Regulation of the Menstrual Cycle

5

INTRODUCTION

Many superstitious beliefs have surrounded menstruation throughout recorded history. Indeed, attitudes and ideas about this aspect of female physiology have changed slowly. Hopefully, the scientific progress of the last few decades, which has revealed the dynamic relationships between the hypothalamic, pituitary, and gonadal hormones and the cyclic nature of the normal reproductive process, will yield a better understanding. The hormone changes, correlated with the morphologic and autocrine–paracrine events in the ovary, make the coordination of this system one of the most remarkable events in biology.

The diagnosis and management of abnormal menstrual function must be based on an understanding of the physiologic mechanisms involved in the regulation of the normal menstrual (ie, ovulatory) cycle. To understand the normal menstrual cycle, it is helpful to divide the cycle into three distinct phases: the follicular phase, ovulation, and the luteal phase. We will examine each of these segments of the menstrual cycle, concentrating on the changes in ovarian and pituitary hormones, processes that govern the pattern of hormone changes, and the effects of these hormones on the ovary, pituitary, and hypothalamus in regulating the menstrual cycle.

THE FOLLICULAR PHASE

In the human ovary, an orderly sequence of events in the follicular phase of the menstrual cycle results in the selection of a single follicle (dominant follicle) from within a group of immature follicles, and which is eventually ready for ovulation. This process, which occurs over the space of 10 to 14 days, features a series of sequential actions of hormones and autocrine–paracrine peptides on the follicle, leading the follicle destined to ovulate through a period of initial growth from a primordial follicle through the stages of the preantral, antral, and preovulatory follicle.

The Primordial Follicle

The primordial germ cells originate in the endoderm of the yolk sac, allantois, and hindgut of the embryo, and by 5 to 6 weeks of gestation, they have migrated to the genital ridge. A rapid mitotic multiplication of germ cells begins at 6 to 8 weeks' gestation, and by 16 to 20 weeks, the maximum number of oocytes is reached: a total of 6 to 7 million in both ovaries.[1] The primordial follicle consists of an oocyte that is arrested in the diplotene stage of meiotic prophase, surrounded by a single layer of spindle-shaped granulosa cells.

Until their numbers are exhausted, follicles begin to grow and undergo atresia under all physiologic circumstances. Follicular growth and atresia are not interrupted by pregnancy, ovulation, or periods of anovulation, including while taking ovulation-suppressing contraception. This dynamic process continues at all ages, including infancy and around the menopause. From the maximum number at 16 to 20 weeks of pregnancy, the number of oocytes will irretrievably decrease. The rate of decrease is proportional to the total number present; thus, the most rapid decrease occurs before birth, resulting in a decline from 6–7 to 2 million at birth and to 300,000 at puberty. From this large reservoir, about 400 follicles will ovulate during a woman's reproductive years.

The mechanism for determining which follicles and how many will start growing during any one cycle is unknown. The number of follicles that start growing each cycle appears to be dependent on the size of the residual pool of inactive primordial follicles.[2,3] Reducing the size of the pool (eg, unilateral oophorectomy) causes the remaining follicles to redistribute their availability over time. It is possible that the follicle that is singled out to play the leading role in a particular cycle is the beneficiary of a timely match of follicle

"readiness" (perhaps prepared by autocrine–paracrine actions in its microenvironment) and appropriate tropic hormone stimulation. The first follicle able to respond to stimulation may achieve an early lead that it never relinquishes.[4] Nevertheless, each cohort of follicles that begins growth is engaged in a serious competition that typically ends, in humans, with only one within the cohort eventually succeeding to attain dominance.

Rescue From Atresia (Apoptosis)

The early growth of follicles occurs over a time span of several menstrual cycles. The ovulatory follicle is one of a cohort that gets recruited at the time of the luteal–follicular transition in the preceding cycle.[5–7] The total duration of time to achieve preovulatory status is approximately 85 days. The majority of this time (until a late stage) involves responses that are independent of hormonal regulation.[8] Eventually, this cohort of follicles reaches a stage where, unless recruited (rescued) by follicle-stimulating hormone (FSH), the next step is atresia. Thus, follicles are continuously available (2–5 mm in size) for a response to FSH. An increase in FSH is the critical feature in rescuing a cohort of follicles from atresia, the usual fate of most follicles, eventually allowing a dominant follicle to emerge and pursue a path to ovulation. In addition, maintenance of this increase in FSH for a critical duration of time is essential.[9] Without the appearance and persistence of an increase in the circulating FSH level, the cohort is doomed to the process of apoptosis, programmed physiologic cell death to eliminate superfluous cells.[10] "Apoptosis" is derived from Greek and means falling off, like leaves from a tree (**Figure 5.1**).

"Recruitment" has been traditionally used to describe the continuing growth of antral follicles in response to FSH. A more useful concept is that the cohort of follicles responding to FSH at the beginning of a cycle is *rescued* from apoptosis. Remember that the very early development of follicles begins continuously and independently from gonadotropin influence. The fate of almost all of these follicles is apoptosis; only those exposed to an increase in FSH stimulation, because of the juxtaposition of their readiness to respond and the increase in FSH during the luteal–follicular transition, have the good fortune to compete for selection as a dominant follicle.

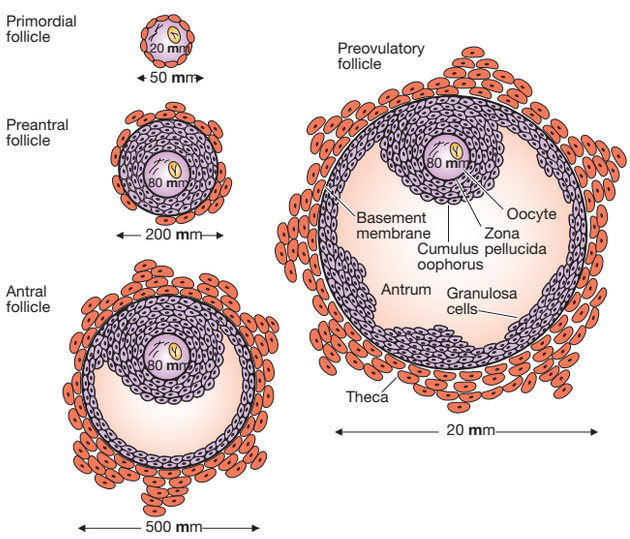

FIGURE 5.1

The first visible signs of follicular development are an increase in the size of the oocyte and the granulosa cells becoming cuboidal rather than squamous in shape. These changes are better viewed as a process of *maturation* rather than *growth*. At this same time, small gap junctions develop between the granulosa cells and the oocyte. Gap junctions are channels that, when open, permit the exchange of nutrients, ions, and regulatory molecules. Thus, the gap junctions serve as the pathway for nutritional, metabolite, and signal interchange between the granulosa cells and the oocyte, setting the stage for a bidirectional communication between the two-cell types. In one direction, inhibition of the final maturation of the oocyte (until the luteinizing hormone [LH] surge) is maintained by factors derived from the granulosa cells. In the other direction, the process of follicular growth is influenced by regulatory factors that originate in the oocyte.

The molecular events that regulate follicle development involve a variety of factors, all locally produced and regulated, including members of the transforming growth factor beta (TGF-β) superfamily of proteins and another family of trophic factors called neurotrophins. Activins, inhibins, antimüllerian hormone (AMH), and bone morphogenetic proteins (BMPs) are members of the TGF-β family of proteins. Activins promote and inhibins hinder primordial follicle development, and their relative local concentrations in the fetal ovary during the time of follicle assembly may determine the size of the ovarian follicular pool.[11] AMH is an important inhibitor of primordial follicle growth, and BMPs exert the opposite effect.[11] Neurotrophins and their receptors are essential for the differentiation and survival of various neuronal populations in the central and peripheral nervous systems, but their presence in the developing ovary suggests that they also play a role in ovarian development. Four mammalian neurotrophins have been identified, including nerve growth factor (NGF), brain-derived neurotropic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin 4/5 (NT-4/5), all of which exert their actions via binding to high-affinity transmembrane tyrosine kinase receptors encoded by members of the *trk* proto-oncogene family (NGF to TrkA, BDNF and NT-4/5 to TrkB, and NT-3 to TrkC).[12] Observations in NGF- and TrkA-null mice indicate that NGF stimulates the proliferation of ovarian mesenchymal cells during the early stages of follicular assembly and promotes differentiation and synthesis of FSH receptors in granulosa cells. Similar experiments with TrkB-null mice suggest that TrkB signaling is required for oocyte survival after follicular assembly and for preantral follicular development.[12] The specific signaling

mechanisms that mediate the effects of activins, inhibins, BMPs, and neurotrophins remain to be established.

Other paracrine factors mediate a bidirectional communication between oocytes and their surrounding granulosa cells. Oocytes are linked to their investment of granulosa cells via gap junctions, which allow the passage of small molecules such as ions (eg, calcium), metabolites (eg, pyruvate, nucleic acids, inositol), amino acids (eg, L-alanine), cholesterol, and intracellular signaling molecules (eg, cyclic adenosine monophosphate [cAMP]) between granulosa cells and oocytes. In mice, targeted deletions of gap junction proteins (known as connexins) disrupt follicular and oocyte development.[13] Oocytes are unable to use glucose as an energy source to support meiotic maturation, cannot transport certain amino acids, and lack both the enzymes necessary for cholesterol synthesis and the receptors for its uptake from carrier-borne sources. Consequently, oocytes are dependent on adjacent granulosa cells to metabolize glucose into a usable energy substrate, such as pyruvate, for transport of essential amino acids, such as L-alanine, and for synthesis and transfer of cholesterol.[14] **To meet their energy needs, oocytes stimulate glycolysis, amino acid transport, and cholesterol synthesis in granulosa cells via paracrine and juxtacrine signals that promote expression of transcripts involved in these metabolic processes, at least in some species**.[14] Candidate signaling molecules include closely related members of the TGF-β family, growth differentiation factor 9 (GDF9), and BMP15; both are expressed robustly in oocytes and appear crucial for normal ovarian follicle development in mammalian species.[15]

Mice that are genetically deficient in GDF-9, a peptide synthesized only in the oocyte after the primordial follicle becomes a preantral follicle, are infertile because follicular development cannot proceed beyond the primary follicle stage.[16,17] Mutations in GDF-9 and BMP-15 are rare causes of ovarian failure.[18–20]

Mutations in *FOXL2*, a gene encoding a transcription factor, cause blepharophimosis/ptosis/epicanthus inversus syndrome, a disorder affecting the eyelid and producing premature ovarian failure.[21,22] This transcription factor has been demonstrated to be essential for granulosa cell differentiation; indeed, mutations are associated with an absence of the very first sign of follicular development, the change in shape of granulosa cells from spindle to a cuboidal.[23]

The gap junction is composed of channels formed by an arrangement of proteins known as connexins, as well as GJAs. The connexin gap junctions are essential for growth and multiplication of the granulosa cells and for the nutrition and regulation of oocyte development.[24] Connexin expression in ovarian follicles is upregulated by FSH and downregulated by LH.[25] In addition, FSH maintains an open channel in the gap junctions, a pathway that is closed by LH.[26] After ovulation, the gap junctions are important again in the corpus luteum, when their function is regulated by locally produced oxytocin.[27]

With multiplication of the cuboidal granulosa cells, the primordial follicle becomes a primary follicle. In early stages of primary follicle development, the granulosa cell arrangement around the oocyte appears pseudostratified. The granulosa layer is separated from the stromal cells by a basement membrane called the basal lamina. The surrounding stromal cells differentiate into concentric layers designated the theca interna (closest to the basal lamina) and the theca externa (the outer portion). The theca layers appear when granulosa proliferation produces 3–6 layers of granulosa cells.[7]

The belief that the initiation of follicular growth from the primordial stage is independent of gonadotropin stimulation is supported by the persistence of this initial growth in gonadotropin-deficient mutant mice and in anencephalic fetuses.[28,29] In the vast majority of instances, this growth is limited and rapidly followed by atresia. In studies of human ovarian follicles, expression of the gene for the FSH receptor could not be detected until after primordial follicles began to grow.[30] Furthermore, in a woman with an inactivating mutation in the β (beta) subunit FSH gene, antral follicular activity was present, although successful growth and ovulation were impossible.[31] Treatment of FSH-deficient women with exogenous FSH results in follicular growth, ovulation, and pregnancy, demonstrating that oocytes and growth of follicles are essentially normal.[31,32]

The general pattern of limited growth and quick atresia is interrupted at the beginning of the menstrual cycle when a group of follicles (after approximately 70 days of development) responds to hormonal changes and is propelled to grow. In young women, this cohort numbers 3 to 11 follicles per ovary.[33] The decline in luteal phase steroidogenesis and inhibin-A secretion in the preceding cycle allows for a progressive late luteal rise in FSH, beginning a few days before menses.[34,35] The timing of this important event was based on data derived from the immunoassay of FSH. Using a sensitive measurement of FSH bioactivity, it has been suggested that increasing bioactivity of FSH begins in the mid- to late luteal phase.[36]

The Preantral Follicle

Once growth is accelerated, the follicle progresses to the preantral stage as the oocyte enlarges and is surrounded by a membrane, the zona pellucida. The granulosa cells undergo a multilayer proliferation as the theca layer continues to organize from the surrounding stroma. This growth is dependent on gonadotropins and is correlated with increasing ovarian production of estrogen. Molecular studies indicate that the granulosa cells in mature follicles are all derived from as few as three precursor cells.[37]

The granulosa cells of the preantral follicle have the ability to synthesize all three classes of steroids; however, significantly more estrogens than either androgens or progestins are produced. An aromatase enzyme system acts to convert androgens to estrogens and is a factor limiting ovarian

estrogen production. Aromatization is induced or activated through the action of FSH. The binding of FSH to its receptor and activation of the adenylate cyclase–mediated signal is followed by expression of multiple mRNAs, which encode proteins responsible for cell proliferation, differentiation, and function. Thus, FSH both initiates steroidogenesis (estrogen production) in granulosa cells and stimulates granulosa cell growth.[38]

Specific receptors for FSH are not detected on granulosa cells until the preantral stage,[30] and the preantral follicle requires the presence of FSH in order to aromatize androgens and generate its own estrogenic microenvironment.[39] Cellular estrogen production is, therefore, limited by its FSH receptor content. Administration of FSH will alter the concentration of its own receptor on granulosa cells (both up- and downregulation), in vivo and in vitro.[40] This action of FSH is modulated by growth factors.[41] FSH receptors quickly reach a concentration of approximately 1,500 receptors per granulosa cell.[42]

FSH operates through the G protein, adenylate cyclase system (described in Chapter 1), which is subject to downregulation and modulation by many factors, including a calcium-calmodulin intermediary. Although steroidogenesis in the ovarian follicle is regulated mainly by the gonadotropins, multiple signaling pathways are involved that respond to many factors besides the gonadotropins. Besides the adenylate cyclase enzyme system, these pathways include ion gate channels, tyrosine kinase receptors, and the phospholipase system of second messengers. These pathways are regulated by a multitude of factors, including growth factors, nitric oxide, prostaglandins, and peptides such as gonadotropin-releasing hormone (GnRH), angiotensin II, tissue necrosis factor-α (alpha), and vasoactive intestinal peptide (VIP). The binding of LH to its receptor in the ovary is also followed by activation of the adenylate cyclase–cyclic AMP pathway via the G protein mechanism.

FSH combines synergistically with estrogen to exert (at least in the nonprimate) a mitogenic action on granulosa cells to stimulate their proliferation. Together, FSH and estrogen promote a rapid accumulation of FSH receptors, reflecting in part the increase in the number of granulosa cells in the dominant follicle. The early appearance of estrogen within the selected follicle allows the follicle to respond to relatively low concentrations of FSH, an autocrine function for estrogen within the follicle. As growth proceeds, the granulosa cells differentiate into several subgroups of different cell populations. This appears to be determined by the position of the cells relative to the oocyte.

There is a system of communication that exists within follicles. Not every cell has to contain receptors for the gonadotropins. Cells with receptors can transfer a signal (by gap junctions), which causes protein kinase activation in cells that lack the receptors.[43] Thus, hormone-initiated action can be transmitted throughout the follicle even though only a subpopulation of cells binds the hormone. This system of communication promotes a coordinated and synchronous performance throughout the follicle, a system that continues to operate in the corpus luteum.

The role of androgens in early follicular development is complex. Specific androgen receptors are present in the granulosa cells.[44] The androgens serve not only as a substrate for FSH-induced aromatization but, in low concentrations, can further enhance aromatase activity. When exposed to an androgen-rich environment, preantral granulosa cells favor the conversion of androgens to more potent 5α-reduced androgens rather than to estrogens (**Figure 5.2**).[45] These androgens cannot be converted to estrogen and, in fact, inhibit aromatase activity.[46] They also inhibit FSH induction

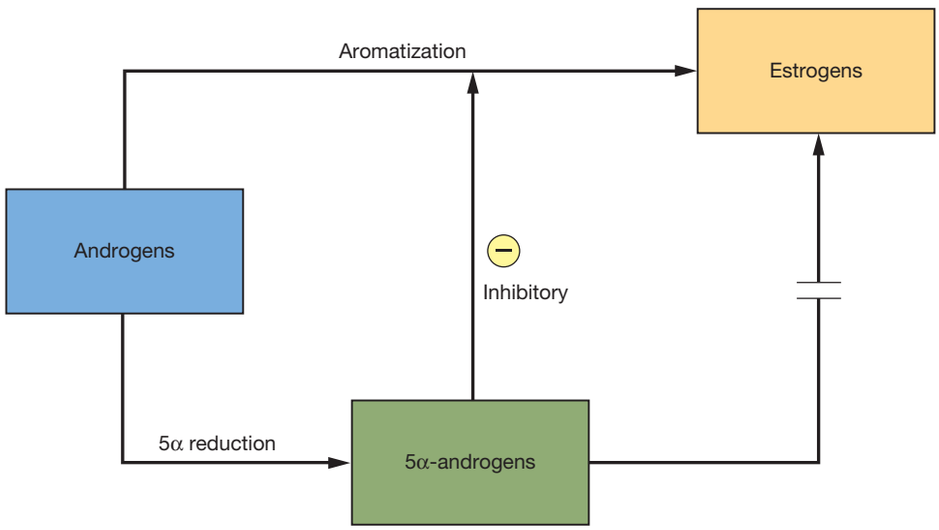

FIGURE 5.2

of LH receptor formation, another essential step in follicular development.[47]

The fate of the preantral follicle is in delicate balance. At low concentrations, androgens enhance their own aromatization and contribute to estrogen production. At higher levels, the limited capacity of aromatization is overwhelmed, and the follicle becomes androgenic and ultimately atretic.[48] The FSH to LH ratio is important for follicular recruitment and development. Follicles will progress in development only if emerging when FSH is elevated and LH is low. Those follicles arising at the end of the luteal phase or early in the subsequent cycle would be favored by an environment in which aromatization in the granulosa cell can prevail. **The success of a follicle depends on its ability to convert an androgen-dominated microenvironment to an estrogen-dominated microenvironment**.[49,50]

Key Points: Preantral Follicle

- Initial follicular development occurs independently of hormone influence.
- FSH stimulation propels follicles to the preantral stage.
- FSH-induced aromatization of androgen in the granulosa results in the production of estrogen.
- Together, FSH and estrogen increase the FSH receptor content of the follicle.

