—Mini Review—

Adverse effect(s) of chronically elevated LH in PCOS

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Abstract: Polycystic ovary syndrome (PCOS) is a common endocrine disorder in reproductive-age women. Hyperandrogenism, chronic anovulation, and infertility are the main features of this heterogeneous condition. The diagnosis of PCOS is based on a combination of clinical, biological, and ultrasound criteria that have been used variably to define PCOS. The usual clinical presentation of PCOS in Asia is slightly different from that in the United States and Europe, with less frequently encountered cases of hyperandrogenism. Moreover, non-obese PCOS is typical in Asian women. The Japanese Society of Obstetrics and Gynecology has recently proposed new, revised diagnostic criteria. Growth arrest of ovarian follicles in the non-obese PCOS is assumed to be associated with an abnormal endocrine environment involving chronically elevated LH. *In vitro* studies currently demonstrate that LH promotes follicular growth during preantral-early antral transition via the increased synthesis and growth promoting action of androgen. However, chronic LH stimulation impairs FSH-dependent antral follicle growth by suppressing FSH receptor expression in granulosa cells, via the modulation of intraovarian regulators. Therefore, the adverse effect(s) of chronically elevated LH on the theca cell/androgen system should also be considered for improved care of non-obese PCOS patients.
Key words: PCOS, LH, ovary, follicle, FSH receptor

Introduction

Polycystic ovary syndrome (PCOS) is a syndrome of ovarian dysfunction with three major symptoms: (1) growth arrest of many small antral follicles in bilateral ovaries (polycystic ovary morphology), (2) menstrual irregularities including oligo- or anovulation, and (3) local androgen excess in ovaries [1]. PCOS affects 5–10% of reproductive-age women, and has remained an important cause of anovulation and infertility, even 79 years after it was first reported by Stein and Leventhal [2]. Current concept suggests that PCOS is not a specific endocrine disorder with a unique cause or pathophysiology, but rather a consequence of chronic anovulation [1]. Since there are many causes of anovulation, there may be many causes of polycystic ovary morphology and PCOS. It has been also suggested that the manifestation of PCOS varies widely among ethnic groups. In this paper, we will review historical changes in the diagnostic criteria for PCOS, and we also present our current results of an *in vitro* model for non-obese PCOS which is typical in Asian women.

Historical changes in the diagnostic criteria for PCOS in the United States and Europe

The most important diagnostic criteria for PCOS has been androgen excess in North America and polycystic ovary morphology by ultrasonography in Europe.

According to the diagnostic criteria first presented in 1990 by the NIH, PCOS is regarded as an ovarian hyperandrogenic syndrome. It is established as having both (1) chronic anovulation and (2) clinical and/or biochemical signs of hyperandrogenism (NIH criteria 1990) [3].

With the accumulation of clinical findings, the possibility has been pointed out that the pathological conditions and manifestations of PCOS might be broader. This led to the establishment of a joint workshop which was held in 2003 by the European Society for Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM), where an agreement was reached on new diagnostic criteria, the presence of two or more of the following three conditions: (1) oligo- or anovulation, (2) clinical and/or biochemical signs of hyperandrogenism, and (3) polycystic ovaries by ultrasonography. They are known as the Rotterdam criteria

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2003 [4, 5].

The Rotterdam criteria 2003 are the most commonly used today, although it is noteworthy that two categories presented below were newly added on transition from the NIH criteria 1990: (1) cases in which polycystic ovaries and hyperandrogenism are observed while the menstrual period is maintained, and (2) cases in which polycystic ovaries and anovulation are observed while hyperandrogenism is not clear.

Some confusion has persisted over the diagnostic criteria for PCOS, even in the United States and Europe where ethnic differences are less prevalent. A North American group, the Androgen Excess Society, which disputes to the Rotterdam criteria 2003, has proposed the AES guideline 2006, which requires both (1) hyperandrogenism (hirsutism and/or hyperandrogenemia) and (2) ovarian dysfunction (oligo-anovulation and/or polycystic ovaries) [6]. Thus, agreement over diagnostic criteria has not yet been reached.

The new criteria for PCOS diagnosis proposed by the Japan Society of Obstetrics and Gynecology

The serum androgen level is known to be lower and the frequency of hyperandrogenism such as hirsutism is also lower in Asian women, including Japanese, than Caucasian women. Therefore, there is a need for the establishment of diagnostic criteria for PCOS that match the current situation in Asia, taking ethnic differences into consideration, but without the contradictions of the criteria used in the United States and Europe. The Japan Society of Obstetrics and Gynecology (JSOG) re-examined its original diagnostic criteria, proposed in 1993 (JSOG criteria 1993), considering several problems and overseas trends, and published new diagnostic criteria in 2007 (JSOG criteria 2007) [7].

