10. REGULATORY MECHANISM OF OVARIAN FUNCTION

Ovarian function is regulated through the hypothalamic-hypophyseal axis and, depending on the type of animal, environmental inputs or behavioural activities further regulate the function of the higher centers (Fig. 10-1).

FOLLICULAR DEVELOPMENT

During the reproductive life of the animals, the ovary contains a large number of follicles at different stages of development. The follicles located in the cortex of the ovary are less developed and grow slower than those more centrally located. Follicular development is a continuous and dynamic process, whereby new follicles are constantly growing however, the majority regress without ever reaching the ovulatory stage (Fig. 10-2).

The proportion of regressing (atretic) follicles increases with growth. Atresia is negligible in follicles with less than two layers of supporting cells, but very significant in those with four or more layers. Follicles grow to the preantral stage under a controlling mechanism that is not well understood. With increasing size, follicles become more dependent on gonadotropins for growth. After formation of the antrum the growing follicle requires stimulation by FSH for further growth. Normally the post-ovulatory FSH rise is responsible for stimulating the following crop.

FSH not only contributes to the formation of the antral follicle, but it is also responsible for the formation of more

![Figure 10-1. General regulatory mechanism for ovarian function](image1)

![Figure 10-2. Follicles are continuously developing and undergoing atresia, only a few fully reach ovulatory stage](image2)

![Figure 10-3. Detailed pathway through which follicles first produce estrogen during folliculogenesis and then, close to ovulation, switches to progesterone production](image3)
cells. Theca cells are located in the periphery of the follicle and are divided into two sub populations: the theca externa and the theca interna layer. The latter is located closer to the basal membrane and plays a fundamental role in steroid synthesis. Granulosa cells are located inside the basal membrane and, in conjunction with the theca interna cells, are essential for steroid production (Figs. 10-3, 10-4, 10-5). As the follicle develops, the type and concentrations of steroids produced vary significantly.

Testosterone, usually converted from circulatory cholesterol, is produced by theca cells in the antral follicle under the influence of LH. Testosterone in these events serves as a precursor for estrogen which diffuses out of the theca cells, through the basal membrane of the follicle into the granulosa cells. LH not only stimulates testosterone synthesis in theca cells, but it also stimulates formation of a large number of LH receptors in these cells. The increase in LH and FSH receptors in theca and granulosa cells respectively, augments the testosterone and estrogen producing capabilities of the follicle. Estrogens, in conjunction with FSH further stimulate the formation of LH receptors in granulosa cells, from an average of 300 at the beginning of folliculogenesis to a maximum of 120,000 per cell prior to ovulation. Furthermore, circulating estrogens stimulate the gonadotropes in the pituitary in such a way that their sensitivity to GnRH is increased. At the same time, estrogens stimulate the ovulatory centre of the hypothalamus through positive feedback and, eventually, make it discharge a large amount of GnRH. This in turn stimulates the already sensitized pituitary to produce a large release of LH. This is the preovulatory surge of LH (Figs. 10-3, 10-6).

As the follicles grow, there is production of a variety of other hormones with paracrine or autocrine modulatory roles in follicular cell differentiation. Among these, we can find the Immunoreactive Growth Factor I (IGF-I), Epidermal Growth Factor (EGF), and Fibroblast Growth Factor (FGF). All of these influence steroidogenesis by granulosa cells.
**SEASONAL BREEDERS**

In seasonal breeders the length of the day measured in hours of light, regulates the secretion of GnRH. The regulation is indirectly made through modifications in the amount of melatonin produced by the pineal gland. In short-day-breeders, melatonin supports secretion of GnRH by the tonic centre of the hypothalamus, while in long-day-breeders it inhibits GnRH. Secretion of melatonin is regulated by the amount of light that impinges in the retina and it is transported through the retinal nerves. Light inhibits the production of melatonin; therefore, short-day-breeders breed in the fall when the days are getting shorter, the reduction in hours of day light triggers an increase in its production. This results in the stimulation of GnRH production with the consequent elevation in gonadotropin production, follicular activity and eventual ovulation (Fig. 10-7).

During the spring, when the days are getting longer, the increasing hours of light inhibit the production of melatonin to the point that there is not enough support for GnRH and, as a consequence, there is not sufficient production of gonadotropins to support follicular development. Ultimately, the follicles fail to ovulate and the animal enters in seasonal anestrus (Fig. 10-8).

**PREOVULATORY LH SURGE AND OVULATION**

The effects of the preovulatory surge of LH in the mature follicle are multiple and simultaneous (Fig. 10-9).

First of all, LH stimulates an increase in blood flow to the Graafian follicle (Fig. 10-10).

