Follicular waves in the human ovary

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ABSTRACT

Follicular waves can be defined as a synchronized growth of a group of antral follicles, among which one or more follicles will be selected for subsequent development and ovulation. These waves occur at regular intervals during the menstrual cycle. The synchronization of the wave beginning and the ovarian stimulation improves the outcomes of the IVF treatment. Follicular waves are a natural phenomenon, and they develop in association with increased concentration of follicle stimulating hormone levels. Studies indicate that the follicular recruitment event occurs only once during the cycle; however, recent studies suggest that the recruitment can occur more than once during the same cycle. Several studies have demonstrated groups of women with two follicular waves and others with up to three waves during the normal menstrual cycle. Follicles recruited during these waves have the potential to ovulate in the presence of an luteinizing hormone surge, providing women, especially the poor responders, a more efficient and less expensive treatment. The majority of studies agree that there is not just a single wave of follicular recruitment during a menstrual cycle and this involves the optimization of treatment of poor responders, expanding the window of action for oocyte retrieval and avoiding expensive treatments.

Keywords: Ovarian follicle, Ovary, Ultrasonography, Follicular waves, Follicular dynamics.

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INTRODUCTION

Reproduction is defined as a life process that allows the living organisms to produce descendants, giving continuity of the species. Originally, human reproduction was described by Hippocrates in the fifth century B.C. as the union of semen with the menstrual blood [1, 2]. Only in 1600, Graaf characterized the ovary as the egg producer, not differentiating the egg from the follicle, and in 1827, Baer individualized the ovarian follicle [2].

After these previous discoveries, the first studies on human folliculogenesis and on the menstrual cycle were performed based on histological and/or endocrinological evaluation of ovarian function [3, 4]. However, ovarian function during the menstrual cycle, as well as follicular growth and atresia, could only be evaluated after the development of transabdominal ultrasonography (USG) in the 1970s, improving dramatically with the transvaginal USG (TV USG) development in the 1980s [5, 6].

TV USG allowed visualization of antral follicles, up to approximately 2 mm, and evaluation of their growth and dynamics during menstrual cycle. Thus, the use of endocrinological, histological, and ultrasonography examinations made it possible to elucidate the antral follicle development, such as the observation of follicular waves during the menstrual cycle. These achievements provided the basis for assisted reproduction (AR) treatments [7, 8].

Treatment effectiveness in AR is mainly achieved by the induction of ovulation in order to increase the number of mature oocytes [9]. To do so, understanding the dynamics of follicular waves allows the recruitment and retrieval of good quality oocytes, a model known as Propitious Moment Theory. Therefore, it results in better fertilization rates, embryo development, and pregnancy [10].

STAGE DEFINITION: PRE-ANTRAL AND EARLY ANTRAL FOLLICLE DEVELOPMENT

Studies based on necropsy or oophorectomy samples estimate that human folliculogenesis from the primordial to the preovulatory phase lasts about 175 days [11].

In the fourth month of fetal life, follicular development begins [4]. At first, the primordial germ cells migrate from the endoderm to the gonads, where they undergo the first meiosis and become primary oocytes. The somatic cells of the primitive gonad involve the oogonium, forming rudimentary ovarian follicles (0.1 mm in diameter) [12]. The ovarian follicular reserve is formed by the oocytes in meiosis I and influences the reproductive potential of each woman. In the 20th week of gestation, there are approximately 7 million of these oocytes and after that they begin to reduce continuously in number throughout the life [4].

The pre-antral follicles (0.1–0.2 mm) develop independently of the action of gonadotrophins and when they reach 0.2–0.4 mm, a fluid-filled cavity begins to form and then they become responsive to gonadotrophins [12, 13].

Still in childhood, follicles develop until the early antral phase; however, due to the immaturity of the hypothalamic-pituitary-ovary axis, they stop evolving [14, 15]. From puberty complete maturity of the hypothalamic-pituitary-ovary axis, the antral follicles continue to develop and reach two or more millimeters in diameter [16, 17]. Visualization of follicles this size is currently possible through modern TV USG.

RECRUITMENT OF ANTRAL FOLLICLES

Antral or Graafian follicles are formed by the development of: a basement membrane between the granular cells and theca layers and the cumulus oophorus wherein the oocyte is located. The Graaf follicle ceases to be dependent on the follicle stimulating hormone (FSH) and begins to respond to the luteinizing hormone (LH), a characteristic necessary for being able to ovulate.

Throughout the menstrual cycle, the presence of antral follicles 2–5 mm in diameter is observed [18]. Three theories have been developed to explain the follicular recruitment during the menstrual cycle, advocating from continuous to cyclical development.