The JSOG criteria 2007 require the presence of all of the following three conditions: (1) menstrual irregularities such as oligo-anovulation; (2) polycystic ovaries by ultrasonography; and (3) high levels of serum androgens or luteinizing hormone (LH) (normal range of follicle-stimulating hormone (FSH)). The JSOG criteria 2007 is compatibile with the Rotterdam criteria 2003. Moreover, the JSOG criteria 2007 is unique in that elevated LH concentration is used as an indicator of hyperandrogenism, based on the interpretation that androgen excess results from increased androgen activity in the pituitary–ovarian axis. It is probably the current optimal solution because it best matches the current circumstances of reproductive clinical practice in Asia, at least in Japan.

The role of androgens in follicular development

The ovarian follicle consists of an oocyte surrounded by granulosa and theca cells. It represents the basic functional unit of the ovary. Follicle growth through primordial, primary, secondary, and preantral stages is independent of gonadotropins, and is tightly regulated by oocyte-granulosa-theca cell interactions including growth factors, cytokines, and steroids [8]. Androgens have long been implicated as an inhibitor of antral follicle growth as they were suspected of inducing granulosa cell apoptosis and follicular atresia [9]. However, recent evidence indicates that the effect of androgens on follicle growth is dependent on the stage of follicular development, and that androgens also have a growth promoting role in early folliculogenesis [8]. Recent results also suggest that this growth-promoting role of androgens is regulated by oocyte-derived factor, e.g. growth differentiation factor (GDF)-9 [10].

The transition of the follicle from preantral to early antral stage is a turning point at which the main developmental regulatory system converts from intraovarian regulators to gonadotropins (especially FSH). In other words, this transitional stage is a critical period for the acquisition of FSH dependency by the follicle (Fig. 1). If hyper-activation of the theca cell/androgen system occurs in this preantral-early antral transition, then it might result in abnormal sensitivity of the follicle to FSH (e.g. poor or no response to gonadotropin stimulation) as described below.

Non-obese PCOS is typical in Asian women

The growth arrest of small antral follicles in PCOS is assumed to be associated with an abnormal endocrine environment involving increased LH stimulation, a hyperandrogenic milieu, and subsequent dysregulated FSH action in ovarian follicles [1]. Because PCOS includes various pathological conditions, there might be ethnic differences in the causes of the disease that enhance the activities of LH and androgens in ovaries. For instance, hyperinsulinemia due to insulin resistance potentiates LH action in the ovaries of obese PCOS, which is typical in Caucasian women [1, 11].

On the other hand, a dysfunctional hypothalamic-pituitary-ovarian axis and subsequent abnormal LH secretory dynamics increase LH stimulation and LH bioactivity in the ovaries of non-obese PCOS, which is typical in Asian women [12, 13]. The frequency of obese PCOS in Japan was 26–33%, and the majority of cases were non-obese PCOS according to a national survey of PCOS by the

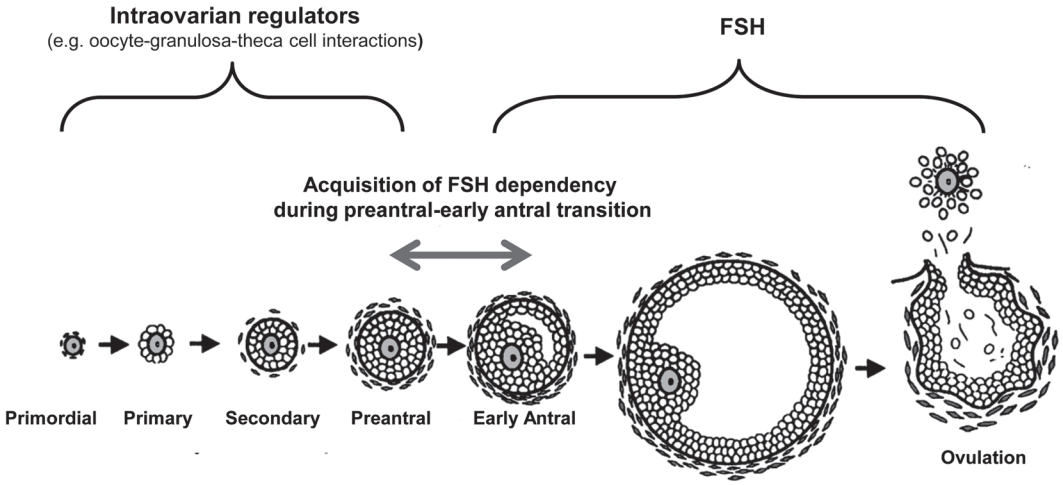

Fig. 1. The preantral-early antral transition is a critical period for the acquisition of FSH dependency by follicles. (Reproduced from Orisaka et al. [8])

