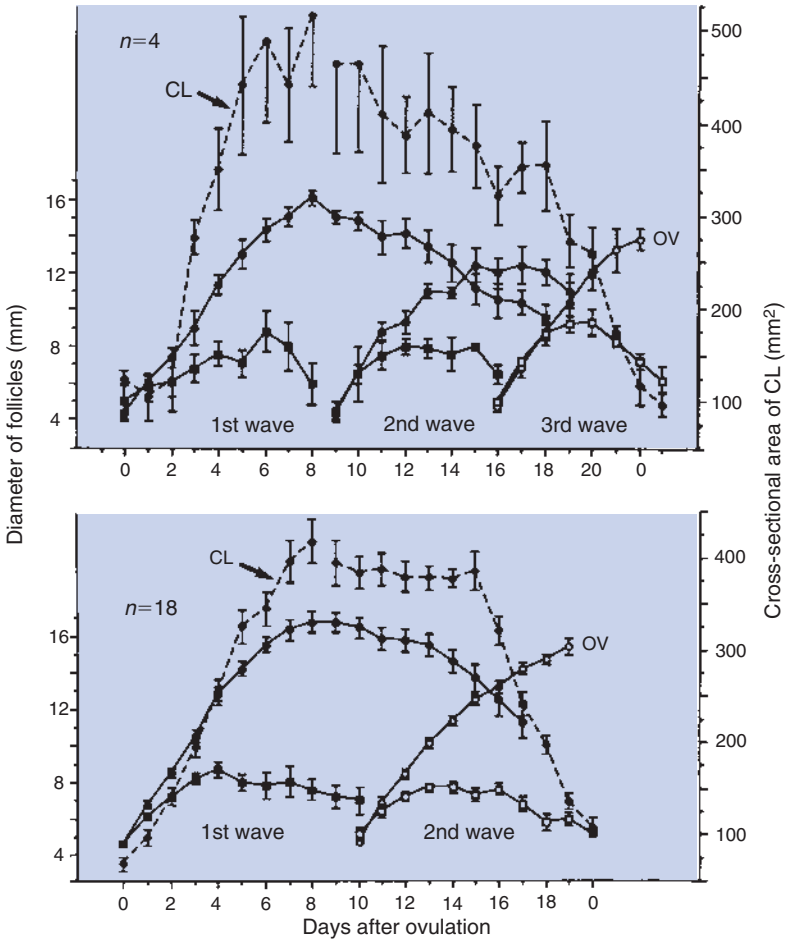

FIGURE 36-1 Mean (± standard error of the mean) profiles of diameters of dominant follicles and the largest subordinate follicle and the cross-sectional luteinized area of the corpus luteum *(CL)* for the interovulatory intervals with three and two follicular waves in cattle. Regression ($P < 0.05$) of the CL began between days 18 and 20 for three-wave intervals and between days 15 and 16 for two-wave intervals. *OV,* Ovulation. (From Ginther OJ, Knopf L, Kastelic JP: Temporal associations among ovarian events in cattle during oestrous cycles with two and three follicular waves, *J Reprod Fertil* 87(1): 223–30, 1989.)

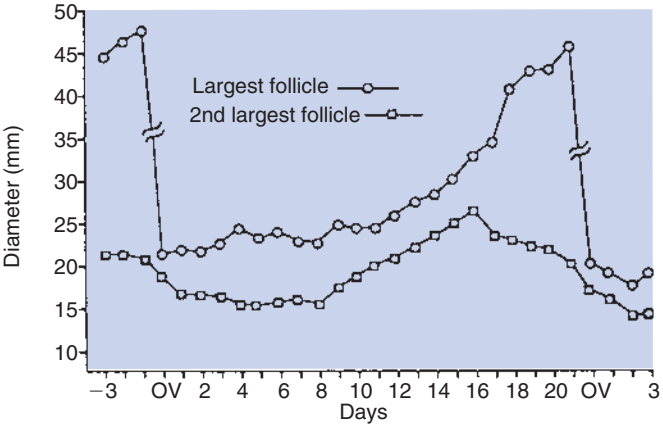

FIGURE 36-2 Development of the dominant and second-largest follicle during the estrous cycle of the mare. Note the divergence in diameter between the largest and second-largest follicles 1 day after ovulation. (From Pierson RA, Ginther OJ: Follicular population dynamics during the estrous cycle of the mare, *Anim Reprod Sci* 14:219, 1987.)

result in release of the oocyte (Figure 36-3). Two important tissues, the oocyte and the granulosa, have been kept under control by the production of inhibitory substances that are probably of granulosa origin. An *oocyte-inhibiting factor* prevents the oocyte from resuming meiosis, and a *luteinizing-inhibiting factor* prevents the granulosa from prematurely being changed into luteal tissue. The impact of the LH surge blocks the production of both these factors. In most animals the resumption of meiosis results in the first division of meiosis (meiosis I), or formation of the *first polar body,* which is complete before ovulation. In animals with the potential for reasonably long reproductive longevity (e.g., cattle), the initiation of the meiotic process could have begun as many as 10 years or more before its completion.

The effect of the LH surge on the granulosa is to allow initiation of the process of *luteinization,* which transforms the cells from estrogen to progesterone secretion. This process is underway before ovulation occurs. With the advent of the LH surge, estrogen secretion declines concomitantly with the onset of progesterone secretion.

Another function of the preovulatory surge release of LH is to cause the granulosa to produce substances, such as relaxin and *prostaglandin* $F_{2\alpha}$ ($PGF_{2\alpha}$), that affect the continuity of the connective tissue of the thecal layers of the follicle. These and other unknown substances disrupt the theca through the development of vesicles (within fibrocytes) that contain hydrolytic enzymes capable of breaking down the collagen matrix of connective tissue. The rupture of the follicle is caused by the disintegration of the connective tissue.

In summary, estrogen is used by the follicle(s) (1) to stimulate the growth and development of the granulosa and (2) to signal the hypothalamus and anterior pituitary as to the readiness of the follicle(s) for ovulation.

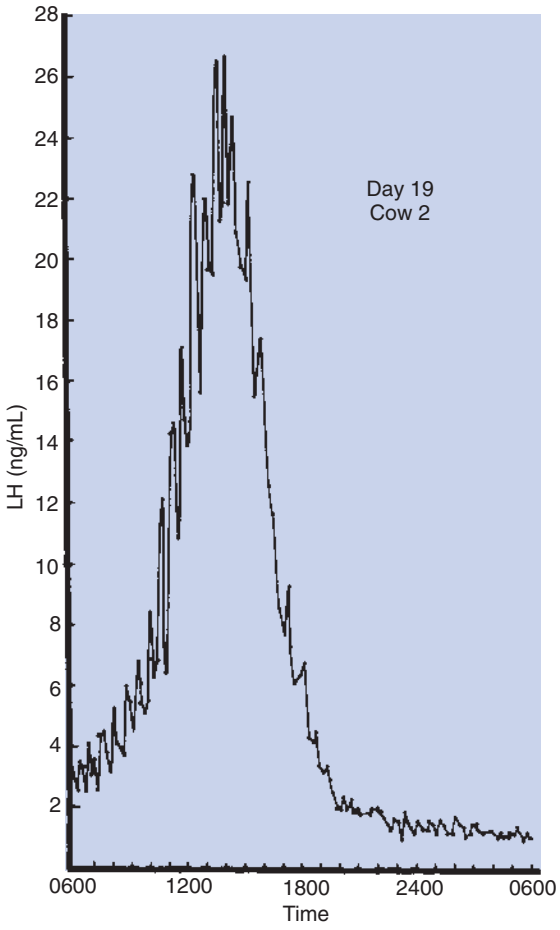

FIGURE 36-3 Preovulatory surge of luteinizing hormone *(LH)* on day 19 of the estrous cycle in a cow. (From Rahe CH, Owens RE, Fleeger JL, et al: Pattern of plasma luteinizing hormone in the cyclic cow: dependence upon the period of the cycle, *Endocrinology* 107(2):498–503, 1980.)

CORPUS LUTEUM

The Corpus Luteum Secretes Progesterone, Which Is Essential for Pregnancy

The main function of the CL is the secretion of progesterone, which prepares the uterus for the initiation and maintenance of pregnancy. The CL forms from the wall of the follicle, which is collapsed and folded after ovulation. With rupture of the follicle, there is a breakdown of the tissues that surround the granulosa, particularly the membrana propria, and hemorrhage into the cavity can occur from vessels in the theca. The folds of tissue that protrude inward into the cavity contain granulosa and theca cells and, importantly, the vascular system that will support cell growth and differentiation. Although the granulosa cell is the dominant cell of the CL, theca cells also contribute significantly to the composition of the structure. The process that granulosa cells undergo during the change from estrogen to progesterone secretion, *luteinization,* begins with the onset of the preovulatory LH surge and accelerates with ovulation.

In most domestic species, significant production of progesterone by the CL begins within 24 hours of ovulation. In some species, including the dog and primates, small amounts of progesterone are produced during the preovulatory LH surge; in the dog, this is important for the expression of sexual receptivity, which occurs as estrogen levels decline while progesterone levels increase.

Luteinizing Hormone Is Important for the Maintenance of the Corpus Luteum

For most domestic animals, LH is the important *luteotropin,* with the CL maintained in either nonpregnant or pregnant animals by a relatively slow pulsatile pattern of LH release (one pulse every 2 to 3 hours). In rodents, prolactin is the important luteotropin; daily biphasic release of prolactin is initiated by copulation, which is essential for the maintenance of the CL. Of the domestic species, prolactin has been implicated as a luteotropin in sheep and dogs.

Normal folliculogenesis, a prerequisite for ovulation, sets the stage for the subsequent development of the postovulatory CL. Thus, more clinical attention is paid to factors controlling the regression of the CL than to luteotropic factors.

Regression of the Corpus Luteum in Nonpregnant Large Domestic Animals Is Controlled by Uterine Secretion of Prostaglandin $F_{2\alpha}$

Regression of the CL is important in large domestic nonpregnant animals so that animals reenter a potentially fertile state as soon as possible. The CL life span after ovulation must be of sufficient duration to allow a newly developing conceptus to synthesize and release factors that allow the CL to be maintained, but it must be relatively short so that a nonpregnant animal can return to a potentially fertile state. In large domestic animals the duration of the luteal phase is about 14 days in the absence of pregnancy. This allows large domestic animals to recycle at relatively frequent intervals, approximately every 3 weeks.

Leo Loeb first showed (in 1923) the importance of the uterus for the regression of the CL through hysterectomy studies that extended the luteal phase in guinea pigs. He concluded that the uterus must produce a substance that terminated luteal activity. This information lay dormant for many years, until hysterectomy studies in cattle, pigs, and sheep in the 1950s produced similar results, that is, a prolongation of the luteal phase of the estrous cycle. Through these studies the concept developed that the uterus is responsible for control of the duration of the life span of the CL, at least in large domestic species (and guinea pigs).

It is now accepted that $PGF_{2\alpha}$, a 20-carbon unsaturated fatty acid, is the uterine substance that causes regression of the CL in large domestic animals, including cattle, goats, horses, pigs, and sheep; $PGF_{2\alpha}$ has no known natural role in CL regression in cats and dogs or in primates. Prostaglandin ($PGF_{2\alpha}$ and PGE) therapy has been used clinically to cause luteolysis in the bitch and queen, for the treatment of pyometra, or to induce abortion. In large domestic species, regression of the CL is initiated by uterine synthesis and release of $PGF_{2\alpha}$ (likely of endometrial origin) at about 14 days postovulation. The mode of transfer of $PGF_{2\alpha}$ from the uterus to the ovary is thought to occur either by *local countercurrent transfer* or *general systemic transfer.* Countercurrent transfer involves the movement of molecules across the blood vascular system from higher concentrations in the venous effluent (uteroovarian vein) to an area of lower concentration (ovarian artery) (Figure 36-4). Systemic transfer involves passage of the molecules through the general circulatory system. In some species (cow and ewe), $PGF_{2\alpha}$ synthesis from a uterine horn only influences the life span of the CL in the ipsilateral ovary. In other species (sow and perhaps mare), $PGF_{2\alpha}$ synthesis from one horn is sufficient to cause regression of CL in both ovaries. This effect likely occurs

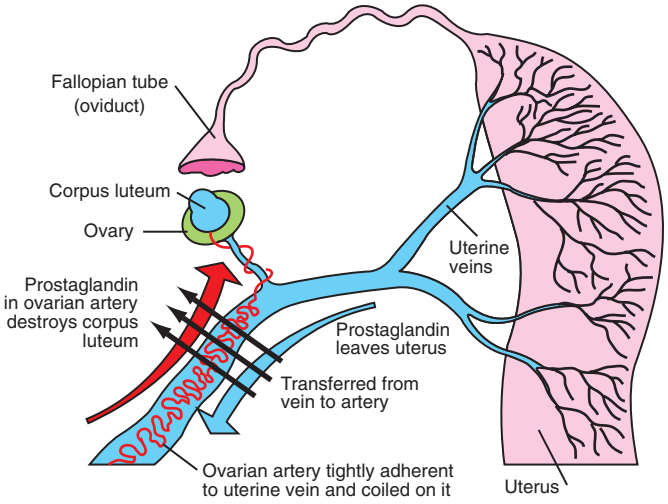

FIGURE 36-4 Postulated route by which prostaglandin secreted by the progesterone-primed uterus is able to enter the ovarian artery and destroy the corpus luteum in sheep. (From Baird DT: The ovary. In Austin CR, Short RV, editors: *Reproduction in mammals,* vol 3, Cambridge, UK, 1986, Cambridge University Press.)

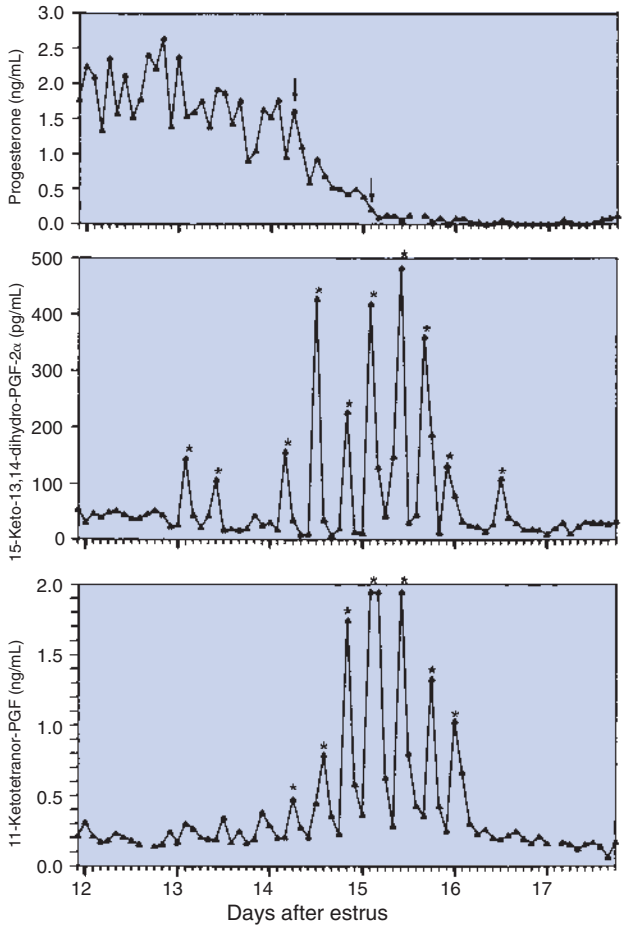

FIGURE 36-5 Concentrations of progesterone, 15-keto-13,14-dihydro-$PGF_{2\alpha}$, and 11-ketotetranor-PGF metabolites in a nonpregnant ewe. Values identified as significant pulses of either $PGF_{2\alpha}$ metabolite are indicated by asterisks. The times of initiation and completion of functional luteolysis are indicated by arrows. *PGF,* Prostaglandin F. (From Zarco L, Stabenfeldt GH, Basu S, et al: Modification of prostaglandin F-2 alpha synthesis and release in the ewe during the initial establishment of pregnancy, *J Reprod Fertil* 83(2):527–36, 1988.)

because of greater production of $PGF_{2\alpha}$ by uterine tissue, as well as a difference in the rate of metabolism of $PGF_{2\alpha}$. $PGF_{2\alpha}$ is rapidly metabolized systemically, with more than 90% changed by one passage through the lungs. Thus the system involving the use of $PGF_{2\alpha}$ as the luteolytic agent in large domestic species requires that $PGF_{2\alpha}$ be conserved through a special transfer system, or that it be produced in relatively large amounts.

The pattern of synthesis and release of $PGF_{2\alpha}$ is essential to its luteolytic effect. For example, $PGF_{2\alpha}$ synthesis and release must be pulsatile, with pulses occurring at about 6-hour intervals, in order for *luteolysis* to be affected (Figure 36-5). The concept has developed that a minimum of four to five pulses within 24 hours is required to cause complete *luteolysis.* If pulse intervals increase significantly before complete luteolysis (e.g., to 12 hours), the CL can recover and continue to function, even if at a lower level of steroid synthetic activity. The uterus must be exposed to estrogen and progesterone to synthesize and release $PGF_{2\alpha}$. Although the initiation of $PGF_{2\alpha}$ synthesis that leads to *luteolysis* is not completely understood, one possible explanation is that estrogen (from an antral follicle) causes the initial synthesis and release of $PGF_{2\alpha}$. In sheep it is thought that interplay occurs between the uterus and ovary after the initial $PGF_{2\alpha}$ pulse. $PGF_{2\alpha}$ affects the CL to cause both a reduction in progesterone production and the release of *luteal oxytocin*. Oxytocin then interacts with receptors within the uterus to initiate another round of $PGF_{2\alpha}$ synthesis. $PGF_{2\alpha}$ synthesis ceases 6 to 12 hours after progesterone concentrations have become basal, that is, with the completion of *luteolysis.* A system for early recycling is not present in nonpregnant dogs and cats as far as regression of CL; the luteal phase is about 70 and 35 days, respectively. Bitches experiencing infertility as a result of frequent estrous cycles may have pathologically shortened diestrus or anestrus.

Changes in Luteal Life Span in Large Domestic Animals Occur Because of Changes in Prostaglandin $F_{2\alpha}$ Synthesis by the Uterus

Significant changes in the length of the life span of the CL in nonpregnant large domestic species occur only because of changes within the uterus. As discussed in Chapter 38, the presence of an embryo results in the blockage of $PGF_{2\alpha}$ synthesis and a continuance in luteal activity. Prolonged luteal phases also typically occur in mares in the absence of uterine infection. This deficit in mares appears to be a genetic propensity toward inadequate synthesis and release of $PGF_{2\alpha}$. The absence of a uterine horn can also result in a lengthened luteal phase in animals in which the ipsilateral horn controls the CL (local control). In this situation (e.g., in the cow), if ovulation occurs in the ovary ipsilateral to the missing horn, the luteal phase is prolonged because of the need for the ipsilateral uterine horn to control the life span of the CL.

In nonpregnant large domestic animals, inflammatory responses of the endometrium caused by bacterial contamination can result in significant synthesis and release of $PGF_{2\alpha}$, leading to premature luteolysis and a shortening of the estrous cycle. It should be emphasized that luteal activity is almost always normal in the absence of uterine abnormality in large domestic species. Thus, short estrous cycles in large domestic animals are pathognomonic for uterine infection.

OVARIAN CYCLES

In Spontaneously Ovulating Animals, Ovarian Cycles Have Two Phases: Follicular and Luteal; Animals That Require Copulation for Ovulation Can Have Only a Follicular Phase

An *ovarian cycle* in a nonpregnant animal is defined as the interval between successive ovulations. The cycle is composed of two phases, an initial *follicular phase* and a subsequent *luteal phase,* with ovulation separating the phases. In most domestic animals and primates, the ovulatory process is governed by internal mechanisms; estrogen from the antral follicle initiates the ovulatory release of gonadotropins. These animals are called *spontaneous ovulators.*

Fundamental differences exist among animals regarding the relationship of the *follicular* and *luteal phases* of the cycle. In higher primates, there is complete separation of follicular and luteal phases, with no significant follicle growth occurring until luteolysis is complete. In large domestic animals, significant follicle growth does occur during the luteal phase of the cycle. For example, in the cow a large antral follicle is present at the onset of *luteolysis,* and in the mare, follicle growth can even result in ovulation of follicles during the luteal phase (about 5% of cycles). Thus, in large domestic animals, much of the follicle growth is telescoped into the luteal phase. This situation results in shorter cycles in large domestic animals versus primates (17 to 21 days versus 28 days); the interval of *luteolysis* to ovulation is shorter in large domestic animals (5 to 10 days) than in primates (12 to 13 days). The period of antral follicle growth leading to ovulation is not appreciably different, however, with the final progression of antral follicle growth requiring about 10 days in large domestic animals and about 12 to 13 days in primates.

Animals that require copulation for ovulation are known as *induced ovulators.* They include cats, rabbits, ferrets, mink, camels, llamas, and alpacas. Copulation replaces estrogen as the stimulus that induces the ovulatory release of gonadotropins. However, these animals require exposure to elevated estrogen concentrations before they can respond to copulation by the release of gonadotropins.

Induced ovulators have follicle growth patterns (in the absence of coitus) in which cohorts of follicles develop, are maintained in a mature state for a few days, and then regress. Follicle growth patterns can be distinctly separated, as in the cat, in which follicles develop and regress over 6 to 7 days, with a minimum of 8 to 9 days between follicle growth waves. Follicle waves can also have some overlap, as in llamas and alpacas (Figure 36-6), or can closely overlap, as in the rabbit.

The Luteal Phase Is Modified by Copulation in Some Species

In rodent species the luteal phase of the ovarian cycle is extended by copulation. The life span of CL is only 1 to 2 days in the absence of copulation. Copulation initiates the release of prolactin, which results in prolongation of luteal activity for up to 10 or 11 days in the absence of pregnancy. This phenomenon is often called *pseudopregnancy.* In the canine, spontaneous regression of the CL marking the end of diestrus occurs in association with increased levels of prolactin, causing clinical pseudopregnancy. Nonpregnant bitches can nest, lactate, and nurture objects during this time. The queen can exhibit pseudopregnancy if copulation occurred with an infertile tom.

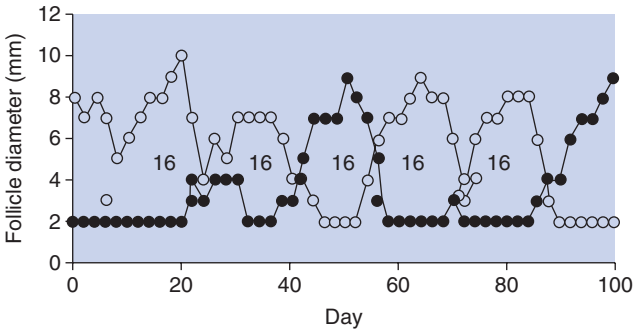

FIGURE 36-6 Ovarian follicular activity over a period of 100 days in a llama, indicating follicle growth alternating between the left *(open circles)* and the right *(solid circles)* ovaries. (From Vaughan JL, Macmillan KL, D'Occhio MJ: Ovarian follicular wave characteristics in alpacas, *Anim Reprod Sci* 80:353–361, 2004.)

CLINICAL CORRELATIONS

INABILITY TO IMPREGNATE A MARE

History. A 14-year-old American Saddlebred mare has been bred two times this season and still is not pregnant. The mare has had three prior foals without any difficulty. This season the owners have had the mare bred by artificial insemination (AI) at the barn where the stallion is kept. The trainer wanted more control over the situation, and she is now going to have the mare kept here. The semen will be shipped here for AI breeding. The trainer has had the semen measured to ensure that the motility is good, which it is. The mare has had a uterine culture and cytology performed, which are within normal limits. On previous ultrasound examinations the mare had no fluid in her uterus and no other abnormalities (e.g., cysts). The stallion is only collected on certain days of the week, so the mare's cycle must be timed so that the semen will arrive before the mare ovulates.

Clinical Examination. The mare is in good body condition. Based on her previous history and diagnostics, it appears that she has a cycle that is difficult to follow. Most mares will ovulate a follicle that is at least 35 mm. Additional parameters to assess when she will ovulate include the following: her cervix will be very relaxed, her edema will maximize and then start to regress, and her progesterone level should be 0 ng/mL. On palpation, the mare has a large corpus luteum (CL) present on one ovary, and she appears to be in diestrus. Because it is desirable to get her back in heat as soon as possible, the mare is given $PGF_{2\alpha}$. This will cause regression of the CL, and the mare should be in heat in 5 to 7 days.

Comment. It is not clear why the mare has not yet become pregnant. The semen appears to be of good quality, and the mare does not appear to have any preexisting conditions that decrease her fertility. The mare will be followed closely to optimize her potential for a pregnancy.

Treatment. The mare is rechecked 4 days after the administration of $PGF_{2\alpha}$. She has a 30-mm follicle on the right, multiple small follicles (MSFs) on the left, but no edema, and the cervix is toned. The mare is rechecked 2 days later, and she has a 35-mm follicle on the right, a 25-mm follicle on the left, edema is 2 (of 3), and cervix is starting to relax. To be sure that she is not missed, progesterone is checked and it is 1 to 2.5 ng/mL. She will be ready to breed soon. The stallion trainer is called because it is anticipated that semen will be needed the following day. The recheck of the mare on the next

day shows a 38-mm follicle on the right, MSFs on the left, and edema of 3, with a relaxing cervix (2). Semen is ordered for the next day. The next morning the mare has a greater than 40-mm follicle on the right, MSFs on the left, 3+ edema, and cervix is a 3. Her progesterone is 0 ng/mL. When the semen arrives, the motility, morphology, and numbers are good, and the mare is bred. She has a 42-mm follicle on the right, MSFs on the left, edema of 3, and cervix is a 3. Although it is anticipated, based on her cycle, that she will ovulate that night, she is given one dose of human chorionic gonadotropin (hCG). This will stimulate FSH and LH to help induce ovulation. When the mare is checked the next morning, she has ovulated. The mare is checked again at 14 days and found to be pregnant. She is rechecked at 24 days, and she is still pregnant. The fetus is growing, and a fetal heartbeat is detected.

PERSISTENT LUTEAL PHASE IN THE MARE

History. You have been called to examine a mare that foaled this spring but was not bred at the foal "heat" because of a retained placenta. It has been 40 days since the foal "heat," and the owner wants to know why the mare has not returned to estrus.

Clinical Examination. The main clinical findings are a cervix that is found (through speculum examination) to be relatively small and tightly constricted and (through palpation per rectum) to have considerable tone. Rectal palpation also reveals a uterus that has considerable tone. The ovaries are normal in size; in fact, one ovary has a 35-mm follicle. This prompts you to ask the owner whether the mare has been vigorously teased by a stallion for the detection of estrus. The owner brings the teasing stallion to the mare to demonstrate the farm's teasing technique, and as predicted, the mare vigorously rejects the stallion.

Comment. A history of a mare that has been previously in estrus and has not returned to estrus within 30 days usually indicates the presence of a persistent corpus luteum (CL). The CL persists because of inadequate $PGF_{2\alpha}$ synthesis and release, which normally occurs approximately 14 days after ovulation and causes regression of the CL in the absence of pregnancy. The incidence of the syndrome may be as high as 15% to 20%. The CL can remain active for as long as 3 months before the mare is able to synthesize and release $PGF_{2\alpha}$ in amounts sufficient to cause regression of the CL. It is difficult to palpate a persistent CL per rectum because it tends to shrink into the interior of the ovary. The structure may be visualized by ultrasonography, but this is not always possible. The appearance of the cervix and the tone of the cervix and uterus suggest that the genital tubular system is under the influence of progesterone; these findings, together with the history, support a tentative diagnosis. A tentative diagnosis can also be made if the mare returns to estrus within a few days after the administration of $PGF_{2\alpha}$. A definitive diagnosis can be made by progesterone analysis of blood; values are often 1 to 2 ng/mL in this syndrome, versus 3 ng/mL or more in mares with normal–estrous-cycle CLs. Additional supportive diagnostics would be to repeat the palpation and ultrasound examination in several days. If the mare maintains her uterine tone, does not have edema, and maintains cervical tone, these findings would also support a persistent CL.

The clinical finding that can be confusing in this syndrome is the presence of a large follicle in the absence of estrus. Ovarian follicles develop in this syndrome, and sometimes ovulation even occurs. However, mares do not show sexual receptivity in the presence of large follicles if luteal-phase concentrations of progesterone are present. Additionally, they do not develop marked uterine edema or cervical relaxation if progesterone is still present. One possibility that should be considered in a differential diagnosis is that ovarian activity has stopped (i.e., the mare has become anestrous). Although this does not occur often in foaling mares, mares that foal early can be adversely affected by the relatively short photoperiod that is present. In this case the clinical signs do not support the diagnosis of anestrus.

