HORMONES 2015, 14(2):190-200

Review

Androgens as double-edged swords: Induction and suppression of follicular development

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ABSTRACT

Androgens, which are mediated via the androgen receptor (AR), play important roles in normal follicular development and female fertility. However, just like a double-edged sword, besides the positive effects of androgen on follicular development, abnormal androgen levels, especially as in hyperandrogenism, seriously suppress normal follicular development. A crucial balance exists between the importance of androgens in follicular development and their negative effects when in excess. As the first meiotic division and epigenetic reprogramming are two critical events in oogenesis, abnormal androgen levels or deficiency in androgen/AR signaling in the ovary may affect these vital events. Oocytes have a tendency to develop genomic instability, thus resulting in an increasing incidence of unpredictable adult diseases. Although many studies have explored the effects of androgens and AR on follicular development, the conclusions are controversial and there has been no thorough review of this topic. This review focuses on the roles of androgens in the physiological process of follicular development, summarizes new insights into the roles of androgens in the arrested development of follicles, and discusses the potential risk of adult diseases originating from abnormal follicular androgen levels or androgen receptor signals, which may determine areas for future studies.

Key words: Androgen, Androgen receptor, Follicular development

INTRODUCTION

Androgens, which are mediated via the androgen receptor (AR), play well-defined roles in male reproductive functions.[1] However, androgen and AR also play important roles in normal follicular development and female fertility. The most powerful evidence of the involvement of androgens in folliculogenesis comes from *in vivo* animal models.[2,3] The global AR knockout (ARKO) and granulosa cell (GC)-specific ARKO female mouse suffers from premature ovarian failure induced by deficiency in folliculogenesis.[2-4] Dehydroepiandrosterone (DHEA), which is largely

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Received 26-12-2014, Accepted 07-04-2015

secreted by the zona reticularis of the adrenal glands, is a crucial precursor for sex steroid biosynthesis in the ovary and other organs.[5] In clinical practice, DHEA is utilized by approximately one third of reproduction centers during *in vitro* fertilization (IVF) treatment, as androgen has been reported capable of improving ovarian functions and increasing pregnancy chances for women with poor ovarian reserve.[6] The roles of androgens in follicle maturation and ovulation induction have increasingly attracted the interest of researchers.[7]

The main sources of circulating androgens in women include direct ovarian production and the peripheral conversion of adrenal sex steroid precursors to active androgens.[8] For women in the gonadotropin-dependent stage, the synthesis of androgens is under the control of luteinizing hormone (LH) from the pituitary.[9] In the production process of ovarian androgen, steroidogenic acute regulatory protein (STAR), and several enzymes, including cholesterol side-chain cleavage cytochrome P450 (CYP11A1), 17α-hydroxylase (CYP17) and 3-β-hydroxysteroid dehydrogenase (HSD3B), are required.[10]

Oogenesis is a complex and highly orchestrated process of differentiation from germ cells to mature oocytes.[11] Follicular development is regulated by endocrine and paracrine hormones, and the oocytes acquire developmental competence within the appropriate intrafollicular microenvironment.[11-13] The biological role of androgens is primarily exerted through transcriptional regulation mediated by the AR.[14] AR is expressed throughout the development of folliculogenesis in the oocytes, GCs, theca cells, and stromal cells.[15] As a transcriptional factor, the AR exerts a variety of effects on the events of folliculogenesis.[16,17]

However, just like a double-edged sword, besides the key positive effects of androgen on follicular development, abnormally high androgen levels suppress normal follicular development (Figure 1). Hyperandrogenism, the typical feature of polycystic ovarian syndrome (PCOS), often manifests as disturbed folliculogenesis, reduced fecundity,[18-20] and the adverse effects of hyperandrogenism on oocyte developmental competence.[20-23] After dihydrotestosterone (DHT) treatment, rats show ovarian follicular developmental and functional inhibition[24] and mice show inhibited preantral follicle growth and estradiol production.[25] In *in vitro* experiments, a larger proportion of pig oocytes were arrested in the metaphase I (MI) stage