The Antral Follicle

Under the synergistic influence of estrogen and FSH, there is an increase in the production of follicular fluid that accumulates in the intercellular spaces of the granulosa, eventually coalescing to form a cavity, as the follicle makes its gradual transition to the antral stage. The accumulation of follicular fluid provides a means whereby the oocyte and surrounding granulosa cells can be nurtured in a specific endocrine environment. The granulosa cells surrounding the oocyte are now designated the **cumulus oophorus**. The differentiation of the cumulus cells is believed to be a response to signals originating in the oocyte.[51] The follicular fluid, rich in hormones, growth factors, and cytokines, provides the milieu that is required for the orderly maturation and development of the oocyte and its surrounding cells.

In the presence of FSH, estrogen becomes the dominant substance in the follicular fluid. Conversely, in the absence of FSH, androgens predominate.[52,53] LH is not normally present in follicular fluid until the midcycle. If LH is prematurely elevated in the plasma and antral fluid, mitotic activity in the granulosa decreases, degenerative changes ensue, and intrafollicular androgen levels rise. Therefore, the dominance of estrogen and FSH is essential for sustained accumulation of granulosa cells and continued follicular growth. Antral follicles with the greatest rates of granulosa proliferation contain the highest estrogen concentrations and the lowest androgen/estrogen ratios and are the most likely to house a healthy oocyte.[54] An androgenic milieu antagonizes estrogen-induced granulosa proliferation and, if sustained, promotes degenerative changes in the oocyte.

The steroids present in follicular fluid can be found in concentrations several orders of magnitude higher than those in plasma and reflect the functional capacity of the surrounding granulosa and theca cells. The synthesis of steroid hormones is functionally compartmentalized within the follicle: the two-cell system.[42,48,53,55,56]

The Two-Cell, Two-Gonadotropin System

The aromatase activity of granulosa cells far exceeds that observed in theca cells. In human preantral and antral follicles, LH receptors are present only on the theca cells and FSH receptors only on the granulosa cells.[57,58] Theca interstitial cells, located in the theca interna, have approximately 20,000 LH receptors in their cell membranes. In response to LH, theca tissue is stimulated to produce androgens that can then be converted, through FSH-induced aromatization, to estrogens in the granulosa cells.

The interaction between the granulosa and theca compartments, with resulting accelerated estrogen production, is not fully functional until later in antral development. Like preantral granulosa cells, the granulosa of small antral follicles exhibits an in vitro tendency to convert significant amounts of androgen to the more potent 5α-reduced form. In contrast, granulosa cells isolated from large antral follicles readily and preferentially metabolize androgens to estrogens. The conversion from an androgen microenvironment to an estrogen microenvironment (a change that is essential for further growth and development) is dependent on a growing sensitivity to FSH brought about by the action of FSH and the enhancing influence of estrogen.

As the follicle develops, theca cells begin to express the genes for LH receptors, P450scc, and 3β-hydroxysteroid dehydrogenase.[59] The separately regulated (by LH) entry of cholesterol into the mitochondria, utilizing internalization of low-density lipoprotein (LDL) cholesterol, is essential for steroidogenesis. **Therefore, ovarian steroidogenesis is LH dependent to a significant degree**. Human ovarian granulosa cells, after luteinization and vascularization that occur following ovulation, can use high-density lipoprotein (HDL) cholesterol in a system that differs from the LDL-cholesterol pathway. The HDL lipoproteins are not internalized, but rather, the cholesteryl esters are extracted from the lipoproteins at the cell surface and then transferred into the cell.[60]

As the follicle matures, the theca cells are characterized by their expression of P450c17, the enzyme step, which is rate limiting for the conversion of 21-carbon substrate to androgens.[61] Granulosa cells do not express P450c17 and

are thus dependent on androgens from the theca in order to make estrogen. Increasing expression of the aromatization system (P450arom) is a marker of increasing maturity of granulosa cells. The presence of P450c17 only in theca cells and P450arom only in granulosa cells is impressive evidence confirming the two-cell, two-gonadotropin explanation for estrogen production.[62]

The importance of the two-cell, two-gonadotropin system in the primate (**Figure 5.3**) is supported by the response of women with a deficiency in gonadotropins to treatment with recombinant (pure) FSH.[63–65] Follicles developed in these women, thus confirming the essential role of FSH, and the lesser role of LH, in recruitment and initial growth, but estradiol production was limited. Some aromatization occurred, perhaps using androgens originating in the adrenal glands, producing early follicular phase estradiol levels, but the usual robust steroidogenesis was impossible without the presence of LH to provide theca production of androgen substrate. This same response has been observed in experiments that use a GnRH antagonist to produce LH-deficient monkeys, followed by the administration of recombinant, pure human FSH.[66,67] **These results indicate that only FSH is required for folliculogenesis, and that in the primate, autocrine–paracrine peptides have assumed the important intraovarian role of modulating gonadotropin response. However, the final stages of maturation are optimized by LH, increasing the amount of androgen substrate for estrogen production and promoting the growth of the dominant follicle while simultaneously hastening the regression of smaller follicles.**[68]

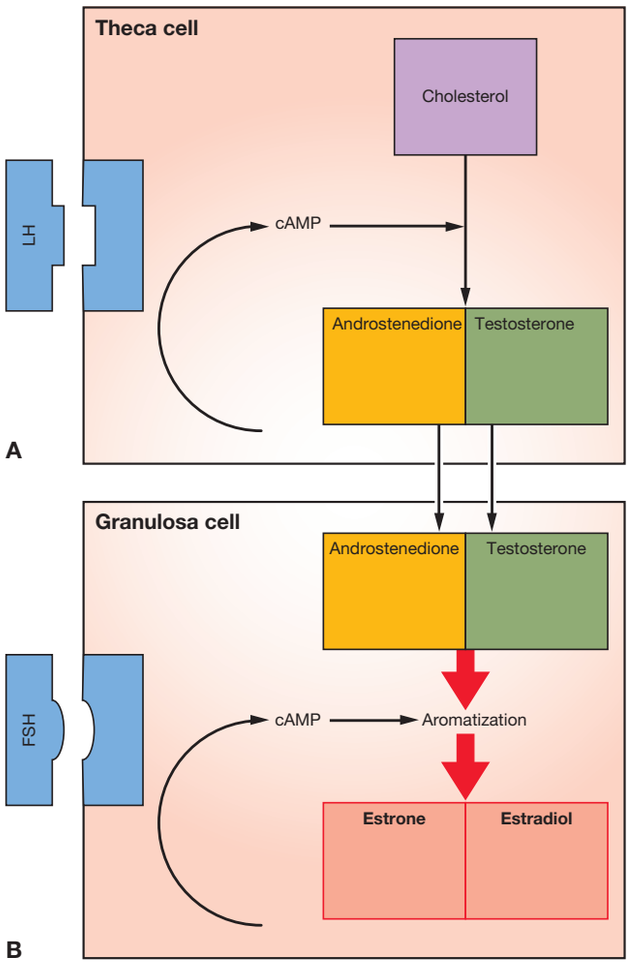

FIGURE 5.3

Selection of the Dominant Follicle

The successful conversion to an estrogen-dominant follicle marks the "selection" of a follicle destined to ovulate, the process whereby, with rare exceptions in humans, only a single follicle succeeds in each cycle.[69] This selection process is to a significant degree the result of two estrogen actions: (1) a local interaction between estrogen and FSH within the follicle and (2) the effect of estrogen on pituitary secretion of FSH. While estrogen exerts a positive influence on FSH action within the maturing follicle, its negative feedback relationship with FSH at the hypothalamic–pituitary level serves to withdraw gonadotropin support from the other less-developed follicles. The fall in FSH leads to a decline in FSH-dependent aromatase activity, limiting estrogen production in the less mature follicles. Even if a lesser follicle succeeds in achieving an estrogen microenvironment, decreasing FSH support would interrupt granulosa proliferation and function, promote a conversion to an androgenic microenvironment, and thereby induce irreversible atretic change. Indeed, the first event in the process of atresia is a reduction in FSH receptors in the granulosa layer.

The loss of oocytes (and follicles) through atresia is a response to changes in many factors. Certainly, gonadotropin stimulation and withdrawal are important, but ovarian steroids and autocrine–paracrine factors are also involved. The consequence of these unfavorable changes, atresia, in the process called **apoptosis**, programmed cell death, is heralded by alterations in mRNAs required for cell proteins that maintain follicle integrity.[70] This type of "natural death" is a physiologic process, in contrast to the pathologic cell death of necrosis.

Once cells have entered the process of apoptosis, their response to FSH is modulated by local growth factors. Tumor necrosis factor (TNF), produced in the granulosa cells, inhibits FSH stimulation of estradiol secretion, except in the dominant follicle.[71] An inverse relationship exists between TNF expression and gonadotropin stimulation of granulosa cells. Thus, as the successful follicle increases its response to gonadotropins, its TNF production decreases. Those follicles with a failing response to gonadotropins increase their TNF production, hastening their own demise.

Although the principal function of AMH is to cause müllerian duct regression during male embryonic sexual

differentiation, AMH is detected in the granulosa cells of early primordial follicles and reaches peak concentrations in the small antral follicles.[72] Its secretion appears to be regulated by the mature oocyte, and AMH decreases when FSH-stimulated follicular growth and estrogen production occur.[73,74] Studies with knockout model mice have indicated that AMH inhibits the recruitment of primordial follicles.[75] The paracrine activity of AMH inhibits FSH-stimulated follicle growth, thus suppressing the growth of lesser follicles and allowing the dominant follicle to emerge. Because of these activities, the circulating level of AMH reflects the number of growing follicles, and the blood concentration of AMH is a recognized measure of ovarian aging and as a prognostic marker for ovarian response during fertility treatment.[76] AMH levels are relatively unaffected by gonadotropins or the sex steroids. Measurement of AMH is reliable on any day in an individual's menstrual cycle,[77] although prolonged periods of ovarian suppression, such as with the use of hormonal contraceptives, can result in spuriously lower circulating AMH levels.[78]

An asymmetry in ovarian estrogen production, an expression of the emerging dominant follicle, can be detected in ovarian venous effluent as early as the fifth day of the cycle, corresponding with the gradual fall of FSH levels observed at the midfollicular phase and preceding the increase in diameter that marks the physical emergence of the dominant follicle.[79] This is a crucial time in the cycle. Exogenous estrogen, administered even after selection of the dominant follicle, disrupts preovulatory development and induces atresia by reducing FSH levels below the sustaining level. Because the lesser follicles have entered the process of atresia, loss of the dominant follicle during this period requires beginning over, with recruitment of another set of preantral follicles.[80]

The negative feedback of estrogen on FSH serves to inhibit the development of all but the dominant follicle. The selected follicle remains dependent on FSH and must complete its preovulatory development in the face of declining plasma levels of FSH. The dominant follicle, therefore, must escape the consequences of declining FSH induced by its own accelerating estrogen production. **The dominant follicle has two significant advantages, a greater content of FSH receptors acquired because of a rate of granulosa proliferation that surpasses that of its cohorts and enhancement of FSH action because of its high intrafollicular estrogen concentration, a consequence of local autocrine–paracrine molecules.** Thus, the dominant follicle is more sensitive to FSH, and as long as a critical duration of FSH exposure was initially present, the dominant follicle continues to develop.[9] As a result, the stimulus for aromatization, FSH, can be maintained, while at the same time, it is being withdrawn from among the less developed follicles. A wave of atresia among the lesser follicles, therefore, is seen to parallel the rise in estrogen.

The accumulation of a greater mass of granulosa cells is accompanied by advanced development of the theca vasculature. By day 9, theca vascularity in the dominant follicle is twice that of other antral follicles.[81] This allows a preferential delivery of gonadotropins to the follicle, permitting the dominant follicle to retain FSH responsiveness and sustain continued development and function despite waning gonadotropin levels. The monkey ovary expresses a potent growth factor (vascular endothelial growth factor [VEGF]) that induces angiogenesis, and this expression is observed at the two development points when proliferation of capillaries is important: the emerging dominant follicle and the early corpus luteum (**Figure 5.4**).[82,83]

In order to respond to the ovulatory LH surge and to become a successful corpus luteum, the granulosa cells must acquire LH receptors. FSH induces LH receptor development on the granulosa cells of the large antral follicles. Here again, estrogen and local autocrine–paracrine peptides serve as the chief coordinators. With increasing concentrations of estrogen within the follicle, FSH changes its focus of action, from upregulating its own receptor to the generation of LH receptors.[84] The combination of a capacity for continued response despite declining levels of FSH and a high local estrogen environment in the dominant follicle provides optimal conditions for LH receptor development. LH can induce the formation of its own receptor in FSH-primed granulosa cells, but the primary mechanism utilizes FSH stimulation and estrogen enhancement.[85,86] The role of estrogen goes beyond synergism and enhancement; it is obligatory.

Evidence from ovarian stimulation for in vitro fertilization (IVF) indicates that LH plays a critical role in the late stages of follicle development, providing support for the final maturation and function of the dominant follicle.[68,87] At least one LH contribution in the late follicular phase is the LH-mediated stimulation of androgen production in the theca to provide for the large amounts of estrogen required at this point in the cycle. In addition, the theca androgens may have a direct beneficial effect on essential growth factors within the follicle. LH presence in the follicle prior to ovulation, therefore, is an important contributor to optimal follicular development that ultimately provides a healthy oocyte.[88,89]

The local action of estrogen within the ovarian follicle was questioned when initial studies failed to detect estrogen receptors in any of the significant ovarian compartments.[90] Subsequently, it was discovered that human granulosa cells and primate oocytes contain only mRNA for estrogen receptor-β.[91–94] The dynamic expression of estrogen receptor-β is consistent with an important local role for estrogen in ovarian follicle and corpus luteum growth and function.

Although prolactin is always present in follicular fluid, there is no evidence to suggest that prolactin is important during normal ovulatory cycles in the primate.

The Feedback System

Through its own estrogen and peptide production, the dominant follicle assumes control of its own destiny. By altering

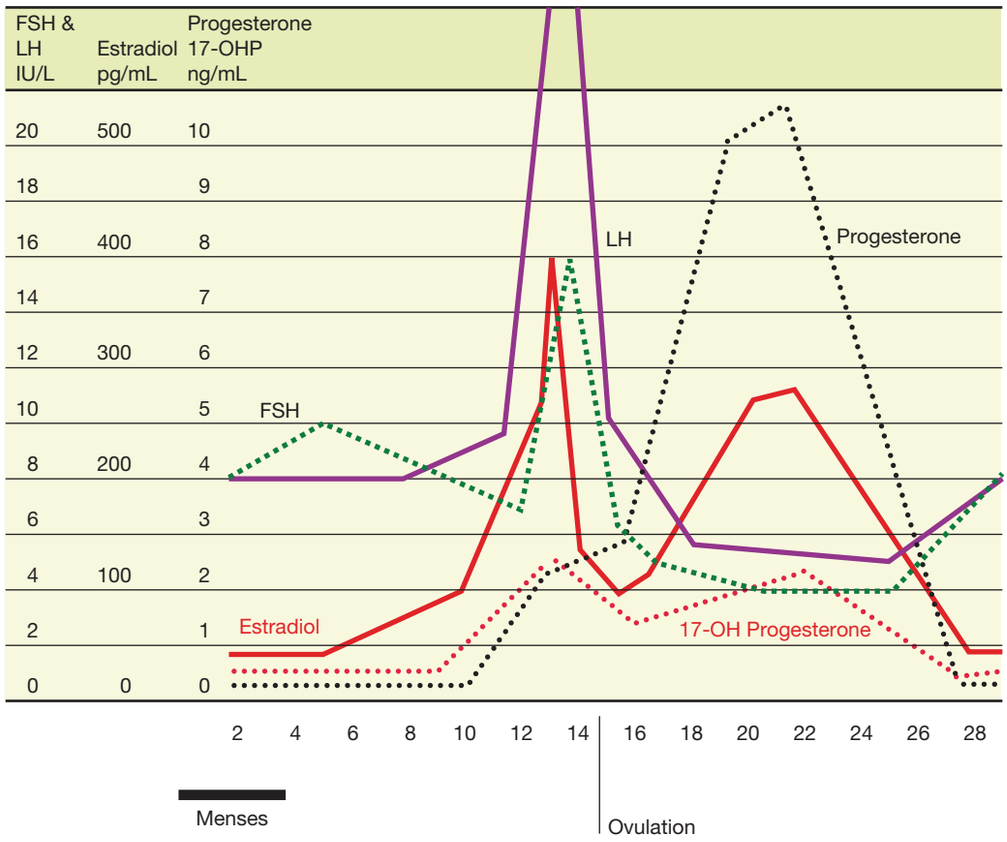

FIGURE 5.4

gonadotropin secretion through feedback mechanisms, it optimizes its own environment to the detriment of the lesser follicles.

As reviewed in Chapter 4, GnRH plays an obligatory role in the control of gonadotropin secretion, but the pattern of gonadotropin secretion observed in the menstrual cycle is the result of feedback modulation of steroids and peptides originating in the dominant follicle, acting directly on the hypothalamus and anterior pituitary.[5] An increase in GnRH accompanying the LH surge, indicating that positive feedback of estrogen operates at both pituitary and hypothalamic sites, has been reported in monkeys but not in humans.[95,96] Positron emission tomography (PET) studies in women have indicated that the positive feedback effects of estrogen on LH occur at the level of the pituitary.[97]

Estrogen exerts its inhibitory effects in both the hypothalamus and the anterior pituitary, decreasing both GnRH pulsatile secretion and the pituitary gonadotropin response to GnRH.[98] Progesterone also operates at these two sites. Its inhibitory action is at the hypothalamic level, and its positive action is directly on the pituitary.[99] As determined by PET studies, the primary site of estrogen-negative feedback on LH is the hypothalamus.[97]

The secretion of FSH is very sensitive to the negative inhibitory effects of estrogen even at low levels. At higher levels, estrogen combines with inhibin for a suppression of FSH that is profound and sustained. In contrast, the influence of estrogen on LH release varies with concentration and duration of exposure. At low levels, estrogen imposes a negative feedback relationship with LH. At higher levels, however, estrogen is capable of exerting a positive stimulatory feedback effect on LH release.