At the same time, or immediately after the LH surge, the cumulus Oophorus of the Graafian follicle is dissociated at the base of the follicle, and meiosis resumes in the ovum (Fig. 10-11). Meiosis is inhibited by a peptide called oocyte meiosis inhibitor (OMI) which is produced within the follicle. OMI appears to be produced only while the cumulus is intact. The other consequence of the LH surge is an increase and further shift in steroid production by granulosa cells from estrogens to progesterone.
These cells develop the ability to synthesize progesterone from either pregnenolone, a derivative of cholesterol, or from circulatory or in situ produced cholesterol, as luteinization starts to take place even before ovulation.

As the follicle is getting ready to ovulate, the aromatase activity in granulosa cells decreases; therefore, no further conversion of androgen to estrogens takes place and; consequently, both, follicular as well as circulatory levels of estrogen decrease (Fig. 10-11).

Follicular edema and the initiation of stigma formation are the immediate consequence of these changes. Progesterone is responsible for the stimulation of the theca collagenase, an enzyme which stimulates the dissociation of cells in the area of the stigma. The last recognized effect of the LH surge is the production of prostaglandins by granulosa cells. Two types of prostaglandins are known to have some role in the process of ovulation. PGE₂, stimulates the plasminogen activator, an enzyme which activates the enzyme plasmin dissolves connective tissue in the area of the stigma. PGF₂α stimulates the rupture of lysosomes in the apex epithelium facilitating further stigma formation; it also stimulates ovarian contractions leading to follicle rupture and; finally stimulates follicle contraction for actual oocyte expulsion (Fig. 10-12).

**Effects of LH surge**

- **Increases follicular blood flow**
- **Cumulus separation**
  - Meiosis resume
- **Stigma formation**
  - Increase P₄, decrease E₂ = edema
  - Increase theca collagenase
- **Granulosa produces PGs**
  - PGE₂ stimulates
    - Plasminogen activator
  - PGF₂α stimulates
    - Rupture of lysosomes
    - Ovarian Contractions
    - Follicular contractions
- **Ovulation**

**Figure 10-10. Specific effects of the LH surge**

**Figure 10-11. Events taking place in the follicle prior to ovulation, as a consequence of the LH surge**

**Figure 10-12. Process of follicle rupture and ovulation**

**LUTEINIZATION**

After the ovum has been released and the follicular fluid expelled, the follicular cavity is filled with blood to form the CH (Fig. 10-13).
This is a mixture of granulosa cells, theca cells, and blood. The blood serves as nutrients for the initial growth and multiplication of the granulosa cells. From this point on, the basal membrane of the follicular cavity is disrupted and the newly formed organ becomes highly vascularized. The granulosa cells become luteinized, which means that they start to produce P₄ instead of E₂, under the influence of the low LH produced in response to GnRH from the tonic centre. The CH is then converted to a CL. Prolactin from lactotropes in the pituitary further supports CL functions (Figs. 10-14, 10-15, 10-16).

**CL REGRESSION (LUTEOLYSIS)**

If fertilization does not take place, several events lead to the eventual destruction of the CL with the consequent reduction in the concentration of circulating progesterone. Under the influence of progesterone, the uterus does not carry out any contractile activity. It seems that in the early stages of the luteal phase, progesterone also prevents the synthesis of PGF₂α by the uterus. Apparently, this is mediated by blocking the ability of the uterus to develop receptors for oxytocin but later in the luteal phase this is reversed and, there is a direct relationship between the number of oxytocin receptors in the endometrium and its ability to produce PGF₂α. In the CL, large luteal cells have the ability to synthesize, store and secrete oxytocin. The secretion is done in a pulsatile
manner. Towards the end of the luteal phase, the frequency and amplitude of the pulses of oxytocin is such that the PGF$_{2\alpha}$ released is sufficient enough to cause inhibition of progesterone synthesis and apoptosis of luteal cells. The pulses of oxytocin are mirrored with the pulses of PGF$_{2\alpha}$ secreted by the endometrium. Following that, there seems to be a positive feedback between luteal oxytocin and endometrial PGF$_{2\alpha}$. To reach the ovary and induce the regression of the CL, the PGF$_{2\alpha}$ being drained from the uterus, has to cross through the endothelial cell of the blood vessels, from the uterine vein into the ovarian artery. Using this counter current mechanism of transfer, PGF$_{2\alpha}$ retains its biological activity, which otherwise would have been lost at the first passage through the lungs. The regressing CL becomes a corpus albicans (CA) which is no longer a steroid secreting tissue (Fig. 10-17). Before the uterus attains the capability to produce PG, it has to be primed by P$_4$ for at least four days in the cow. After this, a new crop of follicles develops for ovulation in the next cycle.

If fertilization takes place, the PGF$_{2\alpha}$ produced in the uterus does not reach the ovary, as it is redirected to the lumen of the uterus and the CL does not regress. After this the CL function is enhanced to produce more P$_4$. This structure becomes the corpus luteum verum or the CL of pregnancy which is responsible for the production of progesterone that will serve to maintain pregnancy in its earlier stages or throughout pregnancy, depending on the species.