Treatment. The administration of $PGF_{2\alpha}$ (or one of its analogues) usually initiates regression of the persistent CL and results in the appearance of estrus within 5 to 7 days. The early return to estrus is based on the fact that ovarian follicles tend to develop on a continuous basis throughout the persistent luteal-phase syndrome. Regression of the CL allows the current dominant follicle to continue to develop and produce estrogen, which brings the mare into estrus. One caveat: If a large follicle (e.g., 40 to 45 mm) is present at treatment, the follicle may ovulate before the mare manifests estrus, and the treatment will be judged as failing. In this case the animal needs to be monitored daily; if ovulation occurs within a few days of treatment, the animal may need to be inseminated artificially if breed rules allow.

PRACTICE QUESTIONS

1. The main hormone secreted by the dominant follicle that allows the follicle to maintain its dominant state is:
   a. Estrogen.
   b. Inhibin.
   c. Oocyte-inhibiting factor.
   d. Progesterone.

2. The factor that is most important in deciding whether a luteal-phase dominant follicle will go on to ovulation is:
   a. Inadequate pituitary stimulation.
   b. Regression of the CL.
   c. Atresia of the follicle.

3. The initiation of the preovulatory LH surge that leads to ovulation in spontaneous ovulators results from:
   a. Estrogen.
   b. Inhibin.
   c. Progesterone.
   d. FSH.
   e. Prolactin.

4. The substance responsible for the regression of the CL in large domestic animals is:
   a. Estrogen.
   b. Inhibin.
   c. Oxytocin.
   d. Prolactin.
   e. $PGF_{2\alpha}$.

5. Ovarian follicle patterns in animals that are induced ovulators—that is, those that require copulation for the induction of ovulation—are as follows:
   a. Ovarian follicle waves greatly overlap
   b. Ovarian follicle waves slightly overlap
   c. Ovarian follicle waves are distinctly separated
   d. All the above

BIBLIOGRAPHY

Austin CR, Short RV, editors: *Reproduction in mammals*, vols 1-6, Cambridge, UK, 1986, Cambridge University Press.

Bocci F, Di Salvo P, Zelli R, et al: *Ovarian ultrasonography and progesterone concentration during the periovulatory period in bitches*. Presented at 5th Biannual Congress, European Veterinary Society for Small Animal Reproduction (EVSSAR), Budapest, Hungary, 2005.

Concannon PW, Morton DB, Weir BJ, editors: Dog and cat reproduction, contraception and artificial insemination, *J Reprod Fertil Suppl* 39, 1989.

Cupps PT, editor: *Reproduction in domestic animals*, ed 4, New York, 1991, Academic Press.

Feldman EC, Nelson RW, editors: *Canine and feline endocrinology and reproduction*, ed 4, Philadelphia, 2009, Saunders.

Hafez ESE, Hafez B, editors: *Reproduction in farm animals*, ed 7, Baltimore, 2000, Lippincott Williams & Wilkins.

Johnson MH, Everitt BJ, editors: *Essential reproduction*, ed 5, London, 2000, Blackwell Scientific.

Neill JD, editor: *Knobil and Neill's physiology of reproduction*, vols 1 and 2, ed 3, Philadelphia, 2005, Elsevier.

Pineda MH, Dooley MP, editors: *McDonald's veterinary endocrinology and reproduction*, ed 5, Ames, 2003, Iowa State University Press.

CHAPTER 37
Reproductive Cycles

KEY POINTS

Reproductive cycles
1. The two types of reproductive cycles are estrual and menstrual.

Puberty and reproductive senescence
1. Puberty is the time when animals initially release mature germ cells.
2. Reproductive senescence in primates occurs because of ovarian inadequacy, not inadequacy of gonadotropin secretion.

Sexual behavior
1. Sexual receptivity is keyed by the interaction of the hormones estrogen and progesterone, via gonadotropin-releasing hormone in the female and testosterone in the male.

External factors controlling reproductive cycles
1. Photoperiod, lactation, nutrition, and animal interaction are important factors that affect reproduction.
2. Inadequate nutrition results in ovarian inactivity, especially in cattle.

REPRODUCTIVE CYCLES

The Two Types of Reproductive Cycles Are Estrual and Menstrual

Two types of reproductive cycles are recognized, *estrual* and *menstrual,* with the term *ovarian cycle* representing the interval between two successive ovulations. These terminologies have developed in order to use certain external characteristics for accurately identifying a particular stage of the reproductive cycle and, most importantly, relating it to the time of ovulation.

Domestic animals have limited periods of *estrus* (or *sexual receptivity*); the term *estrous cycle* is used, and the onset of *proestrus* defines the start of the cycle (Figure 37-1). Primates are sexually receptive during most of the reproductive cycle; the term *menstrual cycle* is used, with the onset of *menstruation* (vaginal discharge of blood-tinged fluids and tissues) designated as the start of the cycle (Figure 37-2). The first day of the cycle for both estrual and menstrual cycles in many species begins shortly after the end of the *luteal phase.* In the dog a normal *anestrous* period separates *diestrus* and *proestrus* (the stages of the cycle are described later).

In domestic animals, proestrus usually begins within 48 hours after the end of the luteal phase; the dog and pig are exceptions, with proestrus in the dog delayed by the anestrus phase (2 to 3 months) and proestrus in the pig not occurring for 5 to 6 days. In primates, menstruation usually begins within 24 hours of the end of the luteal phase. Even though both cycles begin at the same time in relation to the luteal phase (shortly after), the time of ovulation differs. This is because, as previously discussed, luteal and follicular phases are separated in primates, with ovulation occurring at a minimum of 12 to 13 days after the onset of menses. In most domestic animals the *follicular phase* overlaps the luteal phase, and therefore ovulation occurs relatively earlier in the estrous cycle. Ovulation is easier to predict in domestic animals (versus primates) because estrus is usually tightly coupled to the preovulatory release of *gonadotropins* and ovulation. The onset of follicular development in primates can be delayed for a variety of reasons (e.g., stress), making the time of ovulation less predictable for primates than for domestic animals.

The estrous cycle has been classically divided into stages that represent either behavioral or gonadal events (see Figure 37-1). The terminology, originally developed for the guinea pig, rat, and mouse, is as follows:

- *Proestrus.* Period of follicle development, occurring subsequent to luteal regression and ending at estrus.
- *Estrus.* Period of sexual receptivity.
- *Metestrus.* Period of initial development of the corpus luteum (CL).
- *Diestrus.* Period of mature phase of the CL.

The classic terminology is not particularly useful for domestic animals. The common terminologies used for domestic animals involve either *behavioral* or *gonadal* activity. The cycle can be described in a behavioral manner by indicating whether animals are in *estrus* (sexually receptive) or not, including the stages of proestrus, metestrus, and diestrus. The cycle can also be described with reference to the activity of the gonads if differentiation of follicles and the CL is possible. Animals can be in the *follicular phase* (proestrus and estrus) or the *luteal phase* (metestrus and diestrus).

Because the equine CL is relatively difficult to identify by palpation per rectum, horses are usually classified by sexual behavior: estrus or nonestrus. The behavioral classification is also used in other domestic species, including the goat, pig, and sheep, because of the difficulty of determining their ovarian status. The ovarian status of cattle can be determined accurately by palpation per rectum, and cows are usually classified by ovarian status: follicular or luteal. The ovarian status of the dog and cat can be determined by performing vaginal cytology (estrogen effect) and measuring serum progesterone levels. If a CL can be identified, the judgment can be made that ovarian activity is normal in the particular animal, because the CL represents the culmination of follicle growth and ovulation.

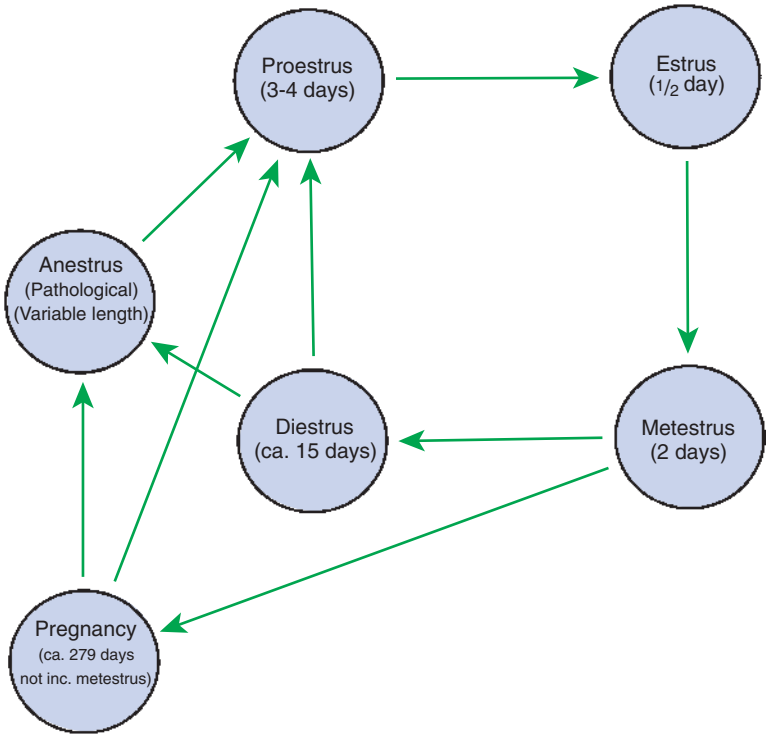

FIGURE 37-1 Various stages of the ovarian cycle of the cow. (From McDonald LE: *Veterinary endocrinology and reproduction,* ed 4, Philadelphia, 1989, Lea & Febiger.)

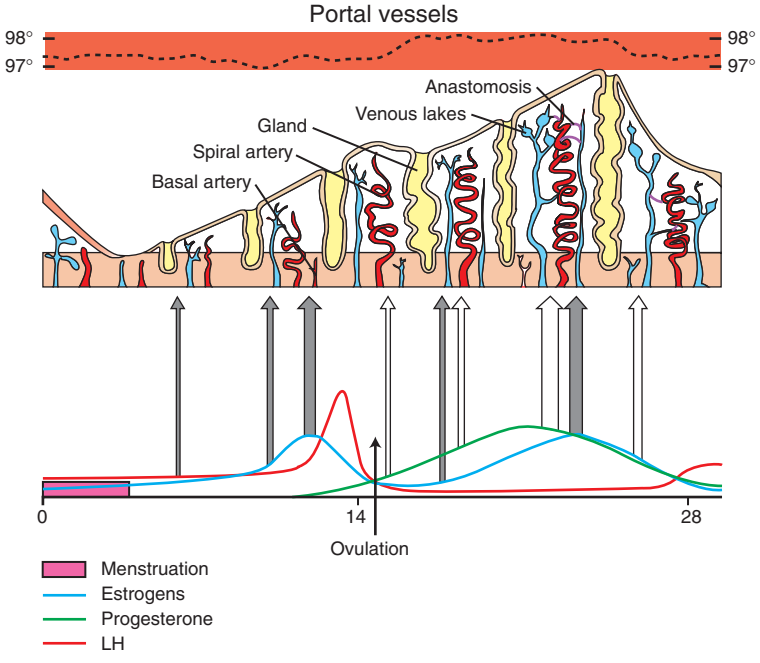

FIGURE 37-2 Changes in human endometrium during the menstrual cycle. Underlying steroid changes are indicated below, and basal body temperature is indicated above. Thickness of *arrows* (estrogens, *shaded*; progestogens, *white*) indicates strength of action. *LH,* Luteinizing hormone. (From Johnson M, Everitt B, editors: *Essential reproduction,* ed 3, London, 1988, Blackwell Scientific.)

PUBERTY AND REPRODUCTIVE SENESCENCE

Puberty Is the Time When Animals Initially Release Mature Germ Cells

For females to begin reproductive cycles, they must undergo a process called *puberty.* The term *puberty* is used to define the onset of reproductive life. For the female, although the onset of sexual activity (in domestic animals) or first menstrual bleeding (in primates) is often used as the onset of puberty, the most precise definition is the time of first ovulation. For all species, there is a critical requirement for the attainment of a certain size in order for puberty to be initiated, in cattle about 275 kg, for example, and in sheep about 40 kg (Figure 37-3). If this critical requirement is not met because of inadequate nutrition, puberty is delayed. The age at puberty for domestic animals is as follows: cats, 6 to 12 months; cows, 8 to 12 months; dogs, 6 to 12 months; goats, 7 to 8 months; horses, 12 to 18 months; and sheep, 7 to 8 months. Classically, bitches have attained 75% of their adult size before puberty occurs.

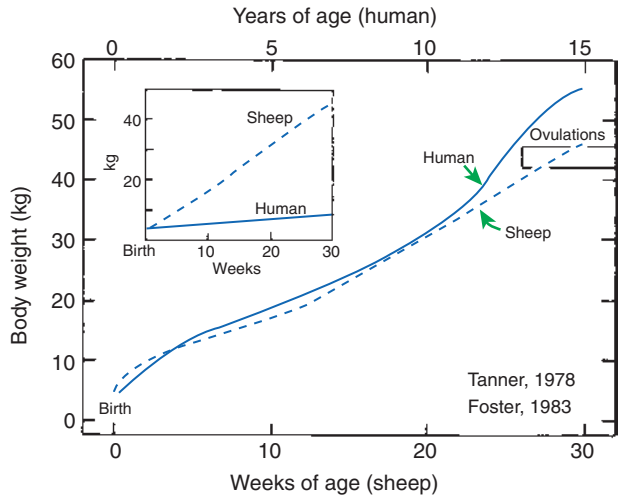

FIGURE 37-3 Body weight from birth through the initiation of ovulation for sheep (mean) and humans (50th percentile). *Inset* shows absolute growth during the first 30 weeks. (From Foster DL, Karsch FJ, Olster DH, et al: Determinants of puberty in a seasonal breeder, *Recent Prog Horm Res* 42:331–84, 1986.)

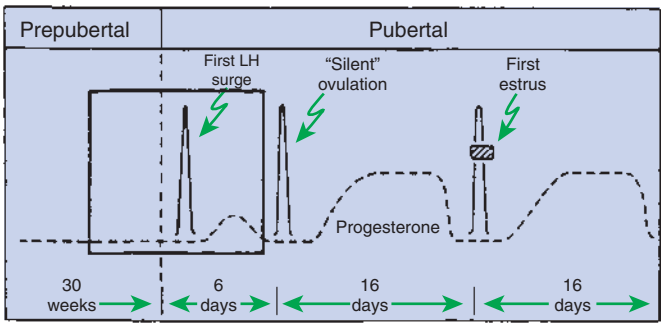

FIGURE 37-4 Schematic overview of major events during the transition into adulthood in the female sheep. *LH,* Luteinizing hormone. (From Foster DL, Ryan KD: Mechanisms governing onset of ovarian cyclicity at puberty in the lamb, *Ann Biol Anim Biochim Biophys* 19:1369, 1979.)

The physiological mechanisms involving control of puberty in domestic animals are best known in sheep. One of the fundamental concepts of the onset of puberty involves an increase in the synthesis and release of *gonadotropin-releasing hormone* (GnRH) from the hypothalamus, which drives *gonadotropin* secretion (in pulsatile form) and follicle growth. Before puberty, GnRH and gonadotropin secretion are kept in check because the hypothalamus is highly sensitive to *negative-feedback inhibition by estrogens.* One of the keys to puberty in lambs is a maturation of the hypothalamus, which results in reduced sensitivity to negative feedback by estrogen. Puberty onset is not held back because of lack of responsiveness of the prepubertal gonads, because ovarian follicle development can be elicited by gonadotropin administration.

Changes in *photoperiod* are important for allowing lambs to enter puberty. It has been shown that lambs must have some exposure to a long photoperiod during their prepubertal development; the period can be as short as 1 to 2 weeks (under experimental conditions). Termination of the long photoperiod, which occurs with the summer solstice, allows the sensitivity of the hypothalamus to decrease in response to negative estrogen feedback. The minimal interval from the end of the long photoperiod exposure to the onset of puberty is 10 weeks under experimental conditions. This aspect agrees well with the timing of spontaneous puberty, in which the first ovulation often occurs in the latter part of September (in the Northern hemisphere), or about 13 weeks from the occurrence of the summer solstice. Note that this concept of the initiation of puberty does not involve decreasing photoperiod; the emphasis is on a turning point that involves the cessation of exposure to a long photoperiod.

With appropriate growth and photoperiod exposure, the secretion of gonadotropins in lambs causes significant follicle growth. This growth is maintained because of decreased sensitivity of the hypothalamus to estrogens produced by growing follicles. The first endocrine event of puberty in the ewe lamb is the appearance of a preovulatory-type surge of gonadotropins, presumably induced by estrogens produced by developing follicles (Figure 37-4). The gonadotropin surge results in the production of a luteal structure, through luteinization of a follicle(s), which has a short life span, 3 to 4 days. After the demise of the initial luteal structure, another gonadotropin surge occurs, leading to ovulation and the formation of a CL, usually of a normal life span. At this time, cyclical ovarian activity is finally initiated in the ewe lamb.

Photoperiod can have a suppressive effect on the timing of puberty in animals whose ovarian cyclicity is controlled by light. Kittens born in the spring may be large enough to enter puberty by late autumn, but puberty could be delayed a few months if the kittens are under the natural photoperiod.

Photoperiod influences the timing of puberty onset in macaque monkeys, depending on the physiological maturity of the individual. The first ovulation, or puberty onset, can occur during the late autumn or early winter, at about 30 months of age (20% of animals) or 12 months later at about 42 months of age (80% of animals). The animals undergoing puberty at about 30 months of age have an earlier maturation of the neuroendocrine system, in which significant gonadotropin secretion begins during the previous spring. Thus, there is a window of opportunity for the onset of puberty in macaques that must be entered within the favorable photoperiod of decreasing light if puberty is to occur at an earlier time; nutrition and growth are likely determinants of the earlier time for onset of puberty.

The onset of puberty usually results in the establishment of cyclical ovarian activity within a relatively short period (i.e., within a few weeks to a month in lambs). Ewe lambs can initiate normal ovarian activity at the onset of puberty, which can lead to pregnancy (if mated) at the first estrus, or they can have false starts with the establishment of limited luteal phases and cessation of ovarian activity for several weeks to a month before they resume ovarian activity. In general, the onset of ovarian cyclicity starts later and ends earlier for ewe lambs compared with adults of the same breed. The earlier cessation of ovarian activity results from an earlier response to negative estrogen feedback.

The initiation of cyclical ovarian activity in pubertal primates takes longer; the first significant follicle growth usually ends in ovulatory failure. In macaque monkeys, 3 to 6 months is usually required after the onset of menarche, or first vaginal bleeding, before the occurrence of the first ovulation of puberty. In humans, follicle growth without ovulation can occur for up to a year before the establishment of normal ovarian cyclicity, including ovulation and CL formation.

For male lambs, the onset of puberty is first keyed when lambs begin to lose their sensitivity to estrogen feedback inhibition,

usually by about 15 weeks of age. For many males, this occurs during the period of increasing, or long, photoperiod, which is in contrast to the ewe lamb. Spermatogenesis (process of sperm production resulting in the presence of mature sperm) usually begins at this time, but because of the length of the process, lambs are usually not capable of successful breeding until about 30 weeks of age or more, or in concert with the onset of puberty in ewe lambs. Thus, puberty is a relatively gradual phenomenon in male sheep compared with the abrupt process in females.

Because adult ewes experience the same double gonadotropin surge at the onset of the breeding season, it has been suggested that adult animals recapitulate puberty each year as they enter the breeding season. Recent studies in adult ewes, however, indicate that *refractoriness* to the long photoperiod experienced by animals during the spring and summer is the most critical aspect for the establishment of ovarian activity. Thus the concept that the renewal of ovarian activity in sheep recapitulates puberty appears not to be accurate, at least in some aspects.

Reproductive Senescence in Primates Occurs Because of Ovarian Inadequacy, Not Inadequacy of Gonadotropin Secretion

The end to ovarian activity that occurs in primates is called *menopause.* In humans, for example, it usually occurs between 45 and 50 years of age. Menopause results from the depletion of oocytes, which has occurred throughout the reproductive life of the individual; in essence, it represents ovarian failure. It is not clear whether follicles fail to develop from their primordial state because of an absolute, or relative, reduction in follicle numbers, or whether the absence of gonadotropin receptors prohibits follicles from entering the gonadotropin-dependent stage of growth. The initiation of menopause often involves cyclical irregularity caused by failure of follicle development and ovulation. Gonadotropin secretion can be increased, or can be normal, because of the lack of estrogen and therefore lack of negative feedback on gonadotropin secretion. In the end, ovarian follicle activity ceases, estrogen concentrations decline, and in the absence of negative-feedback inhibition, gonadotropin concentrations increase dramatically.

Reproductive senescence is not recognized in domestic animals. This is partly because some domestic species have lives that are shortened for economic or humane reasons. Nevertheless, a phenomenon such as menopause clearly does not occur in domestic animals. One effect of age can be noted in the dog: estrous-cycle intervals gradually increase from the norm of 7.5 months to 12 to 15 months toward the end of the life span. Also, litter size diminishes, and increased neonatal mortality, probably associated with dystocia, occurs with increasing age of the dam. Reproductive senescence in the cheetah has been reported to be a consequence of uterine rather than ovarian changes.

SEXUAL BEHAVIOR

Sexual Receptivity Is Keyed by the Interaction of the Hormones Estrogen and Progesterone via Gonadotropin-Releasing Hormone in the Female and Testosterone in the Male

As indicated previously, the establishment of sexual behavior depends on exposure, or lack of exposure, of the hypothalamus to testosterone during the early neonatal period. In essence, testosterone (aromatized to estrogen) causes masculinization of the sexual centers in the hypothalamus; in the absence of testosterone, the hypothalamus becomes feminized. An area within the hypothalamus, the *medial preoptic area,* has been identified in the rat as an area that is modified structurally by exposure to testosterone.

Several principles exist regarding the effects of hormones on sexual behavior of domestic animals. First, the magnitude of change in hormone concentration that affects sexual behavior is small; in the cat, for example, an increase in estradiol-17 concentration from 10 to 20 pg/mL of plasma results in signs of proestrus. Second, synergism between hormones is often important for sexual receptiveness; in the dog, for example, estrogen priming followed by progesterone is important. Third, the sequence of exposure to hormones can be important; in the ewe, for example, progesterone priming is required before estrogen exposure for manifestation of estrus.

Estrogen, from the developing antral follicle, is the one hormone required for sexual receptivity in all domestic animals. *Progesterone,* derived from either the granulosa of the preovulatory follicle or the CL, is also important for estrus in some animals.

In sheep and goats, estrus occurs in response to estrogen only if the animal has been exposed previously to progesterone (through the presence of a previous CL). Estrus usually begins within a short period after the end of the luteal phase (i.e., 24 to 36 hours) because of the presence of large antral follicles at luteolysis; thus the period from last exposure to progesterone and the onset of estrus is short (Figure 37-5). The requirement of progesterone for sexual receptivity means that the first follicular phase of the breeding season, which leads to ovulation in the ewe, is not accompanied by estrus. Most adult ewes show estrus after the first luteal phase. Ewe lambs often require the exposure of two or more luteal phases before they express estrus.

Of the domestic species, dogs are unusual in that sexual receptivity is keyed by progesterone, produced initially by the

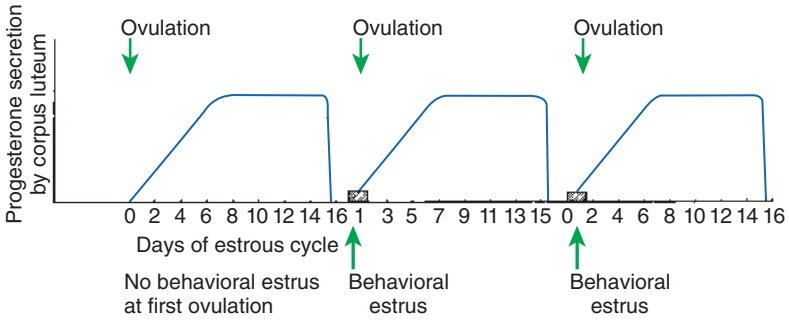

FIGURE 37-5 Estrous cycle of the ewe, showing how the first ovulation of the season is unaccompanied by estrus. Note the short interval between regression of the corpus luteum and the next ovulation. (From Short RV: Oestrous and menstrual cycles. In Austin CR, Short RV, editors: *Reproduction in mammals,* vols 1-6, Cambridge, UK, 1986, Cambridge University Press.)

granulosa during the preovulatory luteinizing hormone (LH) surge and subsequently by the developing CL. Prior exposure to estrogen makes the female attractive to males but does not produce sexual receptivity; estrus requires the additional exposure to progesterone. Estrus is often maintained for up to a week in the presence of a developing luteal phase. In other domestic species, progesterone is inhibitory for estrous activity.

The importance of prior progesterone priming for estrus manifestation has been suggested for dairy cattle by the finding of a reduced incidence of estrus at the first postpartum ovulation (days 15 to 20). Complete progesterone withdrawal occurs in the cow immediately before delivery, and animals would not have been exposed to progesterone for 2 to 3 weeks in this situation. Sows also have a reduced incidence of estrus at the first ovulation, which usually does not occur until after weaning, usually not until at least 45 days after parturition. Other domestic species (i.e., cats, goats, horses) all show estrus with the first ovulation of the season with no apparent requirement for progesterone priming.

Testosterone is important for *libido* in female primates. The theca layer from degenerating follicles forms an active interstitium that secretes the androgens androstenedione and testosterone. Androgens are also essential for the maintenance of libido in males. Occasionally, castrated males, particularly horses, are able to maintain libido despite the lower concentrations of androgens (of adrenal origin) that are present after castration. These animals can sometimes be differentiated from those with retained testicles (*cryptorchid* animals) by testosterone analysis of plasma; however, serum testosterone levels in intact males vary by the minute. A GnRH stimulation test more accurately identifies remaining testicular tissues (2.2 µg/kg intravenously, sampling before and 1 to 3 hours later). When commercially available, serum LH levels are better for differentiating bilaterally cryptorchid individuals (LH <1 ng/mL) from castrated animals (LH >1 ng/mL).

Both experimental and circumstantial evidence indicates that GnRH plays a role in sexual receptivity. The administration of GnRH to ovariectomized rats produced sexual (lordotic) responses, and in prepubertal gilts, GnRH administration resulted in the occurrence of estrus within 24 hours. The circumstantial evidence is that the onset of sexual receptivity in animals is tightly coupled to the onset of the preovulatory gonadotropin surge. Because the preovulatory gonadotropin surge is the result of an increased rate of pulsatile release of gonadotropins driven by increased GnRH synthesis and release, it is likely that this increased GnRH secretory activity affects sexual centers within the hypothalamus for the promotion of sexual receptivity. This allows the onset of the ovulatory process, triggered by the gonadotropin surge, to be tightly coupled with sexual receptivity.

EXTERNAL FACTORS CONTROLLING REPRODUCTIVE CYCLES

Photoperiod, Lactation, Nutrition, and Animal Interaction Are Important Factors That Affect Reproduction

Photoperiod

Photoperiod controls the occurrence of reproductive cycles in a number of domestic species, including cats, goats, horses, and sheep. The result is that these animals have an annual period in which they have continuous (cyclical) ovarian activity, as well as another period of no ovarian activity, termed *anestrus.* The response to photoperiod is different among these species; cats and horses are positively affected by increasing light, and goats and sheep are positively affected by decreasing photoperiod (Figure 37-6).

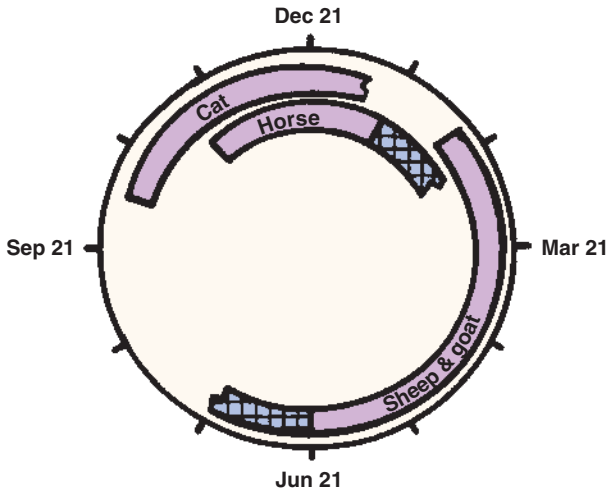

FIGURE 37-6 Diagrammatic representation of the effect of photoperiod on ovarian activity in the typical cat, horse, sheep, and goat. The *bars* represent periods of ovarian inactivity (anestrum). The transitional periods for the horse, sheep, and goat are shown by the *hatched portions* of the bars. (From Stabenfeldt GH, Edqvist LE: Female reproductive processes. In Swenson MJ, editor: *Dukes' physiology of domestic animals,* ed 10, Ithaca, NY, 1984, Cornell University Press.)