The transition from estrogen-mediated suppression to stimulation of LH release occurs as estradiol levels rise during the midfollicular phase. There are two critical features in this mechanism: (1) the concentration of estradiol and (2) the length of time during which the estradiol elevation is sustained. In women, the estradiol concentration necessary to achieve a positive feedback effect on LH release is more than 200 pg/mL, and this concentration must be sustained for approximately 50 hours.[100] In spontaneous cycles, this level of circulating estrogen is achieved approximately when the dominant follicle has reached a diameter of 15 mm.[101] The estrogen stimulus must be sustained beyond the initiation of the LH surge until after the surge actually begins. Otherwise, the LH surge is abbreviated or fails to occur at all.

Within the well-established monthly pattern, the gonadotropins are secreted in a pulsatile fashion with a frequency and magnitude that vary with the phase of the cycle. While the pulsatile pattern of gonadotropins is directly due to a similar pulsatile secretion of GnRH, the amplitude and frequency modulations are the consequence of steroid feedback on both the hypothalamus and the anterior pituitary.[102–104] **Pulsatile secretion of gonadotropins is more frequent but smaller in amplitude during the follicular phase compared to the luteal phase, with a slight increase in frequency observed as the follicular phase progresses to ovulation**.

The pulsatile pattern of FSH is not easily discerned because of its relatively longer half-life compared to LH, but the experimental data indicate that FSH and LH are secreted simultaneously and that GnRH stimulates the secretion of both gonadotropins. Even as late as only 36 to 48 hours before menses, gonadotropin secretion is still characterized by infrequent LH pulses and low FSH levels typical of the late luteal phase.[102] During the transition from the previous luteal phase to the next follicular phase, GnRH and the gonadotropins are released from the inhibitory effects of estradiol, progesterone, and inhibin. A progressive and fairly rapid increase in GnRH pulse secretion is associated with a preferential secretion of FSH compared to LH. The frequency of GnRH and LH pulses increases 4.5 fold during this period and is accompanied by a 3.5-fold increase in the circulating levels of FSH and a 2-fold increase in LH levels.[105]

The GnRH pulse frequency changes in the luteal phase correlate with duration of exposure to progesterone, while pulse amplitude changes appear to be influenced by changes in progesterone levels.[102] Both estradiol and progesterone are required to achieve the low, suppressed secretory pattern of GnRH during the luteal phase.[106] Evidence supports that the circulating steroids influence changes in both frequency and amplitude of gonadotropin pulses in the different phases of the cycle; the frequency of gonadotropin pulses is modulated through effects on frequency of GnRH release at the level of the hypothalamus, whereas the amplitude of gonadotropin pulses is mediated by actions of steroids at the level of the pituitary. The inhibitory action of luteal phase steroids on gonadotropin release appears to be mediated by an increase in hypothalamic endogenous opioid peptides. Both estrogen and progesterone can increase endogenous opiates. Estrogen appears to enhance the stimulatory action of progesterone in the luteal phase on endogenous opioid peptides, creating relatively high levels of endogenous opiates during the luteal phase. Administration of clomiphene (an estrogen receptor antagonist) during the luteal phase increases the LH pulse frequency with no effect on amplitude.[107]

Plasma endorphin begins to rise in the 2 days before the LH peak, coinciding with the midcycle gonadotropin surge.[108] The maximal level is reached just after the LH peak, coinciding with ovulation. Levels then gradually decline until the nadir is reached during menses and the early follicular phase. Monkeys have their highest β-endorphin levels in the hypophyseal portal blood at midcycle.[108,109] **Normal cyclicity requires sequential periods of high (during midcycle and luteal phase) and low (during menses) hypothalamic opioid activity.**

There is another important action of estrogen. A disparity exists between the patterns of FSH and LH secretion as determined by immunoassay and bioassay, indicating that more biologically active gonadotropins are secreted at midcycle than at other times in the cycle.[110] This quality, bioactivity versus immunoreactivity, is determined by the molecular structure of the gonadotropin molecule, a concept referred to in Chapter 1 as heterogeneity of the tropic hormones. There is a well-established relationship between the activity and half-life of glycoprotein hormones and their sialic acid content. The feedback effects of estrogen include modulation of sialylation and the size and activity of the gonadotropins subsequently released, as well as an augmentation of GnRH-stimulated secretory release of biologically active gonadotropin. It certainly makes sense to intensify the gonadotropin effect at midcycle. The positive feedback actions of estrogen, therefore, increase both the quantity and the quality (the bioactivity) of FSH and LH. In addition to the change at midcycle that favors gonadotropin activity at the ovarian follicle, FSH isoforms with greater biologic activity also increase during the late luteal phase, a change that is appropriately geared toward propelling new ovarian follicle growth for the next cycle.[111,112]

There is a diurnal rhythm in FSH and LH secretion.[113] In contrast to the nocturnal rise seen with other anterior pituitary hormones such as adrenocorticotropic hormone (ACTH), thyroid-stimulating hormone (TSH), growth hormone, and prolactin, FSH and LH exhibit nocturnal decline, probably mediated by the endogenous opiates. This diurnal rhythm for LH is present only in the early follicular phase, while FSH maintains a circadian rhythm throughout the menstrual cycle (and, thus, it is not influenced by steroid hormone feedback) and even in the postmenopausal period of life.

Inhibin, Activin, Follistatin

This family of peptides is synthesized by the granulosa cells in response to FSH and secreted into the follicular fluid and ovarian venous effluent.[114–117] Expression of these peptides is not limited to the ovary; they are present in many tissues throughout the body, serving as autocrine–paracrine regulators. Inhibin is an important inhibitor of FSH secretion. Activin stimulates FSH release in the pituitary and augments FSH action in the ovary. Follistatin suppresses FSH activity, by binding activin.

Inhibin consists of two dissimilar peptides (known as α- and β-subunits) linked by disulfide bonds. Two forms of inhibin (inhibin-A and inhibin-B) have been purified, each containing an identical α-subunit and distinct but related β-subunits. Thus, there are three subunits for inhibins: alpha

(α), beta-A (βA), and beta-B (βB). Each subunit is a product of a different messenger RNA, each derived from its own precursor molecule. Mutations of the α subunit have been found in patients with premature ovarian failure.[118,119]

The Two Forms of Inhibin:

1. **Inhibin-A: Alpha-BetaA** (α-βA)
2. **Inhibin-B: Alpha-BetaB** (α-βB)

FSH stimulates the secretion of inhibin from granulosa cells and, in turn, is suppressed by inhibin—a reciprocal relationship similar to its relationship with estrogen.[120,121] Inhibin-B is the form of inhibin predominantly secreted by granulosa cells in the follicular phase of the cycle.[122,123] The secretion of inhibin is further regulated by local autocrine–paracrine control. GnRH and epidermal growth factor (EGF) diminish FSH stimulation of inhibin secretion, whereas insulin-like growth factor-1 (IGF-1) enhances inhibin production. The inhibitory effects of GnRH and EGF are consistent with their known ability to decrease FSH-stimulated estrogen production and LH receptor formation. The two forms of GnRH (GnRH-1 and GnRH-2) along with their receptor are expressed in granulosa cells.[124,125]

The secretion of inhibin-B into the circulation further amplifies the withdrawal of FSH from other follicles, another mechanism by which an emerging follicle secures dominance. Inhibin-B rises slowly but steadily, in a pulsatile fashion (60–70 minute periodicity), reaching peak levels in the early and midfollicular phases and then decreasing in the late follicular phase before ovulation to reach a nadir in the midluteal phase (**Figure 5.5**).[35,122,126,127] An inhibin-B peak the day after ovulation is probably the result of release from the ruptured follicle. This relationship of inhibin B and FSH is supported by the demonstration that inhibin-B levels are lower and FSH levels are higher in the follicular phase in women 45 to 49 years old compared to younger women.[126,128] An ovarian fibrothecoma secreting inhibin-B was predictably associated with secondary amenorrhea and infertility due to suppression of FSH secretion.[129]

With the appearance of LH receptors on the granulosa cells of the dominant follicle and the subsequent development of the follicle into a corpus luteum, inhibin expression comes under the control of LH, and expression changes from inhibin-B to inhibin-A.[130] The circulating levels of inhibin A rise in the late follicular phase to reach a peak level at the midluteal phase.[35,131] Inhibin A, therefore, contributes to the suppression of FSH to nadir levels during the luteal phase and to the changes at the luteal–follicular transition.

Inhibin has multiple, diverse inhibitory effects on gonadotropin secretion. Inhibin can block the synthesis and secretion of FSH, prevent the upregulation of GnRH receptors by

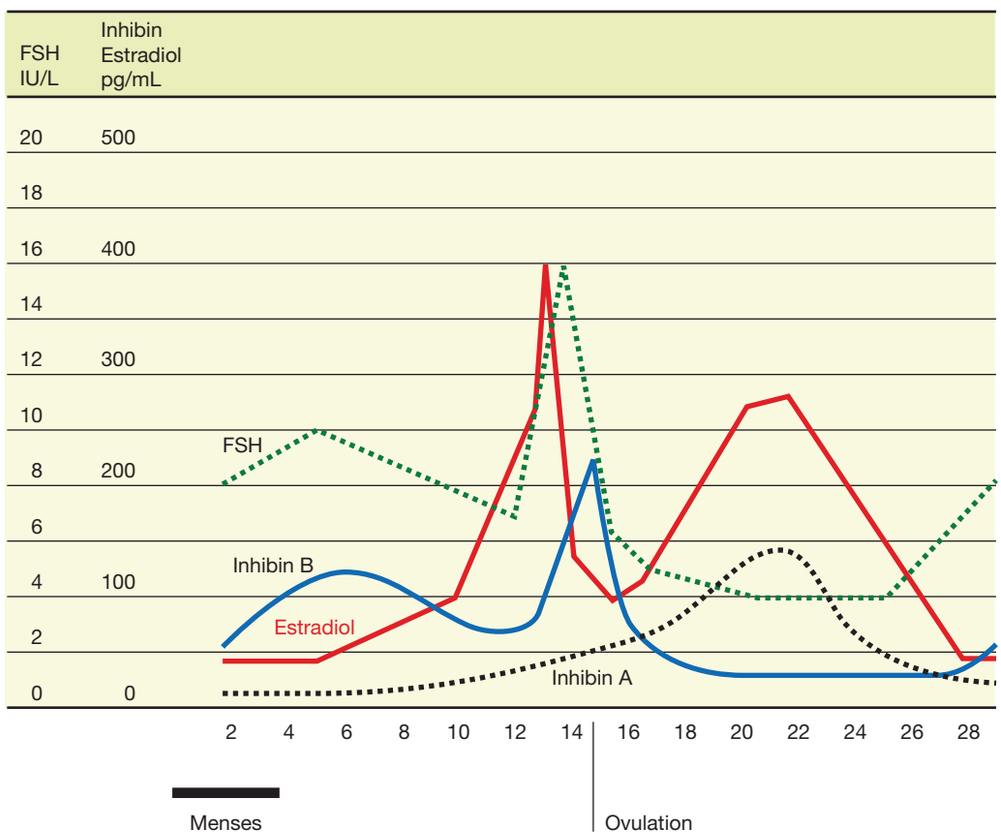

FIGURE 5.5

GnRH, reduce the number of GnRH receptors present, and, at high concentrations, promote the intracellular degradation of gonadotropins.

Activin, derived from granulosa cells but also present in the pituitary gonadotropes, contains two subunits that are identical to the βA and βB subunits of inhibin A and B. In addition, activins have been identified with variants of the β subunits, designated as beta-C (βC), beta-D (βD), and beta-E (βE).[132] The activin βC and βE genes have been demonstrated to be nonessential in mouse knockout models.[133] Activin augments the secretion of FSH and inhibits prolactin, ACTH, and growth hormone responses.[134–137] Activin increases pituitary response to GnRH by enhancing GnRH receptor formation.[138,139] The effects of activin are blocked by inhibin and follistatin.[140] The structure of the activin genes is homologous to that of TGF-β, indicating that these products all come from the same gene family.[141]

The three forms of Activin:

1. **Activin-A:** **BetaA-BetaA** (βA-βA)
2. **Activin-AB:** **BetaA-BetaB** (βA-βB)
3. **Activin-B:** **BetaB-BetaB** (βB-βB)

Activin is present in many cell types, regulating growth and differentiation. In the ovarian follicle, activin increases FSH binding in granulosa cells (by regulating receptor numbers) and augments FSH stimulation of aromatization and inhibin production.[116] Considerable evidence derived from studies using human cells indicates that inhibin and activin act directly on theca cells to regulate androgen synthesis.[142–144] Inhibin enhances the stimulatory action of LH and/or IGF-1, while activin suppresses this action. In increasing doses, inhibin can overcome the inhibitory action of activin. Prior to ovulation, activin suppresses progesterone production by granulosa cells, perhaps preventing premature luteinization. There is a repertoire of cell transmembrane kinase receptors for activin, with differing binding affinities and domain structures.[145] This receptor heterogeneity allows the many different responses elicited by a single peptide. Both activin-A and inhibin-A have been demonstrated to be very potent in stimulating in vitro maturation of oocytes that subsequently yield a high rate of fertilization.[146]

In the male, activin inhibits and inhibin facilitates LH stimulation of androgen biosynthesis in the testicular Leydig cells. In addition, activin stimulates and inhibin decreases spermatogonial proliferation; inhibin is produced in the Sertoli cell, the locus that has the principal role in modulating spermatogenesis. Thus, activin and inhibin play similar autocrine–paracrine roles in both the male and the female gonads.

The anterior pituitary expresses the inhibin–activin subunits, and locally produced activin-B augments FSH secretion. Activin-A has been demonstrated to directly stimulate the synthesis of GnRH receptors in pituitary cells.[147] Follistatin is a glycopeptide secreted by a variety of pituitary cells, including the gonadotropes.[148] This peptide has also been called FSH-suppressing protein because of its main action: inhibition of FSH synthesis and secretion and the FSH response to GnRH by binding to activin and in that fashion decreasing the activity of activin.[149,150] Activin stimulates follistatin production, and inhibin prevents this response. Follistatin is also expressed by granulosa cells in response to FSH, and, therefore, follistatin, like inhibin and activin, functions locally in the follicle and in the pituitary.[151] Circulating levels of activin increase in the late luteal phase to peak at menses; however, activin-A is highly bound in the circulation, and it is not certain it has an endocrine role.[152]

In summary, the pituitary secretion of FSH can be significantly regulated by the balance of activin and inhibin, with follistatin playing a role by inhibiting activin and enhancing inhibin activity. Within the ovarian follicle, activin and inhibin influence growth and development by modulating theca and granulosa cell responses to gonadotropins.

Growth Factors

Growth factors are polypeptides that modulate cell proliferation and differentiation, operating through binding to specific cell membrane receptors. They are not classic endocrine substances; they act locally and function in paracrine and autocrine modes. There are multiple growth factors, and most cells contain multiple receptors for the various growth factors. A major interest in intraovarian injection of autologous platelet-rich plasma (PRP) has recently been explored to enhance follicle development and clinical outcomes in women with diminished ovarian reserve and ovarian failure.[153,154] While the current evidence from randomized clinical trials does not support a clinical benefit,[155,156] PRP contains high levels of cytokines (IL-1β and IL-6) and growth factors (TGF-β, VEGF, IGF-1, EGF, platelet-derived growth factor [PDGF], and fibroblast growth factor [FGF]) and induces changes in cumulus cell gene expression.[157]

Insulin-Like Growth Factors

The insulin-like growth factors (IGFs) (also called somatomedins) are peptides that have structural and functional similarity to insulin and mediate growth hormone action.[158] IGF-1 and IGF-2 are single chain polypeptides containing three disulfide bonds. IGF-1 is encoded on the long arm of chromosome 12 and IGF-2 on the short arm of chromosome 11 (which also contains the insulin gene). The genes are subject to a variety of promoters, and thus, differential regulation can govern ultimate actions.

IGF-1 mediates the growth-promoting actions of growth hormone. The majority of circulating IGF-1 is derived from growth hormone–dependent synthesis in the liver. However, IGF-1 is also synthesized in many tissues where production can be regulated in conjunction with growth hormone or **independently** by other factors.

IGF-2 has little growth hormone dependence. It is involved in fetal growth and development, metabolic disorders, and tumorigenesis. Both IGFs induce the expression of cellular genes responsible for cellular proliferation and differentiation.

Insulin-Like Growth Factor–Binding Proteins

There are seven known nonglycosylated peptides that function as IGF-binding proteins (IGFBPs), IGFBP-1 to IGFBP-7.[159] These binding proteins serve to carry the IGFs in serum, prolong their half-lives, and regulate their tissue effects. The regulating action of IGFBPs appears to be due to binding and sequestering of the IGFs, preventing their access to the cell membrane surface receptors, and, thus, not permitting the synergistic actions that result when gonadotropins and growth factors are combined. The IGFBPs may also exert direct actions on cellular functions, independently of growth factor functions. IGFBP-1 expression rises at least 100 to 200 fold in human endometrial stromal cells following decidualization.[160] IGFBP-1 is the principal binding protein in the amniotic fluid, whereas IGFBP-3 is the main binding protein in serum, and its synthesis, which occurs primarily in the liver, is dependent on growth hormone. Circulating levels of IGFBP-3 reflect the total IGF concentration (IGF-1 plus IGF-2) and carry at least 90% of the circulating IGFs. The IGFBPs change with age (decreasing levels of IGFBP-3) and during pregnancy (decreasing IGFBP-3 due to a circulating protease unique to pregnancy). IGFBPs do not bind insulin.