CONTINUOUS RECRUITMENT

Theory 1:

Histological studies in animals concluded that the early growth of antral follicles occurs continuously throughout the cycle period. Afterward, this theory evolved to explain the human menstrual cycle [19]. This theory proposes that antral follicles, smaller than or equal to 4–6 mm, are recruited continuously, independent of gonadotrophins, at all stages of reproductive life. Also, it postulates that the ovulatory follicle is chosen randomly, since they are at the optimum stage of maturity, being able to respond to increased levels of FSH and, subsequently, LH [20].

CYCLICAL RECRUITMENT

The appearance of a “follicular wave” at regular intervals during the menstrual cycle has been previously described [3, 18, 20, 21]. However, there are conflicting results in the literature regarding the number of these recruitments.

Theory 2—single follicular recruitment:

It postulates the single recruitment during the menstrual cycle of follicles 2–5 mm in size after the regression of the corpus luteum (CL), with decreasing levels of estradiol and inhibin and a transient increase of FSH in the late luteal or early follicular phase, which is known as the privileged phase [21]. It has been postulated that each follicle has an FSH threshold below which no recruitment occurs [22].

The recruited follicles contain low concentration of estradiol and high concentration of androgens [21]. Inhibin B produced by granulosa cells inhibits FSH secretion in the follicular phase [23]. Inhibin A, however, is low in the follicular phase and reaches maximal levels in the luteal phase, suggesting that CL is a source of inhibin A, which is produced to suppress FSH and prevent follicular development [21].

The anti-Müllerian hormone (AMH) is produced by the granulosa cells of the primary, secondary, pre-antral, and early antral follicles (≤4 mm). It inhibits follicular growth by decreasing sensitivity to FSH. However, the action of the AMH is not fully understood [24]

Theory 3—follicular waves:

The growth of a group of antral follicles at regular intervals during the menstrual cycle is defined as follicular wave. The follicles in each wave are of similar but not identical diameters [18]. Two waves have been detected: the first in the follicular phase and the second in the luteal phase. However, the luteal follicles presented fewer granulosa cells and lower levels of estradiol compared to the ones in follicular phase [3, 20]. In addition, those women with regular cycles of 30–35 days presented two follicular waves in contrast with a single follicular wave in women with cycles of 26–30 days [18].

In those women with two follicular waves, an anovulatory wave appeared at the time of ovulation (early luteal phase) followed by the wave of ovulation that developed in the early follicular phase. In women with three waves, an anovulatory wave arose at the time of ovulation and a second anovulatory wave emerged during the mid to late period of the luteal phase. The third wave, ovulatory, appeared at the beginning of the mid follicular phase [18].

An elevation of FSH precedes the recruitment of each follicular wave [18]. Inhibin B produced by the granulosa cells of the recruited follicles inhibits FSH secretion [23]. However, the precise action of inhibin A, inhibin B, and AMH on the appearance of multiple follicular waves in women is unclear [18].

In clinical practice, both oocyte recovery and successful in vitro maturation are observed with ovarian stimulation during the luteal phase, being considered as a procedure for urgent preservation of fertility. Otherwise, by using USG, the day of follicular wave emergence is considered when the largest follicle to be recruited among the follicle pool reaches a diameter of 4–6 mm [25]. The stimulation of ovulation during the luteal phase followed by stimulation of the follicular phase has also been advocated to reduce the time to pregnancy of poor responder patients.

FOLLICLE SELECTION

During the recruitment of follicles, only one follicle called “dominant” is selected for subsequent growth and ovulation, while the other follicles undergo atresia. This process is called “follicle selection” and usually occurs in the early-to-mid-follicular phase [3, 8, 11, 18, 23].

When the dominant follicle reaches a diameter of 10 mm, between days 6–9 of the follicular phase, it suppresses the growth of the other follicles of the same follicular wave and it also suppresses the appearance of a new follicular wave by inhibiting FSH secretion [8, 18, 26, 27]. During the course of follicular selection is observed a concentration variation and the role of some hormones, such as:

FOLLICLE-STIMULATING HORMONE (FSH)

A peak of FSH concentration levels occurs for initial follicular recruitment and, subsequently, a post-surge concentration decrease occurs, being necessary for selection of the dominant follicle [18, 23, 27–29]. The duration of the FSH peak above a critical threshold determines the number of dominant follicles selected from the recruited cohort for preferential growth, a concept known as FSH threshold/window/gate [30–33]. In contrast, if the FSH levels remain above the threshold for a short duration, there is a development of a single dominant follicle. However, if the duration of FSH peak persists, multiple dominant follicles are recruited at the same time, a fact observed during the ovarian stimulation therapy [31].

Regarding the differences among the dominant follicle and the other follicles, it was postulated that the dominant one contains more granulosa cells and FSH receptors, which increases sensitivity to FSH, even at low concentrations. Therefore, with the decrease of FSH levels, the dominant follicle continues to develop while the subordinates are not able to evolve and undergo degeneration by atresia. In this way, the smaller recruited follicles are the ones that undergo atresia first [18, 22, 23, 34–36].