**INDUCED OVULATORS**

The queen and the rabbit are polyestrous induced ovulators. This means that they cycle throughout the year and that ovulation, and formation of the CL does not take place unless copulation occurs. The length of the cycle and other possible events depends on several factors. The more relevant factors are mating, ovulation, conception, pregnancy to completion, and whether or not lactation takes place. The typical cycle length, when no mating takes place, is usually 21 days. If breeding takes place but conception fails, the average cycle takes about 6 weeks (Fig. 10-18).

The estrus period varies with the season in such a way that in spring, a queen may manifest estrus for 5 to 14 days per cycle. In other seasons, a 1 to 6 day estrus period is more typical. When coitus and ovulation take place, the average cycle is less than 6 days while in non-ovulatory cycles, the estrus period lasts about 8 days (Fig. 10-18).

During the non-breeding cycle, the follicles develop normally from the constantly developing follicle pool, but in the absence of an ovulatory surge of LH, they fail to ovulate and regress. A new crop of follicles starts to develop significantly in order to establish the next estrous cycle. Since ovulation does not take place, there is no formation of a CL and levels of progesterone remain undetectable. If breeding takes place, the physical stimulation of the vagina during copulation sends signals through the nervous system to the ovulatory centre in the

**REPRODUCTIVE ALTERNATIVES FOR A QUEEN**

<table>
<thead>
<tr>
<th>Event</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrus</td>
<td>No copulation = Estrus 5 to 16 days later</td>
</tr>
<tr>
<td>Copulation</td>
<td>No ovulation = Estrus 5 to 16 days later</td>
</tr>
<tr>
<td>Ovulation</td>
<td>No conception = Pseudopregnancy for 35 days, Estrus 8 to 10 days later</td>
</tr>
<tr>
<td>Conception</td>
<td>No lactation = Estrus 8 to 10 days later</td>
</tr>
<tr>
<td>Pregnancy 63 days</td>
<td></td>
</tr>
<tr>
<td>Lactation 4 to 6 weeks</td>
<td></td>
</tr>
<tr>
<td>Estrus 7 to 3 days later</td>
<td></td>
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</tbody>
</table>

Figure 10-18. Possible sequence of events taking place in a queen based on several determining factors

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*Figure 10-17. Mechanism to trigger the regression of the corpus luteum if the animal does not get pregnant*
hypothalamus. In response to this input, cells in the hypothalamus release a large discharge of GnRH, which triggers the LH ovulatory surge within 90 mins. This results in ovulation between 24 and 50 hours post coitum. As a result of the ovulatory process, the follicles develop into a functional CL which produces large amounts of progesterone (Fig. 10-19).

If conception does not take place, the animal will normally manifest pseudopregnancy for about 35 days. After this, cyclical activity resumes. If fertilization takes place and pregnancy is maintained, the production of progesterone will peak at about 22 days and thereafter decline towards parturition on the 63rd day.

**MONOESTRUS BREEDERS**

The fox, wolf and the bitch belong to the group of animals designated as monoestrus breeders. This implies that they have approximately one breeding season in the year and that in each breeding season they manifest estrus and can potentially ovulate multiple ova at one time. As pointed out earlier, the bitch cycles more often than once a year, averaging three cycles in two years. The entire estrous cycle is much longer in monoestrus breeders than in food-producing animals and some of the behavioural phases of the estrous cycle overlap with the physiological processes characteristic of a different phase (Fig. 10-20).

**Proestrus**

Proestrus is the period characterized by early signs, such as vulva edema and blood-tinged vaginal secretions, which finish when the bitch accepts the male. During this period, the female is restless, excitable, and disobedient and manifests frequent mounting behaviour. Polydipsia and polyuria are also evident. The vaginal secretions produce a pheromone, methyl-p-hydroxybenzoate which attracts males. At this time she does not accept mounting and can be aggressive towards males. On average this phase lasts about 9 days.

**Estrus**

Estrus is described as the period of male acceptance which also lasts about 9 days. However, longer periods of male receptivity are not uncommon. The first estrus following puberty is normally longer than the subsequent ones. Ovulation is highly variable. It may take place between 2 days before, to 7 days after the initiation of estrus, the most common period being days 1 to 3 after initiation of estrus. Most follicles are ovulated in a period of about 24 hours. Endocrinologically, the ovary may be forming the CL and producing progesterone while the female still accepts the male.
Metestrus
If conception does not take place, metestrus lasts about 75 days and is defined as the period between the end of male acceptance and the regression of the CL.

Anestrus
Anestrus is a period of ovarian inactivity during which there is neither vaginal discharge nor behavioural interest in the male. It lasts approximately 125 days.