Insulin-Like Growth Factor Receptors

The Type 1 receptor preferentially binds IGF-1 and can be called the IGF-1 receptor. The Type 2 receptor can, in a similar fashion, be called the IGF-2 receptor. IGF-1 also binds to the insulin receptor but with low affinity. Insulin binds to the IGF-1 receptor with moderate affinity. The IGF-1 receptor and the insulin receptor are similar in structure: tetramers composed of two α-subunits and two β-subunits linked by disulfide bonds. The intracellular component of the β-subunit is a tyrosine kinase that is activated by autophosphorylation. The IGF-2 receptor does not bind insulin. It is a single chain glycoprotein, with 90% of its structure extending extracellularly. This receptor functions as a receptor coupled to a G protein. The physiologic effects of IGF-1 are mediated by its own receptor, but IGF-2 can exert its actions via both receptors. Indeed, the IGF-1 receptor binds IGF-1 and IGF-2 with equal affinity. In human cells, the IGF-1 receptor and IGF-2 receptor are present in theca and granulosa cells and in luteinized granulosa cells. Ovarian stromal tissue contains IGF-1 receptors.

The Ovarian Actions of IGFs

IGF signaling is recognized to play critical roles in the processes of follicular development, growth, and steroidogenesis (**Figures 5.6–5.8**). IGF-1 has been demonstrated to stimulate the following events in ovarian theca and granulosa cells: DNA synthesis, steroidogenesis, aromatase activity, LH receptor synthesis, and inhibin secretion. IGF-2 stimulates granulosa mitosis. In human ovarian cells, IGF-1, in synergy with FSH, stimulates protein synthesis and steroidogenesis. After LH receptors appear, IGF-1 enhances LH-induced progesterone synthesis and stimulates the proliferation of granulosa–luteal cells. IGF-1, in synergy with FSH, is very active in stimulating aromatase activity in the preovulatory follicles. Thus, IGF-1 can be involved in both estradiol and progesterone synthesis.

In animal experiments, synthesis of IGF-1 by the granulosa cells is dependent on FSH but enhanced by estradiol. Growth hormone also acts synergistically with FSH and estradiol to increase IGF synthesis.

In studies with human ovarian tissue, IGF-2 is highly expressed in both theca cells and granulosa cells; however, the level is highest in the granulosa and increases with growth of the follicle.[161,162] IGF-2 is also synthesized by luteinized granulosa and appears to function locally in an autocrine fashion.[163] These findings indicate that IGF-2 is the primary IGF in the human ovary. Nevertheless, IGF-1 is still a significant product of human theca cells.[164]

Human theca cells express mRNA transcripts that encode receptors for both IGF-1 and insulin.[165] Because insulin and IGF-2 can both activate the receptor for IGF-1, this pathway provides a method for exertion of paracrine influences on granulosa cells and autocrine activity in the theca (augmenting LH stimulation of androgen production). In vitro studies confirm that IGF-2 is capable of stimulating steroidogenesis and proliferation in human theca and granulosa cells.[166–168] These actions are augmented by growth hormone, which increases IGF production and, thus, indirectly enhances gonadotropin stimulation of ovarian follicles.[169]

This primate scenario is supported by finding higher levels of IGF-2, but not IGF-1, in the follicular fluid of developing follicles, with the highest levels present in dominant follicles.[170] The IGF levels in follicular fluid correlate with estradiol levels and undergo a further transient increase after the LH surge. There are no menstrual cycle changes in the circulating levels of IGF-1, IGF-2, IGFBP-1, or IGFBP-3; high levels in the dominant follicle are not associated with an increase in circulating levels.[171]

In human studies, IGFBP-1 inhibits IGF-1–mediated steroidogenesis and proliferation of the luteinized granulosa cells. Synthesis of IGFBPs by human granulosa is inhibited by FSH, IGF-1, and IGF-2.[172,173] These findings fit with the overall idea that the binding proteins counteract the synergism between gonadotropins and growth factors. In general, IGFBP-1 expression is found in granulosa cells of the growing follicles; IGFBP-3 in theca cells and the granulosa of the dominant follicle; and IGFBP-2, -4, and -5 in theca and granulosa of antral and atretic follicles; and relatively

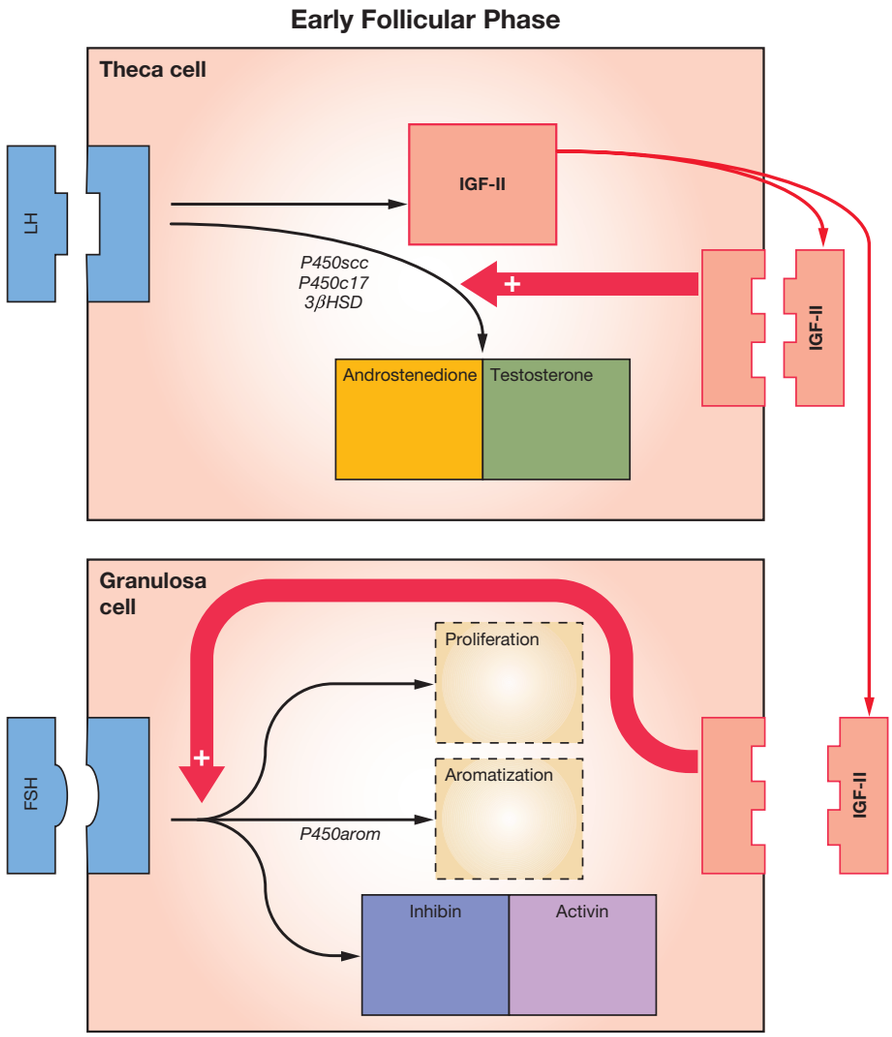

FIGURE 5.6

lower levels of IGFBP-6[174] and IGFBP-7[175] have been found in ovarian cancer cells.[161] The predominant binding protein in the preovulatory follicles is IGFBP-2 in the granulosa and IGFBP-3 in the theca; these increase progressively in the follicle that gains dominance and then decrease in the late follicular phase.[162,176,177] These patterns suggest that IGFBPs -1, -2, and -3 play a role in the growing follicles, whereas IGFBPs -2, -4, and -5 are relevant in the atretic and failing follicles. IGFBP expression in polycystic ovaries is like that seen in atretic follicles. The decrease in IGFBP-3 that occurs in dominant follicles should allow an increase in IGF levels and activity. The increase in IGFBP-2 in the failing follicles probably correlates with sequestering of IGF, depriving the follicle of an important force in gonadotropin augmentation.

Circulating levels of IGFBP-1 decrease in response to insulin, and thus circulating levels (and follicular fluid levels)[178] are decreased in women with anovulation and polycystic ovary syndrome who have elevated levels of insulin.[179] These patients also have increased circulating levels of IGF-1, probably a consequence of LH-stimulated synthesis and secretion in the theca cells. The level of IGFBP-1 in the follicular fluid from polycystic ovaries is decreased; thus, this binding protein is not playing a role inhibiting the action of IGF-1 in the polycystic ovaries. The levels of IGFBPs -2 and -4 in the follicular fluid from follicles in anovulatory patients are increased (as in atretic follicles).[161,180] Even though these changes may play a role in anovulatory pathophysiology, they are consistent with failure in development and thus may not be etiologic factors.

IGF activity may also be modulated by the proteases that regulate the activity of the IGFBPs.[181] Estrogen-dominant follicular fluid contains very low levels of IGFBP-4, in contrast to the high levels present in androgen-dominant follicular

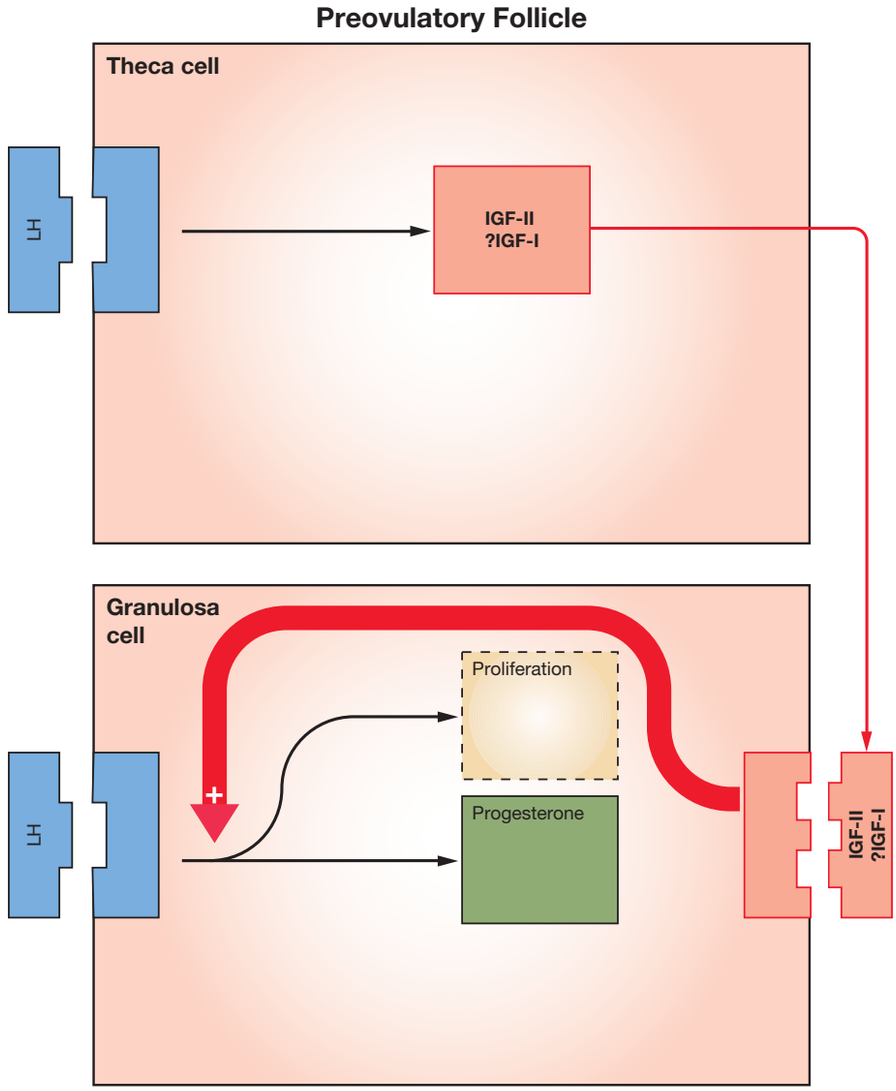

FIGURE 5.7

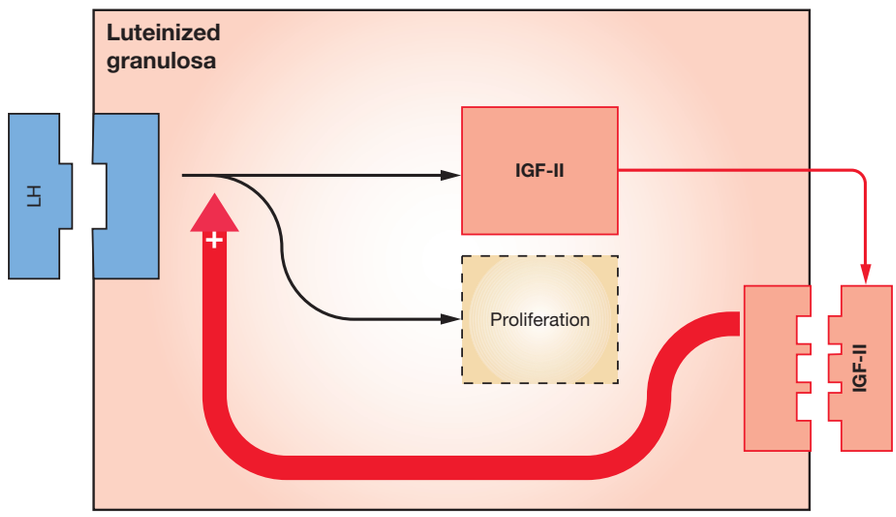

FIGURE 5.8

fluid. The low level of IGFBP-4 in estrogen-dominant follicular fluid is associated with the presence of an IGFBP-4 specific protease. This protease would decrease IGFBP activity and enhance IGF activity, another mechanism for ensuring the success of the dominant follicle.

The IGF story is at once complex, fascinating, and compelling. However, its contribution may be facilitatory but not essential. Laron-type dwarfism is characterized by a deficiency in IGF-1 due to an abnormality in the growth hormone receptor. Despite low levels of IGF-1 and high levels of IGFBP, a woman with Laron-type dwarfism responded to exogenous gonadotropin stimulation with the production of multiple, mature follicles with good estrogen production and fertilizable oocytes.[182] Another explanation for this observation is that IGF-2, rather than IGF-1, is the important factor in the dominant follicle in humans. This possibility is supported by evidence indicating that IGF-2 is the most abundant IGF in human ovarian follicles.[161,162] Another possibility is that the Laron-type dwarf is deficient only in growth hormone–dependent IGF-1 and that ovarian IGFs are not totally dependent on growth hormone.

Key Points: Insulin-Like Growth Factor Action in the Ovary

- IGF-2 stimulates granulosa cell proliferation, aromatase activity, and progesterone synthesis.
- IGF-2 is produced in theca cells, granulosa cells, and luteinized granulosa cells.
- Gonadotropins stimulate IGF production, and in animal experiments, this stimulation is enhanced by estradiol and growth hormone.
- IGF-1 receptors are present in theca and granulosa cells, and only IGF-2 receptors are present in luteinized granulosa.
- IGF-2 activates both IGF-1 and IGF-2 receptors.
- The most abundant IGF in human follicles is IGF-2. In the pig and the rat, the primary IGF is IGF-I.
- FSH inhibits binding protein synthesis and thus maximizes growth factor availability.

Epidermal Growth Factor

EGF is a mitogen for a variety of cells, and its action is potentiated by other growth factors. Granulosa cells, in particular, respond to this growth factor in a variety of ways related to gonadotropin stimulation, including proliferation. EGF suppresses the upregulatory effects of FSH on its own receptor.[41] Amphiregulin and epiregulin, ligands that are similar to EGF, are produced in luteinized granulosa cells in response to LH and induce progesterone synthesis in the corpus luteum.[183–185]

Transforming Growth Factor

TGF-alpha (α) is a structural analog of EGF and can bind to the EGF receptor. TGF-beta (β) utilizes a receptor that is distinct from the EGF receptor. These factors are thought to be autocrine growth regulators. Inhibin and activin are derived from the same gene family. TGF-β, secreted by theca cells, enhances FSH induction of LH receptors on granulosa cells, an action that is opposite that of EGF.[186] While this action can be viewed as a positive impact on granulosa cells, in the theca, TGF-β has a negative action, inhibiting androgen production.[187] GDF-9 is a member of the TGF-β family that originates in the oocyte and is essential for normal growth and development of the ovarian follicle.[17]

Fibroblast Growth Factor

This factor is a mitogen for a variety of cells and is present in all steroid-producing tissues. Important roles in the ovarian follicle include stimulation of mitosis in granulosa cells, stimulation of angiogenesis, stimulation of plasminogen activator, inhibition of FSH upregulation of its own receptor, and inhibition of FSH-induced LH receptor expression and estrogen production.[41,188] These actions are opposite to those of TGF-β.

Platelet-Derived Growth Factor

This growth factor modifies cyclic AMP pathways responding to FSH, especially those involved in granulosa cell differentiation. Both platelet-derived growth factor (PDGF) and EGF may also modify prostaglandin production within the follicle.

Angiogenic Growth Factors

Vascularization of the follicle is influenced by peptides in the follicular fluid, especially VEGF, a cytokine produced in granulosa cells in response to LH.[189,190] Luteal cells respond to human chorionic gonadotropin (hCG) with greater VEGF output, a probable mechanism contributing to the increased vascular permeability associated with ovarian hyperstimulation syndrome that can occur with exogenous gonadotropin administration (Chapter 28).[191] Angiopoietins bind to an endothelial receptor (Tie-2) and provide an inhibitory influence on angiogenesis. Angiopoietin-1 is the active agent, opposed by angiopoietin-2, which competes for the Tie-2 receptor on endothelial cells. Differential expression of these angiogenic factors is involved in the coordinated growth and regression of follicles and the corpus luteum.[192–194] Injection of VEGF and angiopoietin antagonists directly into dominant follicles in the monkey interferes with both the physical process of ovulation and the subsequent function of the corpus luteum.[195]

The Interleukin-1 System

Leukocytes are a prominent component of the ovarian follicle and a major source of interleukins. IL-1 is a member of

the cytokine family of immunomediators. The human ovary contains the complete IL-1 system (ligand and receptor). In the rat, IL-1 stimulates ovarian prostaglandin synthesis and plays a role in ovulation.[196,197] The progression of ovulation has been reported[198] to occur by neutrophil infiltration into theca cells, which induces IL-1β synthesis to stimulate ovulation.[199]

Tumor Necrosis Factor-α

TNF-α is also a product of leukocytes (macrophages). It very likely is a key player in the process of apoptosis, a feature of follicular atresia as well as luteolysis of the corpus luteum. It is also thought to be involved in ovulation via autophagy of granulosa cells.[200]

Antimüllerian Hormone

A member of the TGF-β family, like inhibin and activin, AMH is produced by granulosa cells and may play a role in oocyte maturation (it inhibits oocyte meiosis) and follicular development.[201,202] AMH directly inhibits proliferation of granulosa and luteal cells, as well as EGF-stimulated proliferation. Its paracrine function may be to suppress growth of all but the dominant follicle in each cycle.[72] Experimental evidence suggests that the source of the AMH is the entire cohort of growing follicles except for the dominant follicle, and, thus, the circulating level correlates with follicle number, follicular response to stimulation, and oocyte retrieval yield per cycle.[203,204] With aging and a decrease in the number of follicles, AMH levels decline. AMH can be measured on any day in an individual's menstrual cycle, as there is minimal variability across the different phases of the menstrual cycle. While AMH levels were previously believed to remain unaffected by use of steroid contraception, recent evidence demonstrates that circulating AMH levels may be falsely suppressed in long-term users of hormonal contraceptives, and among the various types of hormonal contraception, this difference (of at least 25% decrease) is seen in all types except levonorgestrel intrauterine device (IUD).[77]

Follicular fluid prevents resumption of meiosis until the preovulatory LH surge either overcomes or removes this inhibition. This action is attributed to **oocyte maturation inhibitor (OMI)**. **Pregnancy-associated plasma protein A**, found in the placenta, is also present in follicular fluid. It may inhibit proteolytic activity within the follicle before ovulation. **Endothelin-1** is a vasoconstrictive peptide, produced in vascular endothelial cells. Endothelin gene expression is induced by the hypoxia associated with the avascular granulosa, and it inhibits LH-induced progesterone production, that is, luteinization of granulosa cells.[205] It is uncertain whether **GnRH-like peptides** have a follicular role or represent sequestered GnRH. **Oxytocin** is found in preovulatory follicles and the corpus luteum. Growth hormone–binding protein is present in follicular fluid and is similar in characteristics to the same binding protein in serum.

