Mechanisms of infertility in polycystic ovary syndrome

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ABSTRACT: It has been proposed that the follicular problem in polycystic ovary syndrome is 2-fold. First, the intra-ovarian hyperandrogenism may promote early follicular growth, leading to a 2-5mm follicle excess. Second, the ensuing excessive number of selectable follicles would inhibit the selection process, presumably through follicle to follicle interaction involving granulosa cell products such as the anti-Müllerian hormone. These factors would induce a reversible refractoriness to the FSH-induced differentiation of granulosa cells. This explanation challenges but does not exclude other hypotheses about the follicular arrest, such as the premature LH action on the granulosa cells of selectable follicles. Hyperinsulinism or insulin resistance would act as a second hit, worsening the follicular arrest either through amplification of the intra-ovarian hyperandrogenism or through dysregulation of the granulosa cells. The loss of cyclic rhythm would prevent the inter-cycle elevation of FSH, thus perpetuating of the ovulation process.

Key Words: Androgens, Anti-Müllerian hormone, Folliculogenesis, Follicular arrest, Polycystic ovary syndrome.

INTRODUCTION

It is widely agreed that the polycystic ovary syndrome (PCOS) is the most common endocrine disorder, affecting up to 10 percent of reproductive age women. However, there is little agreement concerning the underlying molecular mechanisms of PCOS, hence precise mechanism-targeted therapies are not available. Most investigators agree that PCOS encompasses a group of several distinct disorders characterized by oligo-ovulation and hyperandrogenism, generally associated with hyperandrogenemia, increased gonadotropins and polycystic morphology of the ovaries. Insulin resistance, metabolic syndrome X and obesity are common. The past 20 years of clinical investigation have produced new insights. For many years, investigators debated whether the primary defect was in the hypothalamic-pituitary-gonadal axis or in the ovary itself. Most now agree that hyperandrogenemia is primary and, in most patients, is of combined ovarian and adrenal origin.

Polycystic ovary syndrome is the commonest cause (70%) of anovulatory infertility. The primary abnormality seems to be an excess of androgen production within the ovary that leads to the recruitment of large numbers of small preovulatory follicles, which fail to respond to normal concentrations of follicle stimulating hormone (FSH). Thus, a dominant follicle is rarely produced. Women with polycystic ovary syndrome commonly present in their late teens or early twenties with hirsutism, acne, or irregular periods (cycle length > 35days). Even if they ovulate the chance of conception for these women is reduced because fewer ovulatory events occur in a given time frame. Only a third of women with polycystic ovary syndrome are obese, but obesity increases the likelihood of a woman with the syndrome developing anovulation.

It has been proposed to divide the follicular problem of PCOS into two main components: first, the early follicular growth is excessive; second, the selection of one future dominant follicle from this increased pool does not proceed (follicular arrest).
Early follicular growth in PCOS
Polycystic ovaries are characterized by an abnormally rich pool of growing follicles, the number of which is 2-6-fold that of normal ovaries, from classes 1-5, except the pool of primordial follicles which is normal. This excess of follicle could be the consequence of an increase in initiation and/or subsequent follicle growth. The initiation of follicular growth is believed to be a continuous process. Factors triggering initial recruitment are not completely identified but stem cell factor or kit-ligand and basic fibroblast growth factor (bFGF) appeared to be attractive candidates. These factors stimulate some primordial follicles to enter growth, whereas the rest of the follicles remain quiescent for months or years. Follicles then enter the growing pool of large primary follicles, reach the preantral stage after some months, and finally need about seventy additional days to reach 2mm in diameter (mid-antral stage).

Initiation is also under a negative control, exerted by anti-Müllerian hormone (AMH), a member of the transforming growth factor-β (TGF-β) superfamily. Indeed, ovaries of AMH-knockout mice are depleted of their primordial follicles earlier than they are in control mice. This decrease is caused by increased recruitment of primordial follicles in AMH-knockout females, since more preantral and small antral follicles are found in pre-pubertal and adult AMH-knockout mice. These results suggest that AMH may inhibit initiation of follicle growth. However, since it is not produced by resting follicles but by early growing follicles, it appears likely that AMH acts on resting follicles through a paracrine effect from neighbor growing follicles.

