Involvement of androgens in ovarian health and disease

M. Lebbe and T.K. Woodruff

ABSTRACT: In women, ovary and adrenal gland produce androgens. Androgens are essential drivers of the primordial to antral follicle development, prior to serving as substrate for estrogen production in the later stages of folliculogenesis. Androgens play a crucial role in the follicular–stromal intertalk by fine tuning the extracellular matrix and vessel content of the ovarian stroma. Local auto- and paracrine factors regulate androgen synthesis in the pre-antral follicle. Androgen excess is a hallmark of polycystic ovary syndrome and is a key contributor in the exaggerated antral follicle formation, stromal hyperplasia and hypervascularity. Hyperandrogenaemia overrides the follicular–stromal dialog, resulting in follicular arrest and disturbed ovulation. On the other hand, androgen deficiency is likely to have a negative impact on fertility as well, and further research is needed to examine the benefits of androgen-replacement therapy in subfertility.

Key words: androgen / folliculogenesis / stroma / angiogenesis / polycystic ovary syndrome

Introduction

The effect of androgens on female fertility is an emerging field in reproductive science at the interface of endocrinology and gynecology. In the following section, the authors aim to describe androgen action on the early growing follicle and its environment, and highlight new implications and developments in the field.

Follicle unit and ovarian structure

The single follicle is the fundamental unit of the ovary and is composed of an oocyte surrounded by specialized endocrine cells. It produces peptide hormones and sex steroids that modulate the maturation of the oocyte and regulate follicle cell growth and differentiation through local auto- and paracrine-signaling pathways. Secreted into the bloodstream, these hormones also exert endocrine effects and prepare the reproductive organs for fertilization and implantation. Primordial follicles are continuously recruited for growth, and this phenomenon, as well as the subsequent stages of follicular development, is considered to be locally regulated and independent of gonadotrophin action. While the majority of growing follicles are lost in atresia, a small cohort of antral follicles is recruited for further growth, dominance and ovulation under the cyclic stimulation of gonadotrophins.

The outer cortex of the ovary contains immature follicles and is a rigid, avascular environment. It is made up of tightly packed spindle-shaped fibroblasts, vasculature-related cells (smooth muscle cells and endothelial cells), inflammatory cells and precursor theca cells. The inner medulla is more elastic and composed of loose connective tissue and ovarian vasculature. The ovarian stroma consists of connective tissue and its extracellular matrix sustains the ovarian architecture and provides structural support to the growing follicles. The stroma also produces a variety of cytokines, chemokines and growth factors that tightly co-regulate—in an autocrine and paracrine fashion—the early growth phase of its enclosed follicles.

Androgens

In women, the main sources of circulating androgens are the adrenal glands and ovaries (Arlt, 2006). Dehydroepiandrosterone (DHEA), mainly from the adrenal glands, acts as a crucial precursor of sex steroids in the ovary and other target tissues (Labrie, 2010). Depending on the intracellular availability of steroidogenic enzymes in target tissues, DHEA is converted to androstenedione and testosterone, both of which can be aromatized to estrogens. Testosterone can also be converted to the much more potent 5α-dihydrotestosterone (DHT). Testosterone and DHT are the only two hormones that bind and activate the androgen receptor (AR). Serum levels of DHEA and androgens peak in the early reproductive years, followed by a steep decline with age (Davison et al., 2005).

Androgen action

Androgens exert their action mainly through the AR. The AR functions as a ligand-activated nuclear transcription factor (Gelmann, 2002). It has recently become clear that many effects of androgens in non-ovarian target tissues depend on other complex-signaling pathways, including rapid non-genomic pathways. The reported non-genomic effects of androgens...
at physiological concentrations appear to be mediated through the cytosolic AR, involved in the activation of mitogen-activated protein kinase-extracellular signal-related kinase (ERK) pathways (Kousteni et al., 2001). There is evidence that this non-genomic stimulation results in an enhancement of AR transcriptional activation, thereby creating an autocrine loop between AR and its ligand (Heinlein and Chang, 2002a, b). It is currently unclear whether androgens exert non-genomic actions in the ovary. Interestingly, the activation of the PI3-K/Akt pathway in the minutes following testosterone supplementation has been reported in neonatal mouse ovaries, an effect reverted by the AR-antagonist flutamide and suggestive of non-genomic androgen signaling (Yang et al., 2010).

