



OVULATION A SIGN OF HEALTH

THE CASE FOR REPRODUCTIVE HEALTH RESEARCH INSTITUTE

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1. Introduction

Ovulation is the major event of the menstrual cycle. It requires a series of coordinated hormonal events to occur and it shows an adequate activity of the hypothalamic-pituitary-gonadal axis (HPG). In this sense, women should care about it as a sign of health (*Vigil et al., 2017*). The occurrence of ovulation is an activity that begins in adolescence and continues throughout a woman's reproductive life until menopause. Thus, the study of ovulation is a powerful tool in order to assess women's health status. The pattern of ovarian activity can undergo relevant changes during a woman's life: this is known as the ovarian continuum (*Brown, 2010*). This term explains the different variations in ovarian activity, that start during intrauterine life and are observed throughout the life of a woman in response to different physiological, behavioral and environmental conditions. According to this concept, under certain physiological conditions such as pregnancy or breastfeeding, an anovulatory state is considered a normal ovarian activity within the continuum (*Brown, 2010; Pérez, 1998*). Also, some periods of irregular ovulation, such as puberty and perimenopause, can be considered as part of a physiological transition. Nonetheless, certain pathological conditions can disrupt ovulation, such as unhealthy lifestyle habits, stress, endocrine abnormalities, gynecological disorders, autoimmune disorders, genetic disorders, drugs (as hormonal contraceptives) and iatrogenic causes (*Vigil et al., 2017*).

Women can use different approaches in order to identify the occurrence of ovulation. The use of biomarkers, as cervical mucus, helps women to identify ovulation and, therefore, their health

and well-being of women. RHRI has been able to meet this need, but further support is needed in order to continue research and spread this knowledge within healthcare providers.

2. Anatomy of ovulation

There is a series of complex structures that interact with each other in order to achieve ovulation. Next, the most important anatomical and histological aspects involved in this process will be analyzed.

The ovaries, in conjunction with the uterus and the oviducts (uterine tubes or Fallopian tubes), are part of the internal female genitals, located in the pelvis minor. These are the female gonads and are responsible for the production of sex hormones. They are two organs located on either side of the uterus. In the ovaries, we can find the follicles that contain the oocytes, the female germ cell involved in reproduction. The development of the ovaries comes from the genital ridges (gonadal ridges) of the embryo, towards the end of the fourth week of gestation. Considering their histological characteristics we can distinguish a cortex and a medulla and the presence of ovarian follicles on their different stages of development.

Cortex & medulla

Cortex, the external layer of the ovary, is called superficial epithelium or germinal epithelium. Immediately under the superficial epithelium lies a small layer of collagen fibers, the tunica albuginea, which gives the ovarian surface its characteristic pearl white color. On the inside, there is a lax connective tissue, and the

follicles appear in different developmental stages. During reproductive years, the cortex constitutes more than 50% of the total ovarian volume.

The medulla is formed by a connective tissue called stroma that is less fibrous than the cortex. It is also constituted by abundant spiral, thick-walled, blood and lymphatic vessels, as well as nerves, all of which enter and exit the ovary through a region called hilum. The medulla also presents fibroblasts, mastocytes, elastic fibers and myocytes (Kühnel, 2004).

The ovarian follicles

A follicle is a structure formed by the oocyte (the germinal cell) surrounded by somatic cells (granulosa cells). On the outside of this cells, and separated by a basal lamina, are the theca cells (Schoenwolf & Larsen, 2009).

Surrounding the oocyte we found a cellular structure called the zona pellucida, which is formed by glycoproteins. The zona pellucida will play a very important role in fertilization and early development in the cycle.

Ovarian follicles experience changes in their function and in their morphology, a process of development called folliculogenesis. According to their developmental stages, follicles can be classified in: primordial, primary, secondary, tertiary, mature (Graafian), corpus luteum and albicans (Figure 1) (Popa et al., 2008).

Folliculogenesis has two stages: an early stage, called initial folliculogenesis, which is independent of gonadotropin

hormones, and a second stage, which is cyclical and dependent on gonadotropin hormones. The initial folliculogenesis continuously occurs and involves the development of a primordial follicle until it reaches the antral stage. It occurs from intrauterine life until menopause when the ovary is depleted from ovarian follicles. Normally it takes about 10 weeks. The second, or dependent stage, is present from the first ovulation to menopause. It occurs during the so-called follicular phase of the menstrual cycle and involves the growth of a follicle since it has reached the antral stage (tertiary follicle) until ovulation (the rupture of a Graafian follicle). This secondary process takes between 10 to 22 days, with an average of 14 days

3. Role of the central nervous system in ovulation

The central nervous system (CNS) is a specialized structure that controls the functions related to motor, behavioral, cognitive and neuroendocrine coordination of the organism (Table 1). The system can be divided into two parts: the spinal cord and the brain. Hormones have an important effect upon wiring of different brain areas, including the hypothalamus and pituitary gland, which have an essential role in reproduction (Figure 2). The brain is regulated by hormones that feedback it (Kandel et al., 2000). The main structures of the CNS involved in the regulation of ovulation are the hypothalamus and pituitary gland, as will be explained next.

During reproductive age, the cortex takes up more than 50% of the total ovarian volume. The cortex contains primordial follicles.

Follicular atresia: is the degeneration of the germ cell and the follicular epithelium with its subsequent replacement for connective tissue (Lunenfeld & Insler, 1993)

In an advanced phase of development, a follicle about to ovulate is formed by the theca externa, the theca interna, a basal membrane, several layers of granulosa cells, the corona radiata, the zona pellucida, the oocyte and an antral cavity.

IV. Tertiary Follicle: It is characterized by the presence of an antrum rich in steroid and peptide hormones. The granulosa cells are subdivided into the parietal cells arranged in several layers and the cumulus oophorus. The surrounding connective tissue forms inter and external layers rich in blood capillaries and nerve endings.

V. Mature Follicle (or Graafian follicle): Also called preovulatory follicle, it has a significantly higher size and several layers of granulosa cells. It develops the corona radiata (granulosa cells around the zona pellucida).

III. Secondary Follicle: Also called preantral follicle. It contains an oocyte surrounded by a developed granulosa cells layers which synthesize estrogens and an external theca cells layers which produce androgens. It is located closer to the vascularized medulla.

II. Primary Follicle: It is an oocyte surrounded by cuboidal granulosa cells that owes its growth to the influence of androgens, independent of gonadotropins.

I. Primordial Follicle: It is an oocyte surrounded by a single layer of flat epithelial cells. It is located close to the cortex.

VII. Corpus Albicans: It corresponds to degradation of corpus luteum in the absence of pregnancy, where the cells are replaced by connective tissue.

VI. Corpus Luteum: It is formed by the luteinization process (invasion of the theca's blood capillaries in the granulosa cells). It produces high levels of progesterone and medium levels of estrogen.

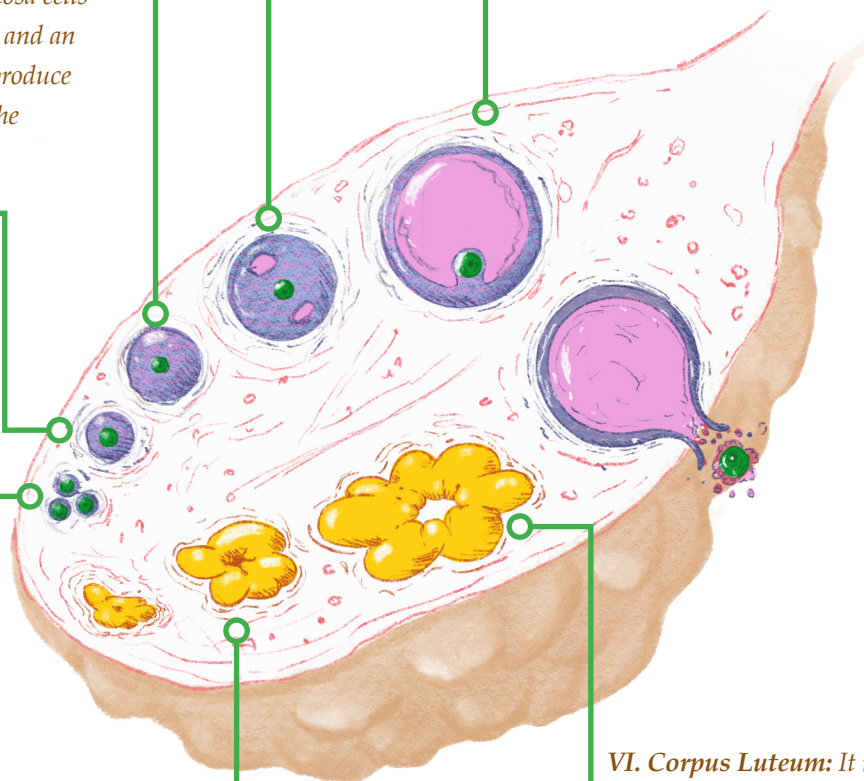


Figure 1: The ovarian follicles. According to their developmental stages, follicles can be classified in: primordial, primary, secondary, tertiary, mature (Graafian), corpus luteum and albicans (Skinner, 2005; Gleicher, 2011).

The hypothalamus

The hypothalamus is a brain region that helps to maintain the homeostasis of the body. It is located in the lower part of the brain, and it is composed of different nuclei, of which the paraventricular, dorsomedial and infundibular nuclei are related to reproduction (Table 1) (Hrabovszky, 2014; Hrabovszky et al., 2010; Skorupskaite et al., 2014).