Initiation of follicular growth
The question is if the initiation of follicle growth is excessive in PCOS. It has been reported that the primordial follicle pool was normal in size, compared to control ovaries. However, it was suggested that the initiation of follicle growth could be stimulated to excess in PCO. Indeed, the mean proportion of primordial follicles was lower than in normal ovaries while the mean proportion of primary follicles was higher. Theoretically, these would induce premature follicle depletion and would accelerate the onset of menopause, which is the case in the AMH-knockout mice but not in PCOS. In addition, the ovarian production of AMH is excessive in PCOS. It is therefore still unclear whether initiation of folliculogenesis is altered or not in PCOS.

Excessive early follicle growth
Another question that arises is if the early follicle growth is excessive. After initial recruitment granulosa cells in primary follicles proliferate. The oocyte continues to grow, the zona pellucida is formed, theca interna cells differentiate, and the vascular supply develops. Compared with the initial recruitment process, substantially more is known about this phenomenon. Oocyte granulosa cells, theca interna cells interactions may play an essential role in the development of early follicles, via their secretions.

In PCOS, a generalized «multifolicularity» beyond the primordial stage has been indicated, which appears as the main feature of PCO. With regard to their important effects on the small follicle growth, the intra-ovarian hyperandrogenism, which is the fundamental feature of PCOS, is designated as the main culprit for this follicle excess.

Although the role of sex steroids in preantral follicle development remains unclear, recent studies highlight the effect of androgens in early follicle growth. This effect on folliculogenesis predominates in small follicles, due probably to their richness in androgen receptors.

In the androgenized monkey model, the gross anatomic appearance of ovaries was very close to the one observed in ovaries from women with PCOS, after only 10 days of exposure to androgens. In line with these experimental data, congenital adrenal hyperplasia, virilizing tumours and exogenous androgen treatment are associated with increased numbers of non-ovulatory antral follicles similar to those seen in women with ‘idiopathic’ PCOS. Many of these ‘cystic’ follicles have healthy steroidogenic and growth characteristics. Furthermore, it has been reported that the 2.5mm follicle number at ultrasound was positively correlated to the serum testosterone and androstenedione levels in patients with PCOS. This supports the hypothesis that the increased number of small follicles is due to the trophic effects of androgens, whether increased locally in the ovary as in PCOS, or systemically as in the other conditions. All...
these evidence support the concept that androgens are not in fact atretogenic in primate and human ovary. Previous data, however, showed that androgens are atretogenic\textsuperscript{32,33}, a cornerstone of the PCOS pathophysiology for a long time. This discrepancy may be explained by significant interspecies differences in ovarian follicular development to different experimental conditions.

Androgen biosynthesis in the human ovary takes place primarily in theca interna cells, whose function is excessive in PCOS. The excess of androgen production can be explained by extra-ovarian, as well as intra-ovarian factors.

**Extra ovarian factors**

As far as extra ovarian factors are concerned, these factors include high plasma LH concentrations and hyperinsulinemia. The increase in plasma LH concentration is attributed to both increased LH pulse frequency and LH pulse amplitude\textsuperscript{34}. The resulting elevated serum LH concentration promotes ovarian theca interna cells steroidogenesis\textsuperscript{35}. The mechanism for the LH hypersecretion remains unclear, but fewer workers now consider it to be a primitive phenomenon. Recent data suggest that it results from an impaired negative feedback on LH secretion, due to excessive androgen action on the hypothalamic-pituitary axis\textsuperscript{36}.

Hyperinsulinemia provides another extra-ovarian determinant of hyperandrogenism by enhancing the effects of LH on theca interna cells steroid production. Experimental evidence\textsuperscript{37} was recently confirmed by the clinical observation that serum androgen concentrations decrease in women in whom insulin concentrations are lowered with insulin sensitizing agents or by weight loss\textsuperscript{38,39}.

**Intra ovarian factors**

The intra ovarian factors include intrinsic theca dysregulation, granulosa dysregulation and oocyte factors.

Using a system of proliferating human theca interna cells in long-term culture, Nelson et al\textsuperscript{40} have shown that enhanced production of androgens is a persistent biochemical phenotype of theca interna cells from PCO. Since these theca interna cells cultures can be maintained through multiple population doublings, the increased steroidogenic activity of theca interna cells from PCO compared with normal theca interna cells is unlikely to reflect the influence of in vivo hormonal stimulation. Consequently, these observations suggest that dysregulation of androgen biosynthesis is an intrinsic property of theca interna cells from PCO. Several experiments suggest that transcription of genes encoding specific steroidogenic enzymes is naturally up-regulated in PCOS theca interna cells, but not all components of the steroidogenic machinery are concerned\textsuperscript{41,42}. It leads to increased production of progestins and androgens. This suggests that hyperandrogenemia is genetically determined, in line with the result of familial studies indicating that hyperandrogenism clusters is a dominant genetic trait\textsuperscript{43}. However, it is unlikely that the hyperandrogenemia of PCOS is principally determined by polymorphisms or mutations in the genes encoding a single steroidogenic enzyme activity\textsuperscript{44-47}.

