MJC 4 (Physiology)
Physiology of Ovarian Cycles

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

1. The Follicular Phase of the Ovarian Cycle

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

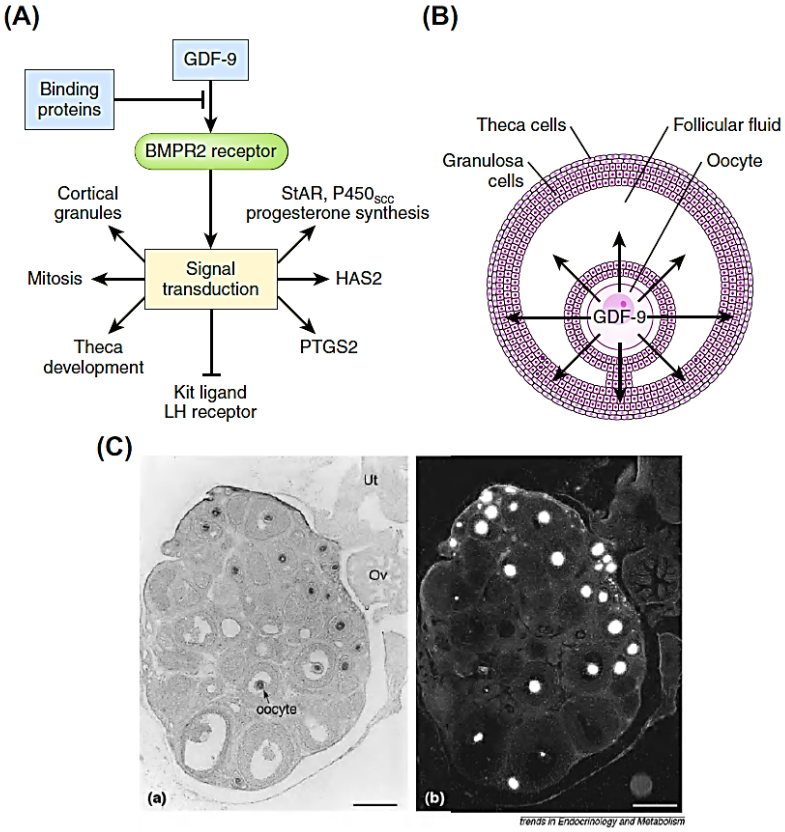


FIGURE 10-14 Growth differentiation factor-9 (GDF-9) is required for folliculogenesis in the mammalian ovary. (A) GDF-9 is produced by the oocyte and binds to surface membrane BMPR2 on follicle cells to initiate a wide variety of functions important for follicle growth including cholesterol transport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