The pituitary gland

The pituitary gland is located at the base of the hypothalamus in the base of the brain, and it is surrounded by a bony cavity called “sella turcica”. It’s composed of two lobes, anterior and posterior. The anterior lobe (adenohypophysis) regulates processes linked to stress,

growth, reproduction, and lactation. The posterior lobe (neurohypophysis) regulates lactation and ion balance in the kidney (Herbison, 2016; Kandel et al., 2000). Regarding ovulation, the communication between the hypothalamus and the pituitary gland depends on kisspeptin and Gonadotropin Releasing Hormone (GnRH) neurons (Figure 3) (Herbison, 2016; Oakley et al., 2009).

Kisspeptin and GnRH

Kisspeptin is a neuropeptide that has been identified in the last decades (Popa et al., 2008). In humans, it is synthesized by a group of hypothalamic neurons. This group of neurons, called the kisspeptinergic system have significant relevance in the onset of puberty (Herbison, 2016). The kisspeptinergic neurons communicate with the GnRH

The hypothalamus and pituitary gland are among the most important structures in the control of reproduction.

The hypothalamic-pituitary axis is the essential connection between the various glands in the body with the nervous system and it contributes to the regulation of the reproductive cycle.

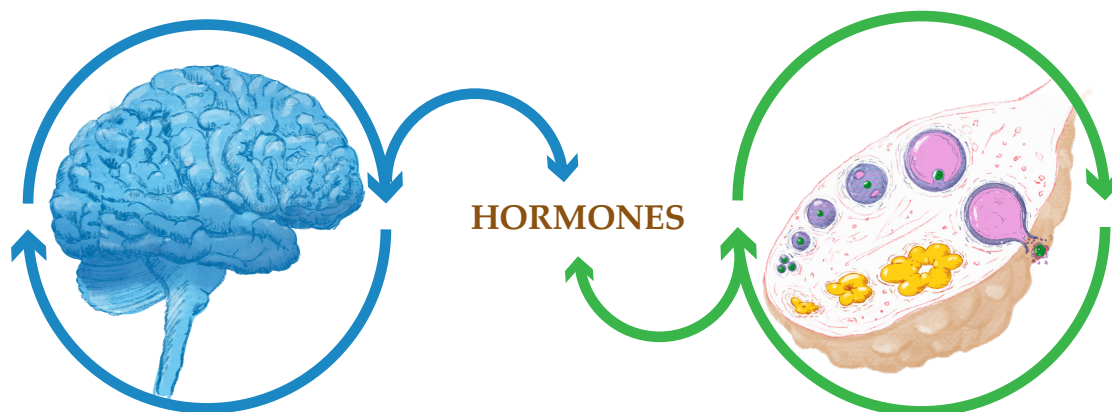


Figure 2: Interaction between hormones and the brain. The brain controls functions related to motor, behavioral, cognitive and neuroendocrine coordination of the organism. Hormones contribute to induce changes in the wiring of different brain areas. This areas are regulated by hormones that feedback it, so there is a permanent communication between the nervous and endocrine system.

Zygote: Cell formed by the union of the male and the female gametes.

Gamete: Male or female reproductive cell of a living being.

Mitosis: Type of division of the cell nucleus in which the genetic information contained in the chromosomes is preserved intact, which thus passes unchanged to the two resulting daughter cells.

Meiosis: Type of division that reduces the chromosomes number by half, creating four haploid cells, each genetically distinct from the parent cells that gave rise to them.

neurons in the hypothalamus. These neurons induce secretion of GnRH into the hypophyseal portal system where it is transported to the anterior pituitary gland, stimulating the production of gonadotropic hormones: Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH). The gonadotropic hormones act upon the gonads: ovaries and testes (Oakley et al., 2009; Popa et al., 2008).

4. Ovarian activity during the life-time of woman

The ovarian continuum has to be understood since the moment of fertilization when the zygote begins to develop. It takes place throughout the entire life of a woman.

Intrauterine life

When the embryo is approximately two months old, the primordial germ cells (precursor cells of the oocytes (PGC)) leave the embryo and migrate to a structure that is known as vitelline sac (yolk sac). This event occurs to avoid experiencing the process of cellular differentiation undergone by the rest of the embryo cells (differentiation into other

tissues such as bone, liver or skin). PGCs remain in the vitelline sac for about four weeks and then return to the region where the future ovary will develop: the genital or gonadal ridge. Here they transform into oogonia, cells that proliferate by mitosis (Schoenwolf & Larsen, 2009). The oogonia that are surrounded by ovarian somatic cells (pre-granulosa cells) will form a primordial follicle and will differentiate into primary oocytes. The remaining oogonias degenerate via an apoptotic process (programmed cell death), called atresia. During the seventh month of intrauterine life, the primary oocytes begin meiosis, arresting this process at the stage of meiosis I (Arrau et al., 1981). Meiosis will be arrested until ovulation, when the meiotic process will be reinitiated. The ovarian follicles, with the oocytes inside, create a population of “reserve” or “resting” primordial follicles with which a girl will be born. Thus, a newborn girl will have 1 to 2 million follicles containing primary oocytes. This initial reserve will give origin to the follicles observed along the ovaries various developmental stages

<i>Hypothalamic Nucleus</i>	<i>Function</i>
<i>Periventricular Nucleus</i>	<i>Related to thermoregulation and sexual behavior.</i>
<i>Paraventricular Nucleus</i>	<i>Commands the regulation of metabolic processes in the body.</i>
<i>Infundibular Nucleus</i>	<i>Regulates the onset of puberty.</i>

Table 1: Hypothalamic regions related to control of the endocrine and reproductive axis.

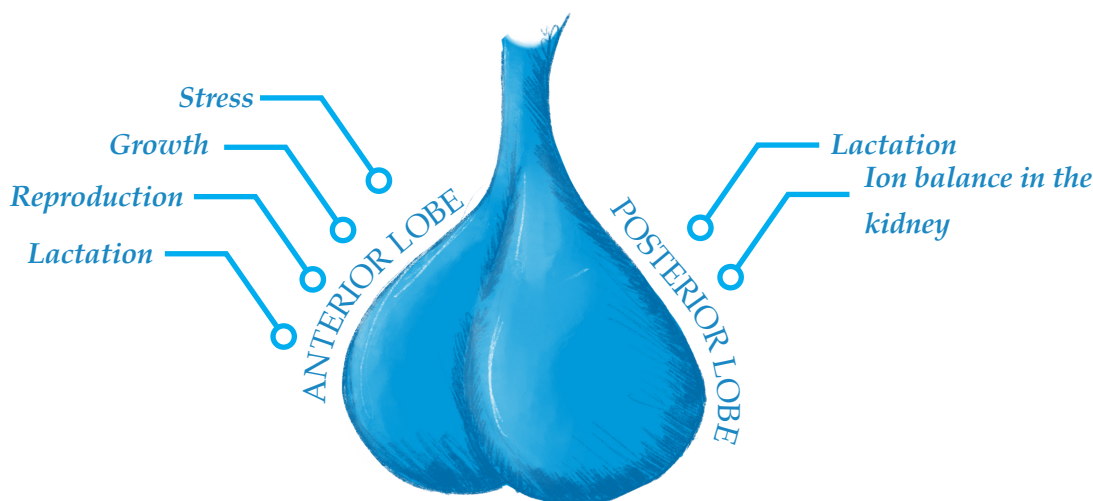


Figure 3: Schematic illustration of the hypophysis. Hypophysis is composed of two lobes, anterior and posterior. The anterior lobe (adenohypophysis) regulates processes linked to stress, growth, reproduction and lactation. The posterior lobe (neurohypophysis) regulates lactation and ion balance in the kidney

(Lunenfeld & Insler, 1993). However, from the moment when the primordial follicles are formed, and during postnatal life, this initial follicle reserve will experience a remarkable decrease in numbers: the primordial follicles that a girl had at birth (1 to 2 million) will be reduced to about 400 thousand in puberty, with only about 500 fully completing folliculogenesis and being ovulated. In other words, from the total primordial follicles developed, only less than 0.01% will be ovulated (Figure 4).

Puberty

Puberty is a process with physical, psychological and social changes through which a child matures into adolescence and adulthood. In general, female puberty starts at the age of 8 to 10, and its accompanying hormonal changes culminate with the expulsion of a mature oocyte from the ovary (i.e. the first ovulation), which generally leads to menarche, the first menses (Grumbach, 2002).

At the beginning of puberty (approximately 8 to 10 years of age) girls should experience the physiological process known as adrenarche, an increase in adrenal androgen secretion. This process can be recognized by the appearance of axillary odor. Along with this, when a girl approaches puberty, the blood concentration of leptin rises, due to a natural increase in fat tissue at this age. At the onset of puberty, higher leptin levels promote kisspeptin secretion, which in turn stimulates GnRH secretion, with a consequent rise in the release of gonadotropins (FSH and LH). As a result of the hormonal stimulation by gonadotropins, the gonads undergo a process of growth, development, and maturation called gonadarche, which leads to higher secretions of sex steroid hormones (estrogens, progestogens and androgens) from the ovaries (Cortés et al., 2015).

Leptin: Protein hormone, mainly produced by white fat cells, which main function is regulation of energy balance, through the control of food intake (Warren et al., 1999; Hoff et al., 1983).

Gonadarche: Gonadal changes during puberty and adolescence in response to pituitary gonadotropins.

FSH (Follicle Stimulating Hormone): Is a gonadotropin, synthesized and secreted by the gonadotropic cells of the anterior pituitary gland and it regulates the development, growth, pubertal maturation, and reproductive processes of the body (Warren et al., 1999; Hoff et al., 1983).

LH (Luteinizing Hormone): Is synthesized and secreted by the gonadotropic cells of the anterior pituitary gland. In females, an acute rise of LH triggers ovulation and development of the corpus luteum (Warren et al., 1999; Hoff et al., 1983).