Key Points: Antral Follicle

- Follicular phase estrogen production is explained by the two-cell, two-gonadotropin mechanism.
- Selection of a dominant follicle is often established during cycle days 5 to 7, and, consequently, peripheral levels of estradiol begin to rise significantly by cycle day 7.
- Estradiol levels, derived from the dominant follicle, increase steadily and, through negative feedback effects, exert a progressively greater suppressive influence on FSH release.
- While directing a decline in FSH levels, the midfollicular rise in estradiol exerts a positive feedback influence on LH secretion.
- The positive action of estrogen also includes modification of the gonadotropin molecule, increasing the quality (the bioactivity) as well as the quantity of FSH and LH at midcycle.
- LH levels rise steadily during the late follicular phase, stimulating androgen production in the theca.
- A unique responsiveness to FSH allows the dominant follicle to utilize the androgen as substrate and further accelerate estrogen production through aromatization.
- FSH induces the appearance of LH receptors on granulosa cells.
- Follicular response to the gonadotropins is modulated by a variety of growth factors and autocrine–paracrine peptides.
- Inhibin-B, secreted by the granulosa cells in response to FSH, directly suppresses pituitary FSH secretion.
- Activin, originating in both the pituitary and granulosa, augments FSH secretion and action.

Follicular Growth and Development in the Primate Ovary

Evidence strongly indicates that autocrine–paracrine peptides, and not estrogen, play the major role in regulating ovarian follicle growth and development in the primate. In monkey experiments, no reduction in the total number or size of follicles resulted when estradiol production was effectively suppressed by treatment with an inhibitor of the aromatase enzyme system or with an inhibitor of the 3β-hydroxysteroid dehydrogenase enzyme.[206–208] Oocyte development was not altered, although the subsequent fertilization rate was reduced by this treatment. Another argument against a major role for estrogen in follicular growth and development is the successful stimulation with gonadotropins of normal follicular growth and development in women with

17α-hydroxylase deficiency (an inherited disorder that prevents the production of androgens and estrogens).[209,210]

A nonessential role for estrogen in follicular growth and development is further supported by the response of women with a deficiency in gonadotropins to treatment with recombinant (pure) FSH.[63–65] Some aromatization occurred, perhaps using androgens originating in the adrenal glands, producing early follicular phase estradiol levels, but the usual robust steroidogenesis was impossible without the presence of LH to provide theca production of androgen substrate. Nevertheless, oocytes were retrieved, and utilizing IVF, pregnancy was achieved. This same response was observed in experiments that used a GnRH antagonist to produce LH-deficient monkeys and then the administration of recombinant, pure human FSH.[66,67]

These results indicate that only FSH is required for early folliculogenesis and that in the primate, autocrine–paracrine peptides have replaced estrogen in the important role of modulating gonadotropin response. Consider the following actions that have been documented in primate ovaries:

1. Inhibin and activin regulate androgen synthesis in human theca cells. Inhibin enhances and activin suppresses the stimulatory action of LH and/or IGF-1, and inhibin can overcome the inhibitory action of activin on theca cells.[142–144]
2. In immature granulosa cells, activin augments all FSH activities, especially aromatase activity (estrogen production).[116,211]
3. In luteinizing granulosa cells, activin has direct mitogenic activity and suppresses steroidogenesis in response to LH, while inhibin has no effect on LH-dependent aromatase in mature granulosa cells.[211,212]
4. In the follicular phase, granulosa production of inhibin is under the control of FSH, but during the late follicular phase a change occurs, culminating in LH control of luteal synthesis of inhibin.[213,214]
5. As the follicle grows, activin production decreases, and inhibin production increases.[215,216] In addition, follistatin levels increase in the follicular fluid with increasing growth of the follicle, a mechanism for decreasing activin activity.[217] In the early follicular phase, FSH and estradiol enhance inhibin-B secretion, probably indirectly by increasing granulosa cell numbers, whereas late in the follicular phase, when LH levels increase, inhibin-A secretion is favored.[218]

These actions come together as follows. In the early follicular phase, activin produced by granulosa cells in immature follicles enhances the action of FSH on aromatase activity as well as FSH and LH receptor formation, while simultaneously suppressing theca cell androgen synthesis (**Figure 5.9**). In the late follicular phase, increased production of inhibin (specifically inhibin-B) by granulosa cells (and decreased activin) promotes androgen synthesis in theca cells in response to LH and IGF-2 to provide substrate for even greater estrogen production in granulosa cells (**Figure 5.10**). In mature granulosa cells within a dominant preovulatory follicle, activin serves to prevent premature luteinization and progesterone production.

The successful follicle is the one that acquires the highest level of aromatase activity and LH receptors in response to FSH and is characterized by the highest estrogen (for central feedback action) and the greatest inhibin production (for both local and central actions). This accomplishment occurs in synchrony with the appropriate activin expression. The highest level of gene activity encoding activin is found in immature antral follicles and the lowest level in the preovulatory follicles. Thus, activin proteins (which enhance FSH activity) are produced in greatest amounts early in follicular development to enhance follicle receptivity to FSH. As for circulating levels of inhibin, inhibin-B is the predominant inhibin in the follicular fluid of preantral follicles, and inhibin-A increases when follicles become large and mature.[219–221] Inhibin synthesis and secretion during the follicular phase are regulated by FSH and growth factors.[222]

The right concentration of androgens in granulosa cells promotes aromatase activity and inhibin production and, in turn, inhibin promotes LH stimulation of theca cell androgen synthesis. With development of the follicle, inhibin expression (specifically inhibin-A) comes under the control of LH. A key to successful ovulation and luteal function is the conversion of inhibin production to LH responsiveness to maintain FSH suppression centrally (**Figure 5.11**) and enhancement of LH action locally.

Responses of ovarian follicles to exogenous FSH and LH stimulation for IVF indicate that the final maturation and function of the dominant follicle prior to ovulation are significantly influenced by LH.[86] Final maturation of the dominant follicle and the health of the oocyte are optimized by the required presence of a threshold level of LH.[68,88,89,223]

A lesser role is assigned to the IGFs in view of the successful production of multiple, estrogen-producing follicles, which yielded fertilizable oocytes in a woman with IGF-1 deficiency treated with gonadotropins.[182] The growth factors assume an important, but perhaps not essential, role as facilitating agents. However, successful pregnancy in a woman with IGF-1 deficiency may indicate the greater importance of IGF-2.

Key Points: Primate Ovarian Follicle

- FSH has multiple activities in the granulosa cell: stimulating aromatization of androgens to estrogens, increasing granulosa cell content of FSH and LH receptors, stimulating proliferation of granulosa cells, and producing autocrine–paracrine factors, especially activin and inhibin.

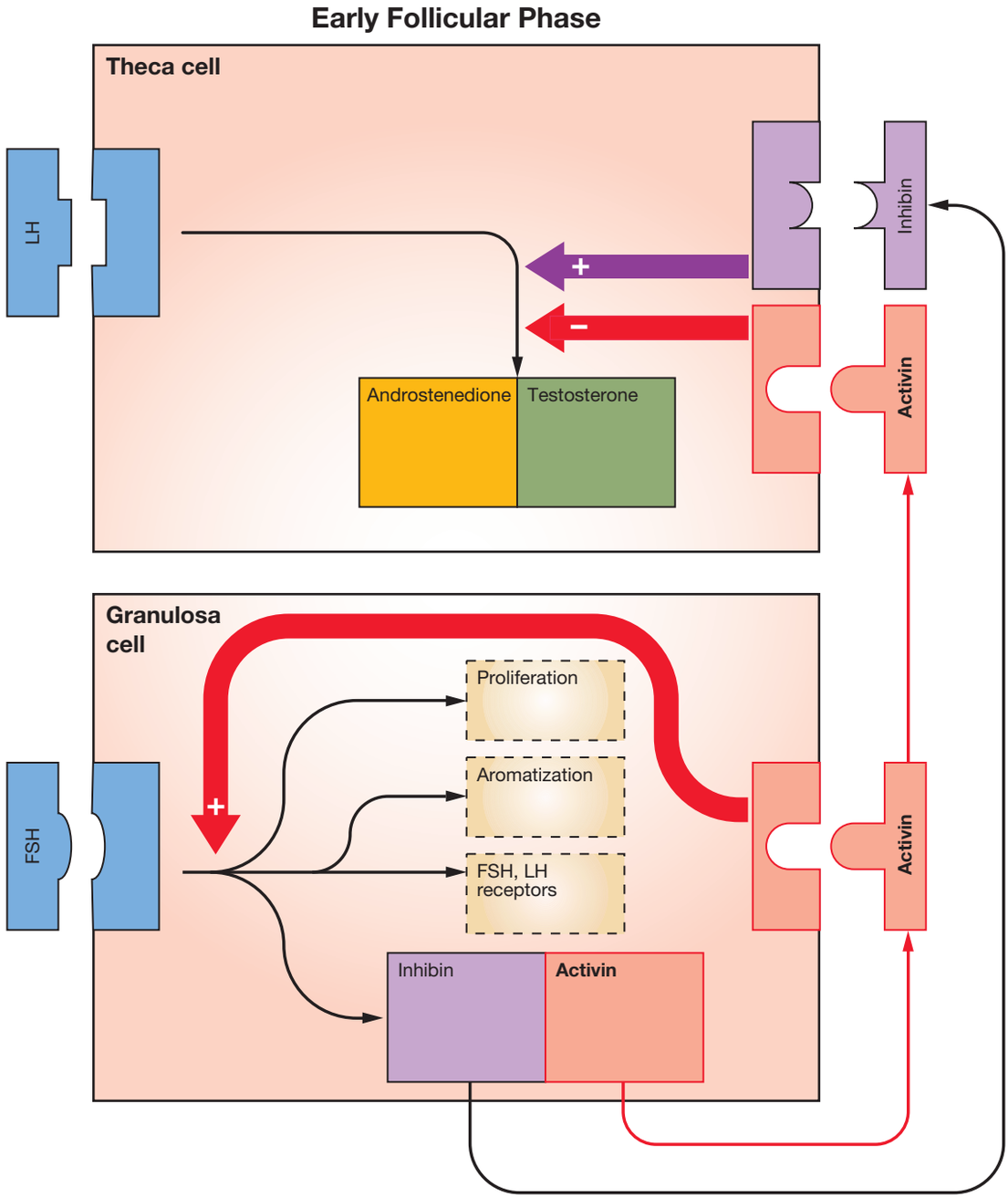

FIGURE 5.9

- In the granulosa cells of the early follicular phase, activin augments FSH activities: FSH receptor expression, aromatization, inhibin/activin production, and LH receptor expression. In the theca cells, activin suppresses androgen production, allowing the emergence of an estrogen-dominant microenvironment.
- Later in the follicular phase, inhibin enhances LH stimulation of androgen synthesis in the theca cells to provide substrate for aromatization to estrogen in the granulosa cells, making available the large amount of estrogen necessary for local follicular actions and to trigger the LH surge.
- Inhibin-B is secreted by the granulosa cells into the circulation, where it acts in a classic endocrine fashion to suppress FSH secretion by the pituitary gland, an important method used to ensure the dominance of a single follicle.

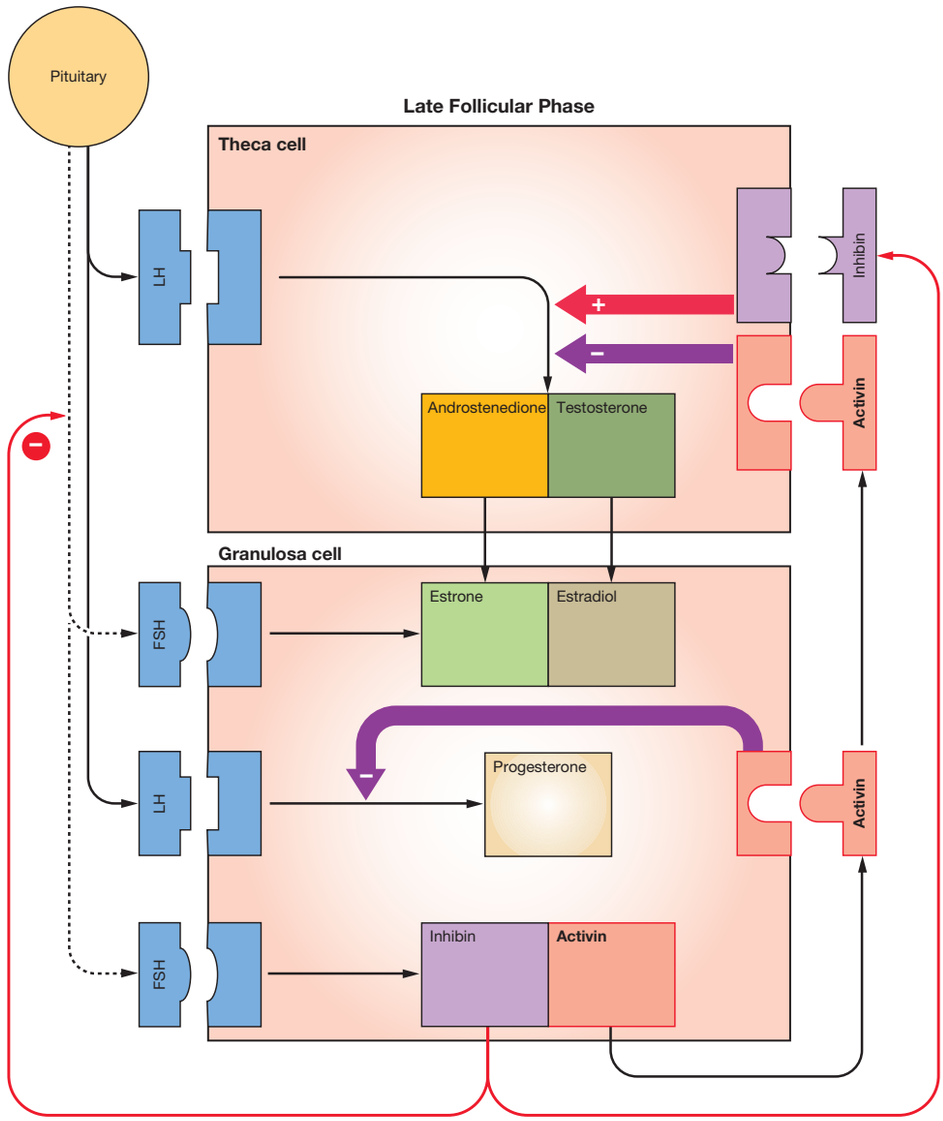

FIGURE 5.10

- With the appearance of LH receptors, inhibin production by the granulosa cells is maintained as it comes under the control of LH.
- Late in the follicular phase, final follicular maturation to yield the most favorable level of steroidogenesis and an oocyte with the best viability requires the presence of a threshold level of LH.
- All functions are modulated by a multitude of growth factors, and IGF-2 may be especially important.

The Preovulatory Follicle

Granulosa cells in the preovulatory follicle enlarge and acquire lipid inclusions, while the theca cells becomes

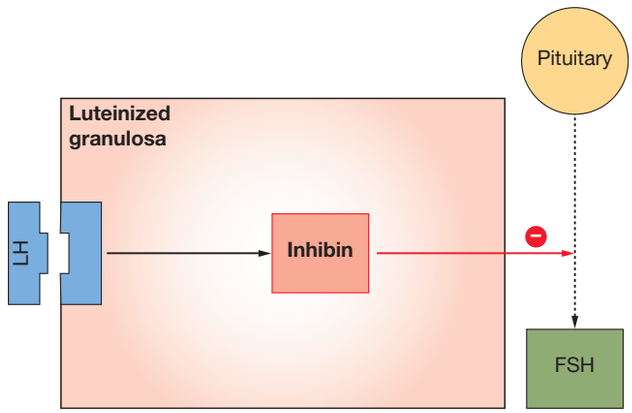

FIGURE 5.11

vacuolated and richly vascular, giving the preovulatory follicle a hyperemic appearance. The oocyte proceeds in meiosis, approaching completion of its reduction division.

Approaching maturity, the preovulatory follicle produces increasing amounts of estrogen. During the late follicular phase, estrogens rise slowly at first, then rapidly, reaching a peak approximately 24 to 36 hours prior to ovulation.[224] The onset of the LH surge occurs when the peak levels of estradiol are achieved.[225] In providing the ovulatory stimulus to the selected follicle, the LH surge seals the fate of the remaining follicles, with their lower estrogen and FSH content, by further increasing androgen superiority.