JSOG [7]. Non-obese PCOS has also accounted for 80% of the PCOS cases we have treated, and obese PCOS was only 20%. In view of these circumstances, treatment for insulin resistance, which is emphasized in the United States and Europe, is inappropriate for PCOS treatment in Asia, at least in Japan. The adverse effect(s) of the chronically elevated LH on the theca cell/androgen system of Asian women with PCOS should also be considered.

LH promotes preantral follicle growth by up-regulating thecal androgen biosynthesis

There is emerging evidence for an intrinsic abnormality of folliculogenesis in PCOS that affects not only the gonadotropin-dependent antral stage but also the preantral stages, raising the possibility that significant abnormalities during preantral follicle development may be the root cause of anovulation in PCOS [14]. However, few studies have examined the relation between early follicular development and LH. Accordingly, we aimed to create an *in vitro* model of non-obese PCOS typical in Asian women. Using a rat preantral follicle culture system, we examined how LH modulates follicular development and steroid production during the preantral-early antral transition [15].

Large preantral follicles isolated from the ovaries of juvenile female rats were stimulated with different concentrations of LH or 5α-dihydrotestosterone (DHT), a non-aromatizable androgen. LH enhanced follicular growth in a concentration-dependent manner. Morphologically, both granulosa and theca cells in LH-stimulated follicles became thickened and multilayered, and the follicular size reached that of early antral follicles (i.e. > 200 μm in diameter). DHT also increased preantral follicle growth in a concentration-dependent manner. However, unlike LH-stimulated follicles, only granulosa cells grew and theca cells remained thin in the DHT-stimulated follicles [15].

LH augments testosterone production in preantral follicles via up-regulating mRNA expression of the rate-limiting enzyme, 17α-hydroxylase. Although LH enhanced preantral follicle growth *in vitro*, this LH-induced follicular growth was completely inhibited by a specific androgen receptor antagonist and a targeted disruption of androgen receptor gene [15].

These findings suggest that LH promotes follicular development, especially during the preantral-early antral follicle transition, by up-regulating thecal androgen biosynthesis. This fact may be relevant to clinical reports that human menopausal gonadotropins (hMG), which consist of LH and FSH, are more effective than recombinant FSH in ovulation induction of older infertile women. In patients over 39 years old undergoing IVF, women treated with hMG require a significantly lower total amount of FSH, and a lower amount of FSH per oocyte than women treated with recombinant FSH [16].

Sustained LH stimulation impairs FSH-dependent follicular growth by suppressing FSH receptor expression

To determine the influence of LH and androgen on follicular sensitivity to gonadotropins during the preantral-early antral transition, real-time quantitative PCR and functional analysis of the FSH receptor (FSHR) were performed on cultured preantral follicles [15]. Sustained