A positive response to a change in the photoperiod usually occurs relatively soon after the occurrence of the summer or winter solstice (i.e., within 1 to 2 months). A negative response to a change in photoperiod usually requires a longer duration for an effect (i.e., 2 to 4 months to suppress ovarian activity after the occurrence of the particular solstice). The net result is that in the absence of pregnancy, cyclical ovarian activity usually occupies more than half the year for these four seasonally breeding species.

In cats, cyclical ovarian activity can range from late January through October (in the Northern Hemisphere). In horses, the usual range of ovarian activity is from March through October. Conversely, sheep and goats have ovarian activity from late July through February or March (depending on the breed). As indicated previously, progesterone priming immediately before follicle development is required for sexual receptivity in sheep. The full length of the reproductive season of sheep is not manifested externally because (1) the first ovulation is not preceded by the presence of a CL, and (2) the last follicle phase may be delayed because of a negative photoperiod, with the priming effects of progesterone lost before follicle growth.

The main translator of photoperiod is the *pineal gland,* which produces *melatonin* in response to darkness. The central nervous system pathway involved with the translation of light includes the retina, suprachiasmatic nucleus, superior cervical ganglion, and pineal gland. Whereas melatonin has been previously described as *antigonadal,* this is obviously not true, because both short and long phases of darkness, with resultant short and long duration of melatonin secretion, can have a positive effect on reproductive cycles. In sheep, however, exposure to increasing darkness may be important only for maintaining ovarian activity. The onset of

ovarian activity is thought to occur in response to the development of refractoriness to the long photoperiod. The development of *photorefractoriness* to a long photoperiod as a requisite to ovarian cyclicity is consonant with the fact that sheep can begin cyclical ovarian activity even before the onset of the summer solstice.

Of the seasonal breeders, the cat is the most sensitive to photoperiod change; estrus, in conjunction with the presence of mature antral follicles, can occur as early as January 15 (in the Northern hemisphere). Initial follicle activity likely begins at least 10 days before the first expression of estrus, or 15 days after the winter solstice. Thus a total photoperiod change as brief as 15 minutes can be perceived and translated by the cat into ovarian activity.

The suppressive effects of photoperiod can be overcome by exposure to artificial lighting regimens. This is relatively easy in the case of cats and horses, in which environments with photoperiods are compatible with ovarian activity (i.e., at least 12 hours of light per day). If the photoperiod is established before the end of ovarian activity in the autumn, cyclical ovarian activity continues through the time associated with anestrus. If mares are allowed to become anestrous in the autumn, it can take a minimum of 2 months of exposure of mares to increased light to reestablish ovarian activity. The usual time for placing mares under lights is December 1 (in the Northern Hemisphere), with cyclical ovarian activity expected by early February.

It is usually not possible to place sheep and goats in light-tight barns to increase their exposure to dark in order to overcome the suppressive effects of increasing light. One recent development in this regard has been the oral or systemic (implant) administration of melatonin to sheep during the spring. This exposure to melatonin has resulted in an early onset of ovarian activity and increased the number of multiple ovulations above that normally observed at the beginning of the breeding season.

Lactation

Lactation can have suppressive effects on ovarian activity. In pigs, suppression of ovarian activity is complete; sows do not come into estrus until after piglets are weaned. Cats can have ovarian activity suppressed throughout lactation, although they occasionally come into estrus during the latter part of lactation. Ovarian activity tends to be suppressed in lactating beef cows, with the first estrus and ovulation not occurring before day 45 postpartum. The suckling process appears important to ovarian suppression; dairy cows are not suppressed by lactation unless it involves a large nutritional deficit.

Goats and sheep usually begin lactation during a photoperiod that is increasingly suppressive for ovarian activity, and therefore the reestablishment of ovarian activity in these species is confounded by the photoperiod. Ewes delivering in the autumn ovulated as early as postpartum day 12 (average, postpartum day 23), indicating that lactation has little suppressive effect on ovarian activity in sheep. Mares usually ovulate by postpartum days 10 to 15, with lactation having no suppressive effect on ovarian activity regarding this ovulatory interval.

One of the concepts of *lactational suppression of ovarian activity* involves the importance of suckling with its related stimulation of prolactin synthesis. Inhibiting factors for prolactin synthesis, including dopamine and the GnRH-associated peptide, need to be suppressed in order for prolactin synthesis to proceed. The sensory input from suckling suppresses the production of these prolactin-inhibiting factors. Because both dopamine and GnRH-associated peptide are essential links in the synthesis of gonadotropins, their reduced output results in reduced ovarian activity through decreased gonadotropin synthesis and release.

Pheromones

Pheromones are chemical compounds that allow communication among animals through the olfactory system. When sexual behavior is affected, the compounds are called *sex pheromones.* Pheromones arise from several tissue sources; the most prominent sources for animals are sebaceous glands, the reproductive tract, and the urinary tract.

Some of the first experiments that demonstrated the potency of male odors to influence reproductive behavior were done in mice. One syndrome, called the *Whitten effect,* involves the synchronization of estrus in female mice through the sudden introduction of a male (or male odor through bedding), with a large number of animals cycling within 3 days of introduction of the male. The effect of the pheromones in this case is to stimulate the synthesis and release of gonadotropins. Another syndrome, called the *Bruce effect,* involves the blockage of pregnancy development by the introduction of a different (strange) male in proximity to a recently bred female. The effect of the odor of the strange male is to block the release of prolactin, the hormone responsible for the maintenance of the CL in association with pregnancy in rodents. Regression of the CL in this case produces fetal loss. Thus, pheromones can strongly affect reproductive cycles.

Pheromones are important for the attraction of the male to the female at the time of sexual receptivity. Sexual attractiveness of the female evolves from the pheromones that she elicits on a limited, cyclical basis in association with estrus. For example, methyl-p-hydroxybenzoate, isolated from the vaginal secretions of dogs in proestrus and estrus, has produced intense anogenital interest by males when applied to anestrous females. Females are also influenced by male odors; sows in estrus assume a breeding *(rigidity)* stance when exposed to the urine of males. Androgens can serve as pheromones, or they can influence the production of substances within the kidney that influence female sexual behavior. The attractiveness of the female to the male involves a change in perception of the male by the female resulting from a changing physiological state within the female, not because of changes that are occurring in the male.

The classic way for males to delineate their territory has been for them to mark the area with urine. In general, pheromones that affect sexual behavior tend to have a musk type of odor. The classic pheromone used by humans is perfume, which is derived from civetone, a cyclical 17-carbon compound obtained from the civet cat.

The Whitten effect has been used to manipulate the estrous cycles of animals. In sheep, males are introduced into a flock of ewes before the breeding season to advance, or ensure, ovarian cyclicity at the beginning of the breeding season. Whereas it was previously thought the effect of the introduction of a male was short-lived (i.e., a gonadotropin response could only be obtained within the first few days from ewes that had antral follicles), it is now clear that the interaction of rams with ewes over extended periods of the anestrus results in earlier ovarian activity.

As discussed, pheromones can account for some of the effect of the male. More recent studies, however, have shown that *sight* of the male by the female, as well as *physical contact,* are important factors that influence gonadotropin secretion and thus ovarian activity.

The Whitten effect has also been used to influence the onset of puberty in pigs. The introduction of males into groups of gilts beginning several weeks before the expected time of puberty (180 to 200 days) has been used to ensure, or advance, the onset of puberty. The *dormitory effect,* the well-recognized synchronization of menstrual cycles in roommate women, occurs in bitches kenneled together as well.

Inadequate Nutrition Results in Ovarian Inactivity, Especially in Cattle

In dairy cattle, genetically selected for high productivity, the ability to produce up to 100 pounds of milk per day is a remarkable achievement. It is almost impossible for dairy cows to consume enough feed during the first part of the lactation cycle to maintain their body weight, and they are often in a negative nutritional balance for up to 100 days postpartum. Animals must have an adequate level of nutrition to initiate ovarian activity, so ovarian activity is suppressed until a positive energy balance is established. If an owner wants a dairy cow to produce large quantities of milk, the owner must be willing to wait for nutrition to "catch up" with milk production.

Inadequate nutrition can affect ovarian activity in the postpartum period. A management practice sometimes used to enhance production efficiency is to maintain beef cows on a marginal plane of nutrition during the winter. This approach forces animals to use fat that has been developed and stored during the grazing season. If pregnant beef cows are not returned to a positive nutritional balance by the last month of gestation, the reestablishment of ovarian cyclicity, which usually occurs between days 45 and 60 postpartum, will be delayed. Another situation that can affect ovarian activity involves pregnant beef heifers. These animals often need extra nutrition in the postpartum period to reestablish ovarian activity because they have requirements for growth as well as for lactation.

CLINICAL CORRELATIONS

SEXUAL ATTRACTIVENESS IN THE SPAYED BITCH

History. You are called by a veterinary colleague who has seen a bitch owned by one of her important clients. The client is upset because the dog is attracting males despite recently having undergone an ovariohysterectomy. You inquire whether the dog allows intromission by males. Although the answer is "no," the owner is sure that a portion of an ovary was left *in situ.* Your colleague is sure she removed the ovaries during the surgical procedure. You are asked to examine the dog as a favor to your colleague.

Clinical Examination. The dog has a vulva that is slightly swollen with a small amount of creamy discharge present. An examination of a vaginal mucosal smear reveals noncornified epithelial cells and an increased number of neutrophils. You indicate to the owner that you believe the (nondiscriminatory) male dogs are being attracted by the presence of an infection in the urogenital tract; the owner needs more convincing. You decide to obtain a reproductive endocrine panel (progesterone level and an LH test) and a urinalysis from the dog. The values for progesterone are low (<1.0 ng/mL) and the LH is positive (indicating lack of feedback) and thus do not support the active presence of ovarian tissue. The presence of white blood cells and bacteria in the urine suggests a urinary tract infection. A culture and sensitivity can be submitted to determine the specific bacteria and its antibiotic sensitivity.

Comment. It is common for bitches with genitourinary infections to attract male dogs, presumably because of the odors generated by the infection. One of the most important points of differentiation as to cause (i.e., bladder infection versus presence of ovarian remnant) is to know about the sexual behavior of the animal. The bitch normally allows intromission by a male only if she has been exposed to progesterone after priming with estrogen. This situation occurs only if an ovarian follicle is present and has begun to luteinize after the preovulatory luteinizing hormone surge. If the animal in question had allowed intromission, the presence of an ovarian remnant would be more likely. Because of the failure of the bitch to allow intromission, you can conclude that the animal almost certainly lacks ovarian tissue. With regard to the endocrine analysis, if the animal was completely ovariectomized, the LH level will be high, indicating a lack of negative feedback from either estrogen (not seen in the vaginal cytology) or progesterone (< 1.0 ng/mL). Nonestrogenized vaginal cytology and a low progesterone level do not rule out the presence of ovarian tissue, but if the sample is obtained when the animal is showing the sexual attractiveness, it can be stated with assurance that the behavior is not caused by hormones or, by extension, activity of ovarian tissue.

Treatment. The bladder infection is treated, and the owner is instructed to keep the female away from males until the infection has cleared.

TRYING TO GET A MARE PREGNANT

History. You work in Minnesota. Your clients have a 4-year-old Thoroughbred mare that they would like to breed early in the season, so that they will have as old of foal as possible, when it is old enough to race.

Clinical Examination. The mare has never been bred before. The breeding soundness exam (which assesses conformation, ultrasonography of her reproductive tract, cytology, and culture of her uterus) is normal.

Comment. If the mare can be bred as early in the year as possible, the owner will hopefully have a foal early in January (in the Northern Hemisphere). This will hopefully result in the foal being at its maximal development to race. The photoperiod is regulated by the pineal gland, which produces melatonin. Processing of light signals through the central nervous system (CNS) is regulated by the retina, suprachiasmatic nucleus, superior cervical ganglion, and the pineal gland. Ovarian activity begins in response to refractoriness of the photoperiod. There are two major methods for stimulating ovulatory activity in mares. One is to increase the total light per day. The other involves pulsatile amounts of light. Alternatively, drugs can be used to alter the cycle.

Treatment. The typical method to stimulate ovulation involves mimicking the photoperiod for 60 days prior to breeding. For this mare, 16 hours of day light should be started in November. In other cases, short periods of "flashes" of light (i.e., 1 hour of light 9.5 hours after onset of darkness) during the photosensitive period (10 hours after onset of darkness) can be used to stimulate ovulatory activity. An alternative option is to stimulate the mare with dopamine antagonists (i.e., domperidone) while increasing the photoperiod (i.e., increase photoperiod for 2 weeks, and then add dopamine antagonist until mare starts to cycle). Using one of these methods, the mare should begin ovulatory activity early in January, and hopefully will be bred and become pregnant for an early January foal.

PRACTICE QUESTIONS

1. The first estrous cycle of the cow subsequent to parturition follows which sequence?
   a. Anestrus, diestrus, estrus, metestrus, proestrus
   b. Anestrus, estrus, diestrus, metestrus, proestrus
   c. Anestrus, metestrus, diestrus, estrus, proestrus
   d. Anestrus, proestrus, estrus, diestrus, proestrus
   e. Anestrus, proestrus, estrus, metestrus, diestrus

2. The usual situation in large domestic animals is for a dominant follicle or dominant follicles to be present at the time of luteal regression, with sexual receptivity manifested within 1 to 2 days after luteal regression; the one large animal species that is the exception to this generalization is the:
   a. Cow.
   b. Doe.
   c. Ewe.
   d. Mare.
   e. Sow.

3. The hormones that form the foundation for sexual receptivity are:
   a. Estrogen and $PGF_{2\alpha}$.
   b. Progesterone and estrogen.
   c. Estrogen and GnRH.
   d. Progesterone and $PGF_{2\alpha}$.
   e. $PGF_{2\alpha}$ and GnRH.

4. Decreasing light turns off cyclical ovarian activity after a number of months, whereas increasing light reverses the process after a number of months, including the development of a transitional period. This description fits which domestic species?
   a. Cat
   b. Cow
   c. Dog
   d. Goat
   e. Horse
   f. Pig
   g. Sheep

5. What response results from the Whitten effect, in which the introduction of a male into a group of noncyclical animals results in the reestablishment of ovarian activity?
   a. Increased estrogen secretion
   b. Increased progesterone secretion
   c. Increased prolactin secretion
   d. Increased follicle-stimulating hormone secretion
   e. Increased luteinizing hormone secretion
   f. Increased follicle-stimulating hormone and luteinizing hormone secretion

6. Which one of the following domestic species requires progesterone priming, in addition to estrogen, to manifest estrus (therefore not manifesting estrus with the first ovarian cycle in the postpartum period)?
   a. Cat
   b. Dog
   c. Goat
   d. Horse
   e. Pig
   f. Sheep

BIBLIOGRAPHY

Austin CR, Short RV, editors: *Reproduction in mammals*, vols 1-6, Cambridge, UK, 1986, Cambridge University Press.

Beach FA: Coital behavior in dogs, *Behavior* 36:544, 1970.

Breen KM, Billings HJ, Wagenmaker ER, et al: Endocrine basis for disruptive effects of cortisol on preovulatory events, *Endocrinology* 146(4):2107–2115, 2005.

Card C: Hormone therapy in the mare. In Samper JC, editor: *Equine breeding management and artificial insemination*, ed 2, St Louis, 2009, Saunders.

Concannon PW, Morton DB, Weir BJ, editors: Dog and cat reproduction, contraception and artificial insemination, *J Reprod Fertil Suppl* 39, 1989.

Cupps PT, editor: *Reproduction in domestic animals*, ed 4, New York, 1991, Academic Press.

Davidson AP, Stabenfelt GH: Reproductive cycles. In Cunningham JG, Klein BG, editors: *Textbook of veterinary physiology*, ed 4, St Louis, 2007, Saunders.

Feldman EC, Nelson RW, editors: *Canine and feline endocrinology and reproduction*, ed 4, Philadelphia, 2009, Saunders.

Hafez ESE, Hafez B, editors: *Reproduction in farm animals*, ed 7, Baltimore, 2000, Lippincott Williams & Wilkins.

Johnson MH, Everitt BJ, editors: *Essential reproduction*, ed 5, London, 2000, Blackwell Scientific.

National Research Council: *Nutrient requirements of dogs and cats*, Washington, DC, 2005, National Academies Press.

Neill JD, editor: Knobil and Neill's physiology of reproduction, vols 1 and 2, ed 3, Philadelphia, 2005, Elsevier.

Pineda MH, Dooley MP, editors: *McDonald's veterinary endocrinology and reproduction*, ed 5, Ames, 2003, Iowa State University Press.

Simpson GM, England GCW, Harvey MJ, editors: *BSAVA manual of small animal reproduction and neonatology*, Gloucester, UK, 2010, BSAVA.

CHAPTER 38
Pregnancy and Parturition

KEY POINTS

Pregnancy

1. The development of an embryo involves fusion of an oocyte and spermatozoon within the oviduct.
2. Extension of the life span of the corpus luteum in large domestic species and cats is essential for pregnancy maintenance.
3. The placenta acts as an endocrine organ.

Parturition

1. Fetal cortisol initiates delivery through increased secretion of estrogen and thus prostaglandin $F_{2\alpha}$.

PREGNANCY

The Development of an Embryo Involves Fusion of an Oocyte and Spermatozoon Within the Oviduct

The development of a new individual requires the transfer of male gametes to the female genital tract for fertilization of the female gamete(s). *Spermatozoa,* which have been concentrated and stored in the epididymis, gradually change from *oxidative* (aerobic) to *glycolytic* (anaerobic) *metabolism* as they progress through the epididymis. In this situation, spermatozoa are in a state of reduced metabolism. Mature sperm are only able to metabolize a special sugar, *fructose,* within the reproductive tract. Lactose, glucose, dextrose, and fructose have all been used in commercially available semen extenders.

Sperm are ejaculated usually into the vagina, although some domestic species (dog, horse, and pig) ejaculate directly into the cervix and uterus. The movement of sperm through the cervix is aided by estrogen-induced changes in cervical mucus, which result in the formation of channels that facilitate movement of sperm. This has been particularly emphasized in primates, in which the thinning of mucus occurs just before ovulation, a factor that can be used to predict the time of ovulation.

The environment of the female genital system is generally inhospitable to the survival of sperm; for example, white blood cells are quickly attracted to the uterine lumen because sperm cells are foreign to the female genital tract. Special reservoirs have evolved in the female tract to aid in the survival of sperm during transport; these include the cervix and oviduct, the latter involving areas at the uterotubule junction and within the ampulla. The reservoirs are progressively filled (from caudal to cranial in the tract), requiring hours before the *oviductal reservoirs* are full. Finally, the reservoir within the ampulla is able to release a few sperm on a continuous basis, so that *fertilization* can occur shortly after the arrival of oocytes within the oviduct.

The first studies in *sperm transport* emphasized the rapidity of the process, with sperm reported passing from the vagina to the fimbriated end of the oviduct within minutes. It is now known that sperm undergoing so-called fast transport are not involved in *fertilization;* in fact, they are damaged by the rapid transport.

Sperm need to undergo changes within the female genital tract that are a prerequisite for fertilization; the process is called *capacitation.* One of the effects of *capacitation* is the removal of glycoproteins from the sperm cell surface.

The glycoproteins, perhaps added for protective purposes, interfere with *fertilization.* This change allows sperm to undergo the *acrosome reaction* when they come in contact with oocytes. The *acrosome reaction* involves the release of hydrolytic enzymes from the acrosomal cap; this may be important for penetration of the sperm through the granulosa and zona pellucida to the oocyte plasma membrane. *Hyaluronidase* causes breakdown of hyaluronic acid, an important component of the intercellular matrix of granulosa cells that surround the oocyte. *Acrosin,* a proteolytic enzyme, digests the acellular coating around the oocyte. Both enzymatic events allow the sperm to penetrate to the oocyte. The *acrosome reaction* also changes the surface of the sperm, which allows it to fuse with the oocyte. The *acrosome reaction* results in tail movements that feature a flagellar beat that tends to drive sperm in a forward direction.

Because of the changes that spermatozoa must undergo within the female reproductive tract before *fertilization,* the deposition of sperm before ovulation is the preferred timing for producing maximal fertility. An exception to this takes place when sperm with reduced longevity are used, such as the case with chilled-extended semen or frozen semen. In these cases, deposition of semen into the female reproductive tract should occur close to the time of ova maturation associated with fertilization. Females are usually sexually receptive for at least 24 hours before ovulation and, in the natural setting (free interaction between genders), insemination usually occurs a number of hours before the occurrence of ovulation. Even with induced ovulators, such as cats, the interval from copulation to ovulation is usually 24 hours or more. In essence, the system has evolved to have ready-to-fertilize sperm at the fertilization site when oocytes arrive. This concurs with the finding that the life span of male gametes tends to be twice that of female gametes.

The presentation of male gametes before female gametes in the oviduct implies that oocytes are ready for fertilization on arrival in the ampulla; this is likely true for a majority of animals. A

prerequisite for fertilization of the oocyte is that it must undergo the first meiotic division before fertilization. Although this occurs in a number of species before ovulation, in the horse and dog the first meiotic division does not occur until after ovulation (in the dog, not for at least 48 hours). In this situation, spermatozoa often wait for oocytes to mature in the oviduct before *fertilization* can occur. One means of adaptation to delayed completion of meiosis is that spermatozoa have a longer life span in the dog (6 to 11 days) and horse compared with other domestic species.

After *fertilization* has occurred, the *embryo* usually develops to the *morula,* or early *blastocyst* stage, within the oviduct before moving into the uterus. This period, lasting usually 4 to 5 days, affords the uterus time to finish its inflammatory response involving the removal of spermatozoa. This period also allows the endometrial glands time to secrete nutrients under the influence of progesterone from the developing corpus luteum (CL); the nutrients are essential for the development of embryos during their preimplantation stage.

An interesting finding in the mare is her ability to distinguish fertilized from unfertilized oocytes; unfertilized oocytes from previous cycles are retained within the oviduct, whereas recently fertilized oocytes (embryos) move through the oviduct to the uterus. It is likely that all animals recognize pregnancy by the presence of an embryo(s) at the early oviductal stage. However, this recognition does not necessarily result in prolongation of the CL and the continued production of progesterone, which is essential for the maintenance of pregnancy. In the bitch, despite ovulation and ova maturation spanning several hours, embryonic ages are synchronized by some mechanism inherent to the bitch's reproductive tract.

Extension of the Life Span of the Corpus Luteum in Large Domestic Species and Cats Is Essential for Pregnancy Maintenance

For those domestic animals (cattle, goats, horses, pigs, sheep) whose luteal activity is controlled by the uterus, modification of *uterine prostaglandin* $F_{2\alpha}$ ($PGF_{2\alpha}$) synthesis and release is critical for the establishment of pregnancy. The embryo apparently produces substances that modify uterine production of $PGF_{2\alpha}$. *Estrogen synthesis* by the embryo is one way the endometrium may be informed regarding the presence of an embryo. A specific protein of embryonic origin called *trophoblastin,* produced before day 14 of pregnancy (or postovulation) in both sheep and cattle, is of immunological interest for the establishment of pregnancy; it has a close structural relationship to the molecule *interferon.* Movement of the embryo(s) in the tract is also important for pregnancy recognition. In the mare the embryo moves throughout both horns before being fixed at day 16. In pigs a minimal number of embryos need to be present (about four), presumably to occupy a sufficiently large area of the endometrium. Litter-bearing animals also use transuterine migration to maximize the opportunity for fetal development, a procedure that aids the recognition of pregnancy process. The end result is either suppression of $PGF_{2\alpha}$ synthesis, as seen in the cow (Figure 38-1), or modification of the secretion mode (*continuous* instead of *pulsatile*), as seen in sheep. The absence of *pulsatile secretion* of $PGF_{2\alpha}$ seems to be critical for the extension of the life span of the CL and the establishment of pregnancy in large domestic species.

In the cat the CL lasts for 35 to 40 days after ovulation regardless of the presence of pregnancy, and thus early modification of luteal activity is not essential for the establishment of pregnancy. Implantation occurs at about day 13, which allows the fetoplacental unit to influence and extend luteal activity that is compatible with pregnancy maintenance. The luteotropic hormone that is responsible for luteal maintenance in the cat is not known. One hormone that likely synergizes with progesterone for the support of pregnancy is relaxin, a placental hormone produced in the cat beginning at about day 20 of gestation (see later discussion).

The dog does not extend its luteal phase during pregnancy; the luteal phase in the nonpregnant animal is often slightly longer (70 days) than in pregnant animals. Nevertheless, enhancement of luteal activity occurs through a placental *luteotropin,* likely *relaxin,* with progesterone secretion enhanced beginning at about day 20 of gestation or a few days after implantation. Early in the *luteal phase,* luteal function in the bitch is likely autonomous. During the second half of the *luteal phase,* luteinizing hormone (LH) and prolactin are likely *luteotrophs* (Figure 38-2).

The rescue of the CL at the onset of pregnancy in primates involves the production of a *luteotropin* called *chorionic gonadotropin* (CG; for humans, hCG), which is produced by trophoblastic cells *(syncytiotrophoblasts)* of the embryo (Figure 38-3). For trophoblast tissue to produce CG, it must have intimate contact with the interstitium of the endometrium. This contact occurs by a type of implantation called *interstitial,* in which the embryo

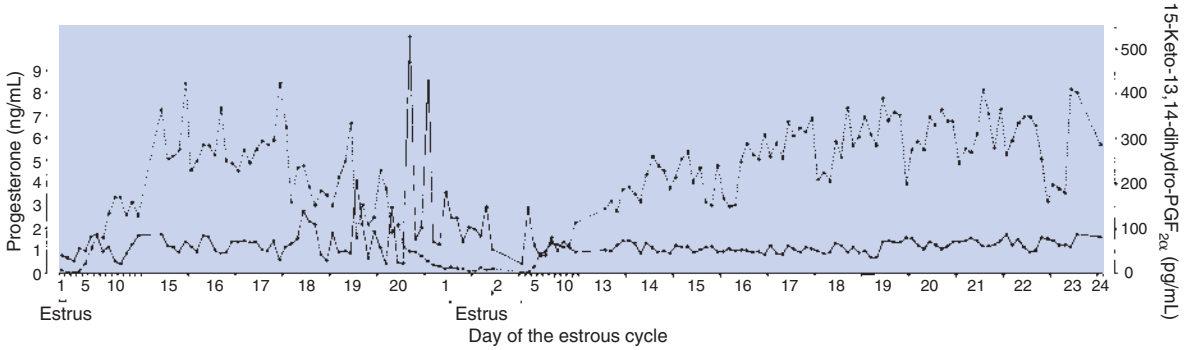

FIGURE 38-1 Relationship between prostaglandin release, as indicated by the measurement of 15-keto-13,14-dihydroprostaglandin $F_{2\alpha}$, and progesterone production by the corpus luteum during a nonfertile cycle and after a conception in the same cow. (From Kindahl H, Edquist LE, Bane A: Blood levels of progesterone and 15-keto-13,14-dihydro-prostaglandin F2alpha during the normal oestrous cycle and early pregnancy of heifers, *Acta Endocrinol (Copenh)* 82(1):134–49, 1976.)

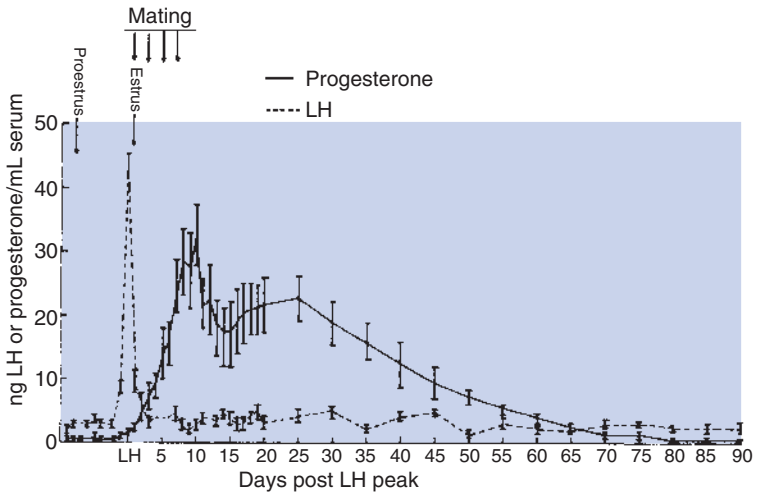

FIGURE 38-2 Luteinizing hormone *(LH)* and progesterone concentrations during pregnancy in nine dogs. *Vertical bars* represent the standard error of the mean. (From Smith MS, McDonald LE: Serum levels of luteinizing hormone and progesterone during the estrous cycle, pseudopregnancy and pregnancy in the dog, *Endocrinology* 94(2):404–12, 1974. Copyright © by The Endocrine Society.)