Reproductive age: The ovulatory menstrual cycle

Once the reproductive system fully matures, women between 12 and 50 years of age normally exhibit regular ovulations characterized by 24- to 36-days cycles with fluctuating plasma estradiol and progesterone values according to the different phases of the cycle. For this, a series of sequential events have to occur in a highly synchronized manner, being ovulation the most important event during the menstrual cycle (Vigil et al., 2017).

• The menstrual cycle

The first day of the menstrual cycle is considered to be the first day of menstruation and the last day is the one preceding the next menses (Brown, 2010; Cortés et al., 2015). It has been shown that 85% to 90% of healthy young women have a menstrual cycle duration that ranges from 24 to 36 days, but the most frequent length is 27 ± 1 days (Fraser et al., 2007).

Despite variability in one woman and among different women, the phases of the menstrual cycle are common for all women during reproductive years.

The menstrual cycle can be divided into two phases (Figure 5): follicular (estrogenic or proliferative phase) and luteal (progestational or secretory phase). The first one is characterized by an increase in estradiol secretion by growing follicles. It starts with menstruation and lasts until ovulation. This phase is the most variable of the cycle, with an average duration of 10 to 22 days for normal cycles. The length can be altered as a result of different pathophysiological conditions (Vigil, 2017).

The luteal phase begins after ovulation and goes until the day before next menses. It is characterized by an increase in progesterone production by the corpus luteum. This phase shows less variability, having a duration between 11 to 17 days (Blackwell et al., 2013; Brown, 2010).

The coordinated hormonal events required for ovulation are reviewed next (Figure 6):

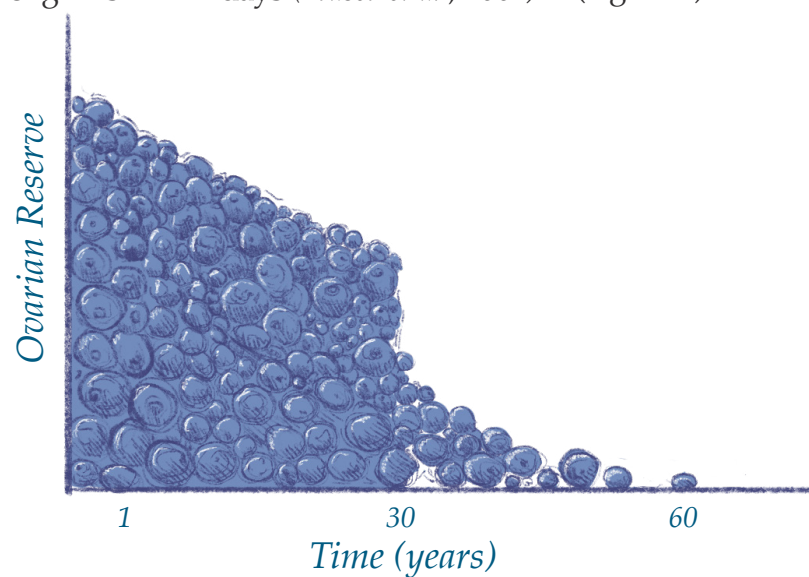


Figure 4: The ovarian reserve over the years. A girl will be born with 1 to 2 million primordial follicles. This initial follicular reserve will experience a remarkable decrease in numbers: the primordial follicles that a girl had at birth will be reduced to about 400 thousand in puberty, with only about 500 being ovulated. In other words, from the total primordial follicles developed, only less than 0.01% will be ovulated.

• **First event:**

At the beginning of each cycle, there is an increase in FSH levels that cause recruitment and development of antral follicles (early tertiary follicles). This recruitment and further development takes place due to the induction of hormone receptors in the follicular cells. FSH also triggers the expression of various enzymes and proteins involved in the biosynthesis of sex steroids (steroidogenesis) which leads to a progressive rise in estrogen production and secretion.

• **Second event:**

After follicular recruitment, estradiol along with inhibin (produced by the developing follicles) exert a negative feedback mechanism upon the HPG

axis that causes a decrease in FSH levels. During this period, estradiol also inhibits kisspeptin expression in the arcuate nucleus of the hypothalamus. Therefore GnRH and gonadotropin production diminishes, enabling the follicle that is able to survive under these unfavorable hormonal conditions, become the dominant one. The rest of the follicles will degenerate (*Lunnenfeld & Insler, 1993*). By this mechanism, a dominant follicle is selected from the follicular cohort that was recruited at the beginning of the follicular phase of the cycle. This dominant follicle will continue to produce estrogen and inhibin in higher concentrations.

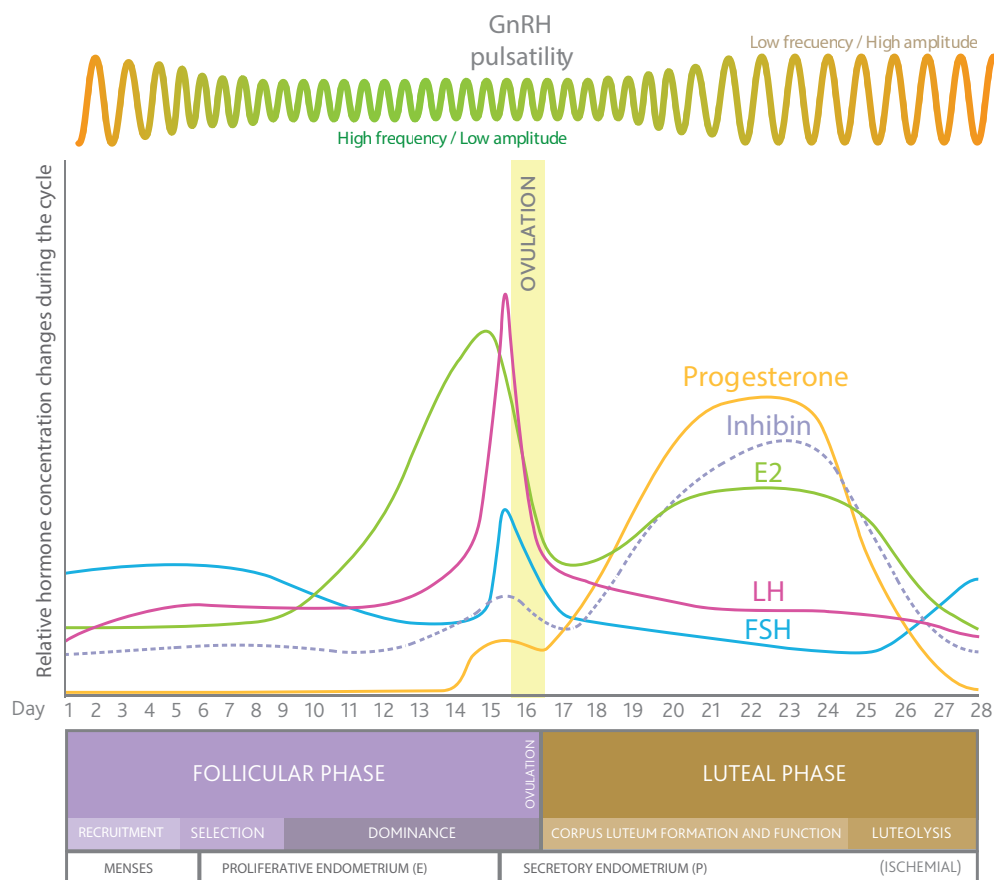


Figure 5: Ovulatory menstrual cycle. Changes in relative levels for each hormone according to cycle phases and endometrial characteristics are shown.