ESTRADIOL

From the fifth to the eighth day of the menstrual cycle, the aromatase activity initiates in the granulosa cells of follicles larger than 6–8 mm and the dominant one produces higher levels of estradiol [37–40]. Thus, the fluid within the dominant follicle contains more estrogen than androgen, while the fluid within the subordinate follicles contains more androgen [14, 41–43].

The LH acts on the theca cells, stimulating the production of androgens that work as the substrate for the production of estradiol in the granulosa [33, 44, 45]. Estradiol, in its turn, promotes negative feedback on FSH secretion, contributing to its decreased levels during follicular growth [46]. After the secretion of estradiol, the LH receptors are expressed in the granulosa of the dominant

follicle, which makes it less dependent on FSH activity and more responsive to LH during the selection process [34, 47, 48].

TRANSFORMING GROWTH FACTOR-BETA (TGF-B)

The interplay between oocyte and cumulus cells regulates both folliculogenesis and oogenesis [49]. The TGF-β family includes inhibin, activin, follistim, TGF-β, and AMH, among others. These molecules present paracrine or autocrine functions that aim to regulate follicular development and oocyte maturation.

All recruited follicles produce inhibin B which acts to decrease FSH levels before the selection process [30, 46, 50]. Activin is produced by granulosa and its role in the follicle selection is not well elucidated; however, it has been proposed that a coordinated transition within the follicular fluid from inhibin B to inhibin A and from activin to follistatin is critical for the development of the dominant follicle [35, 36].

The follicular content of AMH gradually decreases during antral follicle growth until 8–10 mm, when it is close to selection, and remains low. While AMH level decreases, there is a rise in aromatase activity [51]. Finally, it is proposed that the differential exposure to these signaling molecules is one of the ways by which the dominant follicle increases sensibility to FSH.

INSULIN-LIKE GROWTH FACTOR (IGF)

The concentration of IGF increases in the dominant follicle at the time of selection and also stimulates the action of aromatase, estradiol, estrogen, and androgen production [52, 53]. Among the subordinate follicles, there is no response to IGF. Therefore, the steroidogenesis of theca and granulosa is inhibited and atresia occurs [52, 54].

LUTEAL INFLUENCES ON FOLLICLE SELECTION

It has been previously reported that the dominant follicles are recruited contralateral to the CL of the previous ovulation and that these follicles contain a greater amount of estradiol in relation to the follicles that have developed ipsilateral to CL [40]. However, it is postulated that follicle selection and ovulation occur randomly among the right and left ovaries [18, 55].

Follicle selection occurs in the initial or middle follicular phase; however, previous studies demonstrated that selection occurs more than once in the menstrual cycle, reinforcing the follicular wave theory [18, 21].

The role of CL in the regulation of follicular wave dynamics has been studied and no significant differences in CL size, life span, progesterone secretion, or estradiol secretion have been observed among women with two versus three follicular waves [56].

DEVELOPMENT OF PREOVULATORY FOLLICLES

After the late follicular phase, the dominant follicle continues its development process, reaching the preovulatory state between 16 and 29 mm [8, 11, 18, 57]. The ovulatory follicle grows about 1–4 mm per day, and may undergo variation in the days preceding ovulation [8, 21, 57, 59]. The preferential growth of the dominant follicle at this stage is related to increased aromatase activity and a rapid increase in estradiol-17b levels in the circulation and within the follicular fluid [18, 20, 40, 43, 58–60].

The improved response of the dominant follicle to gonadotrophins is mediated by the production of estradiol in its granulosa layer, expression of the LH receptor, and by continuous preovulatory growth [20, 23, 34, 43, 46–48].

The dominant follicle accounts for more than 90% of estrogen production in the preovulatory period [60]. Preovulatory follicle growth is related to both intra-ovarian and endocrine factors, and the increase in aromatase levels is inversely proportional to that of AMH in the follicular fluid [51].

Theca cells produce more androgens influenced by high levels of inhibin A, increasing the amount of substrate for estradiol secretion [36]. In contrast, granulosa cells express IGF-II mRNA in abundance, stimulating aromatase activity [61]. The estradiol peak has a positive feedback action in the hypothalamus stimulating the release of LH. The LH surge is, thereby, necessary to induce ovulation. From this stage, the dominant follicle presenting a high expression of LH receptors is prepared to respond to the LH secretion. Then, ovulation occurs about 24 hours after the LH surge [62].

The interaction between LH and EGF in the granulosa stimulates the expression of protein kinases 1 and 2, leading to the reduction of granulosa layer proliferation and estrogen synthesis. Also, cumulus expansion and progesterone secretion occur as a consequence of ovulation [63]. This is particularly important to reduce the time to pregnancy of poor responders and for oncology patients who need to initiate treatment immediately

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