J. Mamm. Ova Res. Vol. 31 (1), 23–30, 2014

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—Mini Review—

Excessive Androgen Exposure as An Etiological Factor of Polycystic Ovary Syndrome

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Abstract: Polycystic ovary syndrome (PCOS) is a heterogeneous group of disorders characterized by ovulation disorder, hyperandrogenism, and polycystic ovarian morphology (PCOM). Several diagnostic criteria suggest that hyperandrogenism is a core symptom of PCOS. Androgens are believed to cause preantral follicle growth and arrest the growth of antral follicles. This results in accumulation of small antral follicles in the ovaries, thus forming PCOM. Observational studies of patients with female-to-male transsexualism or congenital adrenal hyperplasia indicate that androgen administration to these patients does not produce typical PCOS-like features. However, endogenous androgen exposure in early life may lead to some traits of PCOS in adulthood. To reveal the association between the timing of excess androgen exposure and reproductive function, various animal models have been investigated using androgen administration. Rhesus monkeys exposed to excess androgen during the early fetal period show a PCOS-like phenotype, including metabolic and hypothalamic-pituitary characteristics. This finding implies that exposure to excess androgen during this critical period programs the hypothalamic-pituitary-ovary axis and metabolic organs. Although findings obtained in animal studies will not necessarily be replicated in humans, prenatal androgen excess is the dominant PCOS hypothesis.

Key words: Polycystic ovary syndrome, Excess androgen exposure, Female-to-male transsexualism, Congenital adrenal hyperplasia, Animal model

Introduction

Polycystic ovary syndrome (PCOS) is characterized by ovulatory disorder, hyperandrogenism, and polycystic ovarian morphology (PCOM), and affects approximately 5–10% of reproductive-age women [1, 2]. Endocrinological and metabolic disturbances are also common in PCOS, such as luteinizing hormone (LH) hypersecretion, increased LH pulse frequency and amplitude, insulin resistance, and hyperinsulinemia. The exact etiology of PCOS is unclear, and diagnostic criteria vary in different societies [3–6]. The main manifestation of PCOS is believed to be androgen excess, because all of the accepted diagnostic criteria place high emphasis on hyperandrogenemia or hyperandrogenism. In addition, the various diagnostic criteria also exclude other hyperandrogenic disorders, such as non-classical congenital adrenal hyperplasia (NC-CAH) and androgen-secreting tumors, because hyperandrogenism may elicit the same symptoms as those of PCOS.

The diagnostic nomenclature indicates that PCOM is an important characteristic of PCOS. In the criteria proposed by the Japan Society of Obstetrics and Gynecology, the presence of PCOM is essential for diagnosing PCOS [6]. Previously, androgen was believed to be an important factor of follicular atresia [7]. Therefore, the characteristics of the ovaries of PCOS patients were formerly considered to include aggregation of cystic atretic follicles and subsequent formation of PCOM. Recently, it has been suggested that excess androgen augments the initial recruitment of follicle growth, and arrests follicle maturation into dominant follicles, resulting in accumulation of excess small antral follicles [8]. Therefore, PCOM observed in PCOS patients is now believed to be

a consequence of ovulation disorder. This concept is further supported by evidence that inadvertent use of ovulation induction results in the resumption of the growth of a large amount of viable, but dormant, small follicles, and causes ovarian hyperstimulation syndrome. PCOS patients with PCOM are estimated to be at higher risk of developing ovarian hyperstimulation syndrome than those without PCOM. Therefore, careful management is needed when using ovulation induction agents to treat patients with PCOS who have PCOM.

Small antral follicles of PCOS patients have high concentrations of androgens, and these androgens are thought to cause arrest of maturity [9]. However, recent studies have indicated that anti-Müllerian hormone (AMH) is an important factor in the interruption of follicle maturation which leads to PCOS [10, 11]. Granulosa cells from PCOS patients have a high ability to produce AMH, and small antral follicles contain abundant AMH [12, 13]. AMH is also known to inhibit aromatase activity and contribute to increased androgen concentrations in the small antral follicles of PCOS patients [14]. These findings suggest that a hyperandrogenic state is not part of the pathogenesis of PCOS, but a sequela of AMH overexpression and ovulation disorder.