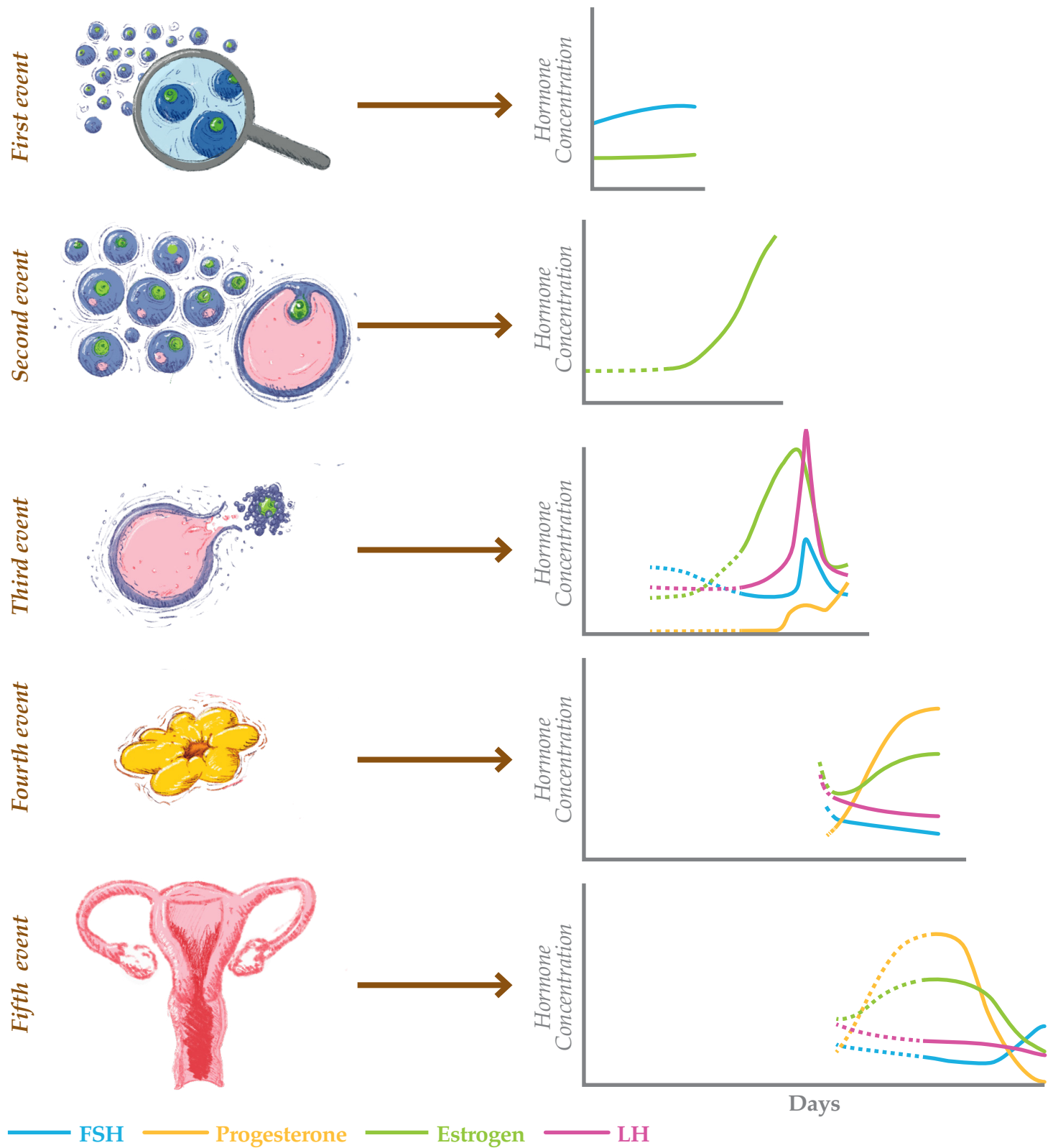


Figure 6: The five events of ovulatory menstrual cycles. Normally, women between 12 and 50 years of age normally exhibit regular ovulations characterized by 24- to 36-days cycles with fluctuating plasma FSH, LH, estradiol and progesterone values according to the different phases of the cycle.

• **Third event:**

The dominant follicle produces increasingly higher levels of estradiol, which stimulates kisspeptinergic neurons in the anteroventral periventricular nucleus of the hypothalamus, thus switching the negative feedback mechanism to a positive one. Kisspeptin induces GnRH secretion and the pre-ovulatory LH peak, which initiates follicular luteinization leading to the formation of the corpus luteum. Before the initiation of the midcycle gonadotropin surge a pre-ovulatory rise in progesterone occurs. This early progesterone rise produced by the pre-ovulatory follicle is critical for: a) follicular rupture, a necessary process for ovulation and b) development of a functional corpus luteum. Through positive feedback, progesterone maintains the LH peak, triggers meiosis resumption and the rupture of the follicle, with the consequent release of the oocyte (*Hoff et al., 1983*). This last event is known as ovulation. The released oocyte will survive typically 12 to 24 hours (*Oakley et al., 2009*).

• **Fourth event:**

The period after ovulation is known as “luteal phase”. Normally it lasts for 11 to 17 days (*Brown, 2010*). During this period, LH and progesterone contribute to the development and maintenance of the corpus luteum, which continues to produce progesterone and estrogen during the luteal phase. The levels of estrogen and progesterone produced by the corpus luteum will exert a negative feedback upon the HPG axis.

• **Fifth event:**

If fertilization does not occur, the corpus luteum starts to regress after 6 days, lasting for 11 to 17 days. This regression causes a drop in estrogen and progesterone levels. The decrease in both hormones eliminates the suppression exerted on the HPG axis and a new cycle begins (*Vigil et al., 2006, 2017*).

5. Biomarkers and knowledge of the fertile window

The fertile window is the period of the menstrual cycle during which conception is most likely to occur. This period is usually considered to begin about 6 days before ovulation and extends past the day of ovulation (*Wilcox et al., 2000, 1995*). This is determined by the lifetime of gametes. Spermatozoa can survive for at least six days in the cervix when estrogenic mucus is present. Mature human ova have a more limited lifespan of 12 to 24 hours (*Royston, 1982*).

Recognizing ovulation enables women and couples to identify the day when the probability of conception reaches its peak. Nonetheless, this knowledge does not allow them to identify the beginning of their fertile window. For this purpose women need to use biomarkers associated with the opening of the fertile window. To recognize both the beginning and the end (including the day of ovulation) of the fertility window enables a woman and her partner to use the information to achieve a pregnancy, postpone a pregnancy or to track her health.

Biomarkers can be used by most women to identify their ovulation

The fertile window is the period of the menstrual cycle during which conception is most likely to occur.

It is always important to consider that the fertile window and ovulation vary in different women and in different cycles in a woman. For this reason, ovulation day should not be calculated based on the first day of menstrual bleeding.

and in this way determine the fertile and infertile periods of the menstrual cycle (Figure 7). This process is often referred to as fertility awareness. Biomarkers as cervical mucus, basal body temperature (BBT) and estradiol, LH and progesterone measured in plasma or urine can be used for this purpose. There are also different apps available that use their own algorithms, but not all of them consider the great variability of the fertile window (Duane et al., 2017; Gross, 1989).

17 hours after the LH peak in plasma and generally the same day or the next day of its peak in urine (Vigil et al., 1992). It is important to consider that the LH surge may be missed with the use of some urinary LH kits (Ecochard, 2001) as its configuration, amplitude, and duration are variable (Alliende, 2002; Park et al., 2007). LH is an important tool for predicting ovulation, but it doesn't identify the beginning of the fertile window and cannot be used to confirm ovulation.

Biomarkers such as cervical mucus, basal body temperature (BBT), estradiol, LH and progesterone can be used by women to identify their fertile window and ovulation.

Useful markers to determine the beginning of the fertile window are estradiol and cervical mucus.

Useful markers to predict ovulation are LH, estradiol, progesterone, cervical mucus and ultrasound.

Useful markers to determine the end of the fertile window are progesterone, cervical mucus, ultrasound and BBT.

Estradiol

The first significant increase either in plasma estradiol or its urinary metabolites (including estrone glucuronide) from baseline values is taken as a biochemical marker for the beginning of the potentially fertile phase of the menstrual cycle (Blackwell & Brown, 1992). A logarithmically increasing rate of plasma estradiol excretion (or of its urinary metabolites) indicates that the dominant follicle has entered its rapid growth phase (Blackwell & Brown, 1992).

Estradiol has a logarithmic increase from baseline of approximately 1.5 times per day for 5 days to reach the estrogen peak, which occurs 24 to 36 hours before ovulation (Boyers, 1980; Vigil 2012). The fall after the peak is a very clear signal for timing ovulation (Brown et al., 1991). It is important to remark that it is possible to find this type of estrogen peak without ovulation.

LH

The LH peak is responsible for triggering the mechanisms that will cause follicle rupture and therefore ovulation. The LH surge lasts 48 hours, and ovulation occurs 32 to 35 hours after its initiation,

Progesterone

Progesterone secretion from follicular cells increases by a factor of 4 before the LH surge (Hoff et al., 1983). This initial increase in progesterone maintains the LH plateau during the LH peak. Progesterone also contributes to the final sequence of follicular events, ending in follicular rupture and ovulation (Baranczuk & Fainstat, 1976).

This small rise in progesterone is also useful to predict ovulation and find the best moment of the cycle to achieve a pregnancy (Blackwell et al., 1998).

After ovulation takes place, a massive rise in progesterone occurs, so ovulation can be confirmed measuring plasma progesterone or pregnanediol glucuronide (PdG) in urine. The rise in the PdG excretion rate over a universal threshold value of 7.0 mmol/24h (Blackwell et al., 1998, 2003, 2013, 2016; Brown et al., 1991; Brown, 2010) is a hormonal marker of the beginning of the post-ovulatory infertile phase and that the fertile window is closed. The PdG cutoff value frequently is reached on the day after ovulation, but this can happen 2 or 3 days after (Brown et al., 1991). The specificity and accuracy of PdG values when used to confirm

