Welcome to chapter 4.
The following chapter is called "Physiology of Ovulation".
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The learning objectives of this lesson are:
- To define and describe the mechanism of ovulation
- To review the different phases of the menstrual cycle and its physiology, and
- To understand the sequence of events occurring during the menstrual cycle
To introduce this issue, let us define the concept of ovulation. Ovulation is the occurrence in the menstrual cycle by which a selected mature follicle breaks and releases a viable oocyte from the ovary. This fact enables the egg to be fertilized by the male sperm cells. Each month, one egg is released in humans; but occasionally, two or more can erupt during the menstrual cycle. In women with regular menstrual cycles, the ovulation takes place approximately two weeks after menstruation. If pregnancy does not occur, the menstruation appears exactly two weeks after ovulation.
Ovulation is the most important event of the menstrual cycle which is commonly divided into three phases:

**Follicular Phase:** At the beginning of each cycle, a group of the most mature follicles (called “antral follicles”) are recruited. Only the follicles most sensitive to follicle stimulating hormone (FSH) undergo a further development. The follicle most sensitive to FSH continuous the development and produces a large amount of estradiol and inhibin B. The remaining follicles become atretic. In the human being, the stock of primordial follicles is about 1 million at birth, and at the beginning of puberty a few hundred thousands. These follicles progress from primordial through primary, secondary to pre-antral and antral follicles from menarche to menopause, but practically all the follicles (about 99%) will be affected by the phenomenon of atresia at variable stages in the course of development.

It has been shown that an average of 85 days – corresponding to 3 ovarian cycles – separates the moment when a follicle becomes preovulatory and the moment when it has differentiated its theca interna (“preantral stage”). This means that a preovulatory follicle enters the preantral stage 85 days earlier, in mid-cycle, at the time of the preovulatory discharge of the two gonadotropic hormones FSH and LH (Gougeon 1990).

**Ovulatory Phase:** In the mid-cycle, the luteinizing hormone (LH) surge triggers ovulation. The wall of the pre-ovulatory follicle is broken due to a cascade of inflammatory responses and the oocyte is released with its cumulus oophorus...
Ovulation is the result of a well ordered series of events. These processes are controlled by the hypothalamic-pituitary-ovary axis. The menstrual cycle is directed by complex functional interactions between the ovaries and the hypothalamus-pituitary system which control each other by means of positive or negative feedback mechanisms.

The main factors responsible for the function of this axis are:

- Firstly the hypothalamic hormones, particularly the gonadotrophin releasing hormone (GnRH),
- Secondly, the pituitary hormones or gonadotropins (FSH and LH), and
- Finally, the ovarian steroid hormones (estradiol and progesterone).
GnRH is a decapeptide which is synthesized and released by specific neuronal endings in the nucleus arcuatus of the hypothalamus.

GnRH is transported through the portal vessels towards the anterior pituitary gland.

The hormone is only detectable in the portal system, being undetectable in the systemic circulation.

Small quantities of GnRH are sufficient to effectuate a release of gonadotropins from the pituitary gland.

The release pattern of the gonadotropins is dictated by the frequency of GnRH release. GnRH must be released in a pulsatile manner, and its effects depend on the frequency and amplitude of these pulses.

If GnRH is released in a constant, non-pulsatile manner, gonadotropin release is suppressed due to an apparent desensitization of the pituitary GnRH receptors.
The next step occurs in the pituitary gland. The varying frequency and amplitude of GnRH release determines the pattern of release of the gonadotropins, FSH and LH, during the menstrual cycle, and, subsequently, controls the ovulation and the ovarian steroid production.

The last step of the hormone cascade is localized in the ovaries, where steroid hormones are synthesized caused by the gonadotropins action. These hormones are estradiol, (produced by the growing follicle and the corpus luteum); and progesterone (produced by the corpus luteum), once ovulation has occurred.
The upper part of this illustration shows how the release of FSH and LH changes in the course of the menstrual cycle. The next slides deal with the role of FSH. The role of LH will be detailed on slide 11 and following.

In the last phase of the previous cycle, FSH level increases due to the drop of estradiol, progesterone and inhibin A levels. This is called positive feedback.

This rise in FSH concentration stimulates the growth of antral follicles, resulting in an increase of estradiol and inhibin B concentrations, producing a negative feedback, i.e. a reduction of FSH concentrations. This decrease of FSH combined with rising estrogen concentrations play an important role in the selection of the dominant follicle.