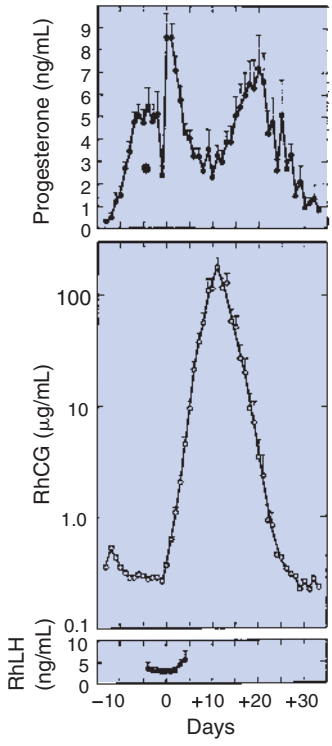

FIGURE 38-3 Summarization of 15 early pregnancies in normal rhesus monkeys normalized to the day of corpus luteum rescue (day 0). Points are means plus or minus standard error. Note the temporal relationship between luteal progesterone production (before day +10) and chorionic gonadotropin output. *RhCG,* Rhesus chorionic gonadotropin; *RhLH,* rhesus luteinizing hormone. (From Knobil E: On the regulation of the primate corpus luteum, *Biol Reprod* 8:246, 1973.)

penetrates the endometrium at about 8 to 9 days after fertilization in humans and nonhuman primates. Secretion of CG begins 24 to 48 hours after implantation, with immediate enhancement of luteal progesterone production. Rescue of the CL in human pregnancy occurs as late as 4 to 5 days before the end of the luteal phase.

As indicated, interstitial implantation is essential to the development of pregnancy in primates. *Implantation* is less invasive in the dog and cat, with the type termed *eccentric.* In the large domestic species, "invasion" of the endometrium is minimal; implantation occurs within special endometrial protrusions called *caruncles* in ruminants and by relatively minor *villus* invasion of the endometrium in horses and pigs. Domestic animals depend more on uterine secretions for the support of pregnancy than do primates. For cattle and horses the first indications of implantation begin about days 25 to 30, and another 7 to 10 days likely passes before a significant amount of embryonic nutrition is obtained through the implantation site. Subclinical uterine infections, or an inadequate number of endometrial glands, can interfere with the establishment of pregnancy in the species in which a long interval exists from fertilization to implantation. The cervix forms an important barrier to contamination of the uterine lumen in both the nonpregnant and the pregnant animal; in the latter the cervix becomes sealed.

The Placenta Acts as an Endocrine Organ

Besides the essential role of providing nutrients and oxygen for embryonic metabolism, the *placenta* functions as an endocrine organ. One of the most important functions of the placenta is the *production of progesterone.* In primates this function is established early in gestation, and the placenta likely can maintain pregnancy within 2 to 3 weeks after implantation in primates. Placental production of sufficient progesterone to maintain pregnancy occurs later in domestic animals (sheep, day 50 of 150-day gestation; horse, day 70 of 340-day gestation; cat, day 45 of 65-day gestation); in some species the placenta never produces enough progesterone to support pregnancy (cattle, goats, pigs, dogs).

The *production of estrogen,* in contrast to that of progesterone, requires interaction between the fetus and the placenta. This interaction has been best described in primates, in particular by the experiments of the Hungarian immigrant to Sweden Egon Dicfaluszy. He and his co-workers found that the primate placenta is unable to produce estrogen from progesterone even though the steroids are only separated by androgens in the steroid biochemical synthetic pathway. The placenta simply does not possess the enzymes necessary for the conversion of progesterone to androgens. Therefore a system has evolved in which the placenta supplies *pregnenolone,* the immediate precursor of progesterone, to the fetus, and the *fetal zone of the adrenal cortex* transforms pregnenolone to a C-19 androgen, *dehydroepiandrosterone.* This is returned to the placenta, which is able to convert dehydroepiandrosterone to an estrogen. In humans the primary

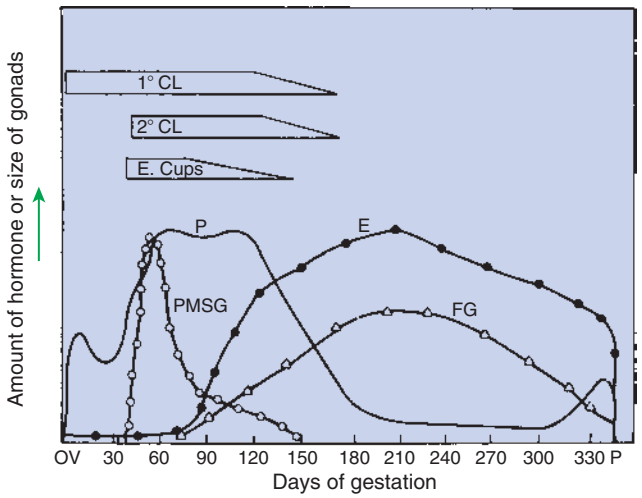

FIGURE 38-4 Summary of the temporal relationships among changes in hormonal concentrations and morphological changes throughout the gestational period of the mare. *1° CL,* Primary corpus luteum; *2° CL,* secondary corpora lutea; *E,* estrogens; *E. Cups,* endometrial cups; *FG,* fetal gonads; *P,* progesterone; *PMSG,* pregnant mares' serum gonadotropin (equine chorionic gonadotropin). (From Daels PF, Hughes JP, Stabenfeldt GH: Reproduction in horses. In Cupps PT, editor: *Reproduction in domestic animals,* ed 4, New York, 1991, Academic Press.)

estrogen of pregnancy is *estriol.* Because the fetus is involved in the production of estriol, the well-being of the fetus can be judged by determining estriol concentrations in the plasma of the mother.

The production of estrogen in the mare also involves an interaction between the placenta and fetus (Figure 38-4). From the work of Pashen and Allen, we know that the fetal gonads replace the fetal adrenals in primates as the key fetal endocrine organ involved in the cooperative synthesis of estrogen. The interstitial cells of the gonads appear to be the interactive cells, with fetal gonads enlarging to a size greater than the maternal gonads during the latter part of gestation. The production of estrogens during pregnancy in other domestic species, occurring relatively late in gestation, may involve the development of placental enzymes that allow progesterone to be metabolized to estrogens without the direct intervention of a fetal endocrine organ. (Fetal cortisol, however, is important for the induction of these placental enzymes, particularly in sheep; see next section.)

The protein hormones that are produced during pregnancy tend to be of placental origin. For example, *relaxin* is a hormone produced by the placenta in the cat, dog, and horse beginning at about days 20, 20, and 70, respectively. Besides its importance for preparing the soft tissues of the pelvic canal for passage of the fetus at birth (see the discussion on parturition), *relaxin* may be important for the support of pregnancy through a synergistic action with progesterone. In exception to the general rule of protein hormone production by the placenta, *relaxin* is produced by the CL in the pig, cow, and primates during pregnancy, with prepartum release occurring in conjunction with *luteolysis.*

The only CG identified in domestic animals to date is *equine CG* (eCG, formerly called *pregnant mares' serum gonadotropin* by its discoverer, Harold Cole) (see Figure 38-4). The eCG is produced by trophoblast cells that initially form as a band on the chorion *(chorionic girdle),* detach themselves around day 35 of pregnancy, penetrate the endometrium, and form associations of cells called *endometrial cups.* The eCG enhances progesterone production by the primary CL of pregnancy and aids in the formation of additional (secondary) CL through the *luteinization,* or ovulation, of preformed follicles. The essentiality of eCG for pregnancy maintenance is not known, because the primary CL is adequate for maintaining pregnancy.

Placental lactogen is another placental protein hormone. Its production increases in primates as CG secretion wanes during pregnancy. Placental lactogen has been reported in goats and sheep, with secretion increasing during the latter part of gestation. The hormone appears to have both *somatotropic* and *lactogenic effects* on the basis of growth hormone–like and prolactin-like properties. In dairy cattle, for example, placental lactogen may be important for mammary gland alveolar development, setting the stage for the next lactation. Another hormone whose production is increased during pregnancy, *prolactin,* also is important for alveolar development during the prepartum period. Prolactin is not a hormone of placental origin; prolactin increases during the latter part of gestation due to the effect of estrogen on its release from the adenohypophysis. Prolactin is luteotrophic in the dog.

PARTURITION

Fetal Cortisol Initiates Delivery Through Increased Secretion of Estrogen and Thus Prostaglandin $F_{2\alpha}$

During pregnancy the uterus progressively enlarges and stretches because of the growing fetus. Progesterone plays an important role in maintaining the quiescence of the myometrium as well as promoting a tightly contracted cervix. During the latter part of gestation, estrogen begins to influence uterine muscle by stimulating the production of *contractile protein* and the formation of *gap junctions;* the former increases the contractile potential of the uterus, and the latter facilitates the contractile process through increased communication among smooth muscle cells. Thus, important changes that set the stage for parturition begin weeks before the actual process begins. In the end, the uterus is converted from a quiescent to a contractile organ, and importantly, the cervix relaxes and opens to allow the fetus to be delivered.

The most important question about parturition concerns what initiates the process. In domestic animals, maturation of the fetus eventually brings about changes that initiate the delivery process. The key organ system of the fetus responsible for initiating the process is the *fetal adrenal cortex,* with the hypothalamus and adenohypophysis playing important supporting roles. This concept came from the work at the University of California (UC)–Davis by Liggins and Kennedy, who showed that destruction of the anterior pituitary of the sheep fetus resulted in prolongation of gestation; Drost subsequently found the same results after fetal adrenalectomy. Critical changes in *cortisol secretion* by the fetus eventually result in the synthesis and release of $PGF_{2\alpha}$ from the uterus, which produces muscle contraction and relaxation of the cervix. The following details of the initiation of parturition emphasize ruminants. It is postulated that elevated cortisol levels also contribute to the initiation of parturition in the dog.

The maturation of the fetal adrenal cortex is of critical importance in the initiation of parturition. The adrenal cortex likely becomes progressively sensitive to fetal adrenocorticotropic hormone (ACTH, corticotropin) (Figure 38-5). The time of adrenal maturation is under fetal genetic control, as shown by studies conducted on fetal lambs of different breeds in the same uterus (produced by embryo transfer) in which the prepartum initiation of cortisol production occurred at times that were

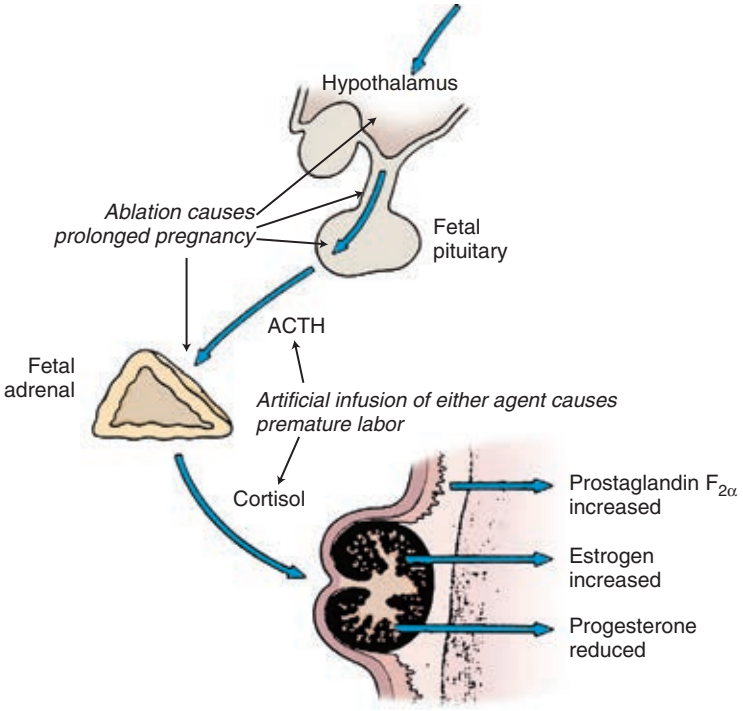

FIGURE 38-5 Diagrammatic summary explaining how the fetal lamb controls the onset of labor. Experimental procedures that lengthen or shorten pregnancy are shown. *ACTH,* Adrenocorticotropic hormone (corticotropin). (Redrawn from Liggins CG: The foetal role in the initiation of parturition in the ewe. In Wolstenholme GEW, O'Connor M, editors: *Foetal autonomy,* London, 1969, J & A Churchill.)

characteristic (and different) for the breed. Fetal cortisol induces placental enzymes (*17-hydroxylase* and *C17-20 lyase*) that direct steroid synthesis away from progesterone to estrogen. This process occurs at different times prepartum in domestic species, beginning at prepartum days 25 to 30 in cattle, 7 to 10 in pigs, and 2 to 3 in sheep. The end result of increased estrogen secretion is the secretion of *prostaglandins,* particularly $PGF_{2\alpha}$. $PGF_{2\alpha}$ is the pivotal hormone for the initiation of parturition; once its secretion begins, the acute phase of delivery is activated. The role of oxytocin in the initiation of delivery is not certain; it likely complements $PGF_{2\alpha}$ when the delivery process has started.

The synthesis of $PGF_{2\alpha}$ is thought to come about through increased availability of the substrate *arachidonic acid,* which is the main rate-limiting step in the synthesis of $PGF_{2\alpha}$. Estrogens are proposed to influence the system by making available the enzyme *phospholipase A,* a membrane-bound lysosomal enzyme that initiates the subsequent hydrolysis of phospholipids and release of arachidonic acid. This likely results from an increasing estrogen/progesterone ratio, with progesterone initially stabilizing, then estrogens destabilizing, lysosomal membranes. The end result is increased availability of arachidonic acid for the synthesis of $PGF_{2\alpha}$. The onset of $PGF_{2\alpha}$ synthesis results in the immediate release of the hormone because $PGF_{2\alpha}$ is not synthesized and stored. The critical effect of $PGF_{2\alpha}$ on the myometrium is to release intracellular calcium ion, which binds to *actin* and *myosin* to initiate the contractile process. Prostaglandins, both PGE and $PGF_{2\alpha}$, also have important effects on the *cervix,* which allow it to relax and dilate, permitting the passage of the fetus. The end result is a direct effect of $PGF_{2\alpha}$ on the intracellular matrix of the cervix in which there is a loss of collagen with a concomitant increase in *glycosaminoglycans,* the latter affecting the aggregation of collagen fibers.

In some animals, such as the cow, goat, dog, and cat, $PGF_{2\alpha}$ synthesis and release initiate regression of the CL beginning 24 to 36 hours before delivery, with complete withdrawal of progesterone occurring 12 to 24 hours before delivery. Although essential for delivery in these species, progesterone withdrawal does not initiate delivery; it is the release of $PGF_{2\alpha}$ that both causes *luteolysis* and drives myometrial contractions.

In the mare, as in primates, delivery occurs even though progesterone concentrations remain elevated during the process. In this situation, $PGF_{2\alpha}$ is able to overcome the suppressive effects of progesterone on myometrial activity. For animals dependent on placental production of progesterone for pregnancy maintenance, it is not possible to turn off one function (i.e., steroid synthesis) and still continue with other functions that are necessary for the support of the fetus through the time of delivery.

Oxytocin is also important to the delivery process (Figure 38-6). Estrogen induces *oxytocin receptor* formation in the myometrium. Recent information indicates that significant amounts of oxytocin are released only with the entry of the fetus into the birth canal. Oxytocin release occurs through the *Ferguson reflex.* The afferent arm of the reflex involves passage of impulses through sensory nerves in the spinal cord to the appropriate nuclei in the hypothalamus; the efferent arm involves transport of oxytocin from the neurohypophysis by the vascular system. Oxytocin is synergistic with $PGF_{2\alpha}$ in promoting contraction of the uterus.

As noted earlier, a hormone important for the preparation of parturition is *relaxin.* This hormone was first identified as being responsible for the separation of the pubic symphysis through relaxation of the interpubic ligament. Relaxin causes the ligaments and associated muscles surrounding the pelvic canal to relax, which allows the fetus to expand the pelvic canal to its fullest potential. In the mare, a well-defined area of muscle softening can be discerned on the midline from the top of the croup through the ventral commissure of the vulva. In the cow, muscles posterior to the hip become relaxed to the point that they undulate as the animal walks in the final 24 hours before parturition. In the cow and pig, the CL is the source of relaxin. In both these species the prepartum release of $PGF_{2\alpha}$ causes luteolysis, with a concomitant decline in progesterone production and the release of preformed relaxin. In other domestic species, such as cats,

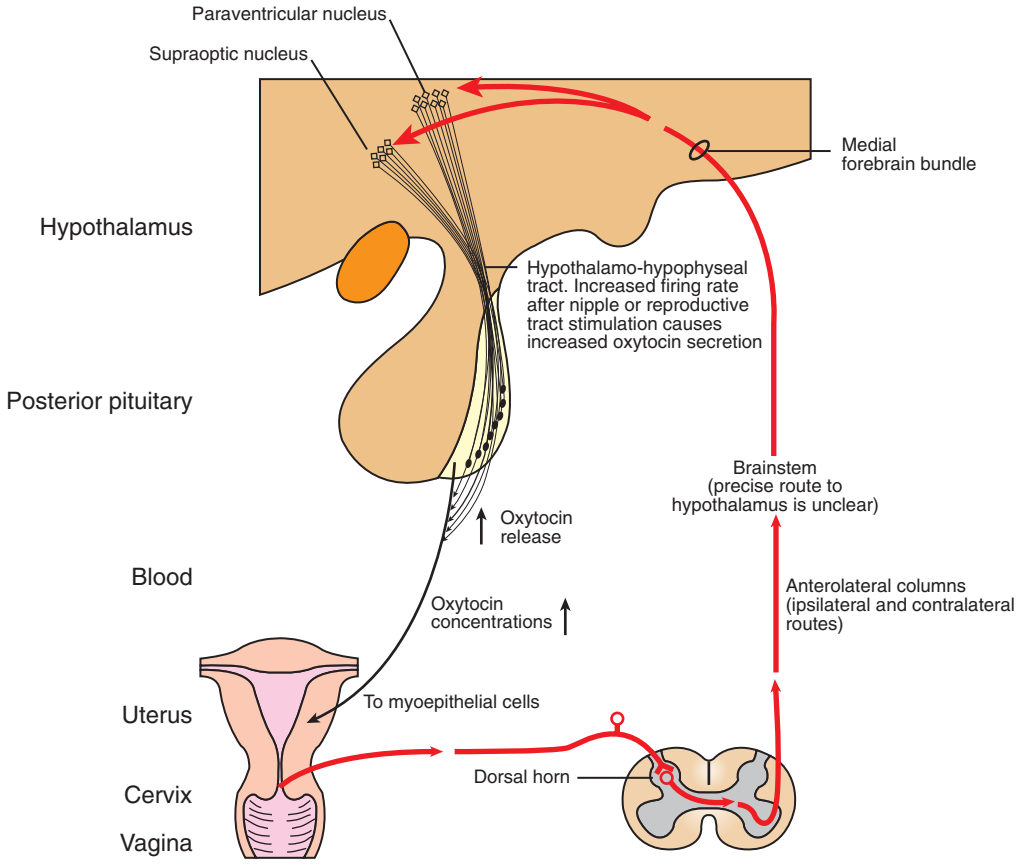

FIGURE 38-6 The neuroendocrine reflex (Ferguson reflex) underlying oxytocin synthesis and secretion. (From Johnson M, Everitt B, editors: *Essential reproduction,* ed 3, London, 1988, Blackwell Scientific.)

dogs, and horses, the source of relaxin is the placenta. In these species, significant relaxin production begins during the first part of gestation, with values sustained through parturition. A relaxin assay has been developed for the diagnosis of pregnancy in the dog, with good accuracy after 25 days of gestation. Relaxin may be important in these species for the maintenance of pregnancy in synergism with progesterone (see Figure 38-2).

The *first stage of parturition* involves presentation of the fetus at the internal os of the cervix. This likely results from increased myometrial activity caused by $PGF_{2\alpha}$ release. When the cervix opens and the fetus passes into the pelvic canal, myometrial contractions become less important for delivery of the fetus; *abdominal press,* accomplished by closure of the epiglottis and contraction of maternal abdominal muscles, becomes the main force involved in the delivery process. The actual delivery process is called the *second stage of parturition.*

The *third stage of parturition* involves the delivery of the fetal membranes. In litter-bearing animals, such as the cat, dog, and pig, the placental membranes are delivered often with, or immediately after, the appearance of each fetus. In single-bearing species, the placenta may be delivered immediately or within a few hours. From studies done on the mare at UC–Davis, we know that major, sustained surges of $PGF_{2\alpha}$ occur in the immediate postpartum period and are important for expulsion of placental membranes and reduction of uterine size through myometrial contraction. $PGF_{2\alpha}$ is likely the most important component of uterine size reduction in the immediate postpartum period for all domestic species. This can be inferred from the episodes of discomfort that parturient animals undergo during the hours immediately after delivery.

The neonate must make a major physiological adjustment to life on the outside. The major change involves the vascular system, in particular the respiratory system. During fetal life, blood bypasses the lungs (except for the perfusion of lung tissue in support of development) by two routes: through the ventricles by way of the *foramen ovale,* and from the pulmonary artery to the aorta by the *ductus arteriosus.* The foramen ovale is closed functionally at birth by a flap of tissue in the left ventricle through the development of higher pressures within the left versus the right ventricle. Although the ductus arteriosus immediately constricts at birth, it requires months before it is completely closed. This course of closure is also true for the *ductus venosus,* which serves as a hepatic shunt during fetal life. The rapid conversion from a fluid to a gaseous environment, as occurs at birth, is a truly remarkable adaptation.

CLINICAL CORRELATIONS

PROLONGED GESTATION

History. You are called to examine a purebred Holstein cow that is 12 days overdue compared with the herd gestation average of 280 days. She was artificially inseminated, was diagnosed pregnant 35 days later, and has not been observed in estrus since insemination. You inquire about the presence of bulls on the dairy farm, but there are none.

Clinical Examination. The cow has a greatly enlarged abdomen. On palpation of the uterus per rectum, you find the presence of a

large calf. The cow certainly appears to be term as far as the size of the calf. You are puzzled, however, by the lack of colostrum in the udder.

Comment. The history and physical examination findings are compatible with an animal that has a fetus that is defective in terms of the initiation of parturition. A normal fetal hypothalamic-pituitary-adrenocortical system is essential for the production of cortisol, which initiates the delivery process. In the cow, this can begin 3 to 4 weeks prepartum, with fetal cortisol directing the increased production of estrogen; this in turn eventually initiates $PGF_{2\alpha}$ synthesis and release. The deficit could be caused by a malformed adrenal gland, pituitary, or hypothalamus. In one syndrome described for Holsteins, the critical defect was a lack of corticotropin-producing cells in the pituitary, which led to inadequate stimulation of the adrenal cortex and inadequate fetal cortisol production. The lack of lactogenesis reflects that the endocrine changes beginning 3 to 4 weeks prepartum as a prelude to delivery are also important for lactogenesis, and in their absence, colostral formation is delayed.

Treatment. The animal can respond to glucocorticoids, with delivery usually occurring 2 to 3 days later. The placenta is normal in this situation, and the systemic administration of glucocorticoids substitutes for fetal cortisol in initiating the endocrine events that lead to parturition. Lactogenesis is usually initiated by glucocorticoid treatment, although the process is usually less advanced than that expected at normal delivery. Because the calf continues to grow in utero in this syndrome, it is often too large to be delivered *per vaginam,* and a cesarean section may have to be performed 2 to 3 days after treatment in concert with dilation of the cervix.

You need to tell the owner that the calf will likely not survive because of inadequate adrenal secretion. If the calf were an extremely valuable bull prospect, one could administer both glucocorticoids and mineralocorticoids for a number of months with the hope the animal would eventually be able to take over its own adrenal support (this actually occurred in one case at UC–Davis). It would be questionable, however, to initiate treatment of the calf on the basis that the disease is an autosomal recessive inherited condition.

EARLY EMBRYONIC DEATH IN A MARE

History. A new client calls; they have a 10-year-old Quarter Horse mare they would like to breed. The mare has previously had a few foals. Last year, she was bred, and determined to be pregnant by ultrasound exam at day 24 post-ovulation, but was not pregnant at day 60.

Clinical Examination. You perform a breeding soundness on the exam and everything appears to be normal. You recommend uterine cytology, culture, and biopsy to determine if there is an apparent cause of the pregnancy loss. The cytology and culture are normal. The biopsy detects some inflammation and fibrosis (category 2b). The inflammation is around the endometrial glands, and it can limit motility and clearance. These changes may be reversible, but a mare with a category 2b uterus has a decreased pregnancy rate (30% to 70%) depending on the severity of inflammation.

Comment. The normal cytology and culture at this time does not rule out that the mare did not have an infection (endometritis) at the time of her pregnancy last year. The biopsy indicates some inflammation, which could limit the ability of the endometrial cups to form and/or placenta to provide sufficient nutrition, oxygen and/or hormones (progesterone). Normally, the corpus luteum (CL) on the ovary will provide sufficient progesterone to maintain the pregnancy. If there is inflammation (i.e., endometritis, colic) present to stimulate release of $PGF_{2\alpha}$, this can cause lysis of the CL and loss of pregnancy. Mares can be checked to determine if they are producing sufficient levels of progesterone. If not, progesterone can be supplemented. The fetoplacental unit takes over production of progesterone at 90 days.

Treatment. The cause of the early embryonic death (EED) is not clear. It is possible that the fibrotic changes in the uterus could limit nutritional support of the fetus. It is also possible that the mare had endometritis last year, and/or the mare did not produce sufficient progesterone to maintain the pregnancy. To increase the chances the most, the mare can be flushed after breeding to decrease inflammation, administer oxytocin to decrease fluid accumulation, and supplement with progesterone.

PRACTICE QUESTIONS

1. Active rescue of luteal activity through suppression of pulsatile prostaglandin synthesis and release by the production of embryonic signals must occur in which of the following species in order for a developing pregnancy to have the early progestational support essential for pregnancy maintenance? (Select all that apply.)
   a. Cattle
   b. Dog
   c. Goat
   d. Horse
   e. Pig
   f. Sheep

2. In primates it has been established that estrogen production during much of pregnancy is a cooperative venture between fetal adrenals and the placenta. The domestic species most extensively studied in this regard is the horse. In this species the main two interactive organs involved in the synthesis of estrogen during pregnancy are the placenta and the:
   a. Fetal adrenals.
   b. Fetal gonads.
   c. Fetal liver.
   d. Fetal hypothalamus.
   e. Fetal pituitary.

3. Which of the following hormones initiates the final process that eventually leads to parturition?
   a. Maternal estrogen
   b. Maternal progesterone
   c. Fetal cortisol
   d. Maternal relaxin
   e. Maternal prostaglandin
   f. Maternal oxytocin

4. The hormone that initiates the myometrial contractile process that acutely initiates parturition is:
   a. Maternal estrogen.
   b. Maternal progesterone.
   c. Fetal cortisol.
   d. Maternal relaxin.
   e. Maternal prostaglandin.
   f. Maternal oxytocin.

5. The hormone released by the passage of the fetus into the pelvic canal through the cervix is:
   a. Maternal estrogen.
   b. Maternal progesterone.
   c. Fetal cortisol.
   d. Maternal relaxin.
   e. Maternal prostaglandin.
   f. Maternal oxytocin.

BIBLIOGRAPHY

Austin CR, Short RV, editors: *Reproduction in mammals*, vols 1-6, Cambridge, UK, 1986, Cambridge University Press.

Concannon PW, Morton DB, Weir BJ, editors: Dog and cat reproduction, contraception and artificial insemination, *J Reprod Fertil Suppl* 39, 1989.

Cupps PT, editor: *Reproduction in domestic animals*, ed 4, New York, 1991, Academic Press.

Feldman EC, Nelson RW, editors: *Canine and feline endocrinology and reproduction*, ed 4, Philadelphia, 2009, Saunders.

Hafez ESE, Hafez B, editors: *Reproduction in farm animals*, ed 7, Baltimore, 2000, Lippincott Williams & Wilkins.

Jackson PGG: *Handbook of veterinary obstetrics*, Philadelphia, 2004, Saunders.

Johnson MH, Everitt BJ, editors: *Essential reproduction*, ed 5, London, 2000, Blackwell Scientific.

LeBlanc MM, Lopate C, Knottenbelt DC, Pascoe RR: The mare. In Knottenbelt DC, Pascoe RR, Lopate C, LeBlanc MM, editors: *Equine stud farm medicine and surgery*, ed 2, Edinburgh, 2003, Saunders.