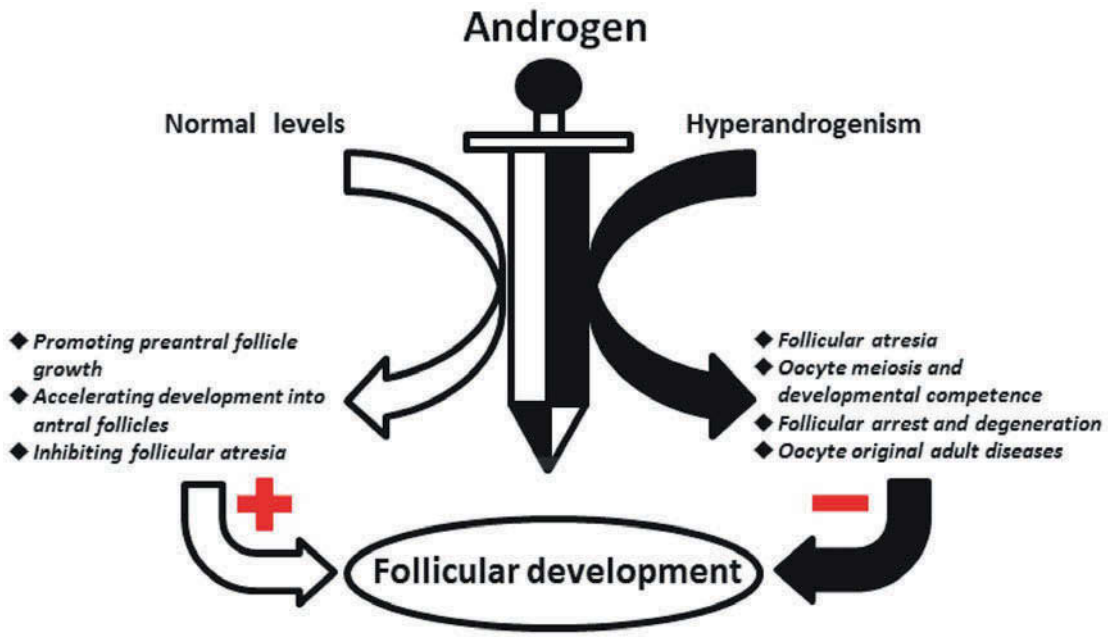

Figure 1. Androgen and androgen receptor (AR) have both positive and negative effects on follicular development, just like a double-edged sword.

after testosterone or DHT supplementation in the culture medium.[26]

Although many studies have explored the effects of androgen and AR on follicular development, the conclusions are controversial,[16,27] and there has been no thorough review of this topic.[14] The present review aims to assess current scientific evidence concerning the positive as well as negative effects of androgens on follicular development.

In order to review the existing studies on the effects of androgens on follicular development, a systematic literature search was performed using the following databases: MEDLINE (1966 to November 2014), EMBASE (1974 to November 2014), SCISEARCH (1974 to November 2014), Cumulative Index to Nursing and Allied Health Literature (1982 to November 2014), the Cochrane Menstrual Disorders and Subfertility Group trials register (November 2014), AMED (Allied and Complementary Medicine) (1985 to November 2014), China Academic Journal Electronic full text Database in China National Knowledge Infrastructure (1982 to November 2014), Wanfang Database (1982 to November 2014), Index to Chinese Periodical Literature (1978 to November 2014) and Thomson Reuters Web of Science. The reference lists of relevant primary and review articles were examined to identify cited articles, which were not captured by electronic searches. We contacted the corresponding authors to obtain the missing information and we did not place any restrictions of language or publication type in our searches. Search terms and key words included: "androgen" or "androgens" or "androgen receptor" or "androgen receptors" or "hyperandrogenism" or "testosterone" or "dehydroepiandrosterone" or "dihydrotestosterone" or "androstenedione" AND "follicular development" or "follicular growth" or "follicular maturation" or "follicular survival" or "ovarian follicle" or "ovary" or "ovarian cysts" or "polycystic ovarian syndrome" or "PCOS" or "polycystic ovary" or "ovary polycystic disease" or "oligoamenorrhea" or "oligo-amenorrhea" or "oligo-ovulation" or "anovulation" or "oligoanovulatory" or "oligo-anovulatory" or "oligohypomenorrhea" or "oligo-hypomenorrhea" or "amenorrhea" or "atretic follicles" or "follicle atresia". All search terms were translated into Chinese terms in order to conduct the searches in Chinese databases.