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

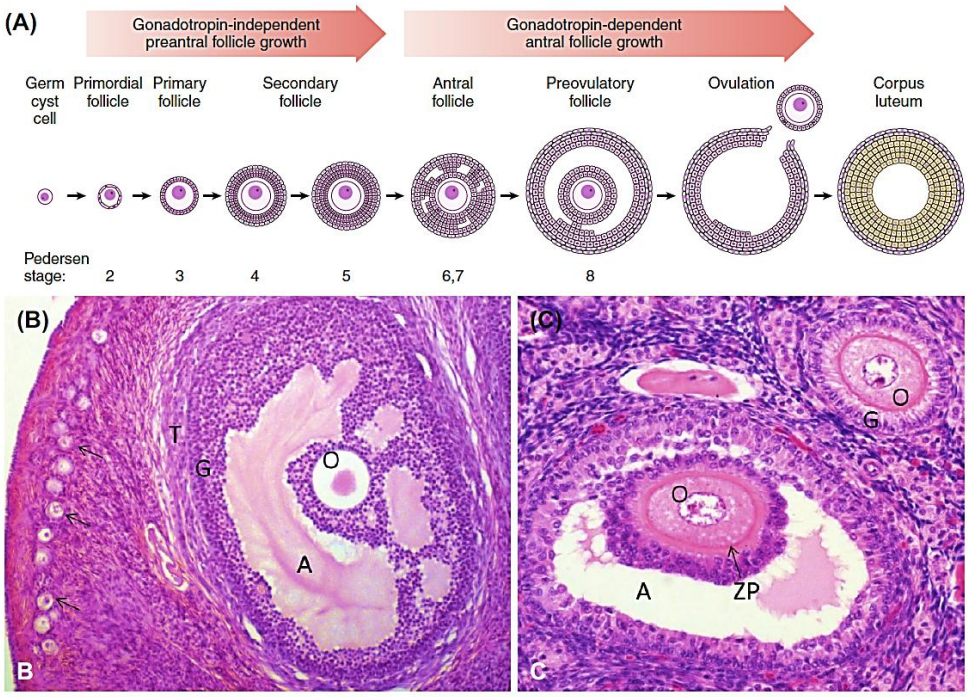


FIGURE 10-15 **Ovarian follicle stages.** (A) The appearance of the oocyte and follicle during oogenesis from germ cell to mature follicle and corpus luteum. (B, C) Sections of rat ovary showing stages of follicular development. (B) Mature or Graffian follicle with a large antrum (A) and clearly defined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

As the follicle grows, the granulosa cells secrete the liquor folliculi or antral fluid that is primarily an ultrafiltrate of blood plasma. Increasing production of antral fluid results in formation and progressive enlargement of a fluidfilled cavity within the follicle, the antrum. The follicle is now called a secondary follicle or an antral follicle. Under the influence of LH and FSH as well as a variety of paracrine factors (see Table 10-6), growing ovarian follicles synthesize and release estrogens, predominantly estradiol (¼ 17β-estradiol), into the general circulation. The synthesis of estrogens in the ovary appears to be a cooperative effort between cells of the theca interna and the granulosa (Figure 10-16). LH stimulates the thecal cells to produce androgens (principally androstenedione) that are aromatized by the granulosa cells to form estradiol. Conversion of androgens to estradiol by the granulosa cells is stimulated by FSH. In turn, FSH increases $P450_{aro}$ levels in these cells. In addition, FSH causes the granulosa cells to produce inhibins, which feed back on the pituitary to selectively inhibit FSH release as was described earlier for males. Inhibin also inhibits $P450_{aro}$ activity locally in granulosa cells, whereas the related peptide activin increases $P450_{aro}$ activity.

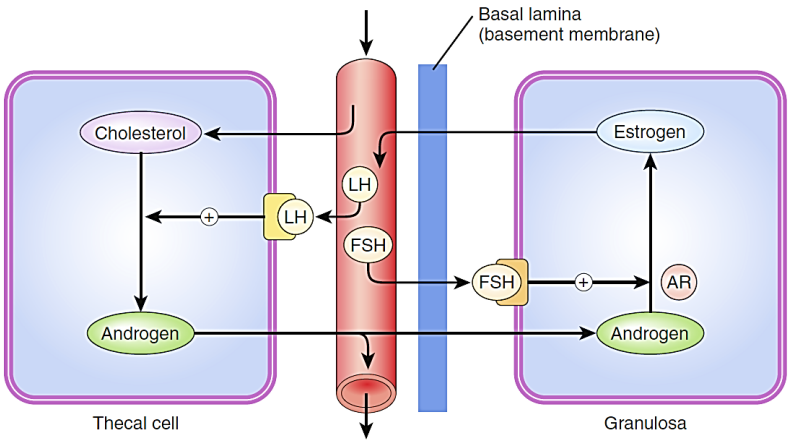


FIGURE 10-16 Two cell model for steroidogenesis. Binding of LH to receptors found only on thecal cells (or ovarian interstitial cells) stimulates androgen synthesis, most of which diffuses through the basal lamina (basement membrane) to the granulosa cell. FSH stimulates aromatase (AR) production, which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

The final stage of follicle growth is the tertiary or mature follicle (also called a graafian follicle). This follicle has reached maximal size and often is characterized by a single large antrum surrounded by a relatively thin layer of granulosa cells with the oocyte relegated to and surrounded by a small mass of granulosa cells, the cumulus oophorus. The final meiotic maturation of the oocyte apparently is influenced by paracrine factors from cells of the cumulus oophorus following ovulation. The mature follicle is located just beneath the surface of the ovary. Most (99%) of the follicles that begin to grow during a given ovarian cycle will exhibit apoptosis and degenerate, a process called atresia (Figure 10-17). These degenerating follicles are called corpora atretica. Atresia can occur at any stage of follicle development. Some of the steroidogenic cells from these atretic follicles will remain active and contribute to what has been called the interstitial gland of the ovary. Androstenedione produced in the interstitial gland by LH stimulation supplements thecal cell contributions for synthesis of estradiol by the granulosa cells.