At mid-cycle, there is a temporary increase in FSH secretion, whose cause and significance is not clear. It may be due to the GnRH surge and may have a function in preparing a cohort of small antral follicles for the next cycle (Homburg, 2005).

In the luteal phase, the corpus luteum secretes both, estradiol and progesterone, resulting in a negative feedback mechanism that suppresses the FSH release until just before the next menstruation.
As mentioned before, the FSH level declines during the mid follicular phase. This decrease of FSH prevents a multiple follicular development, as only the largest of the developing follicles stays above the FSH threshold, has the most FSH receptors, remains most sensitive to FSH and produces most estrogen. The leading follicle becomes less sensitive to the declining FSH concentrations and continues to develop while the remaining follicles fade into atresia resulted by a lack of sufficient FSH stimulation.

The induction of LH receptors on the largest developing follicle enables LH to take a part in the development of the dominant follicle in the late follicular phase and prepare it for the oncoming LH surge.
FSH has many roles:

As it has been mentioned, the rise in FSH concentrations at the beginning of the cycle promotes:

The granulosa cell proliferation and differentiation, as well as, the antral follicle development. It also induces the estrogen production due to the activation of the enzyme aromatase, that converts androgens into estrogens. In addition, it activates LH receptors on the dominant follicle. Finally, FSH enhances the synthesis of inhibin.
During the follicular phase the secretion and, therefore, the concentration, of LH is relatively low. However, it suddenly increases in the late follicular phase. This LH surge induces the ovulation. The duration of the LH surge is 36-48 hours.

The causes of the LH surge are: First, the negative feedback of estradiol at the hypothalamic-pituitary level turns to a positive feedback when estradiol concentrations reach a critical point. Then, the pituitary gland becomes highly sensitive to GnRH stimulation, due to the increase of GnRH receptors. Thus, the GnRH surge produces the LH surge.

Following ovulation, increasing concentrations of progesterone slow down the frequency of LH releasing pulses. Concentrations of LH once again drop to baseline levels.

The corpus luteum maintains itself for 14 days, possessing a considerable intrinsic capacity of self-regulation.
The pre-ovulatory LH surge has a number of key functions:
It triggers ovulation and follicular rupture about 36 hours after the surge, and is responsible for the disruption of the cumulus-oocyte complex. Furthermore, it induces the resumption of oocyte meiotic maturation and the luteinization of granulosa cells.
To understand the role of gonadotropins on the ovary function, it is important to review the well known “two cell – two gonadotropin hypothesis” (Fevold 1941).

The mid-cycle surge of LH induces the production of androgens by cells of the theca follicles. The androgens, androstenedione and testosterone, are then ‘passed on’ to the neighbouring granulosa cells, where aromatase converts them into estrogens, mainly estradiol but also estrone. Aromatase activity, and, therefore, estrogen production, is controlled by FSH.

These events suggest that the function of theca cells and granulosa cells are controlled by LH and FSH respectively.

Moreover, there is also a fine-tuning achieved by other factors such as inhibin and insulin-like growth factors (IGFs) I and II.
In the antral follicle, LH receptors are only present in the theca layer while the granulosa layer only expresses FSH receptors. Later on, due to tonic FSH stimulation, LH and aromatase receptors appear also on the granulosa cells in the preovulatory follicle. As a consequence, FSH and LH synergize their action for estradiol synthesis and therefore oocyte maturation.
To sum up the concept, this slide shows how the follicle becomes sensitive to the gonadotropins as it grows.

Progression from primordial, through primary, secondary to pre-antral follicles does not depend on the gonadotropic stimulation, being a process controlled and induced by the ovary itself.

Nevertheless, the antral follicle already has FSH receptors and grows due to the FSH action. In the last stage, the preovulatory follicle also becomes sensitive to the LH action.
Estradiol is the most important estrogen in the ovulatory cycle.

- During menstruation estradiol concentrations are low, but start to increase as FSH induces follicular development in the mid-follicular phase.
- When estradiol levels reach a critical point, they activate a positive feedback mechanism in the hypothalamus which causes the pituitary to release a massive surge of LH.
- Following ovulation, estradiol concentrations decrease temporarily but are revived caused by corpus luteum activity. With the demise of the corpus luteum, estradiol concentrations drop rapidly to their lowest levels and by a positive feedback, increase FSH levels immediately preceding menstruation.
The key functions of estradiol in the ovulatory cycle are:

• In the mid-late follicular phase, it suppresses the secretion of FSH due to a negative feedback mechanism leading to the selection of a dominant follicle and preventing multi-follicular development.