Although theca dysregulation seems to be the main culprit of intra-ovarian hyperandrogenism, granulosa dysregulation may also play a role, via granulosa cells secretions.

Ovarian granulosa cells produce inhibins which are thought to modulate directly follicular steroidogenesis. Inhibins might be involved in the excess of intra-ovarian androgens in PCOS through a paracrine effect from granulosa cells\textsuperscript{48}. The use of immunoassays that detect serum dimeric inhibins has allowed us to elucidate a peculiar dysfunction of the inhibin system in PCOS. This data could support the hypothesis that inhibins participate in the hyperandrogenism of PCOS\textsuperscript{49}. However, there have been found minimal differences in the levels of inhibin B between controls and patients with PCOS and a decrease in mature dimeric inhibin A\textsuperscript{50}. These results leave the question whether the increase of inhibins is only a marker of theca hyperactivity in PCOS or whether it reflects the exaggeration of a putative relationship between inhibins and androgen production by theca interna cells.

Recent data about AMH, which is produced by granulosa cells, may suggest that it could be implicated in the hyperandrogenism of PCOS. Indeed, the ovarian production of AMH is excessive in PCOS\textsuperscript{51,52}. A positive and significant relationship between AMH
and serum testosterone and androstenedione levels in PCOS patients was found, but not in controls. This could suggest a paracrine positive effect of AMH on theca interna cells. However, further experimental studies are required before speculating on implications of AMH in the theca dysregulation of PCOS.

It has also been proposed that the oocyte may play a role in regulating theca interna cells activity but the available data are still controversial. Growth Differentiating Factor-9 (GDF-9) stimulates both basal and LH-stimulated androgen biosynthesis by rat theca interna cells. In contrast, the treatment of human theca interna cells in vitro with GDF-9 increased their proliferation but blocked forskolin-stimulated progesterone and androgen synthesis. The level of GDF-9 expression has been reported to be reduced in polycystic ovaries and one could speculate that a low level of GDF-9 is one of the causes enhancing androgen synthesis in PCOS follicles. However, these data are too rare presently to cast the oocyte a role in the pathophysiology of hyperandrogenism in PCOS.

In conclusion, the first follicular abnormality in PCOS is an increased number of early-growing and selectable follicles, which is presumably the consequence of intra-ovarian hyperandrogenism. The accumulation of 2-5mm follicles gives the typical aspect of multifollicular ovaries at ultrasonography, which is in close relationship with the androgen serum levels.

**Follicular arrest**

The second component of the follicular problem in PCOS is the follicular arrest. Follicular arrest means that the selection of one dominant follicle is impaired, despite the excess in the number of selectable follicles. This second abnormality in folliculogenesis explains the anovulation of PCOS.

The follicular arrest has not received yet a clear explanation. Furthermore, in contrast to the excess in small follicles, it does not always occur in patients with PCOS and some of them do even ovulate monthly. Lastly, the follicular arrest can be easily reversed in most cases by pharmacological manipulations aiming at increasing the amount of FSH reaching the ovaries. Many selectable follicles can therefore be rescued, which suggest that in PCOS, unknown factor(s) protect(s) them partially from atresia, which normally should happen because of their stagnation. The main clinical consequence of this is the well-known increased risk for ovarian hyperstimulation syndrome in PCOS.

Several mechanisms can be hypothesized to explain the follicular arrest in PCOS. One of these mechanisms could be the absence of inter-cycle FSH rise in PCOS: although normogonadotropic anovulatory patients display bioactive and immunoreactive FSH levels in the range of those during the normal menstrual cycle, these patients lack the inter-cycle FSH rise. The enlargement of the pool of selectable follicles could explain this, via an excessive production of inhibin B, leading to a suppression of FSH release by a negative feed-back effect. However, there is no clear-cut increase in the inhibin B serum levels in PCOS, and several authors have previously reported that the serum FSH and inhibin B levels do not correlate negatively. Therefore, the absence of inter-cycle FSH rise is more likely due to the absence of ovulation and the subsequent absence of luteum corpus and luteolysis during a preceding cycle. Hence, it is rather a secondary phenomenon than a primary defect.