There is little doubt that hyperandrogenism is highly associated with PCOS. However, whether hyperandrogenism is a cause of PCOS or a consequence of ovulation disorder remains unclear. To determine the etiology and relationship of androgens with PCOS, various animal models involving exposure to excess androgens have been developed. Most of the animals used have been rodents because of their small size, short life-span, short estrous cycles, and low costs. Over 50 years ago, dehydroepiandrosterone (DHEA) treatment was used to develop a rat PCOS model [15]. Administration of DHEA to immature female rats is thought to be a surrogate model of premature adrenarche, which is believed to be one of the causes of PCOS in humans. DHEA treatment causes polycystic ovary and anovulation in rats [16]. However, the ovaries of DHEA-treated rats show increased numbers of atretic follicles, thin layers of theca cells, and reduced ovarian volume, characteristics which are dissimilar to those of human ovaries with PCOS. To date, various animal species and several types of androgens from the prenatal to the postnatal periods have been investigated, but rodent models of PCOS do not replicate the endocrinological or metabolic characteristics of this disorder [17]. Recently, a nonhuman primate model of PCOS was thought to be an important breakthrough. Prenatal androgenization of rhesus monkeys fulfilled all of the characteristics of human PCOS [18]. Nonhuman primates have androgen-mediated molecular pathways and follicle growth in the ovaries which are similar to those of humans. However, the results observed in rhesus monkeys will not necessarily be replicated in humans. In this article, we discuss the relationship between androgen exposure and PCOS.

Use of androgen Administration for modeling PCOS in humans

In humans, experimental androgen administration for determining its effects is not ethically justifiable. However, cross-sex hormone treatment to female-to-male transsexual (FTM-TS) patients is legitimate and provides data relevant to the underlying question. At our institution, a gender identity disorder clinic was established in 2003, and over 300 FTM-TS patients have been treated. Among these cases, we investigated 11 FTM-TS patients receiving long-term administration of androgen followed by bilateral salpingo-oophorectomy for sex-reassignment surgery [19]. The patients were initially diagnosed as not having PCOS, although two patients had PCOM. The initial timing of the treatment for all of these cases was older than 18 years, and they received 125–250 mg of testosterone enanthate biweekly for periods ranging from 17 months to 14 years (median: 38 months). In all cases, pituitary gonadotropin levels did not significantly change after androgen administration. Ovaries after these treatments displayed hypertrophy of the cortex and hyperplasia of the stroma, resembling ovaries in patients with Stein–Leventhal syndrome (Fig. 1). Although the numbers of early antral follicles did not increase, compared with normal ovaries, the numbers of atretic follicles increased in the androgen-treated ovaries. In addition, anovulation and hyperandrogenemia caused by androgen administration were transient, and after cessation of androgen treatment menstruation resumed in some patients [20]. These findings indicate that exposing reproductive-age women to a high dose of exogenous androgen does not lead to the development of typical PCOS.

Endogenous Excess Androgen Exposure and PCOS Traits

Classical congenital adrenal hyperplasia (CAH), mostly due to 21-hydroxylase deficiency, is known to cause endogenous hyperandrogenism during the prenatal to prepubertal periods. Interestingly, Barnes et al. reported that women with these disorders develop PCOS in adulthood, even though corticosteroid treatment is successful in early life [21]. They also reported menstrual disturbance,
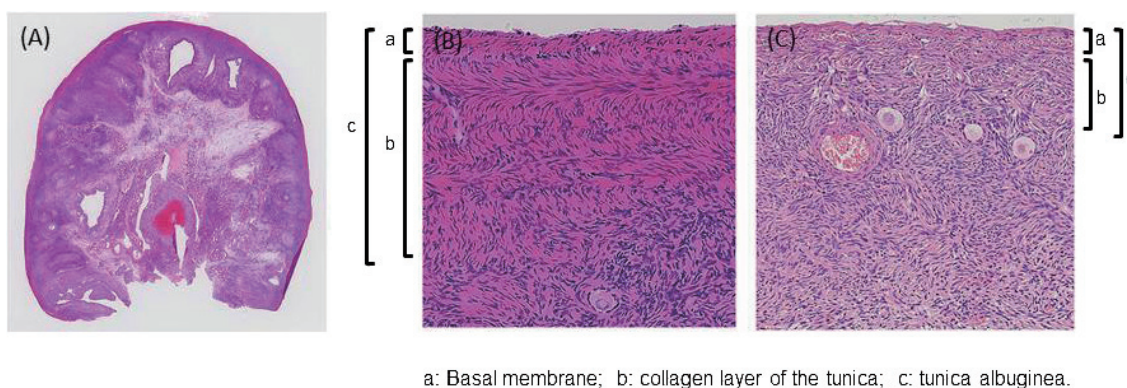
a: Basal membrane; b: collagen layer of the tunica; c: tunica albuginea.