Lennoz-Roland M: *Practical uses of aglepristone: review of a recent expert meeting*. Presented at 5th Biannual Congress, *European Veterinary Society for Small Animal Reproduction (EVSSAR)*, Budapest, Hungary, 2006.

Neill JD, editor: *Knobil and Neill's physiology of reproduction*, vols 1 and 2, ed 3, Philadelphia, 2005, Elsevier.

Olson PN, Nett TM, Bowen RA, et al: Endocrine regulation of the corpus luteum of the bitch as a potential target for altering fertility, *J Reprod Fertil Suppl* 39:27–40, 1989.

Pineda MH, Dooley MP, editors: *McDonald's veterinary endocrinology and reproduction*, ed 5, Ames, 2003, Iowa State University Press.

Silva LD, Verstegen JP: Comparisons between three different extenders for canine intrauterine insemination with frozen-thawed spermatozoa, *Theriogenology* 44(4):571–579, 1995.

Simpson GM, England GC, Harvey MJ, editors: *BSAVA manual of small animal reproduction and neonatology*, Gloucester, UK, 2010, BSAVA.

Stout TA: The early pregnancy. In Samper JC, editor: *Equine breeding management and artificial insemination*, ed 2, St Louis, 2009, Saunders.

Van der Weyden GC, Taverne MA, Dieleman SJ, et al: Physiological aspects of pregnancy and parturition in dogs, *J Reprod Fertil Suppl* 39:211–214, 1989.

CHAPTER 39
The Mammary Gland

KEY POINTS

Anatomical aspects of the mammary gland
1. The milk-secreting cells of the mammary gland develop through the proliferation of epithelium into hollow structures called alveoli.
2. Most of the milk that accumulates before suckling or milking is stored in the alveoli, even though animals have enlarged milk-storage areas called cisterns.
3. A suspensory system involving the udder of the cow allows the animal to carry a large amount of milk.

Control of mammogenesis
1. Initial development of the mammary gland is programmed by embryonic mesenchyme.
2. Proliferation of the mammary duct system begins at puberty, with ducts under the control of estrogens, growth hormone, and adrenal steroids, and alveoli under the control of progesterone and prolactin.

Colostrum
1. Prepartum milk secretion (without removal) results in the formation of colostrum.
2. The ingestion of colostrum is important because of the passive immunity it confers through the presence of high concentrations of immunoglobulins.
3. The time immunoglobulins can be absorbed through the neonatal gut is limited to the first 24 to 36 hours of life.
4. Lipids (particularly vitamin A) and proteins (caseins and albumins) are high in concentration in colostrum; carbohydrates (lactose) are low.

Lactogenesis
1. Prolactin, inhibited by dopamine and stimulated by vasoactive intestinal peptide, is the most important hormone involved in the process of milk synthesis, or lactogenesis; growth hormone is also important for lactogenesis.
2. The release of fat into milk from the alveolar cell involves constriction of the plasma membrane around the fat droplet; fats are dispersed in milk in droplet form.
3. Milk proteins and lactose are released from alveolar cells by the process of exocytosis.

Milk removal
1. Efficient milk removal requires the release of oxytocin, which causes contraction of muscle cells that surround the alveoli (myoepithelial cells), and movement of milk into the ducts and cisterns.

First nursing
1. Carbohydrate stores are good in neonates born as singles or twins, whereas carbohydrate stores are low in neonates born in litters; consequently the former can stand a longer interval to first suckling than can the latter.

Composition of milk
1. Fats are the most important energy source in milk.
2. Lactose, composed of glucose and galactose, is the main carbohydrate of mammalian milk.
3. The main proteins in milk are called *caseins* and are found in curd.

The lactation cycle
1. Milk production peaks at 1 month postpartum in dairy cattle, followed by a slow decline in production; milking usually stops at 305 days of lactation so that the animal can prepare the mammary gland for the next lactation.
2. Lactation can be induced by hormone administration (estrogen and progesterone) and enhanced by growth hormone and increased photoperiod exposure.

Diseases associated with the mammary gland
1. The main diseases that affect the mammary gland directly are mastitis (prevalent in dairy cattle and dogs) and neoplasia (prevalent in intact dogs and cats).
2. The main conditions that involve the mammary gland indirectly are passive transfer of red blood cell agglutinating antibodies by the ingestion of colostrum (mare, queen) and hypocalcemia caused by the transient drain of calcium that occurs with initiation of lactation (dairy cattle) or during the perinatal period (dog).

Animals that belong to the class *Mammalia* are characterized as having bodies that are basically covered with hair, delivering live young instead of eggs (the monotremes are an exception), and, pertinent to this chapter, nurturing their young through the use of structures called *mammary glands*. The ability of mammals to nurture their young through milk secretion by mammary glands during the early part of post-fetal life has given these animals survival advantages. Because the reproductive strategy of mammals involves the production of far fewer young, compared with reptiles, amphibians, and birds, mammary glands have allowed mammals to be much more efficient in the nurture of their young. Egg-laying classes of animals, such as fish, reptiles, and amphibians, depend on favorable environmental factors for the nurture of their young; the offspring are often vulnerable to the vagaries of nature. Mammalian young do not require teeth for the suckling process and thus can be delivered with immature maxillae and mandibles, which facilitates the delivery of the head. The development of teeth coincides with the need to consume food other than milk.

ANATOMICAL ASPECTS OF THE MAMMARY GLAND

The Milk-Secreting Cells of the Mammary Gland Develop Through the Proliferation of Epithelium into Hollow Structures Called Alveoli

Embryonic ectoderm is the source of the mammary glands. The mammary ectoderm is first represented by parallel linear thickenings on the ventral belly wall. The continuity of the ridge that is formed is broken into the appropriate number of *mammary buds,* from which the functional part of the mammary gland will be derived.

The *parenchyma,* or milk-secreting cells, of the mammary gland develops through the proliferation of epithelial cells that arise from the primary mammary cord. The epithelial cells eventually form hollow, circular structures called *alveoli,* which are the fundamental milk-secreting units of the mammary gland (Figure 39-1). In concert with this development, an enlarged area of epithelium, the *nipple,* which is the external connection to the internal milk-secreting system, develops on the surface. In males, although nipples often develop, the underlying primary mammary cord does not develop into substantial glandular tissue.

Most of the Milk That Accumulates Before Suckling or Milking Is Stored in the Alveoli, Even Though Animals Have Enlarged Milk-Storage Areas Called Cisterns

Duct systems connect alveoli with the nipple, or teat, enabling milk to pass from the area of formation to the area of delivery (nipple). The ducts may come together so that there is only one final duct per gland, which has one opening through the nipple, or teat, such as occurs in cattle, goats, and sheep. Two main ducts and associated openings occur in the mare and sow, whereas the cat and dog can have 10 or more openings in the nipple, with each opening representing separate glands (Figure 39-2). Both the cow and the doe (goat) have specialized areas for holding milk, called *cisterns,* which are located in the ventral part of the gland and into which all main ducts empty (Figure 39-3). This has enabled the cow, for example, to synthesize and store larger amounts of milk than would otherwise be possible. Despite this adaptation, it is important to realize that a majority of the milk present at the time of milking is stored in the duct system of the mammary glands.

Mammary glands develop typically as paired structures. The number of pairs in domestic animals varies from one in goats, horses, and sheep; two in cattle; to seven to nine in the sow and seven to ten in the bitch and queen. The position of mammary glands varies in animals, being thoracic in primates; extending the length of the thorax and abdomen in cats, dogs, and pigs; and being inguinal in cattle, goats, and horses. In domestic species, such as cattle, goats, horses, and sheep, pairs of mammary glands are closely apposed to each other; the resulting structure is called an *udder.* In the cow, for example, two pairs of glands (four quarters) compose the udder.

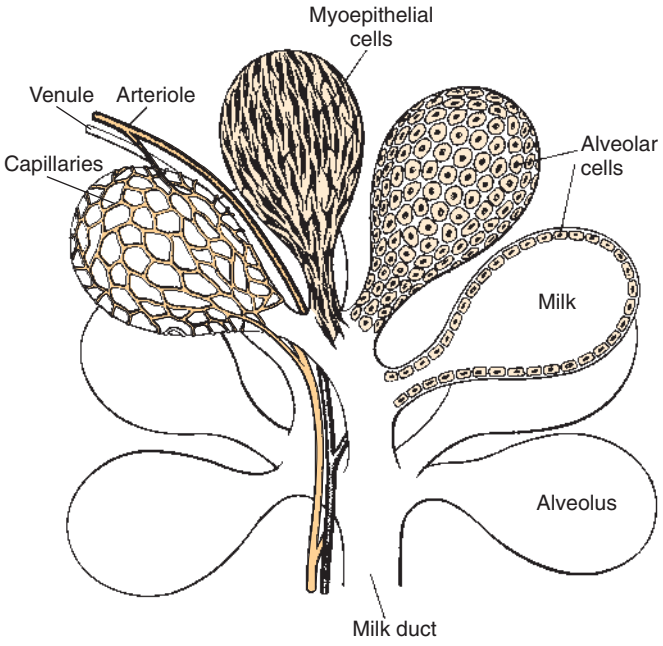

FIGURE 39-1 Diagram of a cluster of alveoli in the mammary gland of a goat. (From Cowie AT: Lactation. In Austin CR, Short RV, editors: *Reproduction in mammals,* ed 2, vol 3, Hormonal control of reproduction, Cambridge, UK, 1984, Cambridge University Press.)

A Suspensory System Involving the Udder of the Cow Allows the Animal to Carry a Large Amount of Milk

One of the important anatomical adaptations of the udder that allows dairy cows to carry large amounts of milk is the development of a suspension system for the udder. This system is formed by the median suspensory ligament (formed between pairs of mammary glands) composed of elastic connective tissue that originates from the abdominal tunic. The lateral (nonelastic) suspensory ligament, which originates from prepubic and subpubic ligaments, enters the glands laterally at various levels to become part of the interstitial connective tissue framework of the udder. It is not unusual for heavy-producing dairy cows to have 25 kg (55 lb) of milk in their udder immediately before milking. If the suspensory support system were not in place, the mammary gland system would soon break down from the weight of the milk.

CONTROL OF MAMMOGENESIS

Initial Development of the Mammary Gland Is Programmed by Embryonic Mesenchyme

The fetal development of the mammary gland is under both genetic and endocrine control. The initial development of the mammary bud is under control of embryonic *mesenchyme* (connective tissue). If mammary mesenchyme is transplanted to another area, mammary bud formation will occur at the site of transplantation. Although little is known about fetal mammary development, it is not thought to be driven by hormones. However, actively secreting mammary glands may be present at birth as a result of exogenous administration of certain hormones to the mother.

Proliferation of the Mammary Duct System Begins at Puberty, with Ducts Under the Control of Estrogens, Growth Hormone, and Adrenal Steroids, and Alveoli Under the Control of Progesterone and Prolactin

Development of the mammary gland in post-fetal life usually starts in concert with puberty. Cyclical ovarian activity results in the production of estrogen and progesterone. Estrogen, with growth hormone and adrenal steroids, is responsible for

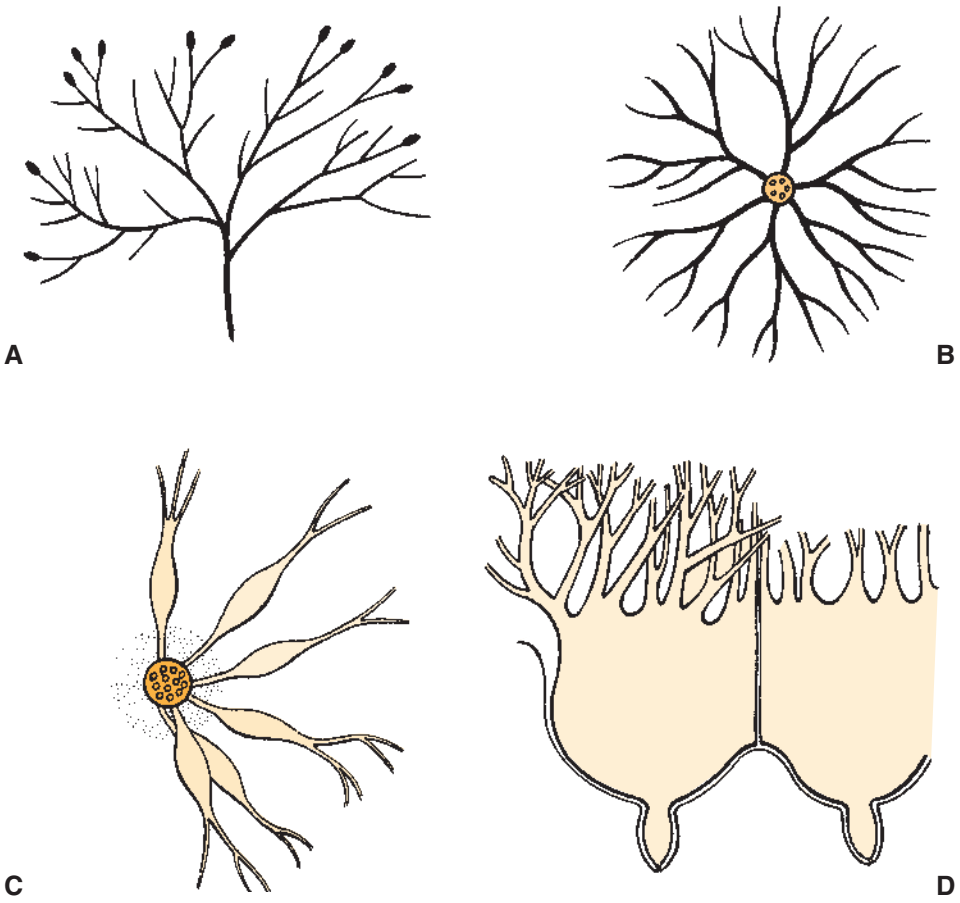

FIGURE 39-2 Diagram showing different arrangements of the mammary duct system. **A,** Cow, goat, sheep. **B,** Horse, pig. **C,** Cat, dog. **D,** Cow, goat cistern. (From Cowie AT: Lactation. In Austin CR, Short RV, editors: *Reproduction in mammals,* ed 2, vol 3, Hormonal control of reproduction, Cambridge, UK, 1984, Cambridge University Press.)

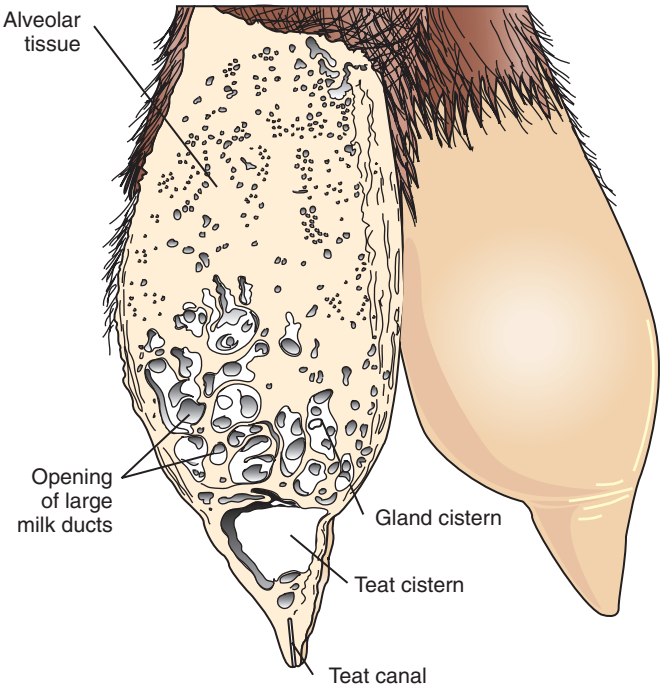

FIGURE 39-3 Depiction of the udder of a goat in which a section of the left mammary gland shows the dense alveolar tissues, the gland cistern with the large ducts opening into it, the teat cistern, and the teat canal. (From Cowie AT: Lactation. In Austin CR, Short RV, editors: *Reproduction in mammals,* ed 2, vol 3, Hormonal control of reproduction, Cambridge, UK, 1984, Cambridge University Press.)

proliferation of the duct system. The development of alveoli from the terminal ends of the ducts requires the addition of progesterone and *prolactin* (Figure 39-4).

Although the development of the mammary gland begins with the onset of puberty, the gland remains relatively undeveloped until the occurrence of pregnancy. In most domestic animals, udder development usually becomes evident by the middle of gestation; the secretion of milk often begins during the latter part of gestation (mainly from increasing *prolactin* secretion) and results in the formation of colostrum, as discussed later. By the end of pregnancy, the mammary gland has been transformed from a structure involving mostly stromal (connective tissue) elements to a structure that is filled with alveolar cells that are actively synthesizing and secreting milk. Groups of adjacent alveoli form *lobules* that further combine into larger structures called *lobes.* Connective tissue bands delineate the lobules and the lobes (Figure 39-5).

COLOSTRUM

Prepartum Milk Secretion (Without Removal) Results in the Formation of Colostrum

The milk formed before parturition is called *colostrum.* Its formation represents a secretory process in which lactogenesis occurs in the absence of milk removal. *Lactation* cannot fully blossom until pregnancy is terminated, however, because of the inhibitory effects of progesterone and estrogen on milk secretion, inhibitory factors that are removed at or just before delivery.

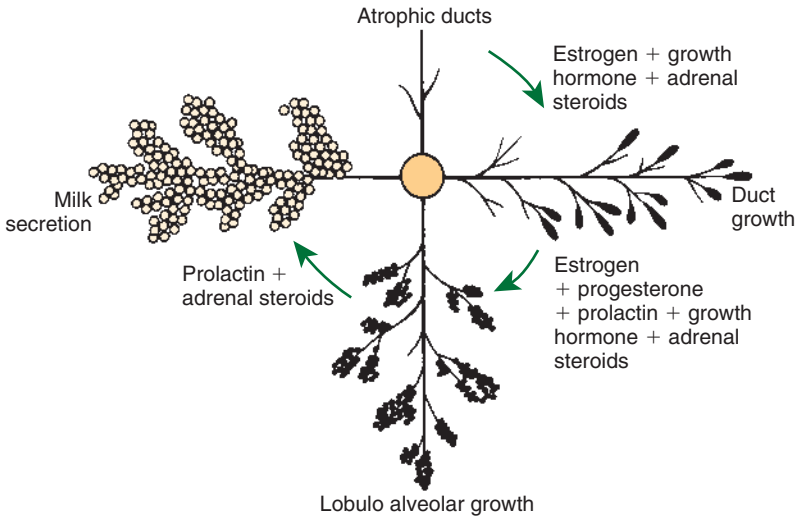

FIGURE 39-4 Hormones involved in the growth of the mammary gland and in the initiation of milk secretion in the hypophysectomized-ovariectomized-adrenalectomized rat. (From Lyons WR: *Proc R Soc B* 149:303, 1958. In Austin CR, Short RV, editors: *Reproduction in mammals,* ed 2, vol 3, Hormonal control of reproduction, Cambridge, UK, 1984, Cambridge University Press.)

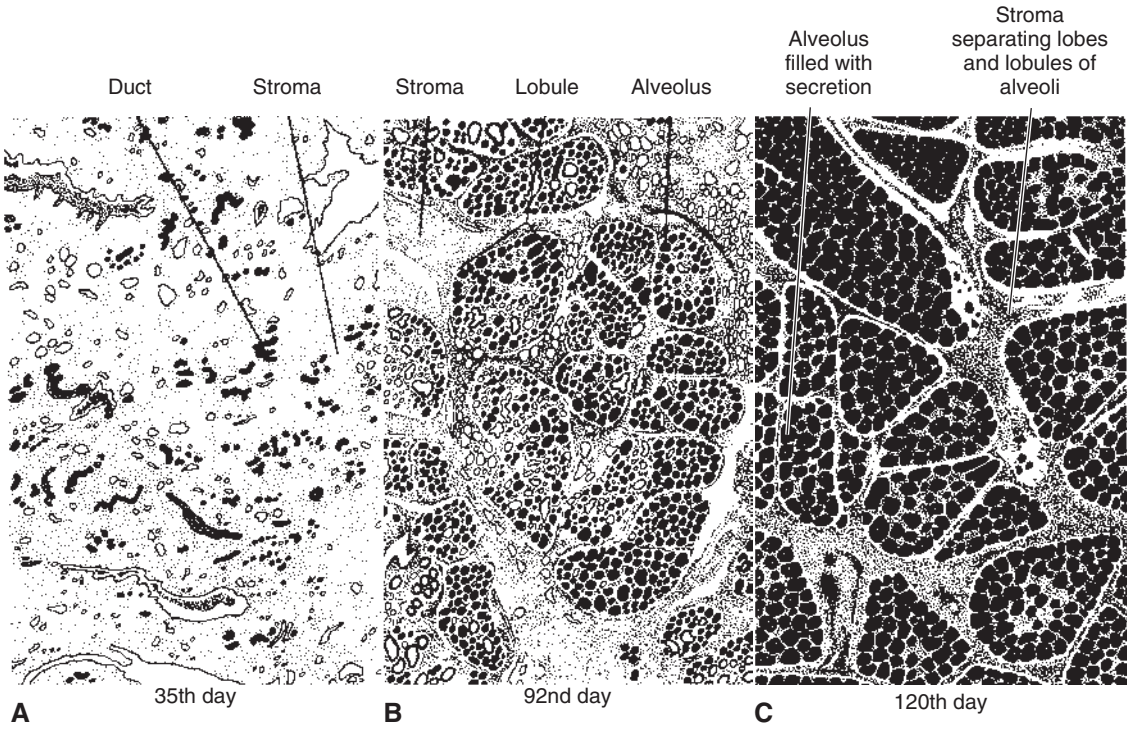

FIGURE 39-5 Drawings of sections of the mammary gland of the goat at three different times during pregnancy (which lasts approximately 150 days). **A,** Note the small collections of ducts scattered throughout the stroma on the 35th day. **B,** On the 92nd day the lobules of alveoli are forming in groups known as lobes; secretion is present in some of the alveolar lumina, and there is still considerable stromal tissue. **C,** On the 120th day the lobules of alveoli are almost fully developed; the alveoli are full of secretion, and the stromal tissue is reduced to thin bands separating lobules and thicker strands between lobes. (From Falconer IR, editor: *Lactation,* London, 1970, Butterworths.)

The Ingestion of Colostrum Is Important Because of the Passive Immunity It Confers Through the Presence of High Concentrations of Immunoglobulins

When colostrum is formed before parturition, certain substances are concentrated in the process. Ingestion of colostrum is important for the well-being of the neonate. In addition to nutrition, colostrum has an important function in temporary, or passive, protection against infectious agents. *Immunoglobulins* (e.g., immunoglobulin A, or IgA) are produced in the mammary gland by plasma cells (derived from B lymphocytes originating in the gut) as a result of exposure of the mother to certain microorganisms. The immunoglobulins gain access to the milk system through the migration of the plasma cells from adjacent tissue sites. The immunoglobulins are highly concentrated in colostrum, and through the consumption of colostrum, the neonate can receive passive immunity against pathogens experienced by

the mother. This allows the young to receive immediate protection from environmental organisms. The neonates of all domestic animals acquire antibodies through the ingestion of colostrum. The absorption of antibodies through milk in domestic animals contrasts with the situation in other species, including humans, rabbits, and guinea pigs, in which a more substantial amount of antibody is passed to the fetus through the placenta.

The Time Immunoglobulins Can Be Absorbed Through the Neonatal Gut Is Limited to the First 24 to 36 Hours of Life

Neonates usually have a limited time (24 to 36 hours) in which immunoglobulins (proteins) can be absorbed through the gut. Thus the feeding of colostrum within this period is important to ensure the presence of immunoglobulins in the newborn. Other antimicrobial factors found in milk that are important for protection against the development of pathogenic enteric bacterial flora include lysozymes, lactoferrin, and the lactoperoxidase system.

Lipids (Particularly Vitamin A) and Proteins (Caseins and Albumins) Are High in Concentration in Colostrum; Carbohydrates (Lactose) Are Low

Colostrum is a rich source of nutrients, especially vitamin A, in addition to immunoglobulins. Placental transfer of vitamin A is limited in domestic animals, with calves and piglets being particularly low in vitamin A at birth. This deficiency is corrected by the ingestion of colostrum. Lipids and proteins, including caseins and albumins, are also present in relatively high concentration in colostrum. One exception is lactose; its synthesis is significantly inhibited by progesterone until about the time of delivery. Nevertheless, at the moment of delivery, the newborn's milk supply is nutritive (high protein, fat, and vitamin A content) and protective (immunoglobulins) (Table 39-1).

TABLE 39-1 Amounts of Selected Components of Bovine Colostrum as Percentage of Level in Normal Milk

	Day(s) After Parturition		
Constituent	**0**	**3**	**4**
Dry matter	220	100	100
Lactose	45	90	100
Lipids	150	90	100
Minerals	120	100	100
Proteins			
Casein	210	110	110
Albumin	500	120	105
Globulin	3500	300	200
Vitamins			
A	600	120	100
Carotene	1200	250	125
E	500	200	125
Thiamine	150	150	150
Riboflavin	320	130	110
Pantothenic acid	45	110	105

From Jacobson NL, McGilland AD: The mammary gland and lactation. In Swenson MJ, editor: *Dukes' physiology of domestic animals,* ed 10, Ithaca, NY, 1984, Cornell University Press.

LACTOGENESIS

Prolactin, Inhibited by Dopamine and Stimulated by Vasoactive Intestinal Peptide, Is the Most Important Hormone Involved in the Process of Milk Synthesis, or Lactogenesis; Growth Hormone Is Also Important for Lactogenesis

Prolactin plays an important role in the secretion of milk, or *lactogenesis.* Prolactin is released in conjunction with manipulation of the teat through either the suckling or the milking process. Sensory stimuli are carried into the hypothalamus, and the synthesis and release of *dopamine,* a major inhibitor of prolactin secretion, is blocked while neurons in the *paraventricular nucleus* are stimulated to produce and release *vasoactive intestinal peptide,* a stimulator of prolactin release (Figure 39-6). A short-lived surge of prolactin secretion occurs immediately after the onset of milk removal; peak values are usually reached within 30 minutes after the initial stimulus. Major surges of prolactin apparently do not need to be elicited on an hourly basis to maintain lactation because 12-hour release intervals, as occur in association with the milking of dairy cows, are sufficient to maintain lactogenesis. Prolactin responses, as judged by the amount of hormone release after mammary gland stimulation, decrease as the lactation period progresses.

Another major hormone required for milk production in ruminants is *growth hormone* (GH). There is now considerable interest in the use of GH to promote additional milk production from cows through exogenous administration of the hormone.

The Release of Fat into Milk from the Alveolar Cell Involves Constriction of the Plasma Membrane Around the Fat Droplet; Fats Are Dispersed in Milk in Droplet Form

The synthesis and release of milk by alveolar epithelial cells is a remarkable physiological process (Figure 39-7). Alveolar cells synthesize fats, proteins, and carbohydrates and extrude the products into the lumen of the alveolus. Fat droplets first accumulate in the basal cytoplasm of the cell and then move to the apex, where the droplet protrudes into the alveolar lumen. The cell membrane constricts about the base of the fat droplet, so fat is dispersed in milk in small droplets, surrounded by cell membranes; the droplet often contains portions of cell cytoplasm.

Milk Proteins and Lactose Are Released from Alveolar Cells by the Process of Exocytosis

Milk proteins are synthesized on the endoplasmic reticulum; the casein molecules pass to the Golgi apparatus, where they are phosphorylated and formed into micelles within the Golgi vesicles. Lactose is also synthesized within the Golgi vesicles and is released in conjunction with milk proteins. The process of extrusion of proteins and carbohydrates is different from that of fat; the Golgi vesicles fuse with the cell membrane, and the release of proteins and carbohydrates occurs by *exocytosis.* Although it is not certain how often cells go through a synthesis and extrusion cycle, it may occur twice daily, particularly in dairy cows that are milked two times per day.