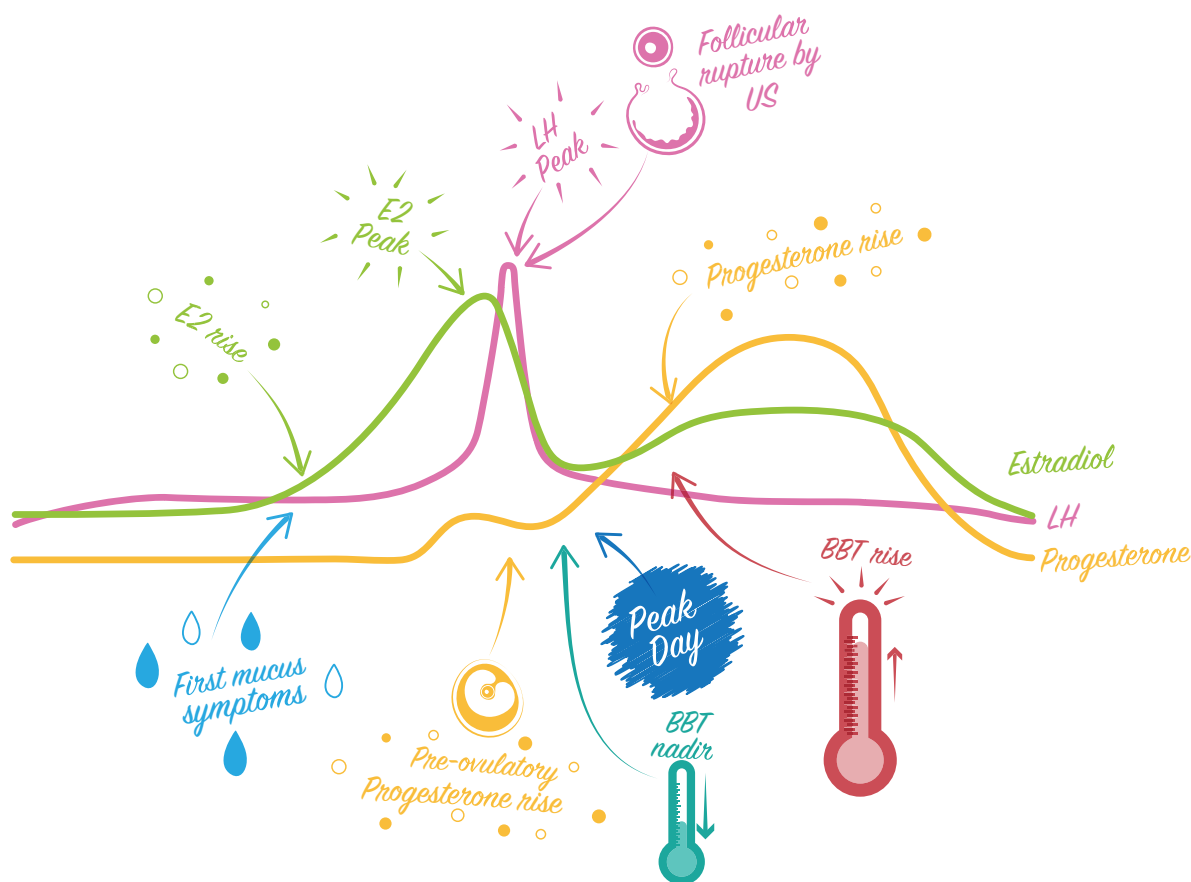


Figure 7: Biomarkers used to determine the fertile window. Biomarkers can be used by most women to identify their ovulation and in this way determine the fertile and infertile periods of the menstrual cycle. Biomarkers as cervical mucus, basal body temperature (BBT) and urine or plasma measurements of estradiol, LH and progesterone can be used for this purpose.

ovulation are nearly 99% (Blackwell et al., 2003).

Cervical mucus

The beginning of the fertile window occurs with the first statistically significant rise in estrogen levels. This causes the secretion of estrogenic cervical mucus with the characteristic changes in the vaginal discharge (Blackwell & Brown, 1992; Billings et al., 1972). During this period, the mucus is aqueous, transparent, fluid and crystalline, giving the woman a slippery sensation at the vulva.

The ultrastructure of cervical mucus varies during the menstrual cycle,

exhibiting a lax network with channels that increase in size as ovulation nears (Chretien & Dubois, 1991; Poon & McCoshen, 1985). In the peri-ovulatory period, this network allows sperm selection and ascent (Vigil et al., 1991, 2008, 2009, 2011). The post-ovulatory rise in progesterone has an anti-estrogenic effect on the cervix, changing the cervical mucus to a form inappropriate for sperm ascent through the cervix (Vigil et al., 1991; Brown, 2010). The fertile window defined by the mucus peak symptoms starts on the first day of the presence of cervical mucus observed or felt at the vulva and ends on the third day after the last day of slippery

Cervical mucus is a crucial biological secretion that performs several functions related to reproductive processes.

The hydration of the cervical mucus will vary depending on estradiol levels.

The observation of changes in cervical mucus is considered a reliable biomarker.

sensation at the vulva. This day closely relates to ovulation day. There is a 96% sensitivity of the cervical mucus symptoms in identifying the entire fertile window (*Ecochard et al., 2015*).

Temperature

During the menstrual cycle, BBT rises after ovulation as a result of ovarian activity, associated with progesterone action (*de Mouzon et al., 1984*). During the follicular phase of the menstrual cycle, BBT remains in a lower range until approximately one day before ovulation, when the BBT often reaches the lowest point (nadir) (*Hsiu-wei et al., 2017*). Ovulation is confirmed when three consecutive temperatures are 0.5-1.0 °F above the highest point of the six previous basal temperatures (*Royston, 1982*). After these three days, the fertile window is considered to be closed.

Measuring her BBT allows a woman to confirm that the fertile window has ended. This method doesn't predict ovulation or the beginning of the fertile window.

Ultrasound

Ultrasound assesses the anatomy of follicular development and its subsequent rupture. There are some ultrasound indicators of ovulation, such as the disappearance of the follicle or decrease in follicular size, free fluid in the pelvis, or a change in endometrial characteristics (*Hsiu-wei et al., 2017*). The combination of ultrasonography and hormone assays, the one providing anatomical information and the other providing functional information, has been a powerful tool for investigating ovarian activity (*Brown, 2010*).

6. The cervical mucus in women's health

The cervical mucus is a crucial biological secretion that performs several functions related to reproductive processes (Table 2). It is a hydrogel formed by mucins, which are proteins that have the ability to form gels. It is produced in the epithelial cells of the cervix of the uterus, and it appears with different rheological properties: sticky or slippery, different viscosities and translucent or opaque (*Morales et al., 1993; Ceric et al., 2005*).

As a hydrogel, cervical mucus has two phases: an aqueous phase and a gel-like phase. The aqueous phase, also known as soluble fraction, is composed of water and other compounds such as electrolytes, fructose, glucose, proteins, and lipids. Its hydration varies between 90 to 99% depending on the levels of estradiol (*Vigil, et al. 1991*). The gel phase, also called solid or insoluble fraction, is made up of mucins, the high molecular mass glycoproteins that give the cervical mucus its characteristic structural and biophysical properties (*Ceric et al., 2005; Gipson, 2005; Sheehan & Carlstedt, 1990*).

Cervical Mucus as a biomarker

The observation of changes in cervical mucus is considered a reliable biomarker (*Gibbons, 1981*), as it has been demonstrated that recognizing mucus patterns can help women to identify the different stages of the ovarian continuum (Figure 8) (*Billings et al., 1972; Vigil et al., 2006*).

Cervical mucus undergoes several modifications during the phases of the reproductive cycle. Increased estrogen levels halfway through the follicular

phase result in a noticeable rise in the secretion of estrogenic mucus.

The last day of clear, slippery and lubricative mucus is defined as the peak day (PD) (Billings *et al.*, 1972). Forty to fifty percent of women ovulate within 24 hours of the PD, but ovulation can occur from three days before to three days after the peak day in 95% of the women (Ecochard *et al.*, 2015). After the peak day, the luteal phase begins.

Progesterone has the opposite effect of estradiol upon cervical mucus (antiestrogenic action). It inhibits production and changes the characteristics of the mucus to an opaque and less fluid mucus and without its ability to crystallize into palm leaf patterns (Odeblad *et al.*, 1994). Due to the rise in progesterone levels, a denser network is observed in the luteal phase, with small pores diameter compared to estrogenic cervical mucus. Spermatozoa in the absence of estrogenic mucus will die within hours or even minutes when placed in the vagina.

Cervical mucus as a tool to recognize ovulation

As we have seen, two main types of cervical mucus have been described: estrogenic and progestative. The first one predominates in the follicular phase. The second one is characteristic of the luteal phase, which normally lasts 11 to 17 days (Brown, 2010).

It has been shown that the luteal phase extends for 11 to 17 days when ovulation is calculated from the estrogen peak to the day before the ensuing bleeding (Brown, 2010; Blackwell *et al.*, 2013). When the luteal phase is calculated from the PD, and knowing the variation of $\pm$ three days between PD and ovulation, the luteal phase length could have an estimated length between 9 to 19 days. In this last scenario, it is important to consider that this variation in the calculated length of the luteal phase is given by the difference that methods have in the accuracy for identifying ovulation, not in the luteal phase per se. The pre-ovulatory rise and fall of estrone glucuronide followed by a pregnanediol glucuronide rise is the most accurate way to identify ovulation

When estradiol levels are high, mucus is aqueous, transparent, fluid and crystalline, tending to form geometric patterns with fern leaves at crystallization.

The last day of clear, slippery and lubricative mucus is defined as the peak day.

Progesterone changes the characteristics of the mucus to an opaque, less fluid and without its ability to crystallize into palm leaf patterns.

<i>Cervical mucus performs critical functions in the reproductive health of women, such as:</i>
<i>Protecting a woman's reproductive tract, by maintaining a moist and lubricated environment.</i>
<i>Participating in sperm transport, facilitating their ascent to the fertilization site.</i>
<i>Being a selective barrier, selecting only the morphologically normal sperm.</i>
<i>Inhibiting the ascent, invasion and proliferation of microorganisms.</i>
<i>Modulating the acrosome reaction.</i>

Table 2: Functions of cervical mucus (Vigil, 2006, 2007, 2009, 2014, 2011; Barros, 1983, 1984; Ceric *et al*, 2005)

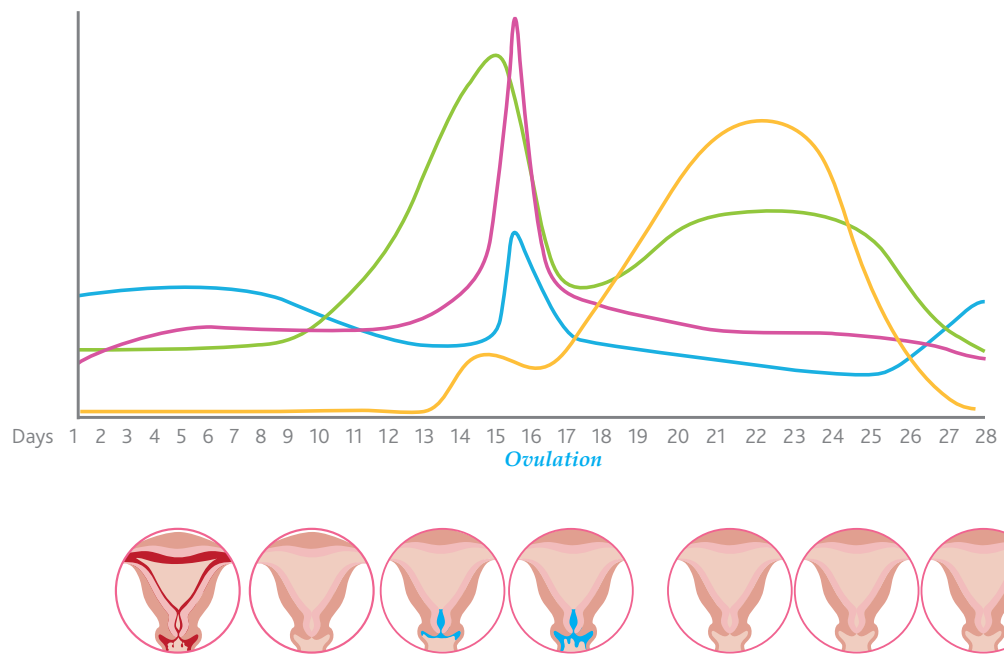


Figure 8: Cervical mucus. Changes in cervical mucus, as perceived by women, during the menstrual cycle (Vigil et al., 2006, 2007, 2009, 2014, 2011; Barros et al., 1983, 1984).