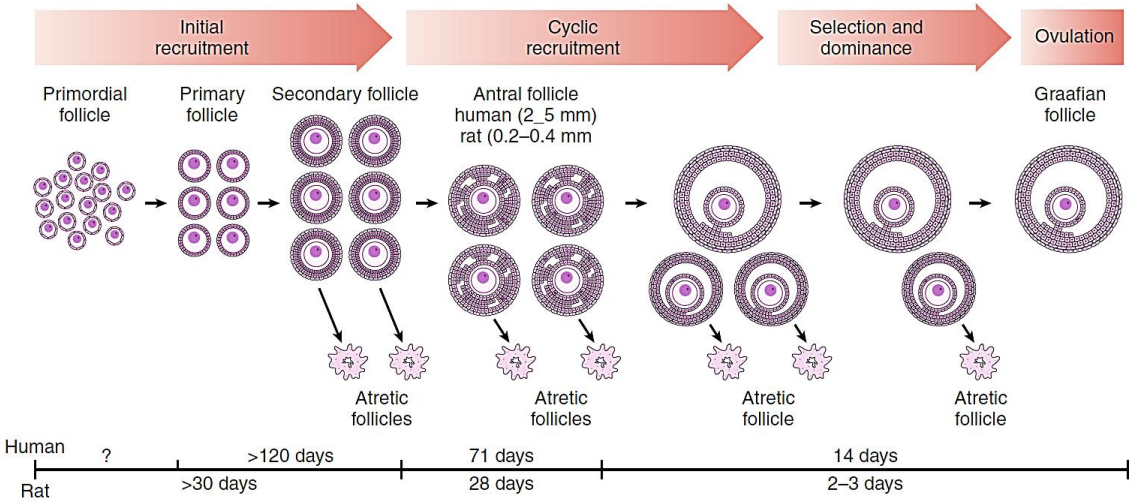


FIGURE 10-17 Recruitment and death (atresia) of ovarian follicles. Beginning at puberty a cohort of follicles selected from primordial follicles begins to grow. The majority of these follicles undergo programmed cell death, and one dominant follicle is selected for ovulation during each ovarian cycle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

2. Ovulation

The process of ovulation involves the rupture of the mature follicle and release of the oocyte from the ovary into the body cavity. This event marks the end of the follicular phase and the beginning of the luteal phase in the ovary and is correlated with estrus. Ovulation occurs as a result of the progressive increase in circulating estradiol that occurs with the growth of the

follicles. Increased estradiol also is responsible for estrous behavior in the female and the enhanced attractiveness of the female to the male at this time. A maximal or critical estrogen level in the blood in most cases activates the surge center in the hypothalamus, which releases a large pulse of GnRH. The pulse of GnRH released results in the LH surge (Figure 10-18) that causes ovulation of one or more follicles within a matter of hours (usually 12 to 24 hours, regardless of the species). The LH surge results in a remarkable series of genetic switches being turned off (FSH gene expression program) and on (genes required for oocyte meiosis; expansion of the cumulus cell oocyte complex, or COC; and corpus luteum formation). The number of follicles that reach maturity and ovulate is species specific, varying from a norm of one in women to a dozen or more in the sow. The determining factors appear to be the amount of GTH available, and increased numbers of mature follicles are produced following supplementation with exogenous GTHs. The physical mechanism by which LH causes the mature follicle to rupture and release the mature oocyte is not understood completely but involves both an increase in growth of the COC and the production of matrix metalloprotease (MMP) enzymes that digest the collagen and elastic fiber components of the extracellular matrix.

