Chapter

Endocrine Characteristics and Regulatory Mechanism of Follicular Development and Ovulation Failure in Mammalian Ovary

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Abstract

In mammals, the follicular development and following ovulation are regulated by reproductive hormones, while polycystic ovary syndrome (PCOS) is an endocrine disorder syndrome with reproductive dysfunction and abnormal glucose metabolism in most PCOS women. Its characteristics are hyperandrogenism, ovarian dysfunction, and the exclusion of other androgen excess or related diseases. Its clinical characteristics are large antral follicle pool from which to recruit and persistent anovulation. The incidence of PCOS in women of childbearing age ranged from 4 to 12%. About one-third of infertility cases had no ovulation, and 90% of them had PCOS. Therefore, further studying the regulatory mechanism of follicular hyperrecruitment and anovulation can provide theoretical basis for exploring the pathogenesis of PCOS and guiding clinical treatment, especially for protecting female fertility and preventing the occurrence of metabolic disorder syndrome. The present article will review the progress in endocrine characteristics and regulatory mechanism of follicular development and ovulation failure in the mammalian ovary.

Keywords: follicle-stimulating hormone, follicular development, luteinizing hormone, ovulation failure, polycystic ovary syndrome

1. Introduction

In mammals, the follicular development mainly includes several periods of primordial follicles, primary follicles, secondary follicles, tertiary follicles, and matured follicles. With the initiation of primordial follicle, primary follicles are gradually formed, then continue to develop to the secondary and tertiary follicles, and finally to matured follicles, which were divided into two stages: the selective follicle stage and the matured follicle stage. The development of follicles undergoes the development of early stage, the growth of antral follicles, the selection of dominant follicles, the maturation, and ovulation of follicles. During an estrous cycle,

when thousands of primordial follicles develop to the ovulation stage, the number of ovulation accounts for only 0.1–0.2% of the total number of primordial follicles. Most of these follicles are atresia during the development, while the hormone regulation is accompanied by the follicular development all the time. Pituitary gonadotropin and steroid hormones promote the growth of oocytes, the proliferation of the follicular cells, and the formation of the follicular cavity, which plays an important role in the follicular development. Polycystic ovary syndrome (PCOS) is an endocrine disorder syndrome with reproductive dysfunction and abnormal glucose metabolism in most PCOS women [1], and its clinical characteristics are large antral follicle pool from which to recruit and persistent anovulation. Further understanding the regulatory mechanism of the follicular development and ovulation in the ovaries will provide theoretical basis for the treatment of PCOS.

2. Hormonal regulation of the normal follicular development

Under physiological conditions, the growth and development of follicles are regulated by a variety of hormones, including pituitary gonadotropins (follicle-stimulating hormone, FSH and luteinizing hormone, LH) and gonadal hormones (estrogen and androgen). Androgen promotes the growth and development of early follicles [2] and plays an important role in the follicular recruitment [3]; FSH recruits the follicles [4] and LH binds to its receptors to promote the androgen synthesis in the follicular membrane cells; FSH binds to its receptors to activate the aromatase activity in the granulosa cells and then to promote the transformation of androgen to estradiol; estrogen promotes the growth and differentiation of follicular cells and cooperates with FSH to promote the development of the follicles; LH is secreted in a peak manner during the late follicular development for the ovulation of matured follicles.

2.1 Hormonal regulation of the early follicular development

It is generally believed that preantral follicles do not need the action of FSH, but studies have found that there are FSH receptors in the granulosa cells of preantral follicles, indicating that these follicles have the ability to respond to FSH very early. The threshold theory proposed by Brown in 1978 shows that the concentration of FSH is very important in the early follicle stage [5]. Once the FSH reaches the threshold, the follicle will enter the growth stage rapidly. The duration of FSH reaching the threshold and the extent of FSH exceeding the threshold will ultimately determine the number of matured follicles [5]. During the pre-antral follicular phase, inhibin-containing follicular fluid was injected into mice and cattle, which reduced the concentration of FSH, caused follicular growth to stop, and delayed the appearance of new follicular waves; and artificial increase of FSH concentration would lead to larger follicular volume and more follicles [6]. Therefore, FSH plays an important role in the initiation and development of primordial follicles during the follicular development [7].