When using the cervical mucus as a biomarker of ovulation the indicator that ovulation has occurred is the identification of a luteal phase according to the peak mucus symptom.

Normal ovulatory activity during reproductive years implies adequate endocrine and gonadal function. Regular ovulation evidences a good health status.

Monitoring the ovulatory cycle should begin in puberty and adolescence. The absence of normal ovulatory cycles can be the first manifestation of some underlying pathology.

and the luteal phase (Dunson et al., 2002). By noting the progression from the basic infertile pattern of post-menstrual dryness to stickiness, wetness, and ultimate lubrication (the “peak day”), a woman can recognize her ovulation (Billings et al., 1972; Vigil et al., 2006). When using the cervical mucus as a biomarker of ovulation the indicator that ovulation has occurred is the identification of a luteal phase according to the peak mucus symptom.

7. Ovulation as a marker of health status

Often, healthcare providers have focused on regularizing bleeding patterns, without paying attention to ovulation in reproductive age women. It has been shown that varying cycle lengths, whether short or long, are associated with decreased fecundity, and that menstrual cycle patterns may predict whether a pregnancy will

survive (Kolstad et al., 1999). However, menstrual cycles with a normal length are not an indicator of proper ovarian function, because these women can also present anovulatory cycles (Prior et al., 2015). Therefore, it is regular ovulation and not regular menstruation which evidences a good health status. Normal ovulatory activity during reproductive years implies adequate endocrine and gonadal function. However, women in conditions such as breastfeeding or pregnancy should also identify their anovulatory state as a healthy part of the ovarian continuum. Periods of transition from anovulation to regular ovulation, such as those found during puberty and perimenopause, can also be identified as a physiological part of the continuum. Monitoring the ovulatory cycle should begin in puberty and adolescence. The absence of normal ovulatory cycles can be the first manifestation of some underlying pathology. For example,

precocious or delayed puberty can be linked to endocrine abnormalities (Stanhope & Brook, 1986).

Because the conditions that alter ovulation during adolescence will only worsen with time, early diagnosis and proper treatment are important for the future prognosis of the underlying health problems (Vigil et al., 2006, 2007; Popat et al. 2008). Importantly, it has been shown that perimenarcheal girls from diverse ethnic and socioeconomic groups are able to learn how to recognize their cervical mucus patterns and to use this information to distinguish normal from abnormal cycles (Klaus & Martin 1989). Normal cycles are those that last 24 to 36 days with an identified luteal phase according to the mucus peak symptom. Abnormal cycles are short cycles (less than 24 days), long cycles (more than 36 days), or normal length cycles with a short luteal phase (less than 9 days), or its absence.

A woman should be referred to a specialist if she identifies three or more abnormal cycles in a year or two consecutive abnormal cycles. As previously mentioned, the first sign of an underlying health problem may be an ovulatory dysfunction, then followed by irregular cycles (Vigil et al., 2006, 2017).

The normal ovulatory activity that should be present during reproductive years will affect the body as a whole, including the central nervous system (CNS). In this way, the same hormones that influence ovulation have an impact upon the brain. This means that a proper hormonal balance during the different stages of life will give women and health care providers an important tool for improving their health, including mental health and well-being.

In the following sections, the most

important causes of ovulatory dysfunction and the relationship between hormones and the brain will be analyzed.

Ovulatory dysfunctions

The first sign of an underlying health problem a woman may experience is an ovulatory dysfunction, followed by irregular cycles or amenorrhea. Indeed, when pregnancy, lactation, or menopause are not the causes, persistent irregularities in the ovulatory cycle can be associated with lifestyle, stress, drugs, and endocrine, gynecological, autoimmune, nutritional, genetic, and iatrogenic disorders (Vigil et al., 2006). The most frequent causes of menstrual irregularities associated with ovulatory dysfunction are hormonal disturbances. These may originate at the hypothalamic, pituitary, thyroid, adrenal, ovarian, or metabolic levels. In this section, the principal endocrine abnormalities leading to ovulatory dysfunction will be examined, with emphasis on the most prevalent etiologies, summarized under the acronym PAInT: Prolactin (hyperprolactinemia), Androgens (hyperandrogenemia), Insulin (insulin resistance), and Thyroid disorders (both hypothyroidism and hyperthyroidism) (figure 9) (Vigil et al., unpublished data).

• Hyperandrogenemia

Androgens are a group of hormones usually linked to development and maintenance of male sex characteristics. In women, androgens also exert important physiological functions related to bone mineralization, muscle development (Notelovitz, 2002), cognition and memory (Hirshman et al., 2004) as well as the appearance of libido (Basson et al., 2010). It has

Abnormal cycles are short cycles (less than 24 days), long cycles (more than 36 days), or normal length cycles with a short luteal phase or its absence.

A proper hormonal balance during the different stages of life will give women and health care providers an important tool for improving their health, including mental health and well-being.

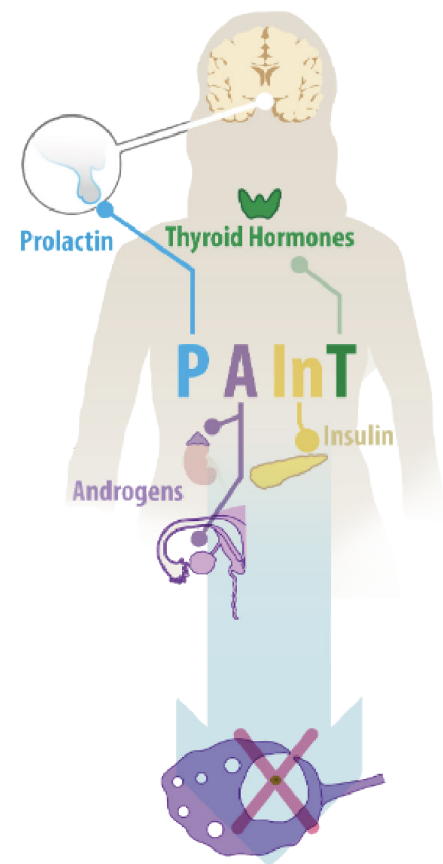
The first sign of an underlying health problem a woman may experience is an ovulatory dysfunction, followed by irregular cycles or amenorrhea.

Androgens in women are mainly produced in the ovaries and in the adrenal glands.

been shown that androgens have an important role in the gonadotropin independent phase of early follicular development (Gleicher, 2011). The main causes of hyperandrogenemia are functional (an increase in the production of androgens by the adrenal gland or the ovaries), peripheral (for example as in hyperinsulinemia), tumoral (as adrenal or ovarian tumours (Azziz et al., 2004; Rosenfield, 1996) and secondary to pharmacological treatments (Azziz et al., 2004).

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women. These patients present ovulatory dysfunction associated with high androgen levels. Symptoms such as acne, hirsutism, alopecia, increased body weight, and mood changes are usually observed.

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in reproductive age women (Amer, 2009), and it is considered a functional cause of hyperandrogenemia. These patients may exhibit acne, hirsutism, alopecia, increased body weight, and mood changes. But the most common perceived symptom is the presence of irregular menstrual cycles and an atypical pattern of cervical mucus. Both symptoms are associated to ovulatory dysfunction (Vigil et al., 2009). Obesity, insulin resistance, and consequent hyperinsulinemia are highly prevalent comorbidities of PCOS and can impair



OVULATORY DYSFUNCTION

Figure 9: The main causes of Ovulatory Dysfunction: Prolactin - Androgens - Insulin - Thyroid (PAInT) (Vigil et al, unpublished data).

The most frequent causes of ovulatory dysfunction are hormonal abnormalities. On a cohort of 251 young women between 18 and 35 years presenting with ovulatory dysfunction the diagnosed endocrine abnormalities were: hyperandrogenemia 50%, insulin resistance 47%, Abnormal thyroid function 28% , hyperprolactinemia 14% and premature ovarian senescence 1%. (Vigil, unpublished data).

ovulation (Vigil et al., 2007; Pauli et al., 2011). Elevated insulin levels are present in about half of these patients and are correlated to body mass index (BMI), but not all PCOS patients are insulin resistant (Vigil et al., 2007). High insulin levels further increase androgen production by stimulating ovarian theca cells to produce more androgens, which lead to premature follicular atresia and even anovulation (Diamanti-Kandarakis, 2006). High levels of testosterone and insulin will decrease SHBG, increasing the free estradiol fraction. This, together with an increase in peripheral production of estrogens by adipose tissue may inhibit

the kisspeptinergic system decreasing GnRH and gonadotropins. PCOS is also associated to an increased risk of type 2 diabetes, metabolic syndrome (Ranasinha *et al.*, 2015), cardiovascular disease, and endometrial, ovarian, and/or breast cancer (Fauser *et al.*, 2012).