THE POSITIVE EFFECTS OF ANDROGENS ON FOLLICULAR DEVELOPMENT

Androgens had long been assumed to be detrimental or dispensable in normal folliculogenesis and ovarian functions.[28-31] However, the assumption was proven mistaken with the development of different types of ARKO mice[32-35] and the evidence of positive effects of androgen supplementation on patients with diminished ovarian reserve undergoing IVF treatment.[6,36,37] Androgens play their biological roles primarily through transcriptional regulation mediated by ARs.[14] The ligand-activated AR is a member of the nuclear receptor gene superfamily and acts as a transcription factor.[38] ARs regulate the expression of androgen-responsive genes by binding the regulatory sequence of target DNA and the androgen-response element.[1] ARs are widely expressed in the hypothalamus, pituitary, and various cells in the ovary.[39,40] AR has been demonstrated to be present in all the stages of follicular development, with the highest expression levels in GCs of preantral and small antral follicles,[41,42] while the expression of AR was seen to gradually decrease when follicles entered the pre-ovulation stage.[15,36] The evidence for the positive effects of androgens on follicular development comes from *in vitro* and *in vivo* studies, especially from the studies on AR knockout mice models.

In vitro studies

In the established *in vitro* follicle culture systems, different androgens, including testosterone, androstenedione, and DHT, were able to stimulate the growth and development of ovarian follicles in mammals.[43-45] As testosterone and androstenedione can be aromatized to corresponding estrogens, it may be difficult to determine the precise molecular mechanisms. Follicular growth during both the preantral phase and the meiotic maturation stage were decreased when mice ovarian follicles were cultured with AR antagonists or androgen antibody.[43,44] These findings show that androgen possesses important and direct stimulatory effects on follicular maturation. Androgens were recently found to enhance the expression of an antiapoptotic microRNA (miR-125b), thereby contributing to androgen-mediated follicular survival by suppressing proapoptotic protein expression and preventing follicular atresia.[7] Androgens

may also up-regulate follicle-stimulating hormone receptor (FSHR) levels in a transcription-independent way, thus sensitizing preantral follicles towards FSH actions and potentially involving androgen-mediated follicle growth.[7]

In vivo studies

Animals treated with short-term exposure to different androgens (aromatizable or non-aromatizable androgens) were found to have increased follicular recruitment and growth, increased proliferation of GCs and theca cells, and enhanced ovulation rates.[46,47] These effects seemed to be mediated by an elevated expression of insulin-like growth factor 1 (IGF1) and IGF1 receptor (IGF1R) as well as the amplification of the IGF system.[48-50] The effects of androgen on ovarian physiological functions and the IGF1 system were fully reproduced with subcutaneous implantation of non-aromatizable DHT, indicating that it is through AR, rather than through conversion to estrogens, that androgens function in the process of follicular development.[48] DHT treatment was also found to improve IGF-1-stimulated proliferation and mitogenic effects of growth differentiation factor 9 (GDF9),[50,51] and these effects could be blocked by an AR antagonist.[51] The follicular growth was positively correlated with AR gene expression, and it was through the AR that androgens were seen to be involved in follicular growth.[52] These findings suggested that the *in vivo* androgen-AR actions might regulate the expression and action of key ovarian growth factors during follicular development.

Ar knockout mice models

The development of ARKO female mice models has provided an important and powerful basis to explore the roles of androgen and AR in fertility maintenance. The global ARKO female mice models were successively established with the Cre/LoxP strategy by targeting deletion of exon 1 ($Ar^{EX1-/-}$), exon 2 ($Ar^{EX2-/-}$), and exon 3 ($Ar^{EX3-/-}$) of *Ar* gene to obtain loss ($Ar^{EX1-/-}$ and$Ar^{EX2-/-}$) or inactivated ($Ar^{EX3-/-}$) Ar protein.[32-35] Although ARKO female mice had normal ovarian and oviductal morphology, they suffered from considerable reproductive defects with fewer pups per litter, defective folliculogenesis, and premature ovarian failure.[32-35] Moreover, GC-specific ARKO (GC-ARKO) mice and oocyte-specific ARKO mice have also been generated.[4,53] GC-ARKO mice were established by crossing *Ar* (exon 2)-floxed miceor *Ar* (exon 3)-floxed mice with the *Amhr2*-Cre mice or *Amh*-Cre mice, respectively.[4,53] GC-ARKO mice presented with almost all the reproductive phenotypes observed in global ARKO mice.[4,53] In contrast to GC-ARKO mice, oocyte-ARKO mice, established by crossing *Ar* (exon 2)-floxed mice with *Gdf9*-Cre mice, had a normal reproductive phenotype.[4] The above-cited evidence indicated that the local androgen actions in GCs were critical contributors to normal follicular development and fertility in female mice.