LH receptor (LHR) is widely distributed in nongonadal tissues besides follicular endometrial cells and Leydig cells. The expression of LHR was later than that of FSH receptor (FSHR) [6, 8]. The expression of LHR mRNA could only be detected 5 days after the birth of the mouse offspring and 7 days after the birth of the rabbits when secondary follicles appeared in the ovaries [8]. In the estrous bovine and rabbit ovaries, LHR began to transcript in the follicular endometrial cells only after the follicles developed into antral follicles and follicular endometrium formed, and later in granulosa cells, indicating that LH had no direct effect on the development of preantral follicles.

2.2 Hormonal regulation of the antral follicles

After FSH initiates the development of primordial follicles, it further promotes the formation of some follicles into the cavity and enters the growth of antral follicles. The continuous growth of antral follicles depends on the support of FSH, while FSH stimulates the growth and development of antral follicles, and at the same time stimulates granulosa cells to produce FSHR. With the increase of the number of FSHR, the response of follicular granulosa cells to FSHR increases, which promotes the continuous development of granulosa cells [9].

Follicular intima began to synthesize LHR, cholesterol side chain cleavage enzyme (P450scc), P450 17alpha hydroxylase (P450 17alpha), and 3beta hydroxysteroid dehydrogenase (3beta-HSD) after the formation of antral follicles. The development of antral follicles is related to the synthesis of P450scc and P450arom enzymes by granulosa cells. LH acts on follicular endometrial cells, P450scc and 3beta-HSD catalyze the conversion of cholesterol to progesterone, and progesterone to androgen under the action of P450 17alpha and P450 C17,20 carbon chain lyase [10].

2.3 Hormonal regulation of the dominant follicles

Some of the first growing follicles were selected to continue to develop into dominant follicles. The remaining follicles in the same group were transformed into secondary follicles and gradually degenerated into atresia [11]. With the advent of dominant follicles, the concentration of FSH gradually decreased to the basic concentration and is maintained until the next peak. During the later selection period, the response ability of dominant follicles to FSH decreased, and the decrease of the peak value of FSH was obviously a necessary factor for the selection of dominant follicles, but not the only factor. LH also played an important role in this process.

During the selection process of dominant follicles, the number of FSH receptors in granulosa cells remained unchanged, while the expression of LHR mRNA was initial, and the number of LHR increased gradually [12]. At the same time, the number of LH binding sites in endometrial cells increased when the dominant follicle was established, so the selection of dominant follicles began to change from FSH-dependent to LH-dependent one, and the number of LH receptors increased rapidly.

Androgen enters granulosa cells, FSH acts on granulosa cells, induces the proliferation and differentiation of granulosa cells, and increases the activity of P450arom, which converts androgen into estrogen [11]. With the increase of estradiol produced by follicles, estradiol has a feedback effect on pituitary gonadotropin, which makes the concentration of gonadotropin decrease slightly, inhibits the development of other follicles, and promotes atresia of other follicles. In addition, FSH has long sensitization to aromatase [13].

Inhibin (INH) is a glycoprotein hormone produced mainly by ovarian granulosa cells. It is a heterodimer composed of two subunits, alpha and beta [14]. INH stimulates androstenedione synthesis in the follicular membrane mediated by LH and enhances aromatase activity, thus increasing estradiol synthesis in granulosa cells. It is of great significance for follicular recruitment and selection of superior follicles. After the formation of dominant follicles, INH and 17beta-estradiol synthesized by granulosa cells increased, and inhibited FSH synthesis and release by blood circulation. On the one hand, the decrease of FSH synthesis restricts the further development of non-dominant follicles and makes them become atresia follicles; on the other hand, INH enhances the sensitivity of dominant follicles to pituitary gonadotropin and avoids follicular stagnation caused by the decrease of FSH synthesis [15].