Another functional cause of hyperandrogenemia is congenital adrenal hyperplasia (CAH). This is a family of disorders caused by mutations in genes that encode for enzymes involved in one of the various steps of adrenal steroid synthesis, leading to an overproduction of androgens (Merke & Bornstein, 2005; Lekarev *et al.*, 2015).

Finally, certain drugs can lead to hyperandrogenemia. The exogenous administration of androgenic derivatives (exogenous testosterone) is the most common cause, but the use of psychiatric medication can also lead to androgen excess. Antiepileptics drugs such as valproate (Rasgon *et al.*, 2005), mood stabilizing agents such as valproate or lithium (Rauchenzauner *et al.*, 2014) and antipsychotic agents such as risperidone and quetiapine, promote the adrenal and ovarian androgen synthesis. This is due to an induced-hyperinsulinemia and insulin-resistant state, together with an enzymatic disruption (Bahtiyar & Weiss, 2007).

• **Insulin resistance**

Hyperinsulinemia is commonly linked to insulin resistance and obesity. Elevated insulin levels generate an increase in androgen production at the ovary, leading to follicular atresia and the formation of multiple ovarian follicular cysts (Diamanti-Kandarakis, 2006). In conjunction, high levels of insulin and androgens will decrease sex hormone-binding globulin (SHBG)

levels and, as a consequence, the free fraction of steroid hormones, such as estrogens, will increase (Kalme *et al.*, 2003). The combination of these alterations produces a hyperestrogenic and hyperandrogenic environment that characterizes hyperinsulinemia. Elevated estradiol levels may inhibit kisspeptin, decreasing GnRH and gonadotropins (FSH and LH).

• **Thyroid abnormalities**

Thyroid hormones also influence ovulation, mainly acting upon folliculogenesis and steroidogenesis at the ovarian level, and by affecting SHBG and GnRH secretion.

Hypothyroidism, an insufficient amount of thyroid hormones, leads to irregular menses, hypermenorrhea, metrorrhagia, spotting and breakthrough bleeding (Krassas *et al.*, 1994, 1999), usually associated with lethargy, depressive symptoms, weight gain, cold intolerance, and hair loss. Hypothyroid women have lower levels of testosterone and estrogens (Krassas *et al.*, 2010). SHBG levels are also diminished on these patients, leading to an increased free fraction of sex hormones. The increased estrogen free fraction triggers negative feedback on kisspeptinergic neurons and the pituitary gland. Additionally, lower levels of thyroid hormones increase thyrotropin-releasing hormone (TRH), which in turn stimulates prolactin, thus inhibiting GnRH (Henderson *et al.*, 2008). Hyperthyroidism is caused by the overproduction of thyroid hormones, and it is linked to irregular menstrual periods and anovulation. In these patients, there is an increase of total testosterone and estrogens, but a decrease in their free fractions due to augmented levels of SHBG. The decrease on free estradiol

Insulin is a peptide hormone produced by pancreatic β -cells. It is one of the main modulators of carbohydrate and lipid metabolism, promoting the entry of glucose to tissues such as muscle, liver and adipose tissue.

Thyroid hormones are secreted by the thyroid gland, an organ located at the base of the neck. Thyroid hormones exert their influence on all cells, tissues and organs, regulating metabolism, growth and cell differentiation.

would cause lack of estrogen negative feedback on kisspeptinergic neurons, augmenting gonadotropins levels (LH and FSH) (Poppe *et al.*, 2007).

Prolactin is a peptide hormone that is mostly produced by the pituitary. Prolactin's best known function is to promote milk production, particularly during lactation.

• **Hyperprolactinemia**

Prolactin is produced by the pituitary gland and is under hypothalamic control. Stress (Johansson *et al.*, 1983), pituitary tumors (prolactinomas) and some drugs (such as antidepressants, antipsychotics, and proton-pump inhibitors) are causes of increased prolactin levels. Hyperprolactinemia inhibits GnRH by negative modulation of kisspeptinergic neurons. Common symptoms and signs of hyperprolactinemia are short luteal phases, anovulation, menstrual irregularities, amenorrhea, galactorrhea, skin dryness, immunological disorders, low libido, hot flashes, and sweaty hands (Higuchi *et al.*, 1984; Araujo-Lopes *et al.*, 2014). High prolactin levels also activate adrenal androgen secretion, causing higher androgen and lower estradiol levels.

It is useful to know that this hormone has an immunostimulatory effect, promoting autoimmunity (Orbach, & Shoenfeld, 2007). The association between hyperprolactinemia and certain immune diseases such as lupus erythematosus and rheumatoid arthritis has been described (Jara *et al.*, 2011). In this way, ovulatory dysfunctions can be an early signal of autoimmune diseases.

• **Premature Ovarian Senescence**

The Premature Ovarian Senescence (POS) refers to the progressive decline of the ovarian reserve earlier than expected for a woman's age. It can be either subclinical or clinical, and it may be present in up to 10% of all women. The subclinical premature depletion

of the ovarian reserve is known as Occult Premature Ovarian Insufficiency (OPOI). One of the first findings is hypoandrogenemia (Gleicher *et al.*, 2013), which leads to suboptimal primary follicle maturation and, consequently, ovulatory dysfunction and low estradiol production. Clinically, this can be evidenced by a dry cervical mucus pattern. The three principal etiologies are autoimmune, genetic, and iatrogenic causes (Wesewich *et al.*, 2020).

The clinical form of POS is known as Primary Ovarian Insufficiency (POI). It is characterized by the presence of amenorrhea or oligomenorrhea associated with markers of low ovarian reserve: low Anti-Müllerian Hormone, low antral follicle count, and elevated FSH (Gleicher *et al.*, 2013). Traditionally, the prevalence of POI has been considered to be around 1%. Nevertheless, this number varies between ethnic groups, usually ranging from 1% to 4%. Nowadays, the prevalence seems higher than in previous years, reaching up to 10% in some cohorts (Li *et al.*, 2023).

• **Nutritional imbalance and Hypothalamic dysfunctions**

Hypothalamic disorders are characterized by a change in the normal pattern of secretion of GnRH, delaying the increase of FSH. Hypothalamic disorders can be caused by nutritional imbalances, stress, excessive exercise, and psychiatric disorders, such as anorexia (Unuane *et al.*, 2011).

Regarding nutritional imbalance, hypoleptinemia has been reported in women with low body weight and body fat, such as in athletes and women with eating disorders. Women with low leptin levels are at high risk of developing amenorrhea (Warren *et al.*, 1999), because

low levels of leptin generate a decrease in the secretion of kisspeptin (*De Bond & Smith, 2014*), and in consequence, a decrease in the activity of GnRH neurons with the consequent reduction in the secretion of gonadotropins (*Clarke et al., 2015*).

On the other hand, hyperleptinemia has been associated with obesity and metabolic syndrome. High leptin levels (like in women with increased adipose tissue) provokes a hypothalamic resistance to leptin (*Sahu, 2002*), also diminishing GnRH and gonadotropin release (*Quennell et al., 2011*).

Hypercortisolism can be induced due to chronic exposure to exogenous corticoids (*Hopkins & Leinung, 2005*), prolonged periods of stress (*Tsigos & Chrousos, 2002*) and mutations in the receptor of this hormone (*Lacroix et al., 2015*). Increased cortisol levels block GnRH secretion and the action of gonadotropins. These disorders may result in hypoeutrogenic cycles, amenorrhea, and anovulation (*Saketos et al., 1993*).

• **Vitamin D**

Vitamin D is a fat-soluble vitamin that can act as a steroid hormone after liver and kidney metabolization. The primary sources of this vitamin are very few foods and the endogenous production, that comes mostly from the conversion of cholesterol by the skin, after exposure to ultraviolet light. This vitamin has a central role in calcium metabolism (*Irani & Merhi, 2014*). Vitamin D is also associated with ovarian function, steroidogenesis and follicular development. Hypovitaminosis D is a prevalent condition, being present in up to 80 to 90% of the female population in North America.

Regarding ovarian function, hypovitaminosis D is present in up to 35% of the patients with ovulatory dysfunction (*Vigil, unpublished data*). A plausible explanation for this result is that low vitamin D levels decrease estrogen and androgen levels (*Irani & Merhi, 2014*), thus affecting ovulation. Added to the above, patients with vitamin D deficiency usually have corpus luteum insufficiency, evidenced by a decrease in progesterone levels. This data reflects that reproductive function and vitamin D are closely related.

Hormones and the Brain

• **Hormones as Neurosteroids:**

The hormonal balance that regulates ovulation acts throughout all our body, including the brain, where steroid hormones have a very active role in the regulation of brain cells (*Figure 10*) (*Schulz & Sisk, 2016*).

Estrogen and progesterone have an effect in the central nervous system and the peripheral nervous system, especially on neurotransmitters such as GABA, serotonin, dopamine, and glutamate (*Irwin et al., 2008*).

Steroid hormones, also known as neurosteroids, regulate different brain areas involved in mood, behavior, and cognition (*Magnaghi et al., 2010; Schiller et al., 2016*). Therefore, the fluctuation of sex hormones during specific reproductive stages of a woman's life correlates with an increased susceptibility to develop mood disorders such as premenstrual dysphoric disorder, postpartum depression, and perimenopausal depression. Endogenous estrogen and progesterone levels also may affect different cognitive processes such as decision making, emotion recognition,

Leptin has a relationship with both waist circumference and the amount of body fat. Changes in leptin levels are related to metabolic, development and reproductive disorders.