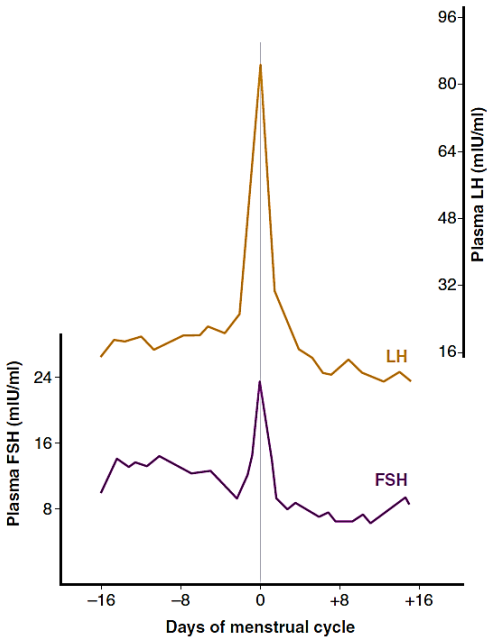


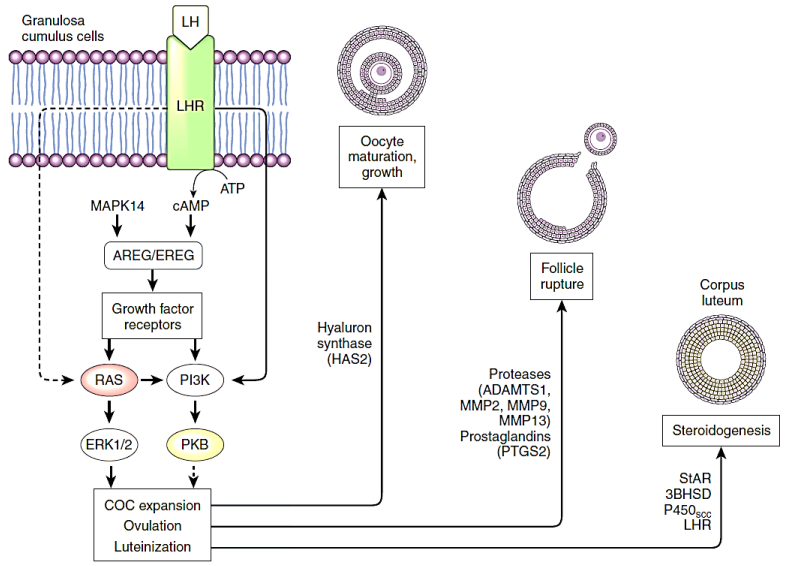
FIGURE 10-18 **Gonadotropin surge in normal women.** The greater release of LH is probably due to the presence of galanin released with GnRH at this time as well as negative feedback effects of inhibins on FSH release at the pituitary level. *Adapted with permission from McCann, S.M. (1974). Regulation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

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

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

3. The Luteal Phase of the Ovarian Cycle

Ovulation marks the onset of the luteal phase of the ovarian cycle as well as the end of the follicular phase. In addition to causing ovulation, the LH surge induces granulosa cells as well as some theca interna cells to differentiate into the corpus luteum (Box Figure 10E-1). This process, known as luteinization, results in the corpus luteum, which functions as an endocrine



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

gland, secreting both estrogens and progesterone into the general circulation. One corpus luteum will form from each ovulated follicle. In addition, other developing follicles may undergo premature luteinization and function as accessory corpora lutea during pregnancy. The corpus luteum begins secreting large quantities of progesterone, along with lesser amounts of estradiol as well as other estrogens and progestogens. Circulating progesterone and estrogens inhibit both the tonic and cyclic hypothalamic GnRH centers during the luteal phase so that additional follicular development is arrested and a second ovulatory episode is prevented. All developing follicles that do not ovulate undergo atresia or form accessory corpora lutea. Some of the follicular cells from the atretic follicles will persist as part of the ovarian interstitial gland that is responsive to LH and synthesizes androstenedione that can be used as a substrate by the corpus luteum to form estradiol.