2.4 Hormonal regulation of ovulation

Ovulation in animals is a complex process, involving a series of changes such as the rupture of matured follicles and excretion of matured oocyte. The hormone that fundamentally affects ovulation is LH. After selecting the dominant follicles, the gonadotropin-dependent transformation was completed, and the estrogen in the follicles increased rapidly, with the peak value of estradiol [16]. With the emergence of estradiol peak, the pituitary response to gonadotropin-releasing hormone (GnRH) gradually increased, and the LH stored in pituitary increased in order to further provide hormones to the LH release pool, which reached the peak before ovulation. LH peak causes follicular wall ischemia to form a necrosis state of "physiological atrophy," leading to ovulation [9].

As in the case of LH, the concentration of FSH in blood increased briefly before ovulation and reached a peak again before ovulation. It is worth noting that LH must cooperate with a certain proportion of FSH in order to promote normal ovulation. LH ruptures all the follicles on the ovary, but when they are used together, only matured follicles are discharged, indicating that FSH has a mechanism to inhibit the rupture of immatured follicles.

The appearance of LH peak activates adenylate cyclase in the follicular membrane, increases cAMP, causes the luteinization of granulosa cells, increases progesterone content in the follicles, thus inhibits the positive feedback of estrogen to promote LH secretion [9], reduces the frequency of LH pulse [9], prolongs the duration of LH rise before ovulation, and ensures the sufficient time for LH and other gonadotropins to initiate follicular maturation and ovulation. At the same time, progesterone activates proteolytic enzymes, amylase, collagenase, and hyaluronidase in the follicles. These enzymes act on the collagen structure of the follicular wall to decrease the tension, increase the expansibility, and finally cause the ovulation.

INH acts as a chemical signal of the pituitary gland to induce the number of developing follicles in the ovary and reduce the release of FSH to maintain the level of species-specific ovulation [17]. Estradiol transmits chemical signals to the hypothalamus during the follicular maturation, so INH is an important inducer of the follicular development, which controls the number of follicles by inhibiting the release of FSH [18].

3. Endocrine regulation of the follicular development in PCOS

PCOS patients are predominantly androgens and the excessive androgens are mainly androstenedione and testosterone [19]. Recent studies have found that other endocrine factors are also involved in the occurrence of PCOS, such as leptin, growth hormone, and so on.

3.1 Androgen

The increase of androgen level in follicular fluid blocks the development of dominant follicles [20], while the main mechanism of androgen excess in PCOS is as following. LH directly acts on the follicular membrane cells, increases the activity of P450 C17 enzyme in the cells, and causes the excessive androgen production in the follicular membrane cells; high level of INS increases the level of LH in PCOS patients, thus promoting the secretion of androgen by ovaries and adrenal glands; insulin-like growth factor (IGF)-I promote androgen production in the follicular membrane cells and adrenal cortical cells; and adrenal hyperfunction also produces a large amount of dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS) [21].

3.2 Gonadotropin

The serum FSH level of PCOS patients was lower than that of normal people, but low FSH level resulted in a large number of follicles accumulated and could not develop into dominant follicles, resulting in anovulation and the changes of polycystic ovarian [22, 23]. Although the persistent high androgen level in the ovary of PCOS patients cannot form dominant follicles, the small follicles in the ovary can still secrete estradiol. At the same time, the increase of estrone converted from androstenedione in the peripheral blood makes the persistent secretion of large amounts of estrogen and a certain level of estradiol act on the hypothalamus and pituitary gland, which has a positive feedback effect on LH, and increases its secretion amplitude and frequency. The level of LH is continuously high, and then stimulates the hyperplasia of follicular membrane, produces excessive androgens, significantly inhibits the role of LH in promoting estradiol secretion, which may be the cause of oocyte maturation disorder in PCOS patients [24].