Cortisol is a glucocorticoid hormone produced in the adrenal gland cortex. This hormone is secreted as a response to stress or to a decline in glucose levels.

The main function of vitamin D is maintaining calcium and phosphate homeostasis. Vitamin D also exerts its influence on the immune, endocrine, cardiovascular and reproductive systems.

Neurosteroids regulate different brain areas involved in mood, behavior, and cognition.

Estrogen and progesterone have an effect in the central nervous system and the peripheral nervous system, especially on neurotransmitters such as GABA, serotonin, dopamine, and glutamate.

consolidation of emotional memory, and fear extinction. For example, women show improved verbal abilities and decreased visual-spatial abilities when estradiol and progesterone levels are high and the opposite occurs when estradiol and progesterone levels are low. Hormonal metabolites have also been shown to function as critical neuromodulators. Allopregnanolone (Allo), a neuroactive metabolite of progesterone, acts as a potent GABA receptor agonist in the central nervous system, exerting important inhibitory and anxiolytic effects. Its concentrations fluctuate across the menstrual cycle in reproductive-aged women, reaching their highest levels during the luteal phase (Kimball et al., 2020). Deficiency of Allo has been associated with neurodegenerative and neurological disorders, abnormalities in pain processing, and mood disturbances such as anxiety and depressive symptoms (Diviccaro et al., 2022). Notably, many of these clinical manifestations are commonly observed in women with ovulatory dysfunction.

These differences can be partially explained via the role that neurosteroids play in the physiological regulation of neurogenesis, neuronal survival, synaptic function and myelin formation, therefore influencing neuronal plasticity. Further studies may make use of them to treat different disorders of the CNS, as recent studies have shown that neurosteroids could be effective in treating psychiatric disorders, such as schizophrenia, depression, and also against neurodegenerative disorders, such as Alzheimer's, Parkinson's, and multiple sclerosis.

In the same way that endogenous steroids influence CNS functionality, steroid

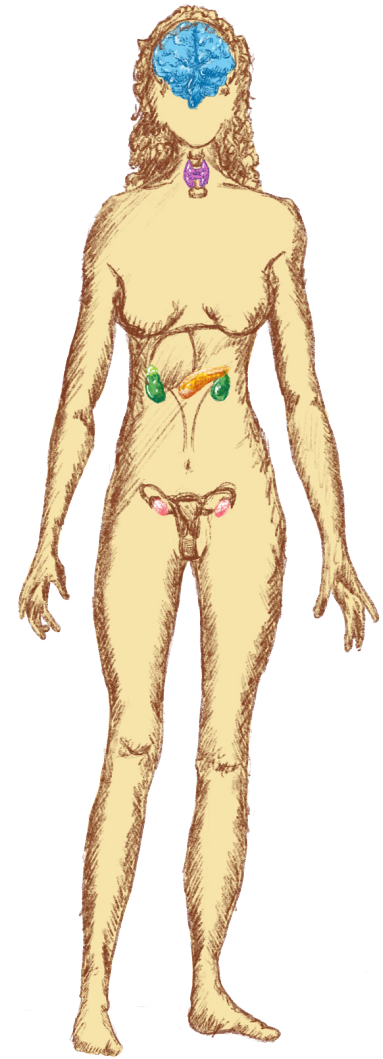


Figure 9: The hormonal balance that regulates ovulation acts throughout all our body in a highly coordinated way.

hormones administered exogenously also exert their actions on the brain. Two of the most common ways in which hormones are administered exogenously to women are: 1) hormonal therapy during menopause and 2) hormonal contraceptives. When facing a need for the administration of exogenous hormones, consideration should be given to the stage of life each woman finds herself in, since exogenous hormones will have different effects on the brain depending on the stage. For example, when treating adolescents, special consideration must

be given to the temporal plasticity window of their developing brain, since it is a period when exogenous hormones may produce both activational and organizational changes in the brain that may have long-term effects. At the other extreme, women over 10 years past menopause must also take precaution when initiating hormone replacement therapy (HRT), since they have been shown to have negative effects on the CNS, increasing the risk of pathologies such as Alzheimer's disease or stroke (Rapp, 2003; Yen et al., 2012). However, it is important to consider that there are many situations when HRT and the administration of exogenous hormones are beneficial. For example, cases such as anorexia nervosa will require, as part of the treatment, the administration of hormones. Similarly, as women age, steroidal hormones decline, and this could have negative consequences, such as hot flushes, osteoporosis, a decrease in libido, and depressive mood. Thus, special consideration for these individuals needs to be addressed.

The activity exerted by steroid hormones on the nervous system emphasizes the notion that achieving hormonal balance is a useful tool in seeking the mental health of women (del Río et al., 2018).

• **Hormones and mental health**

The role of hormonal homeostasis in mental health remains an important clinical and research challenge. Despite increasing evidence demonstrating the detrimental effects of various endocrinopathies on psychological well-being, their impact is still frequently underrecognized (del Río et al., 2024).

Women with polycystic ovary syndrome (PCOS) exhibit a significantly higher relative risk of depressive symptoms

and an increased incidence of suicidal behavior compared with the general population (Wong et al., 2025). Individuals with hypothyroidism also show elevated rates of depression. Patients with hyperprolactinemia have been reported to present higher levels of anxiety and hostility (Reavley et al., 1997), while those with insulin resistance demonstrate increased anxiety and depression scores (Abdelfattah et al., 2025).

8. Conclusion: the need for Reproductive Health Research Institute (RHRI)

Every woman should comprehend and learn how to read her own special signs. Biomarkers, as cervical mucus, will help them identify in which phase of the ovarian continuum they are, whether having ovulatory cycles, anovulatory cycles or both. Understanding the concept of the ovarian continuum and the instruction in fertility literacy allows women and health care providers to recognize if ovulation is occurring or, on the contrary, to recognize an ovulatory dysfunction (Vigil et al., 2017). As the hormonal balance that regulates ovulation influences all the body, the presence of normal ovulatory cycles is a good indicator of a woman's health status. It must be kept in mind that anovulation may be a normal part of the ovarian continuum. Women should learn that this is a normal condition in circumstances such as breastfeeding. But, when no physiological condition explains an anovulatory state, anovulation may be caused by pathological conditions such as unhealthy lifestyle habits, stress, endocrine abnormalities, drugs and gynecological, autoimmune, nutritional, genetic, and/or iatrogenic disorders. As the first sign of an ovulatory dysfunction

Steroid hormones administered exogenously also exert their actions on the brain. Two of the most common ways in which hormones are administered to women exogenously are: 1) hormonal therapy during menopause and 2) hormonal contraceptives.

Understanding the concept of the ovarian continuum and the instruction in fertility literacy allows women and health care providers to recognize if ovulation is occurring or, on the contrary, to recognize an ovulatory dysfunction

The mission of the Reproductive Health Research Institute (RHRI) is to conduct new research on women's health, with a particular focus on reproductive endocrinology.

The medical protocols developed by RHRI offer sensitive diagnosis and treatment for underlying hormonal issues and they cover conditions such as ovulatory dysfunction, polycystic ovaries, thyroid problems, endometriosis, infertility, menopausal disorders, and depression.

is anovulation (followed later-on by irregular menses or amenorrhea), women who know how to recognize their ovulation can receive a timely diagnosis and an early treatment.

Nowadays there is still an unmet need for further research on how to diagnose and treat ovulatory dysfunctions. The mission of the Reproductive Health Research Institute (RHRI) is to conduct new research on women's health, with a particular focus on reproductive endocrinology. It works to improve women's health by developing research, creating clinical protocols and advancing scientific knowledge. RHRI was founded in 2014. It is composed by a multidisciplinary group of researchers that include medical doctors, reproductive endocrinologists, neurobiologists, ecologists, nurses, and psychologists. The Institute is led by Dr. Pilar Vigil (MD, PhD, OB/GYN, FACOG) and Dr. Patricio Contreras (MD, Medical Endocrinologist).

As seen in the present paper, women with ovulatory dysfunction may present symptoms such as irregular cycles, weight gain, hirsutism, acne, pain, headache, anxiety, and fatigue. The medical protocols developed by the RHRI offer sensitive diagnosis and treatment for the underlying hormonal issues causing the mentioned symptoms, and they cover conditions such as ovulatory dysfunction, polycystic ovaries, thyroid problems, endometriosis, infertility, menopausal disorders, and depression. These protocols are scientifically based and standardize the latest advances in biomedical research for clinical use.

Treatment plans help women recover healthy hormonal balance. Depending on the measured deficiencies, treatment protocols can range from a significant

emphasis on diet, exercise and lifestyle changes to pharmacological interventions or immunological support. FEMM collaborates with RHRI in the training of healthcare providers in these protocols around the world.

9. Summary

This text analyzed the anatomy of ovulation and the structures involved in this process. It reviewed the role of the central nervous system and its communication through the neuroendocrine axis with the gonads and endocrine glands involved in ovulation. The concept of ovarian activity in the lifetime of women, the events that occur in intrauterine life, puberty, and during the reproductive age were discussed. It analyzed the concept of the fertile window and the processes that surround it, mentioning certain biomarkers. Special relevance was given to the role of cervical mucus as a tool for recognizing ovulation. Also, the relationship between hormones, the brain and mental health was mentioned. Finally, in relation to future perspectives, we have exposed the need for research in order to improve women's health and well-being, recognizing ovulation as a sign of health.

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