MILK REMOVAL

For lactogenesis to be maintained, milk must be removed from the mammary gland by suckling or milking. If milk is not removed within about 16 hours in dairy cows, the synthesis of milk begins to be suppressed. As indicated previously, most of the milk in the

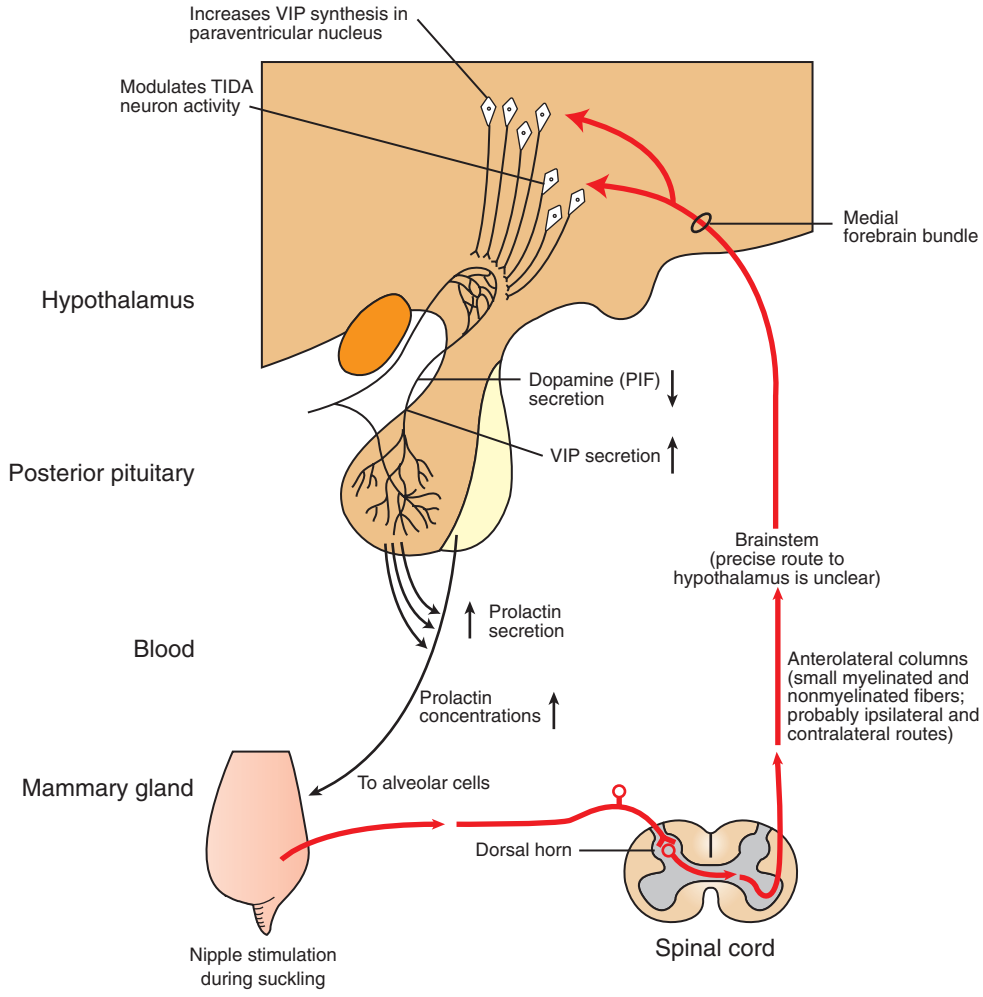

FIGURE 39-6 Somatosensory pathways in the suckling-induced reflex release of prolactin. The exact route taken by sensory information between brainstem and hypothalamus is speculative. Although tuberoinfundibular *(TIDA)* dopamine neuron activity is modulated as a result of the arrival of this somatosensory-derived input (prolactin inhibiting factor, *PIF*), the increased secretory activity of neurons containing vasoactive intestinal peptide *(VIP)* in the paraventricular nucleus is probably also crucial in driving prolactin secretion during suckling. (Modified from Johnson M, Everitt B: *Essential reproduction,* ed 3, London, 1988, Blackwell Scientific.)

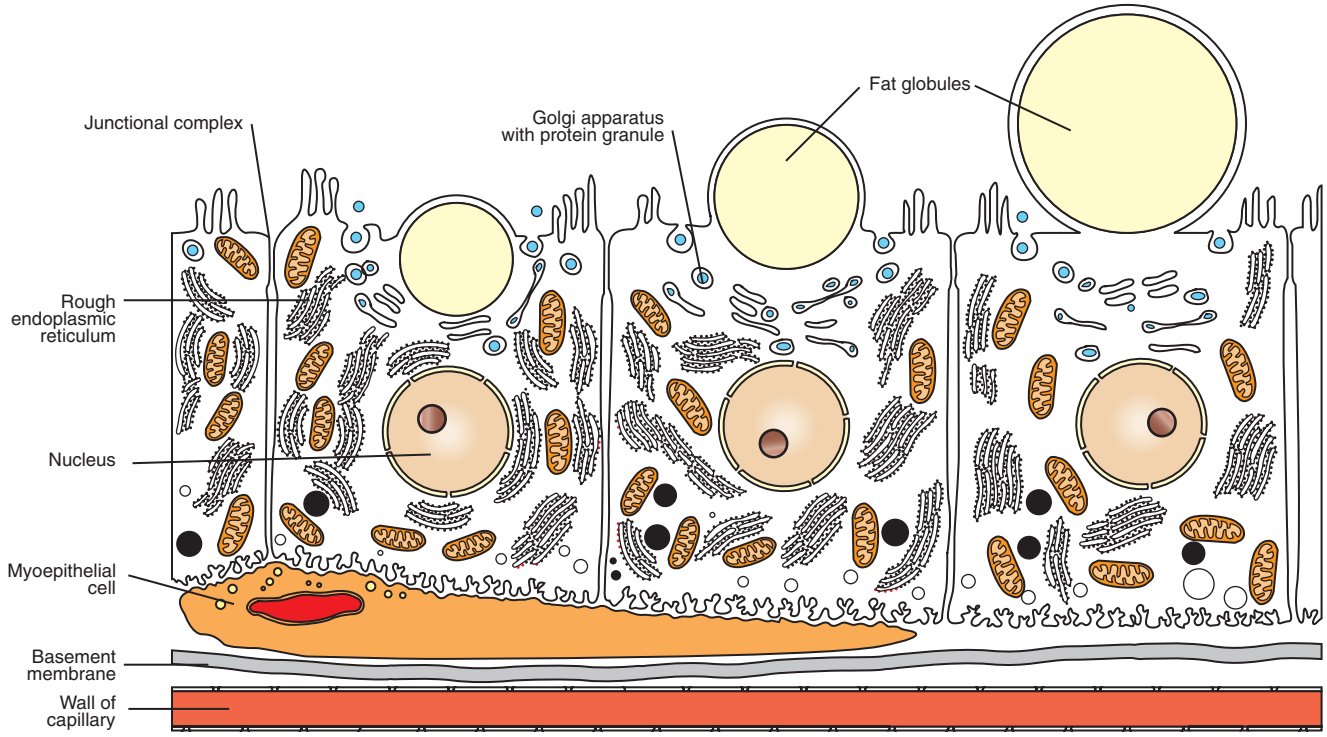

FIGURE 39-7 Diagram of the ultrastructure of three alveolar cells and a myoepithelial cell. (From Cowie AT: Lactation. In Austin CR, Short RV, editors: *Reproduction in mammals,* ed 2, vol 3, *Hormonal control of reproduction,* Cambridge, UK, 1984, Cambridge University Press.)

udder of a dairy cow at the time of milking is located in the ducts and alveoli. The movement of milk into the gland cistern at suckling or milking would be slow, and less milk would be obtained during the milking of a cow, if the drainage of milk were a passive process.

Efficient Milk Removal Requires the Release of Oxytocin, Which Causes Contraction of Muscle Cells That Surround the Alveoli (Myoepithelial Cells), and Movement of Milk into the Ducts and Cisterns

To facilitate the process of milk removal, *myoepithelial cells* surround the alveoli and ducts (see Figures 39-1 and 39-7). The myoepithelial cells are particularly responsive to oxytocin and, in fact, contract when exposed to the hormone. The synthesis and release of oxytocin from the posterior pituitary is elicited by a neuroendocrine reflex involving tactile stimulation of the udder by suckling by the young, or the manual stimulation of washing before milking. The sensory stimuli from the udder are carried through the spinal cord into the hypothalamus. Neurons in the paraventricular and supraoptic nuclei are stimulated to synthesize oxytocin and release it from nerve terminals that impinge on the median eminence (Figure 39-8). Other sensory stimuli that elicit oxytocin release include auditory, visual, and olfactory stimuli that occur near or within the kennel, cattery, or milking parlor. Past societies used various deceptions to have earlier breeds of cattle release their milk. They often allowed the calf to suckle one teat while they milked the other glands. They also knew about the *Ferguson reflex,* if not in name, in which stimulation of the cervix (and release of oxytocin) was elicited by blowing air into the vagina using hollow tubes.

The release of oxytocin occurs within seconds after the stimulus arrives in the hypothalamus; increased pressure within the mammary gland is evident within a minute of stimulation as milk is forced out of the alveoli and ducts because of contraction of the myoepithelial cells. The term used in mammals to describe this phenomenon is *milk letdown.* Increased pressure within the udder is often obvious within a minute of the stimulation. The release of oxytocin lasts only a few minutes, and it is important that the milking process begin soon after milk letdown is complete (Figure 39-9). The milking process, as done by machine or by hand in earlier times, is often completed within 4 to 5 minutes.

It is interesting to compare stimuli that release oxytocin, which initiates the passive part of lactogenesis, with stimuli that release prolactin, which directly influences lactogenesis. Any sensory stimulus that a cow associates with milking has the potential for releasing oxytocin. The neuroendocrine reflex is elicited in the expectation of milk removal because of the environment (kennel, cattery, or milking parlor) to which the animal is exposed. Prolactin, on the other hand, is released only by tactile stimulation of the udder. The latter makes sense, because there is no need to stimulate milk synthesis and release unless the evidence for milk removal (udder stimulation) is strong. Milk removed during hand milking is trapped in the teat and forced out, whereas milk removed by milking machines moves by suction.

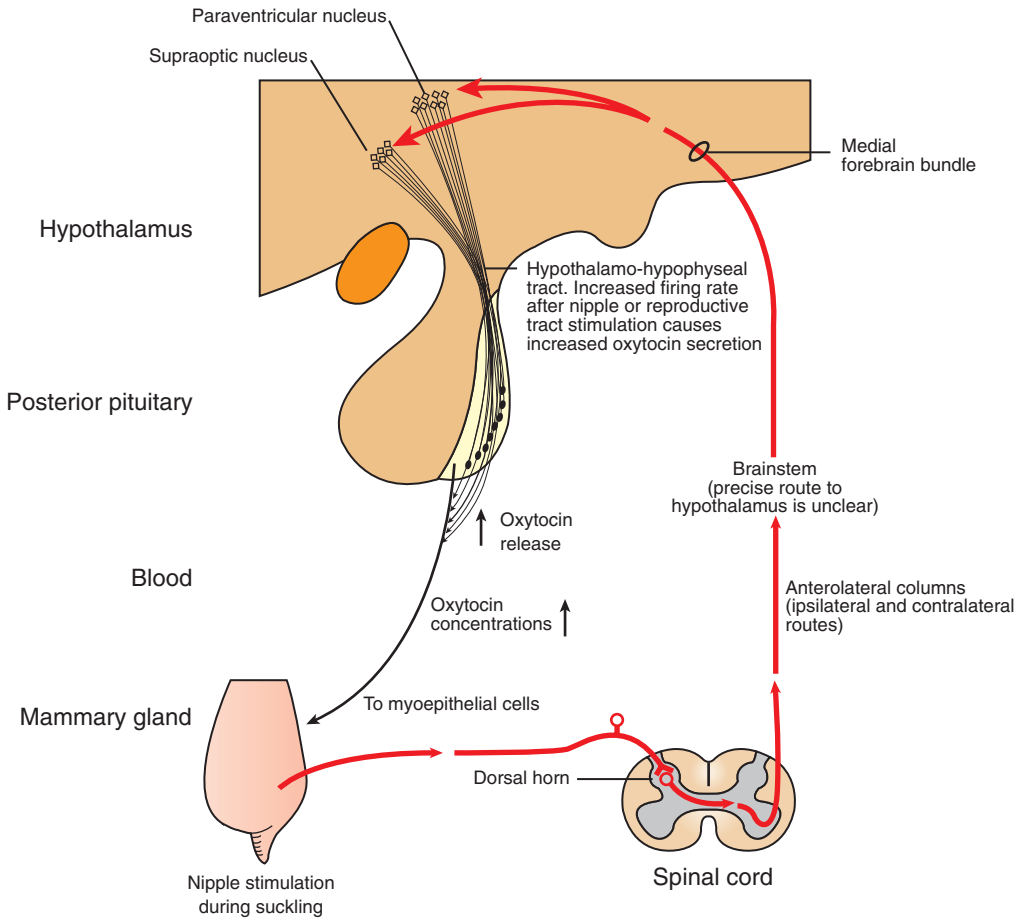

FIGURE 39-8 Somatosensory pathways in the suckling-induced reflex release of oxytocin. The actual pathway of sensory input in the hypothalamus is unknown, but it probably involves the medial forebrain bundle. (Modified from Johnson M, Everitt B: *Essential reproduction,* ed 3, London, 1988, Blackwell Scientific.)

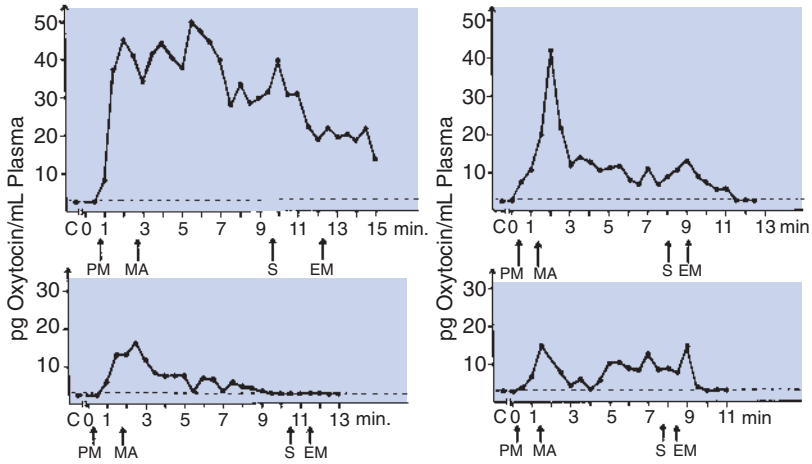

FIGURE 39-9 Oxytocin in the blood of cows before, during, and after milking. *Abscissae* show time in minutes. *C,* Control level; *EM,* end of machine milking; *PM,* preparation for milking; *MA,* application of teat cups; *S,* stripping. (From Schams et al: *Acta Endocrinologica* 92:258–270, 1979.)

FIRST NURSING

Carbohydrate Stores Are Good in Neonates Born as Singles or Twins, Whereas Carbohydrate Stores Are Low in Neonates Born in Litters; the Former Can Stand a Longer Interval to First Suckling Than Can the Latter

In domestic animals that have one or two offspring, such as cattle, horses, sheep, and goats, the young have to be able to stand in order to suckle. In this situation, neonates have reasonably good carbohydrate stores, and suckling may not occur for 1 to 2 hours without adverse effect as the young gain the ability to stand and locate the mammary gland. Young that are part of litters (cats, dogs, and pigs) are usually immediately nestled toward the mammary glands and often will be sucking in less than 30 minutes. This is important for animals born in litters because they tend to be immature at birth and susceptible to hypoglycemia, and suckling delays are often detrimental to their survival. Hypoglycemia results in stasis of the bowel (ileus) and can promote neonatal sepsis (overwhelming infection).

The suckling interval during the neonatal period varies considerably among domestic animals. Species nursing litters, such as cats, dogs, and pigs, often nurse at intervals of 1 hour or less. Goats, horses, and sheep nurse at slightly longer intervals, often up to 2 hours. Rabbits are an exception regarding the time between suckling periods; their young nurse at 24-hour intervals. As can be imagined, baby rabbits are engorged after each suckling period.

COMPOSITION OF MILK

Fats Are the Most Important Energy Source in Milk

Of the components of milk, fat is the most important energy source. Milk fat is composed of a number of lipids, including monoglycerides, diglycerides, triglycerides, free fatty acids, phospholipids, and steroids. Triglycerides are the main component of milk fat. The types of lipid synthesized are complex, with great variations in both chain length and saturation of fatty acids observed on the basis of species. The amount of fat produced varies greatly both within and among species (Table 39-2). Marine mammal milk has a high fat content, with values of about 40% to 50% in seals, 40% in dolphins, and 30% in whales. In these species the high energy content of the milk through fat helps offset the heat loss of the young.

TABLE 39-2 Composition of Milk from Various Species (Percentage)

Species	Fat	Protein	Lactose	Ash
Cat	7.1	10.1	4.2	0.5
Cow	3.5	3.1	4.9	0.7
Dog	9.5	9.3	3.1	1.2
Goat	3.5	3.2	4.6	0.8
Horse	1.6	2.4	6.1	0.5

Modified from Jacobson NL, McGilland AD: The mammary gland and lactation. In Swenson MJ, editor: *Dukes' physiology of domestic animals,* ed 10, Ithaca, NY, 1984, Cornell University Press.

In domestic animals, sheep, swine, dogs, and cats have milk that ranges from 7% to 10% in fat content. Dairy cattle have values that range from 3.5% to 5.5%; goats are similar to cows (3.5%); and mares have lower values (1.6%). In the past, milk was sold on a butterfat basis, and breeds that had a relatively high butterfat content of milk (e.g., the Jersey with 5% butterfat) found more acceptance in dairy operations than is currently the case. Small farms produced mainly cream (for butter manufacture); the fat-concentrated portion of milk was produced by use of a separator that separated cream on the basis of specific gravity and centrifugal force. Because milk is now sold on a solids, not fat, basis, breeds that produce more milk (and protein) are favored, even though the fat content of the favored breed, Holstein-Friesian, is lower (3.5%).

Lactose, Composed of Glucose and Galactose, Is the Main Carbohydrate of Mammalian Milk

Lactose is the main carbohydrate of most mammals. It is composed of glucose and galactose. Blood glucose is the main precursor molecule for lactose, with propionate an important precursor for glucose in ruminants. Lactose is formed under the direction of *lactose synthetase,* an enzyme composed of *α-lactalbumin* (a milk protein) and *galactosyl transferase.* Lactose synthesis is held

in abeyance until immediately before term because progesterone is inhibitory for the formation of α-lactalbumin. Prolactin, on the other hand, is stimulatory for the formation of lactose synthetase. Animals must have the enzyme *lactase* present in the jejunum for lactose to be cleaved (to glucose and galactose) and used. Lactase is present in most mammalian young but is sometimes not present in adult animals, including humans. In the absence of lactase, lactose can have an osmotic effect in the gastrointestinal tract, which can lead to diarrhea.

The Main Proteins in Milk Are Called *Caseins* and Are Found in Curd

The main proteins produced by the alveolar cells are called *caseins.* Caseins can be removed (as a curd) from milk through a process called *curdling* or *coagulation,* with other milk proteins, such as albumins and globulins, remaining in the fluid part of the milk (whey).

THE LACTATION CYCLE

The time required for changeover from colostrum to normal milk secretion varies with each species. In cattle, colostral milk tends to be stringy and yellow for several days postpartum. The complex bovine udder needs time for all areas to be flushed of colostrum. The milk of cattle is withheld from the milk supply for several days because of its unacceptable aesthetic quality, not because of the basic quality of the milk.

Milk Production Peaks at 1 Month Postpartum in Dairy Cattle, Followed by a Slow Decline in Production; Milking Usually Stops at 305 Days of Lactation So That the Animal Can Prepare the Mammary Gland for the Next Lactation

Milk production tends to increase for the first 3 to 4 weeks of lactation and then begins to slowly decline through the end of lactation (Figure 39-10). Cows are usually "dried up" after 305-day lactational periods; pounds of milk and butterfat production rates are calculated on this basis. Dairy animals are forced to stop lactating in order to prepare for the next lactation. The usual procedure is to stop milking. The back pressure of milk within the alveoli gradually inhibits the secretion of milk by the alveolar epithelial cells, with a resultant regression of the alveolar cells and small ducts. The process, called *involution,* often requires at least a month, with a 6-week period usually desired as the minimal interval from drying off to the onset of the next lactational period. Within 1 to 2 months, the secretory (alveoli) and excretory (duct) systems regress and are once again replaced. The process by which epithelial structures regress, yet retain coding for the renewal of duct and alveolar systems, is truly remarkable.

Lactation Can Be Induced by Hormone Administration (Estrogen and Progesterone) and Enhanced by Growth Hormone and Increased Photoperiod Exposure

The *induction of lactation* by hormone treatment is sometimes desired, especially in dairy animals with high-lactation records but poor reproductive performance. The use of a combined treatment of estrogen and progesterone over a relatively short period (1 week) has induced alveolar development sufficiently to result in milk production. Although the amount of milk produced is less than normal, the cows can be maintained in the milking string while efforts to impregnate them continue. To induce lactogenesis by hormonal means, animals should not be lactating at treatment and should have mammary glands free of infection.

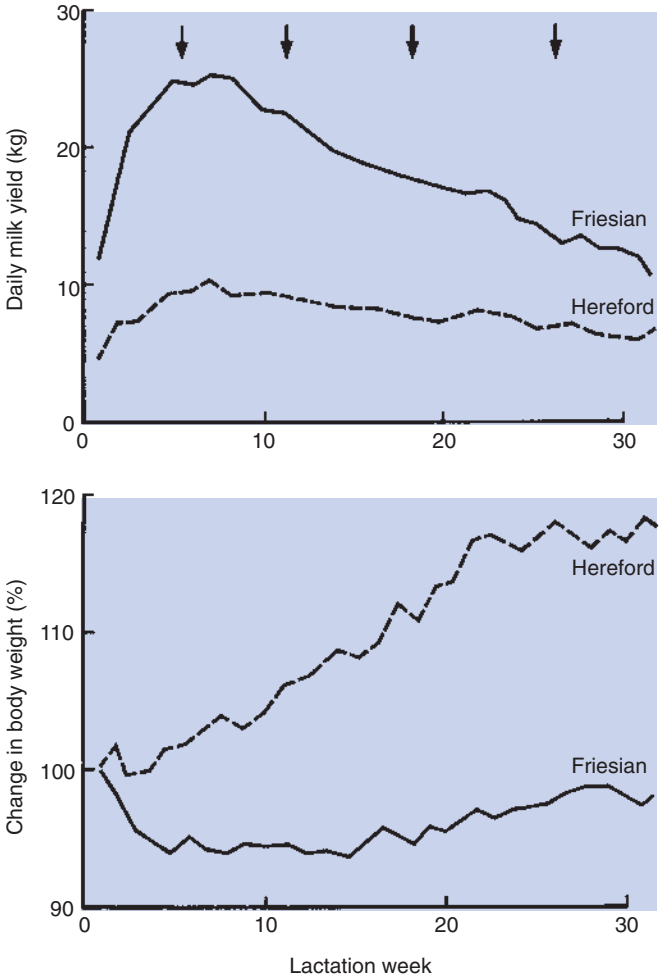

FIGURE 39-10 Average daily milk yield *(top)* and average percentage change in body weight *(bottom)* in seven low-yielding cows *(broken line)* and eight high-yielding cows *(solid line). Arrows* indicate times of blood sampling. (Courtesy Dr IC Hart. From Cowie AT: Lactation. In Austin CR, Short RV, editors: *Reproduction in mammals,* ed 2, vol 3, *Hormonal control of reproduction,* Cambridge, UK, 1984, Cambridge University Press.)

Growth hormone, which is important to the normal lactational process, can be used for the enhancement of lactation when administered over a rather wide range of concentrations (Figure 39-11). The ability to synthesize GH is relatively recent; its availability has expanded interest in its use for increasing the amount of milk produced by dairy cows. In general, GH acts on the postabsorptive use of nutrients so that protein, fat, and carbohydrate metabolism in the whole body are changed, and the nutrients are directed toward milk synthesis. If cows are in early lactation and in a negative energy balance, GH administration results in the mobilization of body fats that are used for milk formation. If cows are in positive energy balance, GH has no effect on the metabolism of body fat. Initially, GH treatment decreases the energy balance of cows; however, this is adjusted by a voluntary increase in feed consumption. Despite the increased feed consumption, GH administration increases the gross efficiency of lactation by as much as 19%. In essence, the effects of exogenous GH do not depend on gross alterations in nutrient

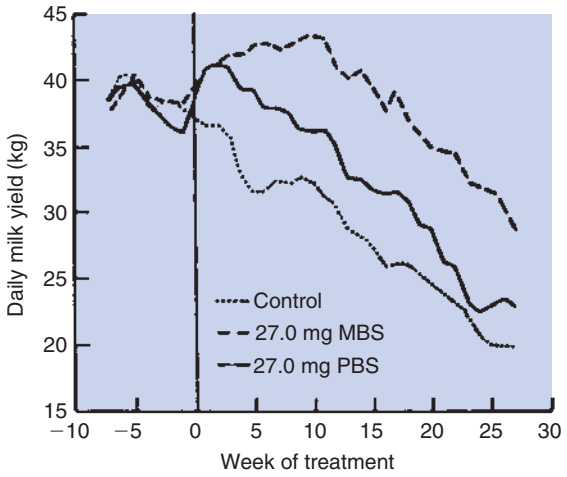

FIGURE 39-11 Average weekly milk yield of cows injected daily with diluent (control), 27 mg of methionyl bovine somatotropin *(MBS),* or 27 mg of pituitary bovine somatotropin *(PBS).* Treatments began at week 0 at an average of 84 ±10 days after parturition. (From Tucker HA: Lactation and its hormonal control. In Knobil E, Neill J, Ewing LL, et al, editors: *The physiology of reproduction,* vol 2, New York, 1988, Raven Press.)

digestibility or on body maintenance requirements. The use of GH may be economically viable, with the increased milk production justifying the expense of the hormone.

An interesting controversy has arisen from the fact that cows treated with GH do not produce "organically" derived milk, despite that synthetic GH is almost identical to endogenously derived GH. Although there is no evidence that increased concentrations of GH occur in the milk as a result of its administration, some consider the resultant milk to be abnormal.

The results with GH are in contrast to the studies in which thyroid hormone administration, in the form of *iodinated casein* (thyroprotein), was used to increase lactation in cows. Although the administration of thyroprotein increased lactation, extra feed was necessary to prevent excessive body weight loss, and milk production declined abruptly when thyroprotein was removed from the diet. In essence, the use of thyroprotein does not affect the efficiency of the lactational process as GH does. In dogs, one differential for gynecomastia (mammary enlargement) is profound hypothyroidism, causing elevated thyrotropin-releasing hormone (TRH) levels, which in turn stimulate prolactin secretion.

Another interesting finding concerning the manipulation of lactation has been that the milk yield in cows can be increased by exposing them to increased light (called a *photoperiod*). Cows under a photoperiod regimen of 16 hours of light (8 hours of dark) produced 6% to 10% more milk than animals under the reverse photoperiod (8 hours of light and 16 hours of dark) (Figure 39-12). Although the mechanism by which light affects lactation is not known, it likely involves prolactin secretion, at least to some extent, in that increased light exposure results in increased prolactin secretion. Similarly, the queen's estrous cycle is affected by photoperiod, mediated by melatonin and prolactin levels. Melatonin and prolactin secretion may play a role in ovarian function in the cat, with lower levels of both hormones present during estrus than during the interestrous period. Protocols exist for inducing improved lactation in postpartum bitches using low-dose oxytocin and metoclopramide (a dopamine D-2 receptor antagonist).

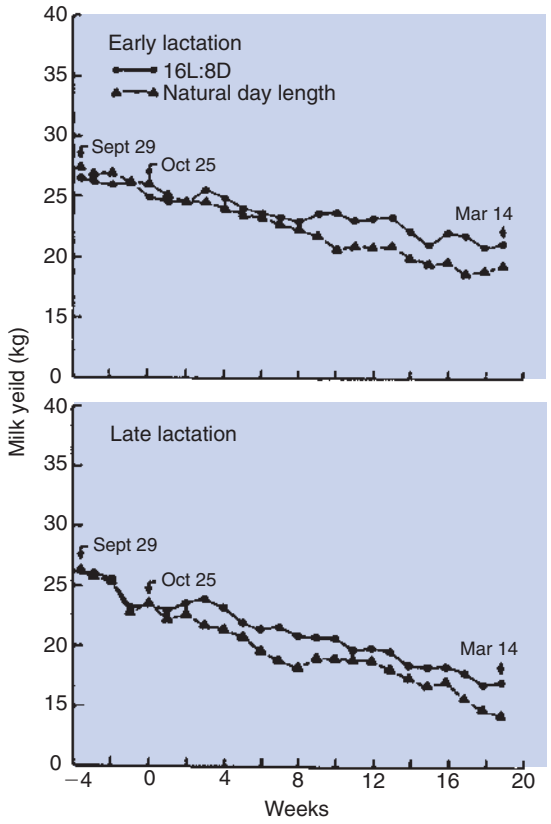

FIGURE 39-12 Influence of day length on milk production of Holstein cows. Between September 29 and October 24, cows at 37 to 74 days (early lactation) or 94 to 204 days (late lactation) after parturition were exposed to natural photoperiods of 12 hours of light per day and standardized diets. Between October 25 and March 14, cows were exposed to natural photoperiod (9 to 12 hours of light daily) or to 16 hours of fluorescent lighting superimposed on the natural photoperiod. *L,* Light; *D,* dark. (From Tucker HA: Lactation and its hormonal control. In Knobil E, Neill J, Ewing LL, et al, editors: *The physiology of reproduction,* vol 2, New York, 1988, Raven Press.)