3.3 Insulin

Insulin (INS) receptors are expressed in ovarian stromal cells, granulosa cells, and follicular membrane cells. INS promotes the follicular recruitment and stimulates the synthesis of steroid hormones by follicular membrane cells and granulosa cells [25]. INS promotes androgen production by follicular membrane cells through the following pathways [26] and plays an important role in the production of hyperandrogenism [27]. INS increases androgen production by enhancing the activity of 17alpha-hydroxylase, increases androgen production by increasing the number of LH receptors or the affinity of LH to receptors, increases free androgen level by reducing the secretion of gonadal hormone binding globulin in the liver, and increases androgen level by inhibiting the secretion of IGF binding protein and enhancing the activity of serum IGF-I. INS resistance exists in PCOS patients, which leads to the hyperinsulinemia and increases androgen production and premature luteinization of granulosa cells, thus causing granulosa cell proliferation and follicular development to stagnate [26, 27].

3.4 Leptin

Leptin is a polypeptide hormone secreted by adipose tissue [28], which plays an important role in controlling reproductive capacity [29]. The disorder of leptin system is related to the pathogenesis of polycystic ovary [29–32]. Relatively high concentrations of leptin in serum and follicular fluid of PCOS patients resulted in lower fertilization, transfer, and pregnancy rates in vitro fertilization-embryo transfer (IVF-ET) [33]. The high concentration of leptin in PCOS patients inhibits the aromatase activity of granulosa cells, prevents the transformation of androgen to estrogen, induces hyperandrogenism, and inhibits follicular development [34]. In addition, leptin may block the selection and development of dominant follicles, leading to anovulation [35].

3.5 Growth hormone

Growth hormone (GH) is a hormone secreted by pituitary gland, which has physiological effects on the growth, development, and metabolism, which stimulates the follicular development, inhibits follicular atresia, and increases ovulation number [36]. GH directly regulates the gene expression of IGF-I or IGF-II and affects the synthesis of ovarian hormones. The impairment of GH secretion and the decrease

of GH level lead to anovulation in PCOS. In addition, INS inhibits the secretion of pituitary GH stimulated by basal and gonadotropin-releasing hormone and also stimulates the production of IGF-II, thus feedback inhibits GH secretion [36].

4. Molecular mechanism regulating the follicular development

Follicular development is regulated by many molecules and related signaling pathways in mammals. For example, prematured luteinization of granulosa cells in mouse follicles is associated with higher LH levels in the follicles, which results in the early meiosis of oocyte and changes in signal transduction, leading to follicular atresia [37]. In addition, activin/inhibin, BMP/Smad, and NPPC/NPR2 signaling pathways also play an active role in the development of ovarian follicles, which will be discussed in the present section.

4.1 Activin/inhibin signaling pathway

Activin is an intercellular signaling molecule secreted mainly by granulosa cells, and also an agonist-stimulating pituitary gland secreting FSH. Activin is involved in many biological functions of mammalian ovaries, including the survival of germ cells and the recruitment of primordial follicles, promotes the proliferation of granulosa cells and the expression of FSHR, delays the luteinization and atresia of follicles, and participates in the luteolysis [38–40]. Activin binds to type II receptor, starts the phosphorylation process, then activates type I receptor, phosphorylates the downstream signal molecule R-Smads, receptor Smad binding to phosphorylated R-Smads occurs the location transfer, and enters into the nucleus to bind with specific receptors, playing a regulatory role. Activin also promotes the proliferation and activity of granulosa cells through smad2/ERK5 signaling pathway, increases the secretion of stem cell factor (SCF, also known as Kit ligand, KL), and then specifically binds to the surface receptor c-Kit of oocytes. The expression level of SCF/c-Kit in rat ovary after binding is increased, thus promoting the development of oocytes [38].

Inhibin is a kind of macromolecule glycoprotein hormone secreted by the gonad, and its structure is similar to activin. Inhibin regulates the synthesis and secretion of pituitary FSH together with activin [41, 42]. During the development stage of dominant follicles, the concentration of inhibin A increased, which increased the sensitivity of dominant follicles to FSH and prevented dominant follicles from atresia. During the luteal formation stage, inhibin A mainly promotes the luteinization of follicles, inhibin B mainly expresses in the small and medium follicles, and enhances FSH to prevent nondominant follicles from entering the preovulation stage, which was conducive to the screening of dominant follicles [43]. It was found that the level of inhibin B in the follicular fluid of PCOS patients decreased significantly [44].