AR expression in the ovary

ARs are expressed in all cell types of the ovarian follicle, including the oocyte, granulosa and theca cells (Sen and Hammes, 2010). Few studies have examined the distribution of AR in the connective tissue or stromal compartment. In bovine, non-human primates and humans, AR is expressed in the cortical stroma (Horie et al., 1992; Weil et al., 1998; Yang and Fortune, 2006). In rodents, cattle, primates and human, increasing concentrations of AR are detected in granulosa cells from the primary stage onward, peaking in the antral stage (Weil et al., 1998; Yang and Fortune, 2006; Rice et al., 2007; Sen and Hammes, 2010). The crucial role of the granulosa AR has been demonstrated by the phenotype of the granulosa cell-specific AR knockout mouse, which is subfertile, has reduced follicle progression, fewer ovulations and reduced litter size (Sen and Hammes, 2010).

Ovarian androgen supply

Primordial and primary follicles within the avascular ovarian cortex rely on passive diffusion of nutrients and growth factors from the surrounding stroma and the systemic circulation. Steroid hormones are lipophilic and can therefore readily enter cells (Oren et al., 2004); however, a central to periphery diffusion gradient is to be expected as steroids produced by growing follicles, and those delivered by the systemic circulation provide from the central medulla. The ovarian stroma seems to be steriodogenically silent in physiological premenopausal conditions (Young and McNeilly, 2010). Studies of fetal human ovaries have shown that the oocyte of the primordial follicle has the steroidogenic machinery in place to synthesize androgens, and that pre-granulosa cells express AR (Fowler et al., 2011). The relevance of this steroidogenic pathway remains currently unknown.

In the secondary stage, theca cells are recruited from the surrounding stroma and participate in providing a vascular network to the growing follicle (Young and McNeilly, 2010). Expression of the required steroidogenic enzymes (Star, P450c11a1, P450c17 and HSD3b), as well as luteinizing hormone (LH) receptor, begin at this stage and allow the theca cells to produce androgens (Logan et al., 2002).

Androgen effects on early stage follicles

Primordial to primary follicle transition

The primordial follicle is the smallest, and contains a meiotically arrested oocyte surrounded by squamous granulosa cells. The primordial follicle pool represents the reserve of follicles available and therefore determines the fertility potential of a female. This follicle reserve is established during fetal development in humans and in the immediate postnatal period in mice (Peters et al., 1975). The number of follicles within the reserve gradually declines via atresia with increasing age. Although this pool of follicles is considered to exist in a resting state, it is also dynamically and tightly regulated with continuous follicular fate decisions: many follicles will be lost to atresia, a large number remain dormant and only a select few are recruited into the growing pool (Tingen et al., 2009; Kim, 2012; Sanchez and Smitz, 2012). The first indication that a primordial follicle has been recruited to enter the growing pool is the change in granulosa cell shape, from squamous to cuboidal, followed by proliferation of the granulosa cells. On a molecular level, the phosphoinoside 3-kinase (PI3K) pathway seems to be central in regulating fate decisions in primordial follicles (Castrillon et al., 2003; Reddy et al., 2008). A basal degree of intra-oocyte PI3K activation is required for survival throughout the long dormancy of these follicles (Reddy et al., 2010).

At the same time, PI3K signaling is inhibited by several molecules, such as PTEN-PDK1 (Reddy et al., 2008; Reddy et al., 2009) and FOXO3 (Castrillon et al., 2003; Liu et al., 2007; John et al., 2008), which ensures sustained dormancy of the primordial follicle pool. Inactivation of these PI3K repressors by KIT ligand and other growth factors plays a crucial role in the recruitment of the primordial follicle, at least in mice (Sanchez and Smitz, 2012).