Acting through its own receptors, LH promotes luteinization of the granulosa cells in the dominant follicle, resulting in the production of progesterone. The LH receptor, once expressed, inhibits further cell growth and focuses the cell's energy on steroidogenesis (these actions are enhanced by IGF).[226] An increase in progesterone can be detected in the venous effluent of the ovary bearing the preovulatory follicle as early as day 10 of the cycle.[79] This small but significant increase in the production of progesterone in the preovulatory period has immense physiologic importance. Prior to the emergence of this follicular progesterone, the circulating levels of progesterone are of adrenal origin.[227]

Progesterone receptors begin to appear in the granulosa cells of the dominant follicle in the periovulatory period.[90] The traditional view has been that progesterone receptors are expressed in response to estrogen through an estrogen-receptor–mediated mechanism; however, this is not the case. Experimental data in the monkey provide excellent evidence that LH stimulates progesterone receptor expression in the granulosa cells.[228] In vitro data with human cells suggest that the preovulatory progesterone and progesterone receptor expression directly inhibit granulosa cell mitosis, probably explaining the limitation of granulosa cell proliferation as these cells gain LH receptors.[229]

Progesterone affects the positive feedback response to estrogen in both a time- and dose-dependent manner. When introduced after adequate estrogen priming, progesterone facilitates the positive feedback response of estrogen by direct action at the level of the pituitary, and in the presence of subthreshold levels of estradiol, it can induce a characteristic LH surge.[230,231] This ability of progesterone to induce an LH surge can explain the surprising onset of ovulation occasionally observed in an anovulatory, amenorrheic woman following administration of a progestin challenge. However, when administered before the estrogen stimulus, or in high doses (achieving a blood level >2 ng/mL), progesterone blocks the midcycle LH surge.

Thus, appropriately low levels of progesterone derived from the maturing follicle contribute to the precise synchronization of the midcycle surge. In addition to its facilitator action on LH, progesterone at midcycle is also responsible for the FSH surge.[231] This action of progesterone can be viewed as a further step in ensuring completion of FSH action on the preovulatory follicle, especially making sure that a full complement of LH receptors is in place in the granulosa layer. In certain experimental situations, incremental estradiol alone can elicit simultaneous surges of LH and FSH, suggesting that progesterone certainly enhances the effect of estradiol but may not be obligatory for the occurrence of the midcycle gonadotropin surge.[232] Nevertheless, blockade of midcycle progesterone synthesis or activity in the monkey impaired the processes of ovulation and luteinization.[233] These actions of estrogen and progesterone in modulating gonadotropin release require the presence and action of GnRH.

The preovulatory period is associated with a rise in plasma levels of 17-hydroxyprogesterone (17-OHP). This steroid precursor does not appear to have a role in cycle regulation, and its appearance in the blood simply represents the secretion of an intermediate product. The preovulatory rise in 17-OHP reflects LH stimulation of the P450scc and P450c17 enzymes that are critical for the production of androgens by the theca cells, which then get aromatized to estrogen by the granulosa cells. After ovulation, some of the theca cells become luteinized as part of the corpus luteum and lose the ability to express P450c17. Other luteinized theca cells retain P450c17 activity and are believed to continue to produce androgens for aromatization to estrogens.

For the lesser follicles that fail to achieve full maturity and undergo atresia, the adjoining theca cells return to their original role as a component of the ovarian stromal tissue, retaining, however, an ability to respond to LH with P450 activity and steroid production. Because the hormonal products of theca tissue are androgens, the increase in stromal tissue in the late follicular phase is associated with a rise in circulating androgen levels at midcycle, when a 15% increase in androstenedione and a 20% increase in testosterone levels can occur.[234] This response is enhanced by the late follicular phase rise in inhibin, known to augment LH stimulation of androgen production in theca cells.

Increasing androgen production in the preovulatory stage in the cycle may serve two purposes: (1) a local role within the ovary to enhance the process of atresia of the lesser follicles and (2) a systemic effect to stimulate libido nearing the time of ovulation.

Intraovarian androgens accelerate granulosa cell death and follicular atresia. The specific mechanism for this action is unclear, although it is attractive to suspect an interference with estrogen and the autocrine–paracrine factors in enhancing FSH activity. Therefore, androgens may play a regulatory role in ensuring that only a dominant follicle reaches the point of ovulation. By activating endoplasmic reticular stress, hyperandrogenism in polycystic ovary syndrome (PCOS) leads to intraovarian accumulation of advanced glycosylation end products (AGE), and an increase in expression of their receptor, receptor for advanced glycation end products (RAGE). Thus, inhibiting RAGE has been shown to be a potential PCOS treatment in a mouse model, as this led

to reduced AGE accumulation in granulosa cells, improved estrous cycle, and a reduction in atretic antral follicles.[235]

It is well known that libido can be stimulated by androgens. If the midcycle rise in androgens affects libido, then an increase in sexual activity should coincide with this rise. Early studies failed to demonstrate a consistent pattern in coital frequency in women because of the effect of male partner initiation. If only sexual behavior initiated by women is studied, a peak in female-initiated sexual activity is seen during the ovulatory phase of the cycle.[236] The coital frequency of married couples has also been noted to increase at the time of ovulation.[237] Therefore, the midcycle rise in androgens may serve to increase sexual activity at the time most likely to achieve pregnancy.

Key Points: Preovulatory Follicle

- Estrogen production by the preovulatory follicle becomes sufficient to achieve and maintain peripheral threshold concentrations of estradiol that are required to induce the LH surge.
- Acting through its receptors, LH initiates luteinization and progesterone production in the granulosa layer.
- The preovulatory rise in progesterone facilitates the positive feedback action of estrogen at the level of the pituitary, which results in the LH surge. Preovulatory rise in progesterone also plays a role in induction of the midcycle FSH peak.
- A midcycle increase in local and peripheral androgens occurs, derived from the theca tissue of the lesser, unsuccessful follicles.

OVULATION

The preovulatory follicle, as detailed in the prior section, provides its own ovulatory stimulus. Considerable variation in timing exists from cycle to cycle, even in the same woman. A reasonable and accurate estimate places ovulation approximately 10 to 12 hours after the LH peak and 24 to 36 hours after peak estradiol levels are attained (**Figure 5.12**).[224,238] The onset of the LH surge appears to be the most reliable indicator of impending ovulation, occurring 34 to 36 hours prior to follicle rupture.[239] A threshold of LH concentration must be maintained for 14 to 27 hours in order for full maturation of the oocyte to occur.[240] Usually, the LH surge lasts 48 to 50 hours.[239] In patients undergoing IVF, oocyte retrieval is performed ~36 hours following HCG or GnRH agonist trigger injection in order to capture the oocytes at full maturation but not risk oocyte loss due to ovulation. Peak levels occur roughly 4 hours after injection.[241]

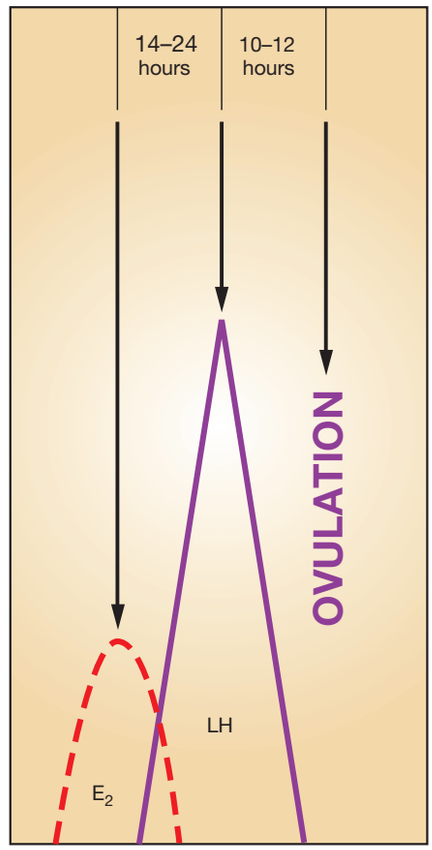

FIGURE 5.12

Over recent decades, increasing utilization of infertility treatments has allowed clarity in our understanding of the timeline of ovarian events following an induced LH surge. Spontaneous LH surge tends to occur at approximately 3 AM, beginning between midnight and 8:00 AM in over two-thirds of women.[100] Seasonal variation in timing of LH surge is recognized.[242] Ovulation occurs primarily in the morning during spring and primarily in the evening during autumn and winter. From July to February in the Northern Hemisphere, about 90% of women ovulate between 4 and 7 PM; during spring, 50% of women ovulate between midnight and 11 AM.

Most of the studies have concluded that ovulation occurs more frequently (about 55% of the time) from the right ovary than in the left. Furthermore, oocytes from the right ovary are suggested to have a higher potential for pregnancy compared to those from the left side.[243] In addition, in stimulated cycles, the right ovary seems to generate a higher number of oocytes. The side of ovulation does not affect cycle characteristics, but cycles with short follicular phases tend to be followed by contralateral ovulation, and ovulation occurs randomly following cycles with a long follicular phase.[244,245] Ovulation alternating between the two ovaries predominates in younger women, but after 30 years of age, ovulations occur more frequently from the same ovary; however, throughout

the reproductive years, more ovulations occur from the right ovary.[245] Contralateral ovulation (ovulating from the opposite ovary as the prior cycle) favors pregnancy more than ipsilateral ovulation (ovulating from the same ovary as the prior cycle), and ipsilateral ovulation increases with increasing age and decreasing fertility.[246]

The gonadotropin surge initiates a cascade of events that ultimately lead to ovulation, the physical release of the oocyte, and its cumulus mass of granulosa cells.[247] Ovulation is not an explosive event; therefore, a complex series of changes must occur, which cause the final maturation of the oocyte and the decomposition of the collagenous layer of the follicular wall with its subsequent breakdown, followed by the release of the follicular contents.[248]

The LH surge initiates the resumption of meiosis in the oocyte (meiosis is not completed until after the sperm has entered and the second polar body is released), luteinization of granulosa cells and progesterone production, expansion of the cumulus, and the synthesis of prostaglandins and other eicosanoids essential for follicle rupture. Premature oocyte maturation and luteinization are prevented by local factors.

An LH-induced increase in cyclic AMP occurs within the follicle just prior to ovulation. Cyclic AMP is transferred from the granulosa cells to the oocyte via the gap junction network, and thus a reduction in cyclic AMP occurs when LH causes a breakdown of the gap junctions. This results in a decrease in the local inhibitory action of OMI. The OMI originates from granulosa cells, and its activity depends on an intact cumulus oophorus.[204] Locally produced activin suppresses progesterone production by the luteal cells, providing yet another means of preventing premature luteinization.[249,250] The propagation of LH-induced changes throughout the follicle depends on growth factors and their receptors, especially members of the EGF-like growth factor family, specifically, LH-induced factors named amphiregulin, epiregulin, and betacellulin.[251] LH induces secretion of EGF-like growth factor from mural granulosa cells, which in turn bind to receptors on cumulus cells and induce expansion of the cumulus cells in preparation for ovulation.[184] Disruption of this pathway interferes with oocyte resumption of meiosis and ovulation.

There is abundant evidence that the oocyte exerts control over granulosa functions, affecting both metabolism and proliferation through the secretion of proteins in the TGF-β family.[51,252–255] These proteins include inhibin, activin, AMH, BMPs, and GDF9, which must be secreted in their active forms after processing of precursor proteins by proteases. The production of the active proteins is regulated by an interaction of the signaling proteins from the oocyte and the granulosa cells, determined by changing responsiveness to FSH as the components of the ovarian follicle develop and differentiate.[256] The differentiation and maintenance of the cumulus cells from the preantral granulosa cells are under the direction of the oocyte.[257,258]

The cumulus oophorus differs from other granulosa cells, lacking in LH receptors and progesterone production; FSH-induced LH receptor expression is suppressed in the contiguous granulosa cells by the oocyte. The oocyte enables cumulus cells to respond to the gonadotropin-induced physical and biochemical changes just before ovulation. The local factors that prevent premature oocyte maturation and luteinization are probably under the control of the oocyte. One mediator of this control system is nitric oxide, which maintains the gap junction system of communication.[259] Nitric oxide resists LH-induced resumption of oocyte meiosis and breakdown of the gap junction network until the massive LH surge overcomes this resistance and communication between the oocyte and the follicular cells is interrupted.

With the LH surge, levels of progesterone in the follicle continue to rise, up until the time of ovulation. The progressive rise in progesterone may act to terminate the LH surge as a negative feedback effect is exerted at higher concentrations.[260] In addition to its central effects, progesterone increases the distensibility of the follicle wall. A change in the elastic properties of the follicular wall is necessary to accommodate the rapid increase in follicular fluid volume, which occurs just prior to ovulation, unaccompanied by any significant change in intrafollicular pressure. FSH, LH, and progesterone stimulate the activity of proteolytic enzymes, resulting in digestion of collagen in the follicular wall and increasing its distensibility. The escape of the ovum is associated with degenerative changes of the collagen in the follicular wall so that just prior to ovulation, the follicular wall becomes thin and stretched.

The proteolytic enzymes are activated in an orderly sequence.[261] The granulosa and theca cells produce plasminogen activator in response to the gonadotropin surge. Plasminogen is activated by either of two plasminogen activators: tissue-type plasminogen activator and urokinase-type plasminogen activator. These activators are encoded by separate genes and are also regulated by inhibitors.

Plasminogen activators produced by granulosa cells activate plasminogen in the follicular fluid to produce plasmin. Plasmin, in turn, generates active collagenase to disrupt the follicular wall. In rat models, plasminogen activator synthesis is triggered by LH stimulation (as well as growth factors and FSH), while plasminogen inhibitor synthesis is decreased.[262] Thus, before and after ovulation, the inhibitor activity is high, while just at ovulation, activator activity dominates, and the inhibitors are at a nadir. A coordinated molecular regulation of these factors is necessary for the complex sequence of events to result in ovulation. Plasminogen activator synthesis in granulosa cells is expressed only at a precise preovulatory stage in response to LH. The inhibitor system, which is very active in theca and interstitial cells, prevents inappropriate activation of plasminogen and disruption of growing follicles. The inhibitor system has been demonstrated to be present in human granulosa cells and preovulatory follicular

fluid and to be responsive to paracrine substances, EGF and IL-1β.[263–265] Physical migration of the preovulatory follicle to the surface of the ovary is an important step in that the exposed surface of the follicle is now prone to rupture because it is now separated from cells rich in the plasminogen inhibitor system. Ovulation is the result of proteolytic digestion of the follicular apex, a site called the stigma. The matrix metalloproteinase (MMP) enzymes and their endogenous inhibitors, tissue inhibitors of metalloproteinases (TIMPs), are increased in response to LH and progesterone and are also involved in this event.[266]

In the rat, the gene that encodes for plasminogen activator contains a promoter region, which has several sequences for known transcription factors, such as the cyclic AMP-responsive element (CRE). The activation of this CRE (which involves a CRE-binding protein) requires FSH stimulation. Thus, both gonadotropins appear to be involved in this process. Studies in the monkey indicate that the activation of plasminogen activator is mediated by prostaglandin E_2.[267]

Prostaglandins E_2 and $F_{2\alpha}$, but mainly prostaglandin E_2, and other eicosanoids (especially HETEs, hydroxyeicosatetraenoic acids) increase markedly in the preovulatory follicular fluid in response to the LH surge, reaching a peak concentration at ovulation.[268–270] Prostaglandin synthesis is stimulated by IL1-β, implicating this cytokine in ovulation.[271] Inhibition of cyclooxygenase-2 (COX-2)-mediated synthesis of these products from arachidonic acid blocks follicle rupture without affecting the other LH-induced processes of luteinization and oocyte maturation.[272–274]

Prostaglandins act to free proteolytic enzymes within the follicular wall; HETEs may promote angiogenesis and hyperemia (an inflammatory-like response).[267,269,275] LH and PGE_2 both activate the EGF-like signaling pathway that leads to cumulus expansion and resumption of oocyte meiosis.[276] Prostaglandins may also contract smooth muscle cells that have been identified in the ovary, thereby aiding the extrusion of the oocyte-cumulus cell mass from the ruptured follicle (Figure 5.13). **This ovulatory role of prostaglandins is so well demonstrated that patients should be advised to avoid the use of drugs that inhibit prostaglandin synthesis while trying to conceive.**[274,277–279] For decades, these medications have been used to inhibit ovulation prior to oocyte retrieval.

A large number of leukocytes enter the follicle prior to ovulation. Neutrophils are a prominent feature in the theca compartment of both healthy and atretic antral follicles.[280] The accumulation of leukocytes is mediated by chemotactic mechanisms of the interleukin system.[281] However, ovulation does not depend on these invading immune cells for the expression of the inflammatory-like response associated with ovulation. Ovarian follicular cells themselves, in response to LH, express the genes involved with immune responses, resulting in the release of the host of products that affect the cellular reactions associated with ovulation and the remodeling process that leads to the corpus luteum.[282]

Estradiol levels plunge as LH reaches its peak. This may be a consequence of LH-mediated downregulation of its own receptors on the periovulatory follicle. Theca tissue derived from healthy antral follicles exhibits marked suppression of steroidogenesis when exposed to high levels of LH, whereas exposure to a low level stimulates steroid production. The low midcycle levels of progesterone exert an inhibitory action on further granulosa cell multiplication, and the drop in estrogen may also reflect this local follicular role for progesterone. Finally, estrogen can exert an inhibitory effect on P450c17, a direct action on the gene that is not receptor mediated.

The granulosa cells that are attached to the basement membrane and enclose the follicle become luteal cells. The cumulus granulosa cells are in immediate proximity and are attached to the oocyte. In the mouse, the cumulus cells are

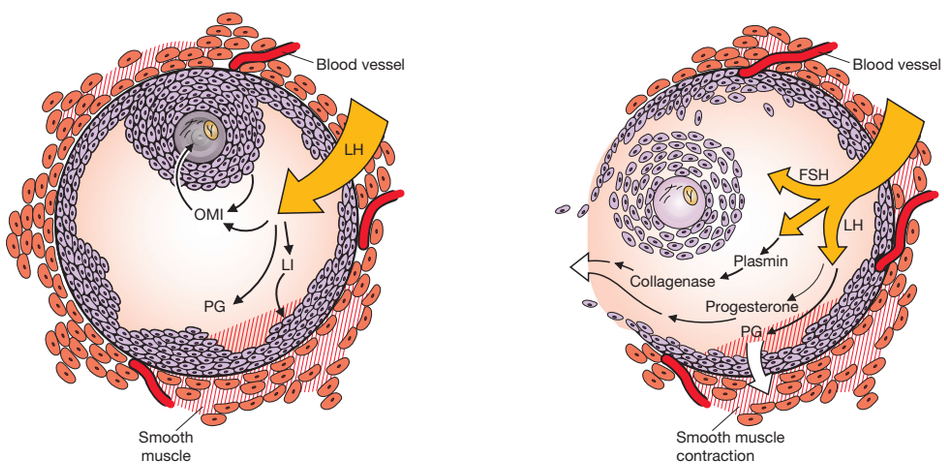

FIGURE 5.13

metabolically linked to the oocyte and respond to the FSH surge by secreting hyaluronic acid that disperses the cumulus cells prior to ovulation. This hyaluronic acid response depends on maintenance of the link with the oocyte, indicating the secretion of a supporting factor. The oocyte further secretes factors that promote granulosa cell proliferation and maintain the structural organization of the follicle.[283] While FSH stimulates mural granulosa cell proliferation, its effect on the cumulus cells is the opposite; proliferation of the cumulus cells is suppressed by FSH.