Based on the various *in vivo* as well as *in vitro* studies during the past two decades and the development of the global or GC-ARKO female mice models, it is now widely recognized that it is through promoting preantral follicle growth, accelerating development into antral follicles, and inhibiting follicular atresia that androgens positively affect follicular development.[16] Androgens function well as essential adjuvants in regulating normal folliculogenesis.[54,55] However, acting in the manner of a double-edged sword, excessive androgens will disturb normal follicular development and ovulation.[54,55]

THE NEGATIVE EFFECTS OF OVARIAN HYPERANDROGENISM ON FOLLICULAR DEVELOPMENT

As described above, androgen and AR are indispensable elements in folliculogenesis. Nevertheless, there is abundant evidence that androgen and AR also exert inhibitory effects on follicular development.[54,55] An inhibition of normal follicular growth and an acceleration of ovarian maturation were found after neonatal androgen administration.[56] The most typical evidence comes from PCOS, the most common endocrine disorder in women, which is characterized by oligo-ovulation/anovulation, hyperandrogenism, and polycystic ovaries.[27] It is reported that sixty to eighty percent of women with PCOS suffer from hyperandrogenism[27] and ovarian hyperandrogenism can induce the dysfunctions of GCs and hamper follicular development.[57,58] In clinical practice, the measurement of androgen levels may be necessary when treating patients with abnormal follicular development.

Which factors are associated with ovarian hyperandrogenism?

Gonadotropins

As is known, the pulse frequency and amplitude of LH will increase with the rapid gonadotropin-releasing hormone (GnRH) secretion, thus resulting in an elevation of plasma LH concentrations and the ratio of LH to FSH,[59] which can enhance the synthesis of ovarian androgen.[9] Conversely, excessive androgen will lead to the hypersecretion of LH through affecting the hypothalamic-pituitary axis and compromising the feedback inhibition on LH secretion by ovarian steroids.[60] However, among adolescent girls with PCOS, androgen excess was not found to reduce hypothalamic feedback inhibition.[61] In concurrence with excessive androgens, FSH fails to stimulate an adequate amount of aromatase to convert androgens into estrogens, thus inducing ovarian hyperandrogenism, which indicates that the relatively decreased FSH levels (in relation to LH) may also play an indirect role in the formation of ovarian hyperandrogenism.[62] Another study found that excessive LH stimulation alone did not lead to ovarian hyperandrogenism and that follicular development was present in the absence of FSH.[63]

Insulin

Insulin resistance is found in fifty to seventy percent of women with PCOS and is implicated in the predisposition to hyperandrogenism in PCOS.[64] Insulin sensitizers, such as metformin, can help to alleviate the hyperandrogenic profile of PCOS patients.[65] Hyperinsulinemia stimulates excessive androgen synthesis in theca cells through the synergy with LH, reduces the production of hepatic sex hormone-binding globulin (SHBG), and increases the bioavailability of testosterone,[66] indicating that insulin might contribute to hyperandrogenism by increasing the sensitivity of the androgenic insulin pathway.[67] In PCOS patients, metabolic pathways stimulated by insulin are generally defective, while the steroidogenic and mitogenic pathways are normally maintained.[68]

Excessive steroidogenic activity

Some steroidogenic enzymes, such as CYP11A, CYP17, and HSD3B2, are involved in the multiple steps of androgen biosynthetic pathways.[69,70] The activities of these enzymes increase in the theca cells of patients with hyperandrogenism.[69,70] GATA6, a transcription factor, is also overexpressed in hyperandrogenism patients.[70] The half-life of CYP17A1 mRNA is increased two-fold in theca cells of hyperandrogenism patients, compared with patients with normal androgen levels, which leads to augmented CYP17A1 mRNA accumulation and enhanced CYP17A1 enzyme activity.[71] In patients with hyperandrogenism, the serine phosphorylation of CYP17A1 enzyme is increased and the protein level of phosphatase 2A decreased to maintain the serine phosphorylation of CYP17A1.[62,72]

AMH

Anti-Müllerian hormone (AMH) is a member of the transforming growth factor-β (TGF-β) superfamily and participates in follicular dynamics.[73] AMH is produced by human GCs from 36 weeks of gestation to menopause and its highest expression level is observed in small antral follicles.[74,75] Serum AMH levels are positively correlated with androgen levels in women with polycystic ovaries.[76] AMH promotes the production of androgen directly through AMH type II receptors (AMHRII) on theca cell membranes and indirectly through inhibiting actions of FSH as well as aromatase in GCs.[62] However, AMH expression levels were recently found to have only a weak correlation with hyperandrogenism in women with PCOS, even though AMH expression was highest in PCOS patients presenting all three main diagnostic criteria (menstrual irregularity, polycystic ovaries, and elevated androgen).[77] Therefore, further studies are needed to elucidate the associations between AMH and ovarian hyperandrogenism.