Another possible mechanism might be a defect in positive local selectors: although it is well recognized that the IGF system is highly involved in the dominant follicle growth, there is as yet no convincing evidence to confirm the hypothesis that a derangement in the IGF system is the central abnormality in PCOS follicular arrest. This is understandable since the mechanisms of the latter apply to selectable and not to selected follicles.

Other mechanism could be an excess of negative local selectors: several experimental and clinical arguments give support to the hypothesis that the follicular arrest is due to an excess of local inhibitor or inhibitors of FSH activity.

Among the potential inhibitors, the presence in serum and follicular fluid of an excessive level of factors inhibiting FSH receptor activation has not been confirmed. IGFBP-4 may be an attractive candidate. However, alterations in IGFBP may sustain the anovulatory steady state in PCOS but are unlikely to initiate development of the syndrome.

One of the still unknown factors secreted local-
ly by the selectable follicles and inhibiting the FSH effects could be the AMH. It has been shown that women with PCOS have significantly higher AMH levels in both serum and follicular fluid than normal women\(^{51,52,60}\). Moreover, the marked increase in serum AMH levels was positively and tightly related to the excess of 2-5mm follicle number and ultrasound. Furthermore, a negative correlation between AMH and FSH serum levels, both in PCOS and controls, was observed\(^{41}\). In addition, an inverse relationship between AMH and estradiol serum levels in women with PCOS was reported\(^{51}\). Thus, it is speculated that the AMH excess is involved in the lack of FSH-induced aromatase activity, which characterizes the follicular arrest of PCOS\(^{67}\). Alternatively, an endocrine action of AMH could be suggested since the increase in the circulating AMH levels in PCOS patients is 2-3-fold the one of normal women\(^{52}\). Nevertheless, the strong and independent positive correlation that has been found between follicle number and serum AMH level argues in favour of the hypothesis that the excess in 2-5 mm follicle number per se is responsible for this excess of AMH production. Altogether, these data concerning AMH make it a good candidate to explain the auto-inhibiting effect of selectable follicles, more particularly on aromatase, thus checking the selection process. Despite the subnormal serum FSH level in PCOS, the negative effect exerted by FSH on AMH would not be sufficient to permit the follicles to escape from the AMH tone and to start to express aromatase.

This hypothesis is supported by the negative correlation that it was found recently between the small (2-5mm) and the larger (6-9mm) antral follicle number at ultrasound, both in normal and PCOS women\(^{68}\). This negative relationship reflects an inhibitory mechanism exerted by the selectable follicles on their further maturation. This physiological phenomenon, involving local inhibitory factors, would be exaggerated in PCOS.

Other mechanisms may also involve the premature action of LH on granulosa cells of follicles in women with PCOS. Physiologically, granulosa cells develop their own LH receptors in the mid-late follicular phase\(^{60}\). Thus, LH takes the control of terminal follicular growth and enhances estradiol and progesterone production by granulosa cells, while inhibiting their proliferation. Although an excessive serum LH level is not always observed in patients with PCOS and does not seem to be necessary to anovulation, premature LH action on granulosa cells is supported by some experimental data\(^{14}\). This hypothesis provides an alternative explanation for the follicular arrest.

In granulosa cell cultures from anovulatory PCOS patients, ovulatory PCOS patients and controls in the presence of LH, it was found that the granulosa cells from anovulatory PCOS patients responded much earlier with estradiol secretion to LH, at a follicle size of 4mm instead of 9.5-10mm, than those from the other two patient categories. These results suggest an earlier LH receptor gain in anovulatory patients\(^{70}\). These data indicate that granulosa cells from anovulatory women with PCOS are at a prematurely advanced stage of development, leading to arrest of cell proliferation, stagnation of follicle growth and anovulation. In line with these experimental data, it has been reported a positive and significant relationship between serum LH and inhibin B levels in women with PCOS but not in controls\(^{71}\), suggesting that in the former, LH is operating earlier on granulosa cells function than in the latter.

Hyperinsulinemia resulting from insulin resistance has been suspected to influence the process of premature differentiation of granulosa cells\(^{66}\). It has been earlier shown in vitro\(^{72}\) that insulin increases the ability of granulosa cells to respond to LH suggesting that raised insulin levels in anovulatory women with PCOS may be a major factor causing the follicular arrest. In this situation, hyperinsulinemia may disturb the FSH to LH ratio that triggers the correct follicular development until ovulation.