Fig. 1. Effect of testosterone administration in FTM-TS.
(A) Low magnification findings. (B, C) High magnification findings of ovarian cortex in FTM-TS (B) and a control (C). The collagen layer is thickened in FTM-TS compared to the control.

ovarian hyperandrogenism, and LH hypersecretion, but the ovarian morphology and metabolic characteristics of these cases were not mentioned. Bachelot et al. reported that the pulse amplitude, frequency, and level of LH in CAH patients were similar to those in controls [22].

A mild form of CAH, called NC-CAH, does not show prenatal virilization, and develops signs of hyperandrogenism during the peripubertal period or later. Therefore, NC-CAH may be a model of juvenile androgenization in humans. In women with NC-CAH, PCOM was observed in 77% of cases, ovarian enlargement in 41%, obesity and overweight in 36.6%, oligomenorrhea in 54.4%, and hirsutism in 80.3% [23, 24]. Unfortunately, other PCOS traits, such as metabolic disturbances, were not assessed in these previous studies. However, it has been reported that patients with NC-CAH have a lower prevalence of PCOM and metabolic dysfunction [25].

Adult women with androgen-secreting tumors may have potential value in the study of the effects of endogenous excess androgens during adulthood on ovarian morphology, ovulation, and metabolic profiles. However, an androgen-secreting tumor is a rare disorder, and the association between androgen-secreting tumors and PCOS is unknown.

Androgen Treatment Animal Models of PCOS

Based on the findings obtained from patients with FTM-TS and CAH, there appears to be a critical period for programming PCOS-related organs in humans. Therefore, androgen administration to humans at various times and duration would be ideal to verify this hypothesis. However, an ethical problem exists because cross-sex hormone treatment cannot be performed under the age of 15 years

according to the guidelines for the diagnosis and treatment of gender identity disorder in Japan. In NC-CAH, the onset of excess androgen secretion may vary. Therefore, it is difficult to elucidate the effects of the timing of excess androgen exposure using human models.

To clarify the effect of exogenous androgen on the perinatal, prepubertal, and pubertal periods, animal models are crucial (Table 1). We studied a juvenile rat model treated with DHEA [26]. The animals used in this study were maintained in accordance with the guidelines of the Animal Resources Center of the Sapporo Medical University School of Medicine. Three-week-old Sprague-Dawley rats were subcutaneously injected with DHEA (6 mg/100 g body weight) every day for 7 (short term), 15 (medium term), and 30 days (long term). Short-term exposure to DHEA tended to produce an increase in total follicle numbers and a reduced proportion of atretic follicles compared with controls (Fig. 2A, B). Medium-term DHEA treatment significantly increased the total follicle numbers and PCOM (Fig. 2C, D). However, the temporarily increased total follicle numbers declined to control levels after long-term androgenization (Fig. 2E, F). In addition, the proportion of primary follicles increased and that of the more advanced stages of follicles decreased. These findings suggest that short- to medium-term exposure to exogenous androgen during the prepubertal period mimics PCOS. However, long-term exposure arrests the growth of primary follicles, which is not a typical feature of PCOS. Other rodent models of androgen exposure with various hormones and timings have failed to produce a complete PCOS phenotype [16, 17]. Prenatal exposure induces irregular reproductive cycles, but PCOM is not present, and the metabolic features of PCOS are missing [17].

Table 1. Human and non-human Models of androgen Exposure

Models	Exposure period	Phenotypes		
		PCOM	HA	LH
Human				
FTM-TS	Reproductive period	(−)	(N/A)	(−)
CAH	Prenatal	(+ ~ −)	(+)	(+ ~ −)
Non-human primates				
Rhesus monkey	Prenatal	(+)	(+)	(+)
Sheep	Prenatal	(+)	(−)	(+)
Rodent				
Rat	Prenatal	(N/A)	(-)	(−)
	Prepubertal	(+ ~ −)	(+)	(−)