Epigenetic and environmental factors

Hyperandrogenism is considered to be a complicated disorder hypothesized to have a genetic basis,[78] which does not present as typical Mendelian inheritance. Epigenetic and environmental factors have been proposed as confounding factors that modulate the phenotype of genetic basis of ovarian hyperandrogenism.[58,79] Alternative splicing has been reported as an intrinsic epigenetic cause of ovarian hyperandrogenism.[80] An unfavorable *in utero* environment may potentially lead to premature adrenarche, and higher levels of dehydroepiandrosterone sulphate (DHEA-

S) and androstenedione, while lower expression of SHBG, might result in hyperandrogenism.[65] In animal models, *in utero* exposure to excessive testosterone may be accompanied by gestational hyperglycemia and hyperinsulinemia, which leads to PCOS-like phenotypes in adulthood.[65] Environmental endocrine-disrupting chemicals may also lead to ovarian hyperandrogenism through interfering with ovarian functions and the associated metabolism.[79] Among those chemicals, bisphenol A (BPA) is a widely-used estrogenic industrial plasticizer, which can be detected in most individuals.[81] BPA has been found to improve androgen production in rat theca cells,[82] and serum androgen levels are positively associated with BPA levels.[83] For women with ovarian hyperandrogenism, the hepatic clearance of BPA was decreased due to the interference of excessive androgen, leading to accumulation of BPA and further augmenting the severity of the phenotype.[83] These factors, especially environmental factors, should be considered in the treatment of patients with abnormal follicular development clinically, and some recommendations may be provided to the patients accordingly.

How does hyperandrogenism negatively influence follicular development?

Follicular atresia

In the ovary, a complex mechanism is involved in follicular development.[84] Follicles progress through primordial, primary, preantral, antral, and preovulatory follicles. Two distinct types of follicular development have been described: initial recruitment and cyclic recruitment.[12] Most follicles undergo atresia during the antral stage and few proceed to ovulation during cyclic recruitment.[85] Follicular atresia is a complicated and multifactorial apoptotic process that depends predominantly on apoptosis of GCs.[86,87] During cyclic recruitment, atresia is promoted by androgens.[12,88,89] A single DHT injection to hypophysectomized immature female rats was often seen to result in decreased ovarian weight, and this was associated with the stimulation of follicular atresia and the reduction in healthy follicles of all types.[90] Moreover, in contrast to the promoting roles of estrogen in follicle survival, androgens blocked the estrogen-induced antiatretogenic effects, increased ovarian apoptotic DNA fragmentation, and promoted cell apoptosis,[91] probably via inhibiting estrogen-induced Ca^{2+} oscillations in oocytes.[92] Atretic follicles are characterized by reduced aromatase activity and accompanied by the accumulation of androgen.[87,93] AR blockers and androgen antibodies can inhibit the production of androgens in GCs, thus suppressing follicle atresia.[89]

Oocyte meiosis and developmental competence

The precise regulation of oocyte meiosis is necessary for female fertility.[94] Oocytes are arrested in the early meiotic cycle until just before ovulation, when ovarian factors trigger meiosis or maturation.[85] In one *in vitro* oocyte study, with the increased concentration of testosterone/androstenedione in the culture medium, the incidence of polar body formation was reduced and more follicles proceeded only to the germinal vesicle breakdown stage or remained meiotically inactive.[95] The predominant effect of hyperandrogenism was to prevent the oocytes from resuming meiotic activity.[28] Androstenedione may inhibit the estradiol-induced Ca^{2+} response of oocytes, which results in harmful effects on oocyte maturation and developmental potential.[92,96] When GCsare is treated with DHT, more cells are arrested in the gap I phase and few proceed to the DNA synthesis phase due to the decreasing expression of cyclin D2.[97] Moreover, follicular testosterone levels are negatively associated with oocyte maturity in human samples.[23]