4.2 BMP/Smad signaling pathway

The signal transduction of bone morphogenetic proteins (Bmps) family can be divided into two main pathways: Smads-dependent pathway and non-Smads-dependent pathway such as phosphatidylinositol 3 kinase (PI3K). Each member of the Smads family performs different functions in signal transduction pathways, which can be divided into three types: receptor-regulated Smads, CO-mediated Smads, and inhibitory Smads [45]. Bmps/Smads signaling pathway plays an important role in regulating follicular growth, granulosa cell growth and differentiation,

oocyte maturation, and ovulation in mammals. Bmps bind to BMPR-II receptor on cell membrane and then make it phosphorylated. Phosphorylated BMPR-II receptor binds to BMP-I receptor to form a complex. BMP-I receptor is activated by corresponding protein kinase and phosphorylated. Then Smads signal molecule is activated. Activated R-Smads binding common CoSmad 4 forms Smad protein complex and enters the nucleus and specificity. DNA sequence binding start the promoter of downstream target gene, make downstream gene begin to transcribe [46–48], downstream signal molecule R-Smads also plays an important role in BMP/Smad signaling pathway. After Smad4 knockout, steroid hormone regulation was blocked, plasma progesterone level increased, and granulosa cells developed premature luteinization, which eventually led to premature ovarian failure [49].

4.3 NPPC/NPR2 signaling pathway

Natriuretic peptide family widely exists in animal brain, heart, and other tissues and organs, which has the functions of maintaining blood pressure and blood volume stability, promoting fat metabolism and cartilage growth. The family consists of three ligands and three specific receptors in mammals. Ligands exist in the form of precursor peptides, namely atrial natriuretic peptide (ANP, also known as NPPA), brain natriuretic peptide (BNP, also known as NPPB), C-type natriuretic peptide (CNP, also known as NPPC), and specific receptors exist in the form of dimers, namely natriuretic peptide receptor A (NPRA, also known as NPR1), natriuretic peptide receptor B (NPRB, also known as NPR2), and natriuretic peptide receptor C (NPRC, also known as NPR3) [50]. NPPC/NPR signaling pathway plays an important role in inhibiting premature maturation of mammalian oocytes. The combination of NPPC and NPR2 produced by granulosa cells of the follicular parietal layer stimulates the production of cGMP, which enters into the oocyte through interstitial links between oocyte and granulosa cells [51], inhibits the activity of phosphodiesterase (PDE3A), and decreases the degree of hydrolysis of cAMP, thus stabilizing at a higher level. The protein kinase PKA dependent on cAMP regulates the activity of maturation-promoting factor (MPF) through phosphatase cell division cycle 25 (CDC25), Wee1 kinase, and myelin transcription factor Myt1. CDC25 dephosphorylated cyclin-dependent kinase 1 (CDK1), Wee1 and Myt1 phosphorylated CDK1, phosphorylated CDK1 and related complexes inactivated, and ultimately inhibited the maturation of oocytes.

5. Conclusion

Nowadays, great achievements have been made in the molecular mechanism of follicular development in mammals. At present, many signaling pathways have been proved to play a very important role during the follicular growth and development, and some of them have been thoroughly studied. However, there are still some problems related to signaling pathways, such as how mammals initiate primordial follicular development and which downstream target genes are involved in signaling pathways. Follicular dysplasia in PCOS patients is closely related to apoptosis of granulosa cells, follicular atresia, and oocyte degeneration. Its mechanism is related to endocrine dysfunction, as well as regulation factors and their receptors in the ovary. Although PCOS patients can obtain a large number of oocytes, the low rate of matured oocytes, the low rate of high-quality embryos, the low pregnancy rate, and the high abortion rate make clear the related factors of oocyte degeneration and atresia regulation, which is of great significance to the application of assisted reproductive technology in PCOS patients. Therefore, further understanding the

molecular mechanism regulating the follicular development in mammals still requires further study on the biology and gene expressions related to the follicular development, which is of great significance for the treatment of mammalian reproductive infertility and other diseases.

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Conflict of interest

The authors declare no conflict of interests.

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