• In mid-cycle estradiol triggers the LH surge due to a positive feedback mechanism when its concentrations rise to a critical level.

• In the follicular phase it is responsible for an increase of thickness of the endometrium.

• In the ovulatory period, estradiol stimulates the glands of the cervix to secrete a particular type of mucus which is essential for the sperm to pass through the cervix to reach the ovum.
Progesterone is the main hormone in the luteal phase. Large quantities are synthesized by luteinized granulosa cells of the corpus luteum following ovulation. Progesterone concentrations rise to a peak 7-8 days following ovulation and fall rapidly with the demise of the corpus luteum. Together with estradiol, progesterone suppresses pituitary gonadotropin release during the luteal phase. The initial rise of progesterone concentrations, immediately preceding the LH surge, may play a role in the triggering of this surge (Homburg 2005).
The main functions of progesterone secreted by the corpus luteum are:
• To induce a secretory endometrium, capable to enhance embryo implantation
• To maintain the endometrium throughout the first weeks of pregnancy
• To modify the endometrial glandular structure (more number, more tortuous) and
• To interfere in the expression of genes needed for implantation at the endometrium level
Oocyte maturation is necessary for an oocyte to become fertilizable. This maturation is governed by FSH and LH and includes changes in the cytoplasm, which will allow the oocyte to sustain further development upon fertilization. Furthermore, oocyte maturation includes a nuclear maturation to activate the resumption of meiosis from the prophase of the first meiotic division and progression to metaphase of the second meiotic division. This event is observed by the disappearance of the oocyte’s nuclear membrane, called germinal vesicle.
Oocyte maturation only occurs within the dominant mature follicle. Until this stage, some inhibit factors such as cyclic adenosine monophosphate (cAMP) and factors increasing its concentration (e.g. FSH), as well as oocyte maturation inhibitor (OMI) and hipoxantine, keep the oocyte in the immature stage.

The mid-cycle LH surge induces a dismantling of the gap junctions between granulosa cells and the oocyte, thus inhibiting the flow of inhibin factors to the oocyte and allowing the flow of maturation inducing factors such as calcium, maturation promotor factor (MPF) and another growth factors.

cAMP is an important mediator in the nuclear maturation of the oocyte. cAMP activates a protein-kinase that suppress the activation of MPF or degrades its subunits. The drop of cAMP levels after LH surge favours oocyte maturation due to the increasing levels of MPF.

Increasing levels of calcium after the LH surge are also necessary to restore the meiotic arrest.
LH surge triggers ovulation throughout a cascade of inflammatory events in the pre-ovulatory follicle that leads to the breakdown of the follicular wall and the release of the complex cumulus-oocyte.

It has been suggested (Speroff 2006; Homburg 2005) that the causes of this rupture are:

- An increase of intrafollicular pressure,
- Proteolytic enzyme activity on the follicular wall,
- Morphological changes in the stigma,
- Perifollicular ovarian smooth muscle contractions and
- Vascular alterations in the perifollicular vessels.

Some of these incidents can be attributed to increased concentrations of prostaglandins in the ovarian follicles and some to a cascade of enzymatic steps resulting in collagenolysis.

It has been suggested that some factors, such as cytokines, oxygen free-radicals, nitric oxide and angiotensin II could contribute to these actions, but this issue is still unclear (Homburg 2005).
The start of ovulation can be detected by various signs. Towards the time of ovulation the pH in the vagina becomes less acidic, the cervical mucus becomes more copious and less viscous, all of which favour the progress of motile sperm towards the released oocyte. Moreover, the action of progesterone increases basal body temperature by one-quarter to one-half degree Celsius (one-half to one degree Fahrenheit). Furthermore, many women also experience secondary fertility signs including Mittelschmerz, i.e. pain associated with ovulation. Many women also refer heightened sense of smell and sexual desire in the several days immediately before ovulation.

### Clinical signs of ovulation

- The pH in vagina becomes less acidic
- Change in the cervical mucus
- Basal body temperature increases after ovulation
- Mittelschmerz (pain associated with ovulation)
- Heightened sense of smell
- Heightened sexual desire immediately before ovulation
References and further reading

References and further reading

- Zeleznik AJ. Follicle selection in primates: "many are called but few are chosen". Biol Reprod. 2001 Sep;65(3):655-9.