The FSH peak, partially and perhaps totally dependent on the preovulatory rise of progesterone, has several functions. Plasminogen activator production is sensitive to FSH as well as LH. Expansion and dispersion of the cumulus cells allows the cumulus–oocyte complex to become free-floating in the antral fluid just before follicle rupture. The process involves the deposition of a hyaluronic acid matrix, the synthesis of which is stimulated by FSH. Finally, an adequate FSH peak ensures an adequate complement of LH receptors on the granulosa layer. It should be noted that a shortened or inadequate luteal phase is observed in cycles when FSH levels are low or selectively suppressed at any point during the follicular phase.

The mechanism that shuts off the LH surge is unknown, with mixed evidence suggesting a role for ovarian, rather than pituitary or hypothalamic factors. Within hours after the rise in LH, there is a precipitous drop in the plasma estrogens. As supported by human studies, the decrease in LH appears to be related to a loss of the positive stimulating action of estradiol or to an increasing negative feedback of progesterone.[284] However, other studies have found that the abrupt fall in LH levels may also reflect a depletion in pituitary LH content due to downregulation of GnRH receptors, either by alterations in GnRH pulse frequency or by changes in steroid levels.[285,286] LH may further be controlled by "short" negative feedback of LH on the hypothalamus. Direct LH suppression of hypothalamic-releasing hormone production has been demonstrated. However, in sheep, the LH surge ends before the GnRH signal begins to decline.[287] Another possibility has been suggested: a so-called gonadotropin surge-inhibiting factor (GnSIF, also known as GnSAF, gonadotropin attenuating factor) originating in the ovary.[288,289] GnSIF is produced in granulosa cells under the control of FSH and reaches a peak level in the circulation in the midfollicular phase. Its major role is believed to be prevention of premature luteinization. It is likely that a combination of all these influences causes the rapid decline in gonadotropin secretion.

The many contributions of progesterone to ovulation are highlighted by the results of experiments in the monkey. Midcycle suppression of steroidogenesis prevented ovulation but not the resumption of oocyte meiosis.[233] Administration of a progestin agonist to this experimental model restored ovulation. In experimental models of mice, knockout of the progesterone receptor gene results in failure to ovulate, although oocyte maturation and luteinization are not impeded.[290,291] These experiments indicate that progesterone receptor-A is the critical isoform necessary for normal ovulation.

An adequate gonadotropin surge does not ensure ovulation. The follicle must be at the appropriate stage of maturity for it to respond to the ovulating stimulus. In the normal cycle, gonadotropin release and final maturation of the follicle coincide because the timing of the gonadotropin surge is controlled by the level of estradiol, which in turn is a function of follicular growth and maturation. Therefore, gonadotropin release and morphologic maturity of the follicle are usually coordinated and coupled in time, and without it, ovulation does not occur. In the majority of spontaneous cycles in humans, the requisite feedback relationships in this system allow only a single follicle to reach the point of ovulation. Nonidentical multiple births may, in part, reflect the random statistical chance of more than one follicle fulfilling all the requirements for simultaneous ovulation.

Key Points: Ovulatory Events

- The LH surge initiates the continuation of meiosis in the oocyte, luteinization of the granulosa, and synthesis of progesterone and prostaglandins within the follicle.
- Progesterone enhances the activity of proteolytic enzymes responsible, together with prostaglandins, for digestion and rupture of the follicular wall.
- The progesterone-influenced midcycle rise in FSH serves to free the oocyte from follicular attachments, to convert plasminogen to the proteolytic enzyme, plasmin, and to ensure that sufficient LH receptors are present to allow an adequate normal luteal phase.

THE LUTEAL PHASE

Before rupture of the follicle and release of the ovum, the granulosa cells begin to increase in size and assume a characteristic vacuolated appearance associated with the accumulation of a yellow pigment, *lutein*, which lends its name to the process of luteinization and the anatomic subunit, the corpus luteum. During the first 3 days after ovulation, the granulosa cells continue to enlarge. In addition, theca lutein cells may differentiate from the surrounding theca and stroma to become part of the corpus luteum. Dissolution of the basal lamina and rapid vascularization and luteinization make it difficult to distinguish the origin of specific cells.

Capillaries begin to penetrate into the granulosa layer after the cessation of the LH surge, reach the central cavity, and often fill it with blood; the corpus luteum at this stage has also been referred to as corpus hemorrhagicum.[292]

Angiogenesis is an important feature of the luteinization process, a response to LH that is mediated by factors such as VEGF and angiopoietins produced in luteinized granulosa cells.[189,190,293] In the early luteal phase, angiogenesis accompanies an increased expression of VEGF, with stabilization of vessel growth maintained by angiopoietin-1 binding to the endothelial Tie-2 receptor.[193,294] With regression of the corpus luteum, VEGF and angiopoietin-1 expressions decrease. This allows for a greater occupancy of the Tie-2 receptor by angiopoietin-2, leading to the vascular breakdown that accompanies luteolysis.

By day 7 after ovulation, a peak vascularization of the corpus luteum is reached, associated with peak circulating levels of progesterone and a secondary peak in estradiol. The corpus luteum has one of the highest blood flows per unit mass in the body. Clinically, this is appreciated as identification of the corpus luteum is performed by applying Doppler ultrasound to visualize the corpus luteum's characteristic peripheral ring of vascularity. On occasion, this ingrowth of vessels and bleeding will result in unchecked hemorrhage and an acute surgical emergency that can present at any time during the luteal phase. Indeed, excessive bleeding following ovulation can be a real risk for women who are anticoagulated; medical suppression of ovulation, such as through the use of a hormonal contraceptive (pill or patch or injection or vaginal ring), should be considered for premenopausal women who are prescribed blood thinners in an effort to minimize the risk of uncontrolled hemorrhage consequent to an otherwise innocuous event of ovulation.

Normal luteal function requires optimal preovulatory follicular development. Suppression of FSH during the follicular phase is associated with lower preovulatory estradiol levels, depressed midluteal progesterone production, and a decrease in luteal cell mass.[295]

Experimental evidence supports the contention that the accumulation of LH receptors during the follicular phase predetermines the extent of luteinization and the subsequent functional capacity of the corpus luteum. Successful conversion of the avascular granulosa of the follicular phase to the vascularized luteal tissue is also of importance. Because steroid production is dependent on LDL transport of cholesterol, vascularization of the granulosa layer is essential to allow circulating LDL cholesterol to reach the luteal cells to provide sufficient substrate for progesterone production. One of the important jobs for LH is to regulate LDL receptor binding, internalization, and postreceptor processing; the induction of LDL receptor expression occurs in granulosa cells during the early stages of luteinization in response to the midcycle LH surge.[296,297] This mechanism supplies cholesterol to the mitochondria for utilization as the basic building block in steroidogenesis (**Figure 5.14**).

The life span and steroidogenic capacity of the corpus luteum are dependent on continued tonic LH secretion. Studies in hypophysectomized women have demonstrated that normal corpus luteum function requires the continuous

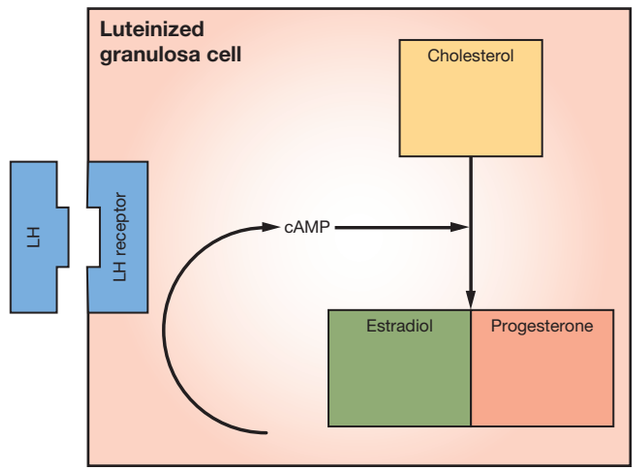

FIGURE 5.14

presence of small amounts of LH.[298] This dependence of the corpus luteum on LH is further supported by the prompt luteolysis that follows the administration of GnRH agonists or antagonists or withdrawal of GnRH when ovulation has been induced by the administration of pulsatile GnRH.[299,300] Hence, the standardization of GnRH agonist triggers usage in ovarian stimulation cycles at risk of hyperstimulation.[301] There is no evidence that other luteotropic hormones, such as prolactin, play a role in primates during the menstrual cycle.[302]

The corpus luteum is a complex and heterogeneous structure. Besides the luteal cells, also present are endothelial cells, leukocytes, and fibroblasts. These nonsteroidogenic cells form the bulk, about 70%, of the total cell population of the corpus luteum. The leukocyte population of the corpus luteum contributes several cytokines, including IL1-β and TNF-α.[303] The many different leukocytes in the corpus luteum are also a rich resource for cytolytic enzymes, prostaglandins, and growth factors involved in processes of angiogenesis, steroidogenesis, and luteolysis.

The corpus luteum is one of the best examples of communication and cross talk in biology. For example, endothelial cells contribute vasoactive compounds, and, in turn, steroidogenic cells contribute factors that influence angiogenesis. The harmonious function of this system is in parallel with its simplicity.

Endothelial cells constitute about 35% of the cells in a mature corpus luteum.[304] As elsewhere in the body, endothelial cells participate in immune reactions and endocrine functions. The endothelial cells are a source of endothelin-1, expressed in response to changes in blood flow, blood pressure, and oxygen tension. Studies have indicated that endothelin-1 may be a mediator of luteolysis.[305,306] Inhibition of VEGF prevents luteal angiogenesis.[307]

Even the luteal cell population is not homogeneous, being composed of at least two morphologically and functionally

distinct cell types, large and small cells.[308,309] Evidence supports that the large cells are derived from granulosa cells and the small cells from theca cells. The small cells are the most abundant. Despite the fact that greater steroidogenesis takes place in the large cells, it is the small cells that contain LH and hCG receptors.[310,311] The absence of LH/hCG receptors on the large cells, presumably derived from granulosa cells that acquire LH receptors in the late follicular phase, requires explanation. Perhaps large cells are functioning at a maximal level, with receptors totally occupied and functional, or because of intercellular communication through gap junctions, the large cells do not require direct gonadotropin support. Thus, the large cells can be functioning at a high level, under the control of regulating factors that originate in the small cells in response to gonadotropins. In addition, the overall function is influenced by autocrine–paracrine signals from the endothelial and immune cells.

Large luteal cells produce peptides (oxytocin, relaxin, inhibin, GnRH, growth factors, and prostaglandins) and are more active in steroidogenesis, with greater aromatase activity and more progesterone synthesis than the small cells.[312,313] Human granulosa cells (already luteinizing, when recovered from spent media of patients undergoing IVF) contain minimal amounts of P450c17 mRNA. This is consistent with the two-cell, two-gonadotropin explanation, which assigns androgen production (and P450c17) to theca cells. With luteinization, expression of StAR, P450scc, and 3-β hydroxysteroid dehydrogenase markedly increases to account for the increasing production of progesterone; continued expression of these essential factors requires LH.[314–316] The aromatase system (P450arom) continues to be active in the luteinized granulosa cells.

Progesterone levels normally rise sharply after ovulation, reaching a peak approximately 8 days after the LH surge. Initiation of new follicular growth during the luteal phase is further inhibited by the low levels of gonadotropins due to the negative feedback actions of estrogen, progesterone, and inhibin-A. With the appearance of LH receptors on the granulosa cells of the dominant follicle and the subsequent transformation of the ovulatory follicle into a corpus luteum, inhibin expression comes under the control of LH, and expression changes from inhibin-B to inhibin-A.[130,310,317] The circulating levels of inhibin-A rise in the late follicular phase to reach a peak level at the midluteal phase.[35,131,318] Inhibin-A, therefore, contributes to the suppression of FSH to nadir levels during the luteal phase and to the changes at the luteal–follicular transition. There is a wave of small follicle growth during the luteal phase, probably in response to the FSH surge at midcycle; however, the luteal phase FSH suppression typically ensures that a mature, large follicle will not emerge.[319,320] This mechanism can become dysfunctional with increasing age, as ovulation of a luteal–phase dominant follicle during menses was reported in a female of advanced reproductive age in which three ovulations occurred within 45 days.[321] In these circumstances, lower levels of progesterone and inhibin-A and reduced luteal growth have been observed.[322]

The secretion of progesterone and estradiol during the luteal phase is episodic, and the changes correlate closely with LH pulses.[102,323] Because of this episodic secretion, relatively low midluteal progesterone levels, which some inappropriately believe are indicative of an inadequate luteal phase, can be found in the course of totally normal luteal phases. The corpus luteum of the primate is unique in its production of estrogen; however, unlike the follicular phase, luteal estrogen synthesis is dependent on LH. Within the corpus luteum, progesterone acts locally to enhance the LH-induced luteinization of granulosa cells, to support its own LH-stimulated synthesis, and to inhibit apoptosis.[324–326]

In the normal cycle, the time period from the LH midcycle surge to menses is consistently close to 14 days. For practical purposes, luteal phases lasting between 11 and 17 days can be considered normal.[327] The incidence of short luteal phases is about 5% to 6%. It is well known that significant variability in cycle length among women is due to the varying number of days required for follicular growth and maturation in the follicular phase. The luteal phase cannot be extended indefinitely even if LH exposure is progressively increased, indicating that the demise of the corpus luteum is due to an active luteolytic mechanism.

The corpus luteum rapidly declines 9 to 11 days after ovulation, and the mechanism of the degeneration remains unknown. In certain nonprimate mammalian species, a luteolytic factor originating in the uterus and stimulated by estrogen (prostaglandin $F_{2\alpha}$) regulates the life span of the corpus luteum. No definite luteolytic factor has been identified in the primate menstrual cycle, and removal of the uterus in the primate does not affect the ovarian cycle. The morphologic regression of luteal cells may be induced by the estradiol produced by the corpus luteum.[328] A premature elevation of circulating estradiol levels in the early luteal phase results in a prompt fall in progesterone concentrations, and direct injections of estradiol into the ovary bearing the corpus luteum induce luteolysis, while similar treatment of the contralateral ovary produces no effect.[329] This action of estrogen may be mediated by nitric oxide. Nitric oxide stimulates luteal prostaglandin synthesis and decreases progesterone production.[330] Nitric oxide and hCG have opposing actions in the human corpus luteum; nitric oxide is associated with apoptosis of luteal cells.[331] The final signal for luteolysis, however, is prostaglandin $F_{2\alpha}$ that is produced within the ovary in response to the locally synthesized luteal estrogen (**Figure 5.15**).[329,332] These relationships are supported by genome studies delineating prostaglandin $F_{2\alpha}$ and hCG effects on gene expression.[333] The early luteal phase in primates is dominated by intraluteal synthesis of the luteotropic prostaglandin, PGE_2; late in the luteal phase, intraluteal prostaglandin synthesis shifts to $PGF_{2\alpha}$.[313]

There is another possible role for the estrogen produced by the corpus luteum. In view of the known estrogen

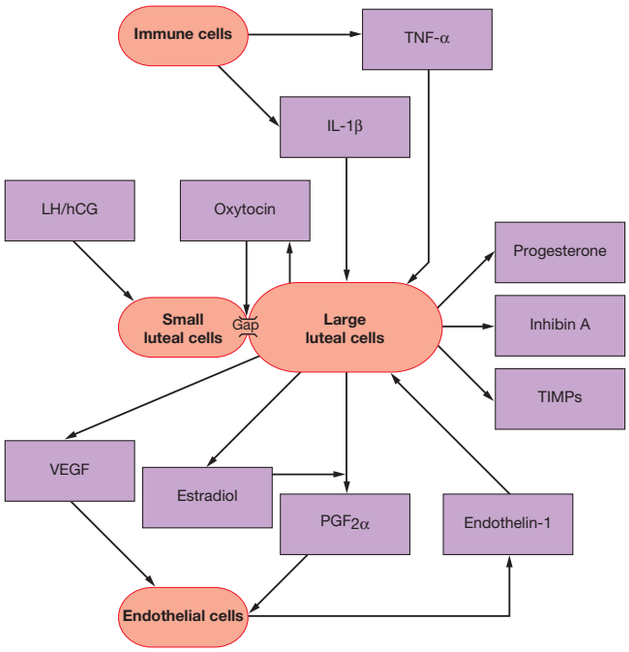

FIGURE 5.15

requirement for the synthesis of progesterone receptors in the endometrium, luteal phase estrogen may be necessary to allow the progesterone-induced changes in the endometrium after ovulation. Inadequate progesterone receptor content due to inadequate estrogen priming of the endometrium is an additional possible mechanism for subfertility; similarly, some early miscarriages (biochemical losses) may represent a form of luteal phase deficiency.