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

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

The predetermined life span for the functional corpus luteum has provided one of the most intriguing mysteries of the ovarian cycle. Apparently, the corpus luteum sows the seeds of its own destruction (Figure 10-20). In female rats, mice, hamsters, rabbits, guinea pigs, and ewes, breakdown of the corpus lutem (luteolysis) requires the production of the prostaglandin PGF2a from the uterine lining. Production of luteolytic pulses of PGF2a requires the coordinated

action of estradiol, OXY, and progesterone on their respective receptors in uterine epithelial cells (Figure 10-20).

First, estradiol secretion from mature follicles increases the expression of progesterone receptors (PRs), OXY receptors (OXTRs), and estrogen receptors (ERs) in uterine epithelial cells. Progesterone action on the PR causes uterine cells to build up phospholipid in order to generate arachidonic acid, the precursor for prostaglandin synthesis. Progesterone action then causes a downregulation or block of ERs and OXTRs during this period of phospholipid buildup followed by an upregulation or release of ERs and OXTRs due to suppression of PR. Pulses of OXY secreted from the posterior pituitary and corpus luteum act on uterine OXTRs to stimulate PGF2a synthesis and luteolysis. The exact mechanism of luteolytic activity caused by PGF2a is not clear, although it may relate to reducing blood flow to the corpus luteum via an interaction locally with angiotensin II and endothelin 1.

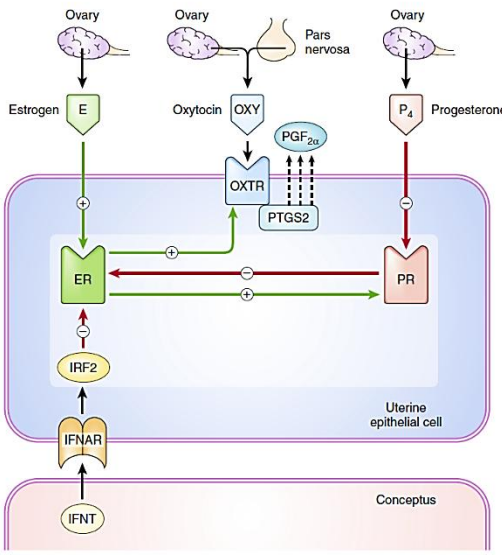


FIGURE 10-20 Mechanism of luteolysis. Estradiol action on uterine epithelial cells leads to increased expression of estrogen receptor alpha (ER), progesterone receptor (PR), and oxytocin receptor (OXTR). Increased PR availability leads to an increase in arachidonic acid, the substrate for prostaglandin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

In primates, the destruction of the corpus luteum toward the end of the luteal phase is not influenced by the uterus but appears to be caused locally by a luteolytic factor (e.g., estrone), produced by the corpus luteum itself. Once fertilization has taken place, there are several mechanisms by which corpus luteum degeneration may be prevented and the life of the corpus luteum prolonged. Degeneration of corpora lutea frees the hypothalamic GnRH centers from the inhibitory influence of estrogens and progesterone, resulting in a moderate increase in circulating GTHs and consequent renewal of follicular development. In fact, increased FSH release occurs in many species during the later stages of the luteal phase so that follicular growth may resume even before regressive uterine events become obvious. The importance of the corpus luteum in maintaining pregnancy varies considerably as does the role of pituitary hormones in stimulating corpus luteum function. For example, in rats, PRL is necessary for maintaining the first half of gestation through actions on the corpus luteum. However, in pigs, the corpora lutea secrete progesterone to maintain the uterine secretory phase during the early portion of the gestation period without aid of any pituitary hormones. In ewes, both LH and PRL are necessary for maintaining corpus luteum function during the first third of pregnancy, but maintenance of pregnancy actually resides in the ability of the conceptus to neutralize the uterine luteolytic factor PGF2a. Estrogens of placental origin apparently are responsible for prolonging the life span of corpora lutea in rabbits as well as promoting progesterone synthesis. If the estrogen-secreting placental cells are damaged (for example, by x-rays), pregnancy is abruptly terminated.

References

- Norris DO & James AC (2013). Vertebrate Endocrinology (5th edition). Academic Press, USA. http://dx.doi.org/10.1016/B978-0-12-394815-1.00001-X.