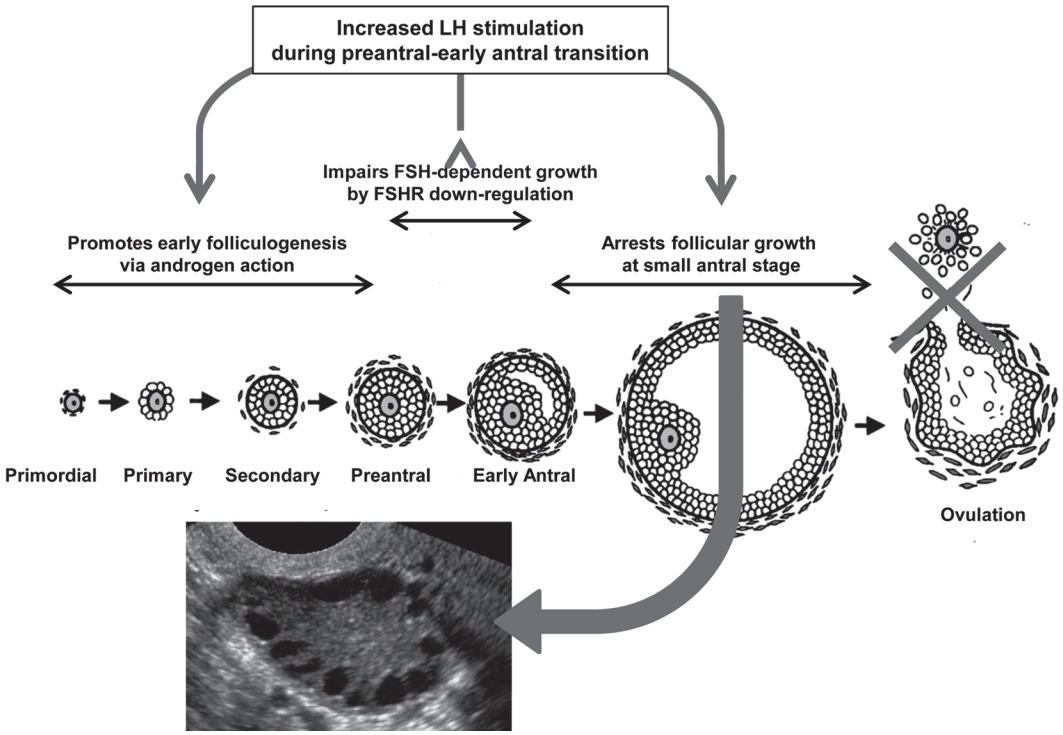

Fig. 2. A hypothetical model illustrating how increased LH stimulation impairs FSH-dependent follicular growth beyond early antral stage. (Reproduced from Orisaka et al. [15])

follicle stimulation by LH, but not by androgen, significantly suppressed mRNA expression and the signaling of FSHR in preantral follicles. Chronic LH stimulation also inhibits FSH-induced follicular development during the preantral-early antral transition [15].

These facts suggest that LH promotes the preantral-early antral transition via the increased synthesis and growth promoting actions of androgen. However, following chronic LH stimulation impairs FSH-dependent antral follicle growth by suppressing granulosa cell FSHR expression via the modulation of intraovarian regulators, including LH-induced thecal factors (Fig. 2).

Conclusion

Ovarian follicular development is controlled by a delicate balance between pituitary gonadotropins and intraovarian regulators. Although FSH plays a central role in the regulation of ovarian folliculogenesis, LH is also essential for normal follicular development, oocyte maturation, and ovulation. Several clinical studies have shown a correlation between hypersecretion of LH and PCOS, infertility, and miscarriage, suggesting that chronically elevated LH may be an important etiological factor in female fertility [17]. Recent research into gonadotropic control of ovarian function and clinical evidence has established that both a threshold and a ceiling for LH concentrations exist during follicular development [18].

The preantral-early antral transition is a critical stage for the acquisition of FSH dependency by the follicle. Exposure to a high-LH environment such as that in non-obese PCOS might promote the preantral-early antral transition via the increased synthesis and growth promoting actions of androgen. However, after chronic LH stimulation, the enlarged follicles which have reached the early antral stage have lost their responsiveness to FSH, and are unable to grow in a FSH-dependent manner. The action of LH on FSHR expression during this transitional stage might be relevant to the narrow therapeutic range of PCOS patients who exhibit resistance to clomiphene citrate but are hyper-responsive to exogenous gonadotropins.

Many anovulatory women with PCOS exhibit abnormal gonadotropin secretary dynamics, and the most typical abnormalities are increased serum LH levels, with normal or slightly suppressed serum FSH concentrations [1]. However, we do not intend to exclusively attribute the primary cause of PCOS to a dysfunctional hypothalamic-pituitary-ovarian axis and subsequent abnormal LH secretary dynamics. These pattern could result from a decrease in hypothalamic inhibition of pulsatile GnRH

secretion [19], or abnormalities in steroid hormone feedback, including a lack of progesterone (due to anovulation) [20], increased circulating androgen levels [21], or disrupted estrogen receptor signaling [22, 23]. Nevertheless, if an increased serum LH level, such as that seen in non-obese PCOS, is left untreated, then the patients' condition could be drawn into a negative spiral in which the ovarian androgen excess deteriorates and dysregulation of follicle growth becomes more severe.

Aside from the causes of PCOS, there has always been a need to clarify the pathological conditions that might require treatment and the development of treatments of PCOS research [14]. Based on the results of recent studies, we have been continuing infertility care and basic research, searching for therapeutic innovation such as the possibility of higher safety and efficacy in ovulation induction with clomiphene citrate or low-dose FSH agents after reduction of the serum LH/androgen levels by progesterone supplementation or estrogen/progesterone supplementation (such as the Kaufmann therapy or low-dose oral contraceptive) at first in non-obese PCOS patients who desire to have children.