Experimental evidence indicates that the luteolytic effect of prostaglandin $F_{2\alpha}$ is partially mediated by endothelin-1.[305,306] Prostaglandin $F_{2\alpha}$ stimulates the synthesis of endothelin-1; endothelin-1 inhibits luteal steroidogenesis, and, in turn, endothelin-1 stimulates prostaglandin production in luteal cells.[334] In addition, endothelin-1 stimulates the release of TNF-α, a growth factor known to induce apoptosis; members of the TNF family, including their receptors, are expressed in the corpus luteum, and their expression peaks at the time of luteolysis.[335,336]

The corpus luteum involves cellular interactions that require cell-to-cell contact. Gap junctions are a prominent feature of luteal cells, just as they are in the follicle before ovulation. When the various cell types of the corpus luteum are studied together, the performance is different compared with studies of single-cell types, with greater steroidogenesis more closely approximating the total function of the corpus luteum.[337] It is believed that communication and exchange of signals takes place through the gap junction structures, explaining how the small cells respond to LH and hCG, but the large cells are the main site of steroidogenesis. Regulation of the gap junction system is influenced by oxytocin, a paracrine role for oxytocin in the corpus luteum.[27]

When ovulation is induced by the administration of GnRH, normal luteal phase demise occurs despite no change in treatment, arguing against a change in LH as the dominant luteolytic mechanism. In addition, LH receptor–binding affinity does not change throughout the luteal phase. Thus, the decline in steroidogenesis must reflect deactivation of the system (producing a refractoriness of the corpus luteum to LH), perhaps through the uncoupling of the G protein adenylate cyclase system. This is supported by studies in the monkey in which alteration in LH pulse frequency or amplitude did not provoke luteolysis.[338]

The process of luteolysis involves proteolytic enzymes, especially the MMPs. These enzymes are held under inhibitory control by TIMPs secreted by the steroidogenic luteal cells, and because TIMP levels do not change in luteal tissue, luteolysis is believed to involve a direct increase in MMP expression. An important part of the rescue mission for hCG is to prevent this increase in MMP expression.[339] hCG can increase TIMP production, and this, too, would inhibit MMP activity and luteolysis.[340] The source of MMPs is the fibroblast cell, and because luteal fibroblasts do not contain LH/hCG receptors, the release of MMPs in the regressing corpus luteum must depend on another signal. One such signal can be locally produced activin-A that acts on the fibroblasts to synthesize and release MMPs.[341] Emerging hCG from a pregnancy can inhibit this activin-A system by increasing follistatin, the glycopeptide that binds the activin. In addition, the human ovary contains the complete IL-1 system, providing another source of cytolytic enzymes.

The survival of the corpus luteum is prolonged by the emergence of a new stimulus of rapidly increasing intensity, the hCG. Blastocysts grown in culture produce and secrete hCG, beginning on days 7 to 8 after fertilization.[342] Messenger RNA for hCG can be found in 6- to 8-cell human embryos.[343] Because the 8- to 12-cell stage is achieved about 3 days after fertilization, it is believed that the human embryo begins to produce hCG before implantation, when it can be detected in the mother as early as 6 to 7 days after ovulation. The embryo is capable, therefore, of preimplantation signaling, and higher levels of estradiol and progesterone can be measured in the maternal circulation even before maternal hCG is detectable, presumably because of stimulation of the corpus luteum by hCG delivered directly from the uterine cavity to the ovary (**Figure 5.16**).[344] Function of the corpus luteum, especially secretion of progesterone, is crucial during the first 7 to 9 weeks of pregnancy, and luteectomy early in pregnancy can precipitate miscarriage.[345] Similarly, early pregnancy loss in primates can be induced by injections of anti-hCG serum.[346] Rescue of the corpus luteum by rising hCG levels from an early pregnancy is associated with maintenance of the corpus luteal vascular system (not new vessel

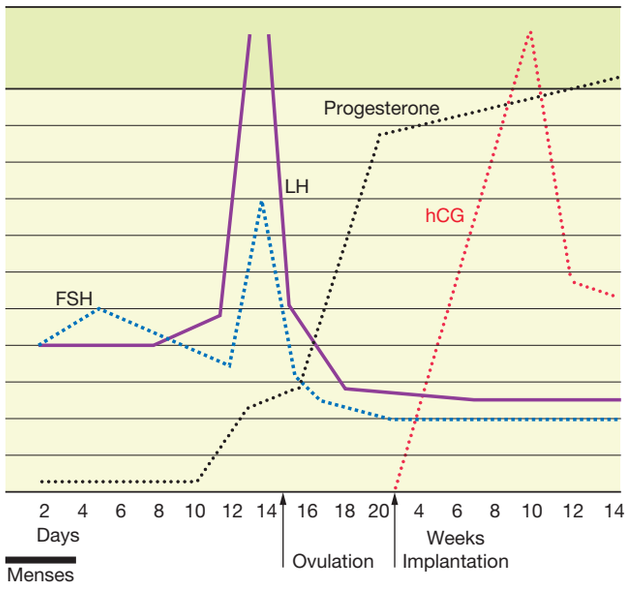

FIGURE 5.16

Key Points: Luteal Phase

- Normal luteal function requires optimal preovulatory follicular development (especially adequate FSH stimulation) and continued tonic LH support.
- The early luteal phase is marked by active angiogenesis mediated by VEGF. New vessel growth is held in check by angiopoietin-1 working through its receptor Tie-2 on endothelial cells.
- Progesterone, estradiol, and inhibin-A act centrally to suppress follicle maturation and ovulation during the luteal phase.
- Regression of the corpus luteum is associated with a decrease in VEGF and angiopoietin-1 expression and an increase in angiopoietin-2 activity and may involve the luteolytic action of its own estrogen production, mediated by an alteration in local prostaglandin and involving nitric oxide, endothelin, and other factors.
- In early pregnancy, hCG rescues the corpus luteum, maintaining luteal function until placental steroidogenesis is well established.

growth), a process that is dependent on the angiogenic factors VEGF and angiopoietin-2.[192,193,294,347]

Unlike the biphasic luteal pattern of circulating progesterone levels (a decrease after ovulation and then a new higher peak at the midluteal phase), mRNA levels for the two major enzymes involved in progesterone synthesis (cholesterol side-chain cleavage and 3β-hydroxysteroid dehydrogenase) are maximal at ovulation and decline throughout the luteal phase.[347] This suggests that the life span of the corpus luteum is established at the time of ovulation and that luteal regression is inevitable unless the corpus luteum is rescued by the hCG of pregnancy. **Therefore, primates have developed a system that requires rescue of the corpus luteum, in contrast to other mammals that use a mechanism that actively causes the demise of the corpus luteum (luteolysis)**.

THE LUTEAL–FOLLICULAR TRANSITION

The interval extending from the late luteal decline of estradiol and progesterone production to the selection of a dominant follicle for the ensuing cycle is a critical and decisive time, marked by the appearance of menses. Equally important but less apparent are the hormone changes that initiate the next cycle, most critically, GnRH, FSH, LH, estradiol, progesterone, and inhibin.

Given the important role of FSH-mediated actions on the granulosa cells, it is appropriate that the recruitment of a new ovulating follicle is directed by a selective increase in FSH that most prominently begins approximately 2 days before the onset of menses (**Figure 5.17**).[348–351] Using a sensitive

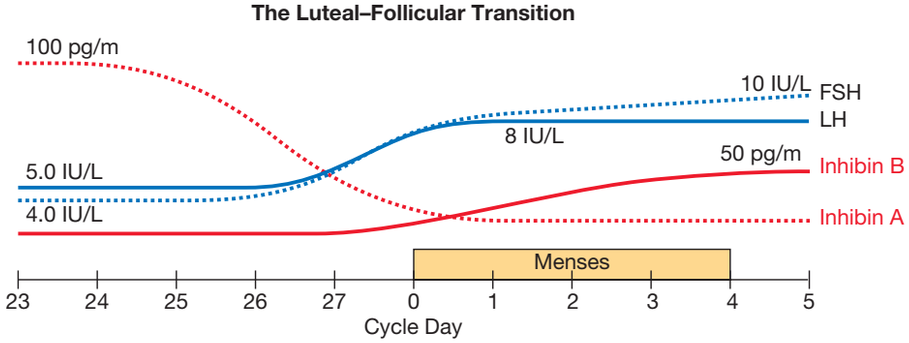

FIGURE 5.17

FSH bioassay, an increase in FSH bioactivity can be measured from as early as the midluteal phase.[36] There are at least two influential changes that result in this important increase in FSH: a decrease in the luteal steroids and inhibin and a change in GnRH pulsatile secretion.

Inhibin-B, originating in the granulosa cells of the corpus luteum and now under the regulation of LH, reaches a nadir in the circulation at the midluteal period.[318] Inhibin-A reaches a peak in the luteal phase and may thus help suppress FSH secretion by the pituitary to the lowest levels reached during a menstrual cycle.[35,318] The process of luteolysis, with resulting demise of the corpus luteum, decreases inhibin-A secretion as well as steroidogenesis. Administration of inhibin-A to monkeys effectively suppresses circulating FSH.[352] Thus, an important suppressing influence on FSH secretion, inhibin-A, is removed from the anterior pituitary during the last days of the luteal phase. This selective action of inhibin on FSH (and not LH) is partly responsible for the greater rise in FSH seen during the luteal–follicular transition, compared to the change in LH. Administration of recombinant (pure) FSH to gonadotropin-deficient women has demonstrated that the early growth of follicles requires FSH and that LH is not essential during this period of the cycle.[63,64]

Inhibin-B levels begin to rise shortly after the increase in FSH (a consequence of FSH stimulation of granulosa cell secretion of inhibin) and reach peak levels about 4 days after the maximal increase in FSH.[35,122,318] Thus, suppression of FSH secretion during the follicular phase is an action exerted by inhibin-B, whereas escape of FSH inhibition during the luteal–follicular transition is partly a response to decreasing inhibin-A secretion by the regressing corpus luteum.

Circulating levels of activin increase before ovulation to a peak in the luteal phase.[152,318] This timing is right for activin to contribute to the rise in FSH during the luteal–follicular transition. GnRH activity is enhanced by activin and suppressed by follistatin. Evidence in vivo and in vitro indicates that gonadotropin response to GnRH requires activin activity.[353] Activin specifically acts synergistically with GnRH to stimulate gene expression in the pituitary for the FSH β-subunit.[354]

The selective rise in FSH is also significantly influenced by a change in GnRH pulsatile secretion, previously strongly suppressed by the high estradiol and progesterone levels of the luteal phase exerting a negative feedback effect at the hypothalamus.[106,355] A progressive and rapid increase in GnRH pulses (as assessed by the measurement of LH pulses) occurs during the luteal–follicular transition.[105] From the midluteal peak to menses, there is a 4.5-fold increase in LH pulse frequency (and presumably GnRH) from approximately 3 pulses/24 hours to 14 pulses/24 hours.[105] During this time period, the mean level of LH increases approximately 2 fold, from approximately a mean of 4.8 to 8 IU/L. The increase in FSH is, as noted, greater than that of LH. FSH pulse frequency increases 3.5 fold from the midluteal period to the time of menses, and FSH levels increase from a mean of approximately 4 IU/L to up to 15 IU/L.

An increase in GnRH pulse frequency has been associated with an initial selective increase in FSH in several experimental models, including the ovariectomized monkey with destruction of the hypothalamus. Additionally, treatment of hypogonadal women with pulsatile GnRH results first in the predominance of FSH secretion (over LH). This experimental response and the changes during the luteal–follicular transition are similar to changes observed during puberty with a predominance of FSH as GnRH pulsatile secretion begins to increase.

The pituitary response to GnRH is also relevant in the luteal–follicular transition period. Estradiol suppresses FSH secretion through its classic negative feedback relationship at the pituitary level. The decrease in estradiol in the late luteal phase restores the capability of the pituitary to respond with an increase in FSH secretion.[356]

Key Points: Luteal–Follicular Transition

- The demise of the corpus luteum results in a nadir in the circulating levels of estradiol, progesterone, and inhibin.
- The decrease in inhibin-A removes a suppressing influence on FSH secretion in the pituitary.
- The decrease in estradiol and progesterone allows a progressive and rapid increase in the frequency of GnRH pulsatile secretion and removal of the pituitary from negative feedback suppression.
- The removal of inhibin-A and estradiol and increasing GnRH pulses combine to allow greater secretion of FSH compared with LH, with an increase in the frequency of the episodic secretion.
- The rising FSH is instrumental in rescuing an approximately 70-day-old group of ready follicles from atresia, initiating a new wave of follicular recruitment, growth that is intended for attainment of dominance of a single follicle in the ensuing cycle.

FOLLICULAR WAVE THEORY

Through the utilization of ultrasound technology in our field, a new concept of progression of antral follicular development within one menstrual cycle has emerged. As discussed, it was previously thought that recruitment of antral follicles occur solely in the late luteal or early follicular phase via stimulation and prevention of atresia of follicles by FSH. This concept is exemplified by the ability to amplify and extend the duration of FSH stimulation in IVF cycles and allow for multiple follicles to grow to maturity. The follicular wave

theory came about when it was noted that multiple waves of antral follicle recruitment are seen throughout the menstrual cycle, likely also preceded by an FSH rise. One study found that 100% of the 50 reproductive age females had multiple waves of follicular development, evaluated via ultrasound to assess follicle count and diameter. In this study, 68% developed two waves throughout an average 27-day cycle and 32% developed three waves in an average 29-day cycle. Of females found with two follicular waves, on average, the waves occurred on days −0.5 and 14.2, with day 0 being the day of ovulation. In females with three follicular waves, waves started on days −0.3, 11.6, and 18.2.[357]

The authors subsequently characterized each wave within these measured cycles and categorized them as major or minor waves.[358] They characterized major waves as those in which a dominant follicle develops, being one that developed to at least 10 mm with ongoing growth beyond the diameter of the next largest follicle in the wave by at least 2 mm. Minor waves were characterized as those not meeting the criteria for dominant follicle development. While females could have multiple major waves in one cycle, the final major wave was the one resulting in successful ovulation; thus, of course, all minor waves are anovulatory. In their analysis, they found these proportions of characterized waves in each cycle: minor then major (in 58% of women), major then major (in 10%), minor then minor then major (20%), minor then major then major (6%), and finally major then major then major (6%).

Clinically, this theory has been applied to a novel, but now commonly accepted, techniques of ovarian stimulation: "random-start," including luteal phase start, and "double ovarian stimulation protocols" (follicular phase start, then retrieval, followed immediately by luteal phase start and a second retrieval within the same menstrual cycle). In patients who cannot afford to wait up to a month for the start of another menstrual cycle, such as those with cancer who seek fertility preservation, these protocols have proven to deliver healthy oocytes with equivalent embryo quality regardless of when stimulation was started and served to prevent delays in their oncologic treatments.[359] Studies are otherwise mixed as to whether these protocols have any additional benefit to patients seeking fertility care for other indications, such as advanced reproductive age or poor ovarian reserve.

THE NORMAL MENSTRUAL CYCLE

Menstrual cycle length (duration between day 1 of menses to the first day of subsequent menses) is determined by the rate and the quality of follicular growth and development, and it is normal for the cycle length to vary somewhat in individual women.[360,361] Subtle alterations in cycle length are appreciated with advancing age, and cycle lengths are the shortest (with the least variability) in the late 30s, a time when subtle but real increases in FSH and decreases in inhibin are occurring (**Figure 5.18**).[128,327,362–365] This can be pictured as

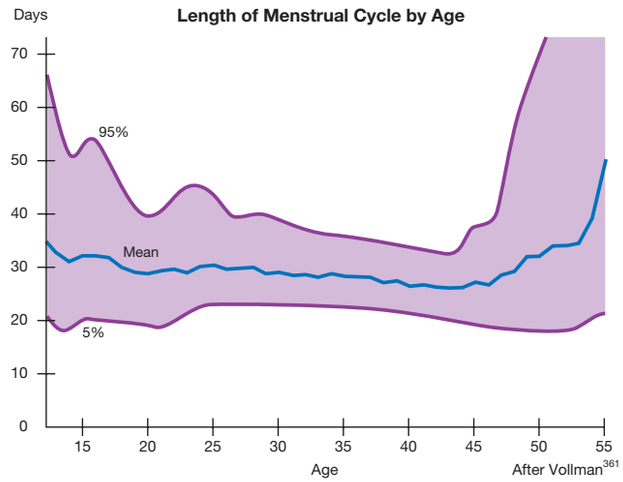

FIGURE 5.18

accelerated follicular growth (because of the changes in FSH and inhibin-B). At the same time, fewer follicles grow per cycle as a woman ages.[366] Approximately 2 to 4 years prior to the final menstrual period (marking the end of reproductive phase and the onset of menopause), the cycles lengthen again. In the last 10 to 15 years before menopause, there is an acceleration of follicular loss.[3] This accelerated loss begins when the total number of follicles reaches approximately 25,000, a number reached in normal women at age 37 to 38. Eventually, menopause occurs because the supply of follicles is almost depleted.[367]

The changes in the later reproductive years reflect either lesser follicular competence as the better primordial follicles respond early in life, leaving the lesser follicles for later, or the fact that the total follicular pool is reduced in number (or both factors).[368] Arguing in favor of a role for a reduced follicular pool is the observation that follicular fluid obtained from preovulatory follicles of older women contains amounts of inhibins A and B that are similar to that measured in follicular fluid from young women.[369]

Variations in menstrual flow and cycle length are common at the extremes of reproductive age, during the early teenage years and in the years preceding the menopause. The prevalence of anovulatory cycles is highest in women under age 20 and over age 40.[370,371] Menarche is typically followed by approximately 5 to 7 years of relatively long cycles that gradually decrease in length and become more regular. Although menstrual cycle characteristics generally do not change appreciably during the reproductive years,[371] overall cycle length and variability slowly decrease. A study looking at over 600,000 menstrual cycles, as reported through the use of apps, found that mean follicular phase length was 16.9 days and the mean luteal phase length 12.4 days, and the mean cycle length decreased by 0.18 days, with a decrease in follicular length by 0.19 days with each increasing year

of a female's age (ages 25–45 years).[372] On average, mean cycle length and variability reach their lows at about age 40 to 42.[361,373] Over the subsequent 8 to 10 years before menopause, the trend is reversed; both average cycle length and variability steadily increase as ovulation becomes less regular and more infrequent.[360,361,374,375] Mean cycle length is greater in women at the extremes of body mass and composition; both high and low body mass index (BMI) are associated with anovulation with an increased mean cycle length, as mean variation in cycle has been shown to be 0.4 days (14%) higher in women with BMI over 35 relative to women with normal BMI (18.5–25).[376,377]

In general, variations in cycle length reflect differences in the length of the follicular phase of the ovarian cycle. Women who have a 25-day cycle ovulate on or about cycle day 10 to 12, and those with a 35-day cycle ovulate approximately 10 days later. Within a few years after menarche, the luteal phase becomes extremely consistent (13–15 days) and remains so until the perimenopause.[356,360] At age 25, over 40% of cycles are between 25 and 28 days in length, increasing to 60% from age 25 to 35. Although it is the most often reported intermenstrual interval, only approximately 15% of cycles in reproductive-aged women are actually 28 days long. Less than 1% of women have a regular cycle lasting less than 21 days or more than 35 days.[378] Most women have cycles that last from 24 to 35 days, but at least 20% of women experience irregular cycles.[373]

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