To date the oocyte abnormalities are certainly the least documented. It has been reported\(^{73}\) that in prenatally androgenized monkeys undergoing ovarian stimulation for IVF, oocyte developmental ability is impaired, as indicated by a decreased percentage of zygotes developing into blastocysts. An apparent explanation is an impaired acquisition of maternally derived proteins and/or transcripts that are important for embryonic gene activation.

A primary oocyte abnormality may also be in-
volved in PCOS disturbed folliculogenesis since the level of growth differentiation factor-9 mRNA appeared to be reduced in primary oocytes from PCO during their growth and differentiation phase. However, it remains to be determined whether the reduced GDF-9 mRNA levels participate in the PCOS follicular arrest or are simply the consequence of another primary abnormality. Nevertheless, the possibility of intrinsic oocyte abnormalities opens a new track, and GDF-9 as well as other oocyte factors may be added to the list of suspects.

A very important subject in the area of PCOS is the fact that the follicular arrest is inconstant: PCOS is a cause of oligo-ovulation, rather than anovulation. From time to time for unknown reasons, a dominant follicle is able to escape from the inhibitory intra-ovarian influence and proceeds towards ovulation and formation of a corpus luteum. Because of these random ovulations, the fertility rate in untreated patients is not null, although that is less than in normal women. Moreover, some patients with PCOS ovulate regularly and have a normal fertility, despite the presence of a clinical and/or biological hyperandrogenism. Nevertheless, a recent meta-analysis of human IVF data in PCOS women did not show any difference in oocyte fertilization, pregnancy and miscarriage rates between PCOS and non-PCOS women.

Attempts to elucidate the factor or the factors which differentiate ovulatory from anovulatory women with PCOS can provide important clues, but they are seldom in the literature.

It is clear that obesity and/or hyperinsulinemia have a negative effect on the ovulation rate. However, rather than being the primary cause of anovulation in PCOS, hyperinsulinemia and/or insulin resistance may be viewed as a «second hit» that non-specifically worsens the follicular arrest. The précised target of this hit remains to be confirmed. Is it at the time of early follicular growth, through enhancement of the follicle number via insulin-induced theca interna cells hyper-function and ovarian hyperandrogenism? Is it at the time of selection, through deleterious effects of hyperinsulinemia and/or insulin resistance on granulosa cells?

Another hypothesis of an auto-blocking effect within the pool of selectable follicles is that the excess in the follicle number and/or in AMH is less in ovulatory patients.

Patients’ age could also differentiate ovulatory from anovulatory women with PCOS it has been reported recently that the decrease in the size of the follicle cohort due to ovarian ageing seemed to be largely responsible for the higher incidence of regular menstrual cycles in women with PCOS aged more than 35 years. Likewise, it is known that patients with regular cycles were significantly older than those with either oligo- or amenorrhea. Jonard et al reported that the age was a negative determinant of the 2-5 mm follicle number in women with PCOS. Finally, it has been referred that the probability of women with PCOS to be resistant to clomiphene citrate is much higher when the baseline 2-5 mm follicle number is more than 20.

From all these data, it appears therefore that ovulation in PCOS, either spontaneous or induced, is highly regulated by the degree of small follicle excess. This supports the hypothesis that the latter is principally responsible for the follicular arrest, and that its negative effect might be exerted through excessive AMH production.

**CONCLUSIONS**

It is possible that the follicular problem in PCOS is 2-fold. Firstly, the intra-ovarian hyperandrogenism promotes early follicular growth and leads to a 2-5mm follicle excess. Secondly, an impaired action of FSH and/or a premature LH action prevent the selection of a dominant follicle from this increased pool.

Actually, these two abnormalities may be only one, at least in some patients with PCOS. With regard to recent pathophysiological data the intra-ovarian hyperandrogenism may be the main culprit for the follicular arrest. Indeed, it seems to lead to follicle excess, which in turn increases the AMH intra-ovarian level and then could exert an inhibiting effect on the FSH-induced aromatase activity. Thus, the follicular arrest may be the consequence of the follicle excess, via a phenomenon of auto-inhibition within the cohort due to follicle interactions.

This hypothesis does not exclude that hyperinsulinemia may play a role in prematurely advancing
granulosa cells differentiation, leading to a worsening of follicular arrest. Intrinsic oocyte abnormalities, such as GDF-9 expression, might be involved in PCOS follicular arrest as well as in defective embryo survival.

Finally, research in genetics, which has not contributed very much so far to this specific topic, perhaps will allow the play entitle «The Disturbed Folliculogenesis of PCO» to proceed a gain in the near future, with new actors and a new plot.

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