FTM-TS: Female-to-male transsexualism. CAH: Congenital adrenal hyperplasia. PCOM: Polycystic ovarian morphology. HA: Hyperandrogenism. LH: LH hypersecretion. '+: present, –: absent, N/A: not available in publication.
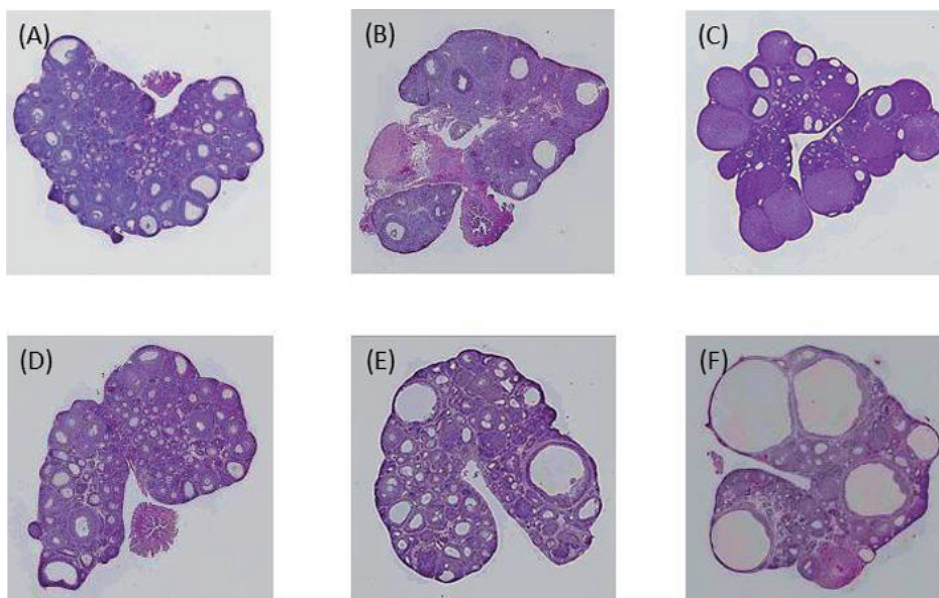

Fig. 2. Light micrographs of hematoxylin and eosin-stained whole rat ovary sections. (A) Seven-day control, (B) 15-day control, (C) 30-day control, (D) 7-day DHEA administration, (E) 15-day DHEA administration, and (F) 30-day DHEA administration. Both 7-day ovaries are similar. The 15-day DHEA ovary has more small follicles than the control ovary. The 30-day DHEA ovary has a higher amount of cystic, atretic follicles compared to the control, and decreased antral follicles compared to the 15-day DHEA ovary.

The phenotypes of androgen-exposed rodent models are disappointing. However, prenatally exposed rhesus monkeys display a typical PCOS phenotype [18]. Androgen excess during early gestation results in intermittent or absent menstrual cycles, ovarian hyperandrogenism, and PCOM. All of these traits result in a diagnosis of PCOS by every set of diagnostic criteria. In addition, metabolic dysfunction, LH hypersecretion, an elevated LH/follicle-stimulating hormone ratio, and increased LH responsiveness to exogenous gonadotropin-releasing hormone occur in this model.

Discussion

PCOS was first reported in 1935 by Stein and Leventhal [27], and many researchers continue to seek its

underlying cause. Nevertheless, the pathogenesis of PCOS is enigmatic. Some etiological hypotheses, including hypothalamic neuroendocrine disorder, insulin resistance, genetic predisposition, and excess androgen exposure, have been suggested [28–33]. Among these hypotheses, androgen exposure is the most interesting.

Testosterone treatment for FTM-TS patients is believed to be a model for evaluating excess androgen exposure in women of reproductive age. In our previous study, androgen administration to FTM-TS patients did not result in PCOM. Surprisingly, FTM-TS patients with no prior androgen treatment showed PCOM in 36.7% of patients, hyperandrogenemia in 38.3%, and fulfilled the Rotterdam criteria of PCOS in 32.0% [34]. Our study advocated excess androgens as an eradicator of PCOM. In contrast to our findings, previous studies indicated that androgen-exposed ovaries of FTM-TS patients showed PCOS-like features (i.e., thickened ovarian cortex and increased cystic follicles) [35–37]. However, these studies may have had methodological flaws because they were not case-control studies or lacked appropriate statistical analyses. The results of our study support previous findings that rhesus monkeys treated with testosterone or dihydrotestosterone have numerous growing preantral and small antral follicles up to 1 mm in diameter, but they do not show PCOM [19, 38]. Another study showed that serum androgen levels are not correlated with antral follicle numbers in patients with PCOS according to the Rotterdam criteria [39]. In addition, androgen-administered FTM-TS patients tend to have attenuated insulin resistance and decreased serum LH levels compared with control women [34]. Therefore, there is a discrepancy between the features of excess androgen exposure in adulthood and those of typical PCOS.