References

1) Fritz MA, Speroff L eds. (2011) Clinical gynecologic endocrinology and infertility. 8th ed. Philadelphia: Lippincott Williams & Wilkins
2) Stein IF, Leventhal ML (1935) Amenorrhea associated with bilateral polycystic ovaries. Am J Obstet Gynecol 29, 181–191.
3) Zawadzki JK, Dunaif A (1992) Diagnostic criteria for polycystic ovary syndrome: towards a rational approach. In: Dunaif A, Givens JR, Haseltine FP, Merriam GR (eds) Polycystic ovary syndrome. Blackwell Scientific, Boston, pp. 377–384.
4) Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group (2004) Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod 19, 41–47.
5) Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group (2004) Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril 81, 19–25.
6) Azziz R (2006) Diagnosis of polycystic ovarian syndrome: The Rotterdam criteria are premature. J Clin Endocrinol Metab 91, 781–785.
7) Kubota T (2013) Update in polycystic ovary syndrome: new criteria of diagnosis and treatment in Japan. Reprod Med Biol 12, 71–77.
8) Orisaka M, Tajima K, Tsang BK, Kotsuji F (2009) Oocyte-granulosa-theca cell interactions during preantral follicular development. J Ovarian Res 2, 9.
9) Billig H, Furuta I, Hsueh AJ (1993) Estrogens inhibit and androgens enhance ovarian granulosa cell apoptosis. Endocrinology 133, 2204–2212.
10) Orisaka M, Jiang JY, Orisaka S, Kotsuji F, Tsang BK (2009) Growth differentiation factor 9 promotes rat preantral follicle growth by up-regulating follicular androgen biosynthesis. Endocrinology 150, 2740–2748.
11) Franks S, Gilling-Smith C, Watson H, Willis D (1999) Insulin action in the normal and polycystic ovary. Endocrinol Metab Clin North Am 28, 361–378.
12) Marshall JC, Eagleson CA, McCartney CR (2001) Hypothalamic dysfunction. Mol Cell Endocrinol 183, 29–32.
13) Blank SK, McCartney CR, Marshall JC (2006) The origins and sequelae of abnormal neuroendocrine function in polycystic ovary syndrome. Hum Reprod Update 12, 351–361.
14) Franks S, Stark J, Hardy K (2008) Follicle dynamics and anovulation in polycystic ovary syndrome. Hum Reprod Update 14, 367–378.
15) Orisaka M, Hattori K, Fukuda S, Mizutani T, Miyamoto K, Sato T, Tsang BK, Kotsuji F, Yoshida Y (2013) Dysregulation of ovarian follicular development in female rat: LH decreases FSH sensitivity during preantral-early antral transition. Endocrinology 154, 2870–2880.
16) Mohamed MA, Sbracia M, Pacchiarotti A, Micara G, Linari A, Tranquilli D, Espinola SM, Aragona C (2006) Urinary follicle-stimulating hormone (FSH) is more effective than recombinant FSH in older women in a controlled randomized study. Fertil Steril 85, 1398-1403.
17) Regan L, Owen EJ, Jacobs HS (1990) Hypersecretion of luteinising hormone, infertility, and miscarriage. Lancet 336, 1141–1144.
18) Balasch J, Fabregues F (2006) LH in the follicular phase: neither too high nor too low. Reprod Biomed Online 12, 406–415.
19) Barnes RB, Lobo RA (1985) Central opioid activity in polycystic ovary syndrome with and without dopaminergic modulation. J Clin Endocrinol Metab 61, 779–782.
20) Berga SL, Yen SS (1989) Opioidergic regulation of LH pulsatility in women with polycystic ovary syndrome. Clin Endocrinol (Oxf) 30, 177–184.
21) Eagleson CA, Gingrich MB, Pastor CL, Arora TK, Burt CM, Evans WS, Marshall JC (2000) Polycystic ovarian syndrome: evidence that flutamide restores sensitivity of the gonadotropin-releasing hormone pulse generator to inhibition by estradiol and progesterone. J Clin Endocrinol Metab 85, 4047–4052.
22) Couse JF, Yates MM, Walker VR, Korach KS (2003) Characterization of the hypothalamic-pituitary-gonadal axis in estrogen receptor (ER) Null mice reveals hypergonadism and endocrine sex reversal in females lacking ERalpha but not ERbeta. Mol Endocrinol 17, 1039–1053.
23) Taniguchi F, Couse JF, Rodriguez KF, Emmen JM, Poirier D, Korach KS (2007) Estrogen receptor-alpha mediates an intraovarian negative feedback loop on thecal cell steroidogenesis via modulation of Cyp17a1 (cytochrome P450, steroid 17alpha-hydroxylase/17,20 lyase) expression. FASEB J 21, 586–595.