DISEASES ASSOCIATED WITH THE MAMMARY GLAND

The Main Diseases That Affect the Mammary Gland Directly Are Mastitis (Prevalent in Dairy Cattle and Dogs) and Neoplasia (Prevalent in Intact Dogs and Cats)

The most important problems involved in the production of milk are those caused by inflammation of the gland *(mastitis).* One fundamental cause of mastitis is injury to the teat canal from the repeated stretching that occurs with the milking process. Organisms that ordinarily would be excluded from the gland are able to make their way past the barrier located within the teat canal; with repeated microorganism exposure, an infection is established.

One of the adverse consequences of mastitis is the formation of connective tissue within the udder as a result of the attempt of the gland to wall off the infection. The presence of connective tissue limits the area into which ducts and alveoli can proliferate, thus reducing the milk-producing potential of the gland. The mammary gland is an example of an organ (the eye is another example) in which the elicitation of an inflammatory response is often detrimental to the function of the organ. Thus, therapies directed toward the treatment of mastitis often combine antiinflammatory and antibacterial agents.

Another process that disturbs the structure of the mammary gland is *neoplasia.* In domestic animals, the dog is most susceptible to the occurrence of mammary tumors. The exposure of the mammae to the ovarian hormones estrogen and progesterone greatly increases the chance of neoplasia. The incidence of mammary tumors is relatively low if the dog is ovariectomized before the first estrous cycle, but it increases progressively through exposure to two ovarian cycles; ovariectomy has little effect on neoplasia if done after the third or fourth cycle. Some owners want their dogs to have one or two cycles before they are ovariectomized. It is important for veterinarians to point out the beneficial aspects of ovariectomy before the onset of puberty because of the incidence of mammary neoplasia, as well as the usual benefits of fertility and behavioral control.

The Main Conditions That Involve the Mammary Gland Indirectly Are Passive Transfer of Red Blood Cell Agglutinating Antibodies by the Ingestion of Colostrum (Mare, Queen) and Hypocalcemia Caused by the Transient Drain of Calcium That Occurs with Initiation of Lactation (Dairy Cattle) or During the Perinatal Period (Dog)

An immunological disease associated with the mammary gland involves the transfer of red blood cell agglutinating antibodies to the neonate through the milk. The situation is most common in the horse, in which fetal red blood cells (RBCs) pass into the maternal system and elicit antibody formation against the fetal RBCs. These antibodies tend to be concentrated in the colostrum along with other immunoglobulins. At birth the foal is able to absorb the RBC agglutinating antibodies (as well as other beneficial immunoglobulins) for up to 48 hours. Foals often go into a hemolytic crisis between 24 and 48 hours after delivery and can die unless given vigorous therapy, including blood transfusions. If fetal RBC antibody formation is suspected in a mare, the disease can be handled by muzzling the foal at birth through 48 hours and feeding with colostrum saved (frozen) from other preparturient mares. A similar condition has been reported in group A blood type kittens (usually purebred) born to group B blood type queens bred to toms with type A blood type. Unfortunately, no feline colostrum is commercially available, but kittens can be given serum or plasma from another blood type A queen for immunoglobulin transfer, while being prevented from nursing from their dam.

Diseases associated with the mammary gland and life threatening to the dam are *hypocalcemia, puerperal tetany,* or *eclampsia.* At parturition the acceleration of lactogenesis causes a great increase in the movement of calcium from the blood into the milk. Both cows and dogs are particularly susceptible, with some dams unable to respond immediately to the calcium drain from the blood by the mobilization of calcium. As a result, the animals lose their ability to maintain normal muscle activity; cows are often unable to stand, and become prostrate with the appearance of being comatose. Bitches develop tremors progressing to seizures. The syndrome occurs in cows at parturition and in dogs during the last weeks of pregnancy or the first few weeks postpartum, when lactation reaches its peak. Inappropriate prenatal nutrition, often with calcium supplementation, sets bitches up for this condition by inhibiting normal parathyroid gland development, necessary to meet the demands for mobilization of calcium by lactation. The systemic administration of calcium to hypocalcemic cows or bitches often produces a dramatic recovery in 10 to 20 minutes.

CLINICAL CORRELATIONS

PREGNANT MARE THAT DOES NOT HAVE SUFFICIENT MILK OR UDDER DEVELOPMENT

History. A 13-year-old pregnant Arabian mare is due to foal in the next week, based on breeding dates. This is her second foal; the first foal did well. She has had limited udder development. The mare is current on vaccinations and deworming; she has no previous medical problems. She has been on pasture with all the other horses. She is supplemented with 2 pounds of 14% mare and foal feed per day. She is given grass hay, and she is in good body condition. The farm is located in eastern Tennessee.

Clinical Examination. The mare is pregnant. The foal is viable based on movement and heartbeat. The foal is in the pelvic inlet, indicating that the mare will foal soon. All other parameters of the examination are normal. The mare is in good body condition.

Comment. With a mare close to parturition that has had limited udder development, one concern is whether the mare has been fed fescue. The owners are asked about this and say that she has been receiving a grass hay with fescue in it. They did not know that fescue could cause problems. The clients are informed that fescue can contain an endophytic fungus, *Neotyphodium coemophialum,* which produces alkaloid toxins. These toxins are dopaminergic and inhibit prolactin. Additionally, the increase in dopamine activity directly decreases prolactin. Normally, neurons in the proventricular nucleus would release prolactin, which would stimulate lactogenesis. In some animals, progesterone levels are also decreased.

Treatment. Domperidone can be given because it inhibits the dopaminergic effects. If domperidone is started 5 to 10 days before parturition, this is often sufficient time for the mare to develop adequate milk for the foal. If domperidone is not started until up to 24 hours after foaling, it must be given for 10 to 14 days. Some mares will respond and will increase the milk production, whereas some mares will not respond. Although this treatment is often useful in stimulating milk production by the mare, it does not necessarily reverse other complications associated with fescue. Mares that have been given fescue may have foals that appear dysmature, are weak, or have prolonged gestation. The placenta from these affected mares is often thickened. To prevent these effects of fescue, clients are encouraged to keep the mares off fescue-containing feeds for at least 30 to 60 days before foaling. In addition, endophyte-free fescue grasses are available, but expensive.

NEONATAL ISOERYTHROLYSIS

History. You are called to examine a mare, 7 months pregnant, that has a previous history of having conceived and delivered a normal foal after her first pregnancy; the foal was subsequently suckled and was sold as a weanling. The mare had no trouble conceiving and carrying the next two pregnancies, but the foals died within 2 to 4 days of birth, even though they were healthy and vigorous at birth and the mare had colostrum and milk. The previous owner became discouraged because of these deaths and sold the mare to the current owner at a bargain price.

Clinical Examination. You perform a general physical examination of the mare and find all organ systems to be normal. Palpation of the uterus per rectum reveals the presence of a viable fetus that appears to be of the correct size for a pregnancy of the purported

duration. Both the external genitalia and the mammary glands are normal in appearance.

Comment. From the history, and because the mare appears to be undergoing a normal pregnancy, you conclude that there is nothing wrong with the reproductive process. The fact that the previous two foals were healthy at birth and yet weakened rapidly and died within 4 days indicates that something likely happened to them after delivery. If the deaths were caused by an issue associated with the mare, the most likely cause of these deaths would be *neonatal isoerythrolysis.* In this situation the mare becomes exposed to the red blood cells (RBCs, erythrocytes) of the fetus during pregnancy, or the mare could have been exposed to RBCs from a stallion, whose erythrocytes are recognized as foreign. If the mare is exposed to the RBCs from the stallion or the fetus, those RBCs enter the circulation of the dam. She responds by making antibodies to the RBCs because of the presence of foreign antigen on the fetal erythrocytes that were inherited from the sire. In the mare, these antibodies do not pass through the placental barrier, so the fetus is protected from these antibodies during pregnancy. The antibodies do pass into the colostrum and are concentrated during the process of colostrum formation. Therefore, when the foal suckles the colostrum, it acquires the antibodies that will react to its own RBCs. The foal develops a type II hypersensitivity reaction in which the antibodies destroy the foal's RBCs through different mechanisms.

Treatment. The foal needs to be prevented from suckling the mare for the first 2 to 3 days of life. During the first 1 to 2 days, the foal is able to absorb large protein molecules, including the important immunoglobulins that enable the foal to ward off infections, as well as, in this case, antibodies against fetal RBC antigens. The gut epithelium closes to the passage of large protein molecules by 36 to 48 hours of life; at this time, or shortly thereafter, the foal can be allowed to suckle without risk of absorbing the antibodies. The key is to prevent the foal from suckling during the first 2 to 3 days of life to prevent absorption of the antibodies that will react to its own RBCs. The mare needs to be monitored closely before parturition so that the foal can be muzzled shortly after delivery. The foal does need nourishment during the first 2 to 3 days of life; thus it is important that the foal be fed colostrum obtained from other mares (usually maintained frozen). If the foal does receive the mare's colostrum containing antibodies that will react to its own RBCs, the foal can still be treated. The foal should not nurse the mare for the first 3 to 5 days of life, and the foal can be given packed RBCs from a donor if necessary. To anticipate the potential risk, blood typing of stallions and mares is now available.

PRACTICE QUESTIONS

1. The development of the duct system in the mammary gland is under the control of estrogens, growth hormone, and adrenal steroids. If the duct system is to develop functional milk-secreting units, called *alveoli,* which of the following hormone(s) are essential to this development?
   a. Progesterone
   b. Prolactin
   c. Relaxin
   d. Prolactin and progesterone
   e. Prolactin and relaxin
   f. Progesterone and relaxin

2. The hormone that is most important for the maintenance of lactation (lactogenesis) is:
   a. Estrogen.
   b. Oxytocin.
   c. Progesterone.
   d. Prolactin.
   e. Relaxin.

3. Sensory inputs (including sound, sight, and smell, but not necessarily touch) elicit the release of what important hormone required for the lactation process in the cow?
   a. Estrogen
   b. Oxytocin
   c. Progesterone
   d. Prolactin
   e. Relaxin

4. The contraction of what anatomical structure is of fundamental importance for the release of milk from the udder of the cow?
   a. Alveoli
   b. Duct
   c. Myoepithelial cell
   d. Duct cistern
   e. Teat cistern

5. The most important energy source in milk is:
   a. Carbohydrates.
   b. Lactose.
   c. Lipids.
   d. Proteins.

BIBLIOGRAPHY

Bogaerts P: *Clinical approach to genital and mammary pathologies in cats*. Presented at 5th Biannual Congress, *European Veterinary Society for Small Animal Reproduction (EVSSAR),* Budapest, Hungary, 2006.

Cowie T: Lactation. In Austin CR, Short RV, editors: *Reproduction in mammals*, vols 1-6, Cambridge, UK, 1986, Cambridge University Press.

Feldman EC, Nelson RW, editors: *Canine and feline endocrinology and reproduction*, ed 4, Philadelphia, 2009, Saunders.

Leyva H, Madley T, Stabenfeldt GH: Effect of light manipulation on ovarian activity and melatonin and prolactin secretion in the domestic cat, *J Reprod Fertil Suppl* 39:125-133, 1989.

Neill JD, editor: Knobil and Neill's physiology of reproduction, vols 1 and 2, ed 3, Philadelphia, 2005, Elsevier.

Park CS, Lindberg GL: The mammary gland and lactation. In Reece WO, editor: *Dukes' physiology of domestic animals*, ed 12, Ithaca, NY, 2004, Comstock Publishing.

Peterson ME, Kutzler MI, editors: *Small animal pediatrics: the first 12 months of life*, Philadelphia, 2011, Saunders.

CHAPTER 40
Reproductive Physiology of the Male

KEY POINTS

Functional anatomy
1. The male reproductive system consists of many individual organs acting in concert to produce spermatozoa and deliver them to the female's reproductive tract.
2. Normal spermatogenesis requires maintenance of uniform testicular temperature 2° to 6° C lower than core body temperature.
3. Emission is the release of spermatozoa and accessory gland fluids into the pelvic urethra, whereas ejaculation is the forceful expulsion of semen from the urethra.

Spermatogenesis
1. Spermatogenesis is a lengthy orchestrated process in which diploid stem cells divide by mitosis to maintain their own numbers and cyclically produce progeny that undergo meiotic division and differentiation into haploid germ cells.
2. Testicular size can predict daily sperm production.

Hypothalamic-pituitary-testicular axis
1. The reproductive system of the male is regulated by the hypothalamus, which is hormonally linked to the anterior pituitary and testes by luteinizing hormone and follicle-stimulating hormone.

Puberty
1. Puberty is not synonymous with sexual maturity.
2. Puberty results from a continuous process of endocrine changes that are initiated shortly after birth.

Anabolic steroids
1. Anabolic steroids are androgen derivatives that exert negative feedback on the hypothalamic-pituitary-testicular axis.

FUNCTIONAL ANATOMY

The Male Reproductive System Consists of Many Individual Organs Acting in Concert to Produce Spermatozoa and Deliver Them to the Female's Reproductive Tract

The male reproductive system is made up of a number of individual organs acting in concert to produce spermatozoa and deliver them to the reproductive tract of the female. This concerted effort involves both the neuroendocrine (hypothalamus and anterior pituitary glands) and the genital system. The genital organs consist of two testes, each suspended within the scrotum by a spermatic cord and external cremaster muscle; two epididymides; two deferent ducts; accessory sex glands; and the penis. The accessory sex glands include paired ampullae, paired seminal vesicles (vesicular glands), a prostate gland, and paired bulbourethral glands (Cowper glands). The presence of individual accessory glands, the testicular orientation, the type of penis, and the site of semen deposition in the female are dependent on the species (Table 40-1).

Normal Spermatogenesis Requires Maintenance of Uniform Testicular Temperature 2° to 6° C Lower Than Core Body Temperature

Normal *spermatogenesis* in most mammals is dependent upon maintenance of uniform testicular temperature between 2° to 6° C lower than core body temperature. Elevated testicular temperature reduces the numbers of live, normal spermatozoa. Thermoregulation of the testes in domestic animals is maintained by the pendulous scrotal sac, the testicular vasculature, the dartos and cremaster muscles, and the scrotal skin. A pendulous scrotum facilitates thermoregulation by using several mechanisms such as conduction, convection, and evaporation. The internal spermatic artery of many mammals is highly convoluted and in farm animals the coiling is so extensive that the artery forms a vascular cone on the dorsal pole of the testis. The testicular vascular cone is composed of a venous pampiniform plexus network surrounding the highly coiled testicular artery. This ramification reduces the mean blood pressure and permits transfer of heat from the testicular arteries (high temperature) to the veins (low temperature) by a counter-current heat-exchange system. This mechanism of heat exchange is possible because the spermatic artery is extensively coiled and in close proximity to the venous pampiniform plexus. In addition, there are periarterial veins and arteriovenous shunts that facilitate heat transfer as well as the transfer of hormones such as testosterone from the veins to the arteries. The dartos and cremaster muscles can increase or reduce the exposure surface area of the scrotum and move the testes closer to or farther from the abdomen, depending on their state of contracture. The scrotal skin is usually thin, generally lacks subcutaneous fat, has relatively little hair or wool, and contains numerous sebaceous and sweat glands. The blood and lymphatic system in the scrotal skin is very extensive, with blood vessels near the skin surface, facilitating radiation of heat. In hot environments the blood flow in the scrotal skin increases and the evaporation per unit area of scrotal skin is greater than the evaporation from the general body surface. In the scrotum, the number and volume of the sweat glands per unit skin surface is greater than

TABLE 40-1 Male Reproductive Parameters

	Bull, Buck, and Ram	Stallion	Boar	Dog	Tom	Llama/Alpaca
Testis orientation	Vertical cauda down	Horizontal	Perineal cauda up	Horizontal	Perineal cauda up	Perineal cauda up
Ampullae	+	+	–	+	–	+
Seminal vesicle	+	+	+	–	–	–
Bulbourethra	+	+	++	–	+	+
Prostate	+	+	+	+	+	+
Penis type	Fibroelastic sigmoid	Vascular	Fibroelastic sigmoid	Vascular	Vascular	Fibroelastic sigmoid
Semen deposition	Vagina	Uterus	Cervix/uterus	Vagina	Vagina	Uterus

other body regions. In addition, the scrotal skin has thermoreceptors that trigger a local and a systemic response in the presence of an increase in local temperature. Locally the blood flow and scrotal sweating will increase. The systemic response will increase the number of breaths per minute (polypnea).

As previously mentioned, in domestic mammals, normal testicular function, especially normal spermatogenesis, is temperature dependent and requires an environment that is lower than core body temperature. Therefore, in normal domestic males, the testes are located outside the abdominal cavity, in the scrotum. Failure of one or both of the testes to descend into the scrotum is known as *cryptorchidism*. Although the cryptorchid testis is still capable of producing *androgens*, it is incapable of producing normal spermatozoa. Consequently, a bilaterally cryptorchid male would be sterile. The cryptorchid testis is more prone to torsion of the spermatic cord and 10 times more likely to be neoplastic. Cryptorchidism appears to be genetic, although the exact mechanism is not completely understood and may vary among species. It is most common in boars, dogs, and stallions and least common in bulls, rams, and bucks. Descent of the testes into the scrotum normally occurs in domestic animals during the following time periods:

- *Horse.* 9 to 11 months of gestation
- *Cattle.* 3.5 to 4 months of gestation
- *Sheep.* 80 days of gestation
- *Pig.* 90 days of gestation
- *Dog.* 5 days after birth
- *Cat.* 2 to 5 days after birth
- *Llama/alpaca.* Usually present at birth

For the majority of domestic species, passage of the testes through the internal rings by 2 weeks after birth is necessary for a final scrotal position to occur. Many animals may have testes in the inguinal region at birth, and the testes may remain there for weeks or months before descending into the scrotum. In the dog, testicular descent is uncommon after 14 weeks of age and does not occur after 6 months of age. In the stallion, although it is considered abnormal, descent of inguinally retained testes has been known to occur as late as 2 to 3 years of age.

The testis is the pivotal organ of the male reproductive system. It must be remembered, however, that all testicular functions are profoundly influenced by the neuroendocrine system. The testis is responsible for steroidogenesis, primarily the production of androgens, as well as the generation of haploid germ cells by spermatogenesis. These two functions occur in the Leydig cells and the seminiferous tubules, respectively.

Functionally, the testis is considered to have three compartments. The interstitial tissue compartment, containing the Leydig cells, surrounds the seminiferous tubules and bathes them with testosterone-rich fluid. The other two compartments reside within the seminiferous tubules. The basal compartment contains spermatogonia, which divide through mitosis, whereas the adluminal compartment represents a special environment where spermatocytes undergo meiosis and continue their meiotic divisions to differentiate into spermatids and finally into spermatozoa. Within the seminiferous tubules, the Sertoli cells, which provide support and nourishment to the developing germ cells, extend from the basal compartment into the adluminal compartment. Tight-junctional complexes between the Sertoli cells separate the basal and adluminal compartments and form the major component of the blood-testis barrier, which functionally prevents many compounds found in the blood and interstitial fluid from entering the adluminal compartment.

The seminiferous tubules empty their contents into the rete testis, which subsequently transports the spermatozoa and seminiferous tubular fluid into the epididymis. The epididymis is a single tortuous duct of considerable length (from 2 m in the cat to 80 m in the stallion) that is anatomically divided into three segments: head, or caput; body, or corpus; and tail, or cauda. The epididymis not only is a conduit for spermatozoa but also provides a special environment in which spermatozoa are concentrated, undergo maturation, and acquire fertilizing capacity. Spermatozoa that enter the caput from the rete testis are immotile and incapable of fertilization. Only after they undergo migration and maturation through the caput and corpus are both motility and the capacity for fertilization achieved. The cauda epididymis and the deferent duct, into which the cauda empties, serve as a storage depot for mature spermatozoa; together, these are known as the extragonadal sperm reserves. The spermatozoal transit time through the caput and corpus epididymis is not altered by *ejaculation* and is similar (2 to 5 days) for domestic species. Storage time in the cauda epididymis is more variable among species (3 to 13 days) and can be reduced by several days in sexually active males. Animals that rest sexually for 7 to 10 days have a maximum number of spermatozoa in the cauda epididymis, and this reserve is reduced by at least 25% with daily or every-other-day ejaculation.

The deferent ducts, or *vasa deferentia,* pass through the inguinal rings into the abdomen and connect the cauda epididymis with the pelvic urethra. In most species the terminal portion of the deferent ducts enlarges to form prominent ampullae such as those found in ruminants and the stallion. In other species the ampullae either are absent or are anatomically indistinct from the vasa deferentia. The ampullae serve as an additional storage depot for spermatozoa, and in some species, such as the bull, stallion,

TABLE 40-2 Seminal Characteristics from Domestic Animals

Parameter	Bull	Ram	Buck	Boar	Stallion	Alpaca/Llama	Dog*	Tom
Ejaculate volume (mL)	5-8	0.7-1.3	0.7-1.4	150-250	50-100	0.7-3.0	2.0-25	0.03-0.3
Spermatozoa concentration (millions/mL)	800-2,000	2,000-3,500	2,000-4,500	200-300	150-300	80-250	60-500	1,700-2,900
Motile spermatozoa (%)	40-75	60-80	60-85	50-80	40-75	40-70	50-90	40-90
Normal spermatozoa (%)	65-95	80-95	75-95	70-90	60-90	55-85	50-90	50-90

*The ejaculate of the dog consists of three fractions.

and dog, ampullary glands add to the ejaculate. Along with spermatozoa, ejaculated semen is composed primarily of accessory gland secretions that add volume, nutrients, buffers, and a number of other substances whose exact functions are unknown. The contribution to the ejaculate by each of the accessory glands varies with the species and is responsible for the variation in concentration, volume, and character between ejaculates. The seminal vesicles lie lateral to the ampullae near the neck of the bladder. In the bull, ram, and buck, these organs are firm and lobulated with a narrow lumen, whereas in the stallion and boar, they are more saclike. The dog and tom lack seminal vesicles but have relatively prominent prostate glands, especially the dog. The prostate gland is present in all domestic males and is intimately associated with the pelvic urethra, but it varies in size and appearance among species. The bulbourethral glands of the tom are almost as large as the prostate, but these glands are absent in the dog. In the stallion and bull, the bulbourethral glands are small, round to ovoid structures that lie adjacent to the pelvic urethra near the ischial arch, whereas those of the boar are large and cylindrical. The male llama/alpaca lacks seminal vesicles, and the bulbourethral and prostate glands are present.

The copulatory organ of the male is the penis. It is more or less cylindrical in all species and extends from the ischial arch to near the umbilicus on the ventral abdominal wall, except in the tom and the llama/alpaca, in which the penis points posteriorly in the relaxed state. The body of the penis is surrounded by a thick fibrous capsule (the tunica albuginea) that encloses numerous cavernous spaces (the corpus cavernosum penis) as well as the corpus spongiosum penis, which immediately surrounds the urethra. Erection is a psychosomatic event that involves mutually occurring actions of the vascular, neurological, and endocrine systems. Contraction of the ischiocavernosus muscle during erection results in occlusion of venous outflow. At the same time, the parasympathetically mediated relaxation of corpus cavernosum and corpus spongiosum results in these cavernous spaces becoming engorged with blood, and the penis becomes elongated and turgid.

Emission Is the Release of Spermatozoa and Accessory Gland Fluids into the Pelvic Urethra, Whereas Ejaculation Is the Forceful Expulsion of Semen from the Urethra

Emission is the release of spermatozoa and accessory gland fluids into the pelvic urethra as a result of sympathetically mediated thoracolumbar reflex contraction of the smooth muscle in the ductus deferens and accessory glands. Ejaculation is the forceful expulsion of semen from the urethra and is prompted by a parasympathetically mediated sacral reflex that induces rhythmic contractions of the bulbospongiosus, ischiocavernosus, and urethralis muscles. After ejaculation, a sacral sympathetically mediated increase in the smooth muscle tone of the cavernous spaces increases the outflow of blood, and contraction of the retractor penis muscle withdraws the penis into the prepuce. The seminal characteristics of the different species are listed in Table 40-2.

SPERMATOGENESIS

Spermatogenesis Is a Lengthy Orchestrated Process in Which Diploid Stem Cells Divide by Mitosis to Maintain Their Own Numbers and Cyclically Produce Progeny That Undergo Meiotic Division and Differentiation into Haploid Germ Cells

Spermatogenesis is a lengthy orchestrated process in which diploid stem cells at the base of the seminiferous tubules (spermatogonia) divide through mitosis to maintain their own numbers. These cells also cyclically produce progeny that undergo further meiotic division and differentiation into haploid spermatids, which are released as spermatozoa (Figure 40-1). Spermatogenesis is generally divided into three major events: spermatocytogenesis, meiosis, and spermiogenesis. Spermatocytogenesis accomplishes two important functions. First, the mitotic divisions of type A spermatogonia produce other spermatogonia that are not yet committed to the immediate spermatozoal production process, thus maintaining a population of stem cells. These stem cell divisions are responsible for the ability of the male to continuously produce spermatozoa throughout his adult life. Second, type A spermatogonia become type B spermatogonia, which further divide through mitosis to produce primary spermatocytes. The primary spermatocytes enter into the pool of meiotically dividing cells and ultimately produce spermatozoa.

Meiosis occurs only during the processes of oogenesis and spermatogenesis, in which the haploid condition results after two cell divisions with only one chromosomal duplication. During meiosis, homologous chromosomes pair up, and this facilitates the exchange of genetic material between chromosomes. At the first meiotic division, the homologous chromosomes segregate into the two resulting cells, creating a haploid condition. In the male the resultant haploid cells are the secondary spermatocytes with duplicated chromatids. In less than 1 day after their formation, secondary spermatocytes divide to form spermatids that contain one chromatid from each of the haploid chromosomes.

The newly formed spermatids continue to differentiate without dividing to form mature spermatids through the process of spermiogenesis. Spermiogenesis occurs just before spermatids are released as spermatozoa at the luminal surface of the seminiferous tubule (spermiation). The major features of spermiogenesis

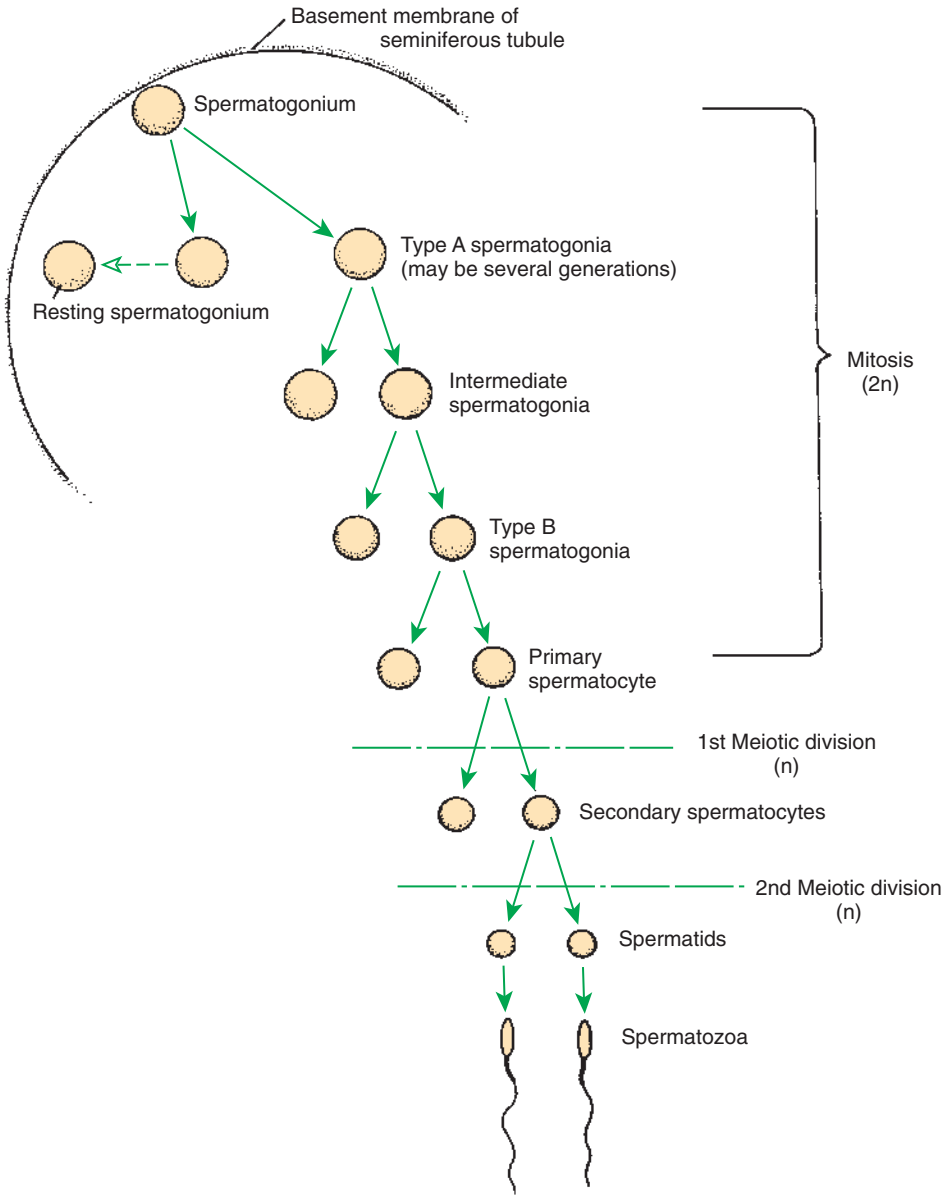

FIGURE 40-1 Diagram of spermatogenesis. (From McDonald LE, Pineda MH, editors: *Veterinary endocrinology and reproduction,* Philadelphia, 1989, Lea & Febiger.)

include formation of the acrosome from the Golgi apparatus, condensation and elongation of the nucleus, formation of the flagellum, and extensive shedding of cytoplasm. The spermiated *spermatozoon* consists of a head, middle piece, and tail (Figure 40-2). The head contains the genetic material to be combined with that of the oocyte during fertilization. Overlying the head is the acrosome, which contains hydrolytic enzymes necessary for penetration of the oocyte. The middle piece contains mitochondria, which provide the energy for microtubules extending into the tail to slide back and forth, past each other, thus producing tail movement.