Oocyte developmental competence involves not only the capability of oocytes to complete meiosis, but also fertilization, embryogenesis, and late development.[13] After prenatal androgen treatment, adult monkeys show increased 5α-reductase activity and decreased aromatase activity, which are accompanied by abnormal oocyte development or cleavage arrest after fertilization of *in vitro* matured oocytes.[22] Prenatal exposure to androgens also reduced the percentage of zygotes developing into blastocysts.[20] PCOS patients with hyperandrogenism typically present higher rates of oocyte immaturity and poor fertilization rates, although more oocytes may be retrieved during IVF.[21] Pregnancy is associated with a higher estradiol/testosterone ratio in follicular fluid, and follicles with a lower estradiol/testosterone ratio tend towards implantation failure or cleavage failure *in vitro*.[21,23,98,99]

Follicular arrest and degeneration

Administration of DHT to female monkeys induces a PCOS-like morphology, which is characterized by increased numbers of primary, secondary, and small antral follicles without the selection of dominant follicles, suggesting direct effects of androgen on follicle arrest and blockage of dominant follicle selection.[46] After anti-androgenic therapy, the ovarian morphology of polycystic ovaries was significantly ameliorated and ovulation was restored.[100] It has been reported that inhibin plays important paracrine roles in normal follicular development,[101] and inhibin deficiency is associated with follicular arrest.[102] Androgen was negatively associated with inhibin levels in follicular fluids,[101] implying that androgen indirectly promotes follicular arrest. In hypophysectomized immature rats, testosterone propionate administration causes degenerative changes in the ovarian follicle and oocytes, which are characterized by non-attached cumulus cells around the oocytes.[103] Meanwhile, hyperandrogenism has been hypothesized to be a contributor to the epigenetic alterations of several important transcriptional factors which are involved in follicular development.[58]

Oocyte original adult diseases

The abnormal development of gamete/embryo, or any adverse event during gametogenesis/embryogenesis, might induce higher risks for some chronic diseases in adulthood.[104] Oogenesis and fertilization occur during the period of epigenetic reprogramming, which involves the erasure and re-establishment of the imprinted genes.[105,106] Formation and maturation of oocytes last for decades and are vital for epigenetic modifications of the gamete.[107] Epigenetic alterations may be involved in the development of the fetus and energy metabolism.[108] Animal studies demonstrate that maternal androgen levels influence the development of the offspring.[109,110] Human data indicate that elevated maternal testosterone levels are associated with lower birth weight and length.[111] The higher prevalence of small for gestational age (SGA) newborns to mothers with PCOS cannot be completely attributed to pregnancy complications and seems to be more closely related to the hyperandrogenic status of the mothers.[112] Compared with daughters of healthy women, daughters born to women with PCOS exhibit increased ovarian volume and the higher levels of AMH, a marker of growing follicles,[113,114] indicating that the daughters of mothers with PCOS show evidence of altered follicular development. These alterations are likely caused by intrinsic molecular defects in the oocytes in combination with androgen excess. Exposure to hyperandrogenism during oogenesis may result in permanent modifications of the offspring's epigenome and alterations of their gene-expression patterns, which may increase susceptibility to diseases in adulthood.[106,115] Therefore, it is concluded that hyperandrogenism in the follicles can affect the establishment and maintenance of maternal imprinting and lead to epigenetic alterations in the offspring. In clinical practice, to improve maternal health and the safety of the offspring, more effective preventive interventions should be developed to ameliorate hyperandrogenism and to correct the oocyte development process.

CONCLUSIONS

For many years, it was believed that elevated androgen levels in women are associated with poor reproductive health. However, the establishment of ARKO female animal models, along with various *in vivo* and *in vitro* studies, has illustrated the significance of androgen and ARs in maintaining female fecundity. Androgens play pivotal roles in the physiological and pathophysiological process of follicular development. Dysfunction of androgen/AR signaling will perturb normal reproductive development. It is becoming increasingly clear that a crucial balance exists between the essentiality of androgens in follicular development and their negative effects in androgen excess conditions. The balance regulates women's health and reproductive performance. If oocytes are exposed to hyperandrogenism or deficiency in androgen/AR signaling, the oocytes will have a predisposition to developing disorders of genomic imprinting, thus resulting in an increasing incidence of unpredictable adult diseases. Consequently, studies are urgently needed to find more effective preventive strategies to rectify hyperandrogenism or deficiency of androgen/AR signaling and to correct the oocyte development process, which will not only improve maternal health, but will also enhance the safe development of the offspring.

GRANTS OR FELLOWSHIP SUPPORTS

China Scholarship Council (No. 201308330139).

CONFLICT OF INTEREST

The authors have nothing to disclose.

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