CAH and NC-CAH are other models of excess androgen exposure in humans. Previous studies have indicated that the characteristics of patients with CAH or NC-CAH are divergent, and a subset of patients with CAH (or NC-CAH) show typical features of PCOS, in contrast to findings in androgenized FTM-TS patients. With regard to gonadotropin secretion, LH levels are elevated in NC-CAH patients, but they do not reach those of PCOS patients. LH pulse frequency is not increased but pulse amplitude is increased [40]. In this regard, gonadotropin abnormalities of NC-CAH appear to be intermediate between those of controls and PCOS. The diversity of phenotypes in CAH and NC-CAH patients may reflect the heterogeneity of PCOS. The differences between CAH (or NC-CAH) and FTM-TS might be due to the quantity of androgen exposure, the timing of excess androgen exposure, and the source of androgens (endogenous or ex-ogenous). According to previous studies of FTM-TS and CAH, endogenous hyperandrogenemia may cause some traits of PCOS, but it does not result in a completely replicated form of typical PCOS. Additionally, it is unclear whether the amount of excess androgen in early life linearly correlates to the symptoms of PCOS.

To further study the relationships between the timing of androgen exposure (critical periods) and reproductive phenotype in adulthood, several animal models have been analyzed [16, 17]. DHEA, testosterone, and dihydrotestosterone have been administered at various time schedules. However, the hormonal profiles and ovarian morphology are not identical across the different models. In postnatally-androgenized rodent models, a PCOS-like condition is transient and cystic atretic follicles, which are not typical features of PCOS, are prominent. In prenatally-androgenized rodent models, PCOS-like features, such as LH hypersecretion, hyperandrogenemia, and PCOM, are lacking. A more severe hyperandrogenic state has been suggested to be responsible for the more severe reproductive phenotype in a mouse model [41]. The inconsistencies in the results of the various animal experiments may partially arise from differences in the loading dose and timing of androgen administration. Additionally, rodents are multi-ovulatory and androgen action may be different from that in primates [42]. Currently, there are limits to the usefulness of rodent models in the study of the pathogenesis of PCOS.

Non-human primates are believed to be superior to rodents for the study of the effects of androgens. Abbott et al. reported that the mechanisms of anovulation induced by androgen exposure are different among primates and non-primate mammals [18]. Androgen excess in non-primates causes dysregulation of the hypothalamic-pituitary unit resulting in the inhibition of LH secretion, which is not observed in primates. Surprisingly, prenatal androgen exposure in rhesus monkeys results in phenotypes similar to PCOS. Excess androgens during the developmental stage are thought to result in programming of the reproductive and metabolic features of PCOS (Fig. 3). In addition, androgen excess in the early prenatal period results in a slightly different phenotype from that in the late prenatal period. Monkeys administered androgens in the late prenatal period do not display LH hypersecretion and insulin resistance in contrast to administration in the early prenatal period [18]. These findings suggest that female reproductive function can be altered over a long period of time. However, the development of gonadotropin-releasing hormone neurons and organs regulating metabolism might be completed in early gestation. Therefore, androgen programming of PCOS seems to
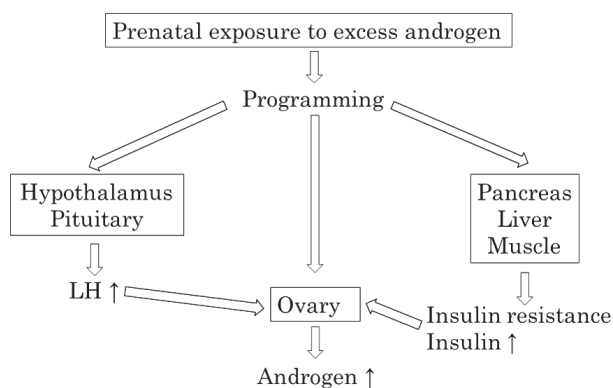
Fig. 3.   Hypothetical mechanisms of androgen excess and its effect on fetal programming.

occur only during specific time windows in fetal life.

At the present time, prenatal androgen excess is the dominant hypothesis explaining the pathogenesis of PCOS. Based on the prenatal androgen excess hypothesis, there are several concomitant features of PCOS. First, PCOS displays a familial tendency, but there is currently no identified gene that is associated with a strong risk of developing PCOS [43]. Second, pregnant women with PCOS have high serum androgen levels, and serum androgen levels of the fetus are positively correlated with those of their mothers [44, 45]. These findings may explain fetal hyperandrogenemia in women with PCOS. However, the question arises as to what might be the source of excess androgen in the fetus because placental aromatase degrades maternal androgens to prevent their transfer to the fetus in humans. Recently, an interesting study reported that the placentas of women with PCOS had diminished aromatase activity compared with placentas of control women [46]. Another author suggested that fetal ovaries in PCOS are genetically predisposed to secrete higher than normal levels of androgens [47]. These findings may be a breakthrough in resolving the PCOS enigma.

There are still some problems to be resolved in how the data obtained in studies of rhesus monkeys should be applied to humans. In addition, the origin of excess androgen supply needs to be determined. Further studies are required to verify these findings in humans.

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