Taking epididymal transit time into account, the interval from type A spermatogonia to ejaculated spermatozoa is approximately 60 to 70 days for the ram and the bull and 50 to 60 days for the boar, the dog, and the stallion. Therefore the interval from an event that may adversely affect the testis or epididymis to a decline in seminal quality may be as short as a few days to as long as 2 months. Similarly, at least 60 days would likely be required for an ejaculate to return to normal after a toxic insult to the testis.

In theory, 16 primary spermatocytes and 64 spermatozoa develop from one type A spermatogonium in the bull and the ram. However, a percentage of potential sperm production is lost to degeneration during the normal course of spermatogenesis. In humans, approximately 40% of the sperm production potential is lost during the latter stages of meiosis. Daily sperm production is the number of spermatozoa produced per day by the testes. It is highly correlated with testicular size and is not affected by frequency of use for breeding.

Testicular Size Can Predict Daily Sperm Production

Testicular size is an important trait of medium to high heritability that provides an accurate estimate of the amount of sperm-producing parenchyma in the testis. Because of the influence of testicular size, there is a wide range in daily sperm production among domestic species. For example, daily sperm production has been calculated to be 0.37×10^9 in the dog and 16.2×10^9 in the boar. Within a species, both individual and breed variation in testicular size can also influence daily sperm production.

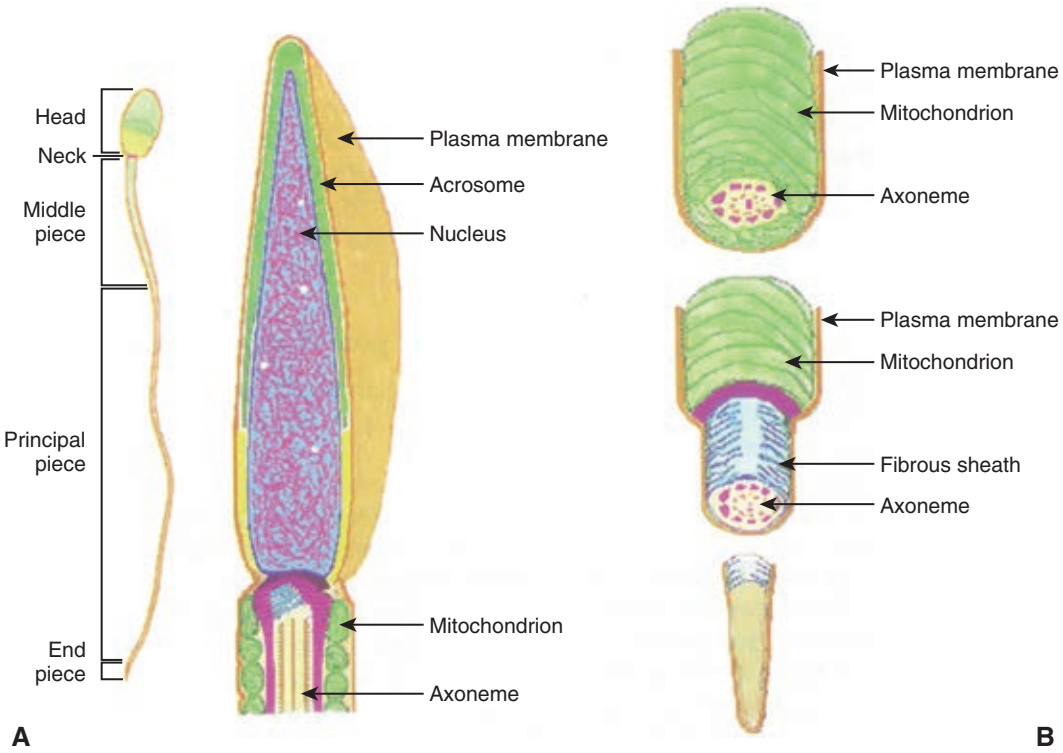

FIGURE 40-2 **A,** Major elements of the mammalian spermatozoa. **B,** Middle piece *(top),* principal *(middle),* and end piece *(bottom)* of a spermatozoon viewed in cross section. (From Robaire B, Pryor JL, Trasler JM: *Handbook of andrology,* Lawrence, Kan, 1995, Allen Press.)

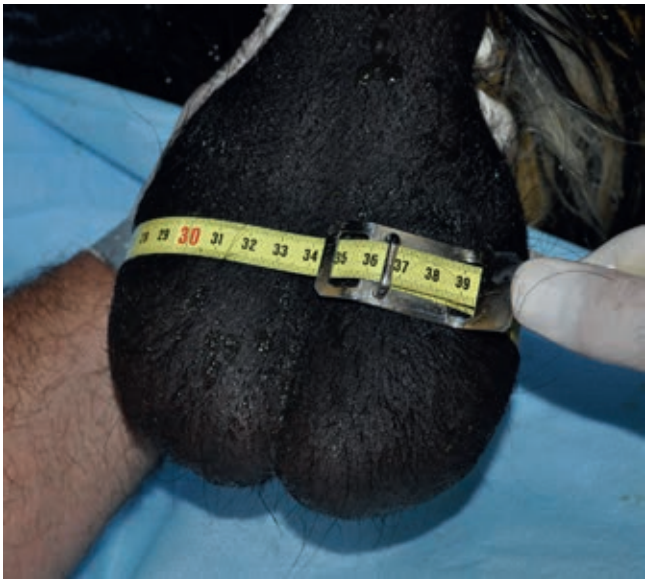

FIGURE 40-3 Measurement of scrotal circumference in a bull by using a scrotal tape.

FIGURE 40-4 Measurement of total scrotal width in the stallion using calipers. (From Brinsko SP, Blanchard TL, Varner DD, et al: *Manual of equine reproduction,* ed 3, St Louis, 2010, Mosby.)

Testicular size cannot be measured directly; therefore, an indirect measure commonly used in ruminants is *scrotal circumference* (Figure 40-3). In other species with more horizontally oriented testes, total scrotal width (Figure 40-4) or testicular volume as determined by ultrasonographic measurements is used. Testicular size is influenced by species, breed, age, and body condition score. Each gram of normal testicular parenchyma produces the same quantity of spermatozoa according to the species but differs among species (Table 40-3). Therefore, males with larger testes will produce more spermatozoa than males with smaller testes for the same age and species. In ruminants, scrotal circumference is also an accurate predictor of the age of puberty onset and of the percentage of normal seminiferous tubules. In cattle, there is a negative correlation between scrotal circumferences and age of puberty in the female offspring; this means, bulls with larger scrotal circumferences will produce females that attain puberty at an earlier age.

TABLE 40-3 Body Weight, Testicular Weight, Spermatogenic Efficiency, and Daily Spermatozoa Production

	Body Weight (kg)	Pair Testes Weight (Grams)	Spermatogenic Efficiency[a]	Daily Spermatozoa Production ($\times 10^9$)[b]
Alpaca	65	20	NA	NA
Boar	150	750	23	17.3
Bulls	600	600	11	6.6
Dog	15	30	17	0.5
Lama	115	30	NA	NA
Ram	100	550	21	11.6
Stallion	500	350	16	5.6
Tom	5	20	16	0.3

NA, Not available.
[a]Spermatozoa produced per gram of testicular parenchyma ($\times 10^6$).
[b]Spermatozoa produced daily by the two testes.

HYPOTHALAMIC-PITUITARY-TESTICULAR AXIS

The Reproductive System of the Male Is Regulated by the Hypothalamus, Which Is Hormonally Linked to the Anterior Pituitary and Testes by Luteinizing Hormone and Follicle-Stimulating Hormone

The reproductive system of male mammals is regulated by intricate feedback mechanisms involving the hypothalamus, anterior pituitary, and testes (Figure 40-5). The hypothalamus synthesizes and secretes the *decapeptide gonadotropin-releasing hormone* (GnRH). Secreted in a pulsatile manner, GnRH acts directly on gonadotropic cells in the anterior pituitary. On stimulation by GnRH, these gonadotropes synthesize and secrete the gonadotropins follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Both FSH and LH are heterodimeric glycoproteins made up of two noncovalently linked polypeptides. The alpha (α) subunit protein is common to both FSH and LH, whereas the beta (β) subunit is specific for each. Individual gonadotropes have the ability to synthesize and secrete FSH, LH, or both. The release of FSH and LH depends on the pulsatile pattern of GnRH secretion. Irregular, low-amplitude GnRH pulses result in FSH release, whereas high-frequency GnRH pulses induce the release of LH.

Within the testis, LH binds to membrane receptors on the Leydig cells and stimulates them to convert cholesterol to *testosterone*. Synthesized androgens diffuse into blood and lymph, where they are bound to androgen-binding protein (ABP) produced by the Sertoli cells. High local concentrations of androgens within the testis are considered essential for normal spermatogenesis to occur. ABP enhances the accumulation of testosterone and dihydrotestosterone in high concentrations within the seminiferous tubules and the interstitium of the testis. Within the testis, the target cells for testosterone are the peritubular myoid cells and the Sertoli cells, which envelop and support the developing sperm cells. ABP also facilitates the transport of androgens from the testis to the epididymis, where these hormones influence epididymal transit and the further maturation of spermatozoa.

Studies have demonstrated that FSH specifically targets receptors on the Sertoli cells within the seminiferous tubules. FSH and testosterone stimulate a variety of Sertoli cell functions, including the synthesis and secretion of ABP, inhibin, activin, estrogen, and several products (e.g., transferrin) that are involved in the transfer of nutrients to germ cells; meiosis; spermatocyte maturation; spermiation; and Leydig cell function. Sertoli and Leydig cells appear to interact in a paracrine fashion. Steroid production of Leydig cells can be stimulated by a product released by Sertoli cells, the secretion of which is enhanced by FSH. A potential candidate for such a substance is inhibin, which is produced by Sertoli cells in response to FSH and stimulates steroidogenesis in Leydig cells. Inhibin, a glycoprotein hormone, along with testosterone, is involved in the complex feedback regulation of pituitary function. Gonadal steroids are known to suppress FSH release, but inhibin appears to be the most potent inhibitor of FSH secretion from the pituitary. Testosterone, dihydrotestosterone, and estrogen regulate LH synthesis and secretion through negative feedback exerted at the level of the hypothalamus or the anterior pituitary gland. Because FSH and LH are necessary for high testicular concentrations of substances responsible for normal spermatogenesis, exogenous administration of testosterone or inhibin to enhance fertility would be contraindicated since they would impede the secretion of those factors responsible for maintaining an optimal spermatogenic environment.

PUBERTY

Puberty Is Not Synonymous with Sexual Maturity

Puberty in the male is when he is first able to produce sufficient numbers of sperm to impregnate a female. For practical reasons, for bulls, boars, rams, and stallions, this could be defined as the age when the ejaculate contains 50×10^6 spermatozoa, of which 10% or more are motile. It must be remembered that puberty is not synonymous with sexual maturity, which can occur months to years later, depending on the species.

Puberty Results from a Continuous Process of Endocrine Changes That Are Initiated Shortly After Birth

The pituitary gland, gonads, and steroid-dependent target tissues are capable of responding to stimulatory hormones before puberty; therefore the hypothalamus is considered to play a pivotal role in the initiation of puberty. Puberty appears to be the end result of a continuous process of endocrine changes that are initiated shortly after birth. Some investigators theorize that puberty occurs when the animal's hypothalamic-pituitary complex becomes desensitized to the feedback inhibition of gonadal steroids. This desensitization would apparently allow

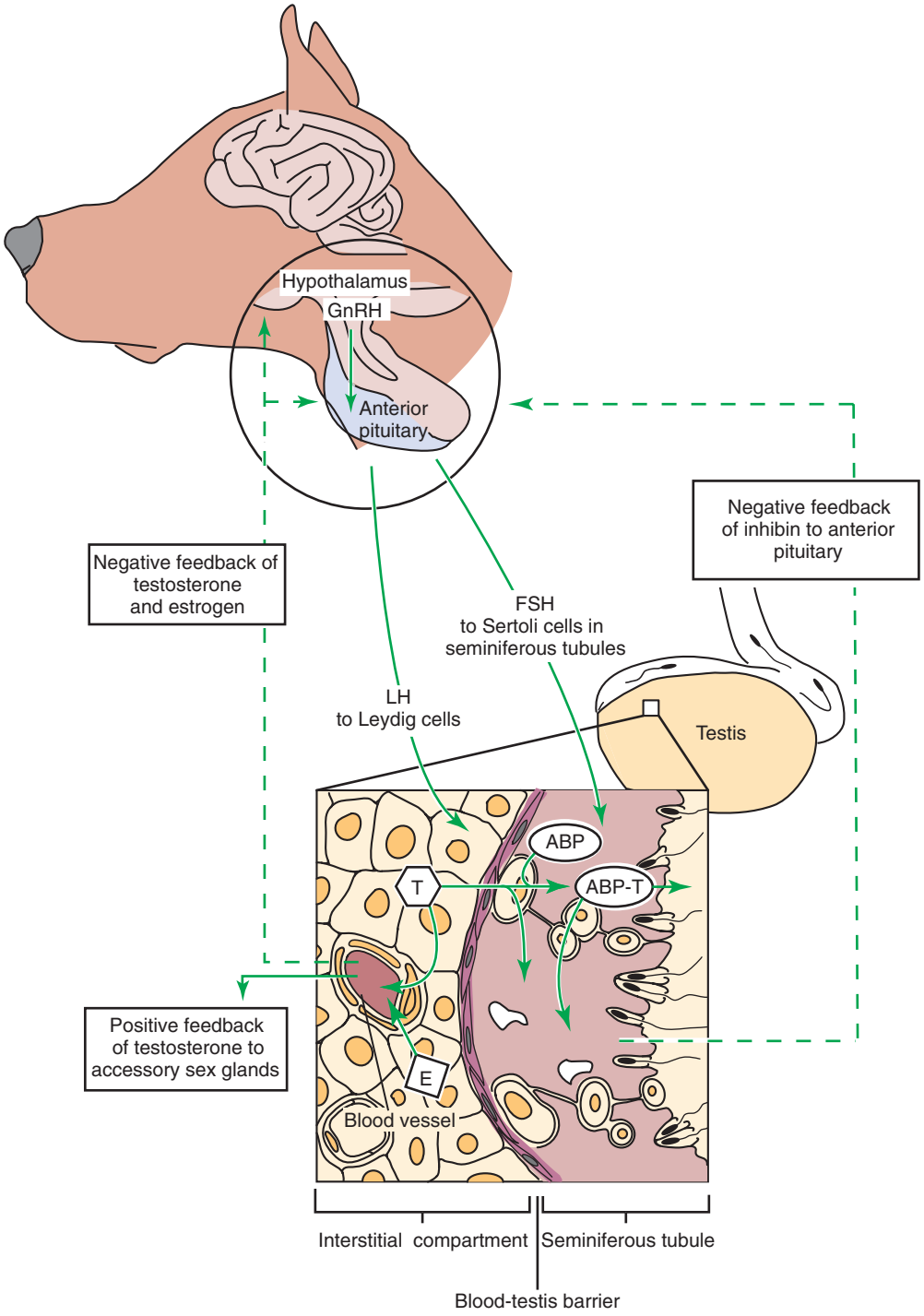

FIGURE 40-5 The reproductive system of male mammals is regulated by intricate feedback mechanisms that involve the hypothalamus, anterior pituitary, and testes. *GnRH,* Gonadotropin-releasing hormone; *FSH,* follicle-stimulating hormone; *LH,* luteinizing hormone; *ABP,* androgen-binding protein; *ABP-T,* androgen-binding protein–testosterone; *E,* estrogen; *T,* testosterone.

increased discharge of GnRH from the hypothalamus and a greater response of the pituitary to GnRH. Although numerous factors can influence the modulation by the central nervous system of the endocrine system, the major factors that affect age at puberty in domestic animals are breed, energy intake, and season of birth.

The hypothalamic-pituitary-gonadal system in humans differentiates and functions during fetal life and briefly during infancy; it then is suppressed during childhood and is reactivated during puberty after almost a decade of low activity. Inhibition of the hypothalamic-pituitary-gonadal system in prepubertal children is mediated through the suppression of GnRH synthesis and pulsatile secretion. Progressive pulsatile stimulation of the pituitary by GnRH and the gonads by LH and FSH is required for the initiation and progression of puberty. Prepubertal children secrete small amounts of FSH and LH from the pituitary, indicating that

the hypothalamic-pituitary-gonadal axis is functional, but at a low level. This low level of gonadotropin secretion rapidly decreases when sex steroids are administered. Therefore, a highly sensitive negative-feedback mechanism appears to exist in young prepubertal children, and a similar mechanism is likely to exist in prepubertal domestic animals.

ANABOLIC STEROIDS

Anabolic Steroids Are Androgen Derivatives That Exert Negative Feedback on the Hypothalamic-Pituitary-Testicular Axis

The use of *anabolic* steroids has become widespread in human and animal athletes as many attempt to increase performance. Testimonials from veterinarians, physicians, human athletes, and trainers indicate that improvements in the athlete's mental attitude, stamina, and physical strength are attained after anabolic steroids are administered. The major concern is that many individuals receiving anabolic steroids are peripubescent or prepubescent. Anabolic steroids are androgen derivatives that have been altered to maximize their anabolic action and to minimize their androgenic side effects. It is not yet possible, however, to produce anabolic steroids that are devoid of androgenic activity, and many of the undesirable side effects of these drugs are caused by their androgenic activity. The adverse reproductive side effects observed with anabolic steroid use are similar to those associated with testosterone administration. Sustained testosterone or anabolic steroid administration affects pituitary function and leads to long-lasting impairment of testicular endocrine function. Potential side effects of the use of anabolic steroids in young animals may lead to incomplete development of the hypothalamic-pituitary-gonadal axis. The long-term side effects of anabolic steroid use on reproductive parameters in sexually immature animals are not yet known.

A high percentage of colts and stallions in training or racing receive androgenic drugs, including anabolic steroids, and these horses have smaller testicles than similar horses not receiving such drugs. Only two anabolic steroids, stanozolol and boldenone undecylenate, are approved by the U.S. Food and Drug Administration (FDA) for use in horses. Neither of these is approved for use in stallions. The administration of anabolic steroids to stallions has been shown to reduce seminal quality, daily sperm output, daily sperm production, and testicular size. These effects most likely result from a negative-feedback mechanism on gonadotropin release from the pituitary. Alterations in seminal parameters are also observed, including depression of sperm concentration, sperm motility, and total number of sperm per ejaculate. Histological examination of the testes demonstrates a reduction in the number of developing germ cells other than type A spermatogonia. In addition, the mean diameter of Leydig cells is decreased, and changes indicative of testicular degeneration (including marked cytoplasmic vacuolization, shrunken tubules, and Leydig cells) and phagocytosis of spermatids by multinucleated giant cells have been observed. These adverse effects on the testes tend to be more severe in younger stallions. Repeated implantation of anabolic steroids in prepubertal bulls also results in decreased testicular size. Effects on testicular growth depend on the type of anabolic steroid administered, the age of the patient, and the dosage and duration of therapy.

Human studies, which did not directly investigate the effects of anabolic steroids on spermatogenesis, also found reduced levels of circulating gonadotropins, testosterone, or both. Individuals using high doses of testosterone and anabolic steroids for only 3 months still had hypogonadotropic hypogonadism 3 weeks after the cessation of drug use. The presence of atrophic testicles and low LH, FSH, and testosterone levels after drug withdrawal indicates that long-term androgenic or anabolic steroid use affects pituitary function and leads to long-lasting impairment of testicular endocrine function.

Questions regarding the possibility of permanent sterility or testicular atrophy with long-term anabolic steroid use have not been answered for adults, and even less is known about the effects in prepubertal or peripubertal individuals. Indirect evidence suggests that prepubertal individuals may be at a higher risk for permanent derangements from anabolic steroid use than adults. Therefore the indiscriminant use of anabolic steroids in males intended for breeding should be strongly discouraged.

CLINICAL CORRELATIONS

INFERTILITY IN A STALLION

History. You are asked to perform a "breeding soundness" examination on a 3-year-old Quarter Horse stallion that was mated with 10 mares last year and impregnated only one mare. The stallion is in demand because of his bloodlines and because his muscular and mature appearance contributed to winning a number of shows as a yearling. All the mares mated to this young stallion have been shown to be free of reproductive abnormalities. You ask whether the horse has had any illness or febrile episodes or has received any medication recently. The answer to all these queries is "no."

Clinical Examination. The stallion demonstrates normal *libido* when exposed to a mare in estrus, and two ejaculates are obtained 1 hour apart by means of an artificial vagina. Examination of both semen samples reveals poor spermatozoal concentration, low sperm numbers, and a high percentage of morphologically abnormal spermatozoa and immature germ cells. The stallion has a normal penis and prepuce, but his testes are small and soft. You ask if the stallion has ever received anabolic steroids, and with some reluctance the owner admits that the trainer did give anabolic steroids to the horse in preparation for the yearling futurity and for shows thereafter.

Comment. The use of anabolic steroids in performance animals is not uncommon. Even so, many owners and trainers are reluctant to admit to their use. Many colts are administered these drugs to give them a competitive edge in the show ring or on the racetrack so that they will later be in demand as sires. Unfortunately, because they are testosterone derivatives, the negative-feedback effects of anabolic steroids adversely affect the fertility of these animals, sometimes permanently. It is not known how severe or long-standing the adverse effects will be if anabolic steroids are given in the peripubertal period. Because this animal's testes are so small and soft, he apparently received high doses of anabolic steroids for a prolonged period during the development of the hypothalamic-pituitary-testicular axis, and the effects are most likely irreversible. It must be noted that as a 3-year-old, the horse is not yet sexually mature, and in the future he may still be able to produce sufficient numbers of normal spermatozoa to impregnate a small number of mares per season, but certainly not a "full book."

Treatment. Other than time, no known treatment will reverse the detrimental side effects caused by anabolic steroid use in adult males. Even less is known about the long-term effects in young

animals. Depending on the percentage of normal, progressively motile sperm, this animal may be able to breed a limited number of mares, most likely through artificial insemination. The owners could also have the stallion reexamined in several months or longer to see if there is any improvement in sperm morphology.

INFERTILITY IN A BULL

History. You are asked to perform a "breeding soundness" examination at your veterinary clinic on a 5-year-old Brangus bull before the breeding season that will start in 1 month. This bull was used in a single sire mating system in the last breeding season with 20 females (75 days exposure) producing 90% of calf crop. The bull was vaccinated against *Clostridium* and respiratory virus with killed products and dewormed 3 months ago. Forty days ago this bull had a short but intense febrile respiratory illness, which resulted in recumbency and lasted 3 days. The bull was treated with antibiotics and antiinflammatory drugs for 5 days and the problem resolved promptly.

Clinical Examination. This bull is in good body condition score (6; scale between 1 and 9) without foot/leg problems detectable when walking and the general physical examination was unremarkable. The accessory sexual organs examined by palpation per rectum were normal. The scrotal contents were within normal limits and the scrotal circumference was 42 cm. Semen was collected via *electroejaculation* and normal penile protrusion and erection was observed. No abnormalities in the penis were detected. Semen evaluation revealed 20% of the spermatozoa to be motile with 50% of the spermatozoa having abnormal morphology. Most of the morphologic abnormalities consisted of detached heads, bent tails, and distal and proximal droplets among other defects.

Comment. Testicular function requires a low testicular temperature compared to systemic temperature in order to allow normal spermatogenesis. This bull had two instances that affected the testicular thermoregulatory function: fever and recumbency. The abnormalities noted in the semen are in agreement with the process of testicular degeneration. The spermatogenesis requires approximately 60 days. The degree of testicular compromise depends on the degree and duration of the insult as well as the inherent natural susceptibility of the male. Remember every ejaculate that you collect today is a snapshot of a process that began at least 60 days ago. Therefore the recommendation would be to re-evaluate this animal at least 60 days from the last day of the illness. It is also important to remark that there is variability among males in the degree of response to this insult.

Treatment. No known treatment, other than time, will reverse the detrimental effects caused by the fever. Due to the fact that spermatogenesis will require approximately 60 days in bulls (54 days plus epididymus transit time), the recommendation would be to not use this bull for breeding until the next evaluation is performed. Moreover, because this bull is used in single sire breeding season, the owner needs to find a new satisfactory potential breeder for this upcoming breeding season. Based on your recommendation you can educate your client that the best time to perform a breeding soundness examination is at least 2 months before the breeding season.

PRACTICE QUESTIONS

1. For most domestic species, the duration of spermatogenesis is approximately:
   a. 120 days.
   b. 10 days.
   c. 60 days.
   d. 6 months.
   e. 21 days.

2. Normal spermatogenesis in domestic mammals requires a testicular temperature that is:
   a. Higher than core body temperature.
   b. Lower than core body temperature.
   c. The same as core body temperature.
   d. Above freezing but below boiling.
   e. Conducive to testosterone metabolism.

3. Normal spermatogenesis requires an intratesticular testosterone concentration that is:
   a. The same as circulating levels.
   b. Lower than circulating levels.
   c. Static and unchanging.
   d. Much higher than circulating levels.
   e. Able to change rapidly with the maturational stage of the spermatozoon.

4. Puberty in the male:
   a. Occurs at about the same time for all species.
   b. Is influenced only by the age of the animal.
   c. Is synonymous with sexual maturity.
   d. Is defined as when he is first able to produce sufficient numbers of sperm to impregnate a female.
   e. Is independent of GnRH secretion.

5. Anabolic steroids are testosterone derivatives and therefore:
   a. Should be helpful in treating infertile males.
   b. Have no effect on male fertility.
   c. Enhance testicular function.
   d. Are only approved for use in stallions.
   e. Should not be used in males intended for breeding because of negative-feedback effects.

BIBLIOGRAPHY

Amann RP, Schanbacher BD: Physiology of male reproduction, *J Anim Sci* 57(suppl 2):380–403, 1983.

Coulter GH, Kastelic JP: Testicular thermoregulation in bulls, *Proc 15th Conf AI Reproduction N.A.A.B.* 28–34, 1994.

Neill JD, editor: *Knobil and Neill's physiology of reproduction*, vols 1 and 2, ed 3, Philadelphia, 2005, Elsevier.

Robaire B, Chan P: *Handbook of andrology*, ed 2, Lawrence, Kan, 2010, Allen Press.

Roberts SJ: Veterinary obstetrics and genital diseases. In *Theriogenology*, ed 3, Woodstock, Vt, 1986, David & Charles.

Strauss JF, Barbieri RL: *Yen and Jaffe's reproductive endocrinology: physiology, pathophysiology, and clinical management*, ed 6, Philadelphia, 2010, Saunders.