Testosterone rapidly increases the intra-oocyte PI3K/Akt/FOXO3 pathway in mouse primordial follicles, increasing by >2-fold the ratio of primary to primordial follicles (Yang et al., 2010). Similar non-genomic activation of the PI3K/Akt pathway by androgens has been described in other target cells (Baron et al., 2004; Kang et al., 2004; Cinaret et al., 2007). In rhesus monkeys, testosterone treatment appears to promote primordial follicle activation, with elevated intra-oocyte IGF1 signaling (Vendola et al., 1999a, b). It is unclear how androgens exert this effect, as AR is not detected, or is below the limit of detection, in this follicle class (Weil et al., 1998). Nevertheless, it is interesting that IGF1 receptor-mediated protection from apoptosis relies on PI3K activation (Shelton et al., 2004).

Primary to secondary follicle transition

Once activated, the oocyte begins to grow, forms the zona pellucida and establishes cell–cell contact with the surrounding granulosa cells. Inter-cellular communication occurs through gap junctions and is of paramount importance for the follicle, which must remain coupled and coordinated unit throughout development. In fetal bovine ovaries, mid-gestational exposure to the anti-androgen flutamide decreases connexin 43 expression and the number of granulosa cell-oocyte gap junctions (Knapczyk-Stwora et al., 2013). In contrast, in a luteinized granulosa cell line, androgen excess decreases connexin 43 expression (Wu et al., 2010). Differences in granulosa differentiation could possibly explain this dual effect.

The secondary follicle is characterized by the acquisition of a second layer of granulosa cells. Testosterone stimulates, in a dose-dependent manner, the primary to secondary follicle transition in fetal bovine ovaries, and this effect is inhibited by the AR antagonist flutamide (Yang and Fortune, 2006). The mitogenic properties of androgens could be mediated partly through increased glucose metabolism via Glut4 signaling (Sato et al., 2008). In rhesus monkeys, testosterone administration up-regulates the expression of its own receptor in the granulosa cell (Weil et al., 1998); similar findings have been reported in ovaries from testosterone-treated transsexual women (Chadha et al., 1994).
Pre-antral to antral follicle transition

As the granulosa cell layers expand, the expression of follicle-stimulating hormone (FSH)- and LH receptors is detected, and although growth remains under control of intra-ovarian regulators at this stage, the follicle becomes gonadotrophin responsive. At this point, an antrum begins to form and granulosa cells differentiate toward mural and cumulus cell phenotypes. The most important development at this stage is theca cell recruitment and differentiation, with acquisition of steroidogenic function and neo-angiogenesis (Young and McNeilly, 2010), allowing the follicle to interact with systemic endocrine factors and enter the gonadotrophin-dependent phase.

Theca cell function is initially under auto/paracrine control, mainly by members of the tumor growth factor (TGF)-beta superfamily. One member of this family is GDF-9, an oocyte-derived growth differentiation factor. GDF-9 knockout mice fail to establish a theca cell layer (Dong et al., 1996). GDF-9 is also essential for inducing P450c17 expression and androgen synthesis in pre-antral follicles (Vitt et al., 2000b; Orisaka et al., 2009), while suppressing P450c19 (aromatase) activity (Vitt et al., 2000a), thereby ensuring an androgen-rich environment. Other members of the TGF-beta superfamily, the bone morphogenetic proteins (BMP-4, BMP-6, BMP-7), act via insulin-like peptide 3 to down-regulate members of the TGF-beta superfamily, the bone morphogenic proteins (BMP-4, BMP-6, BMP-7), act via insulin-like peptide 3 to down-regulate P450c17 and have shown to be potent suppressors of basal and LH-induced thecal androgen production (Glister et al., 2005; Glister et al., 2013). Several local-binding proteins, such as gremlin, chordin and follistatin, can reverse BMP proteins’ function (Glister et al., 2005).

In antral follicles, granulosa cells produce activin that suppresses androgen synthesis in pre-antral follicles (Vitt et al., 2000b), thereby ensuring an androgen-rich environment. Other members of the TGF-beta superfamily, the bone morphogenetic proteins (BMP-4, BMP-6, BMP-7), act via insulin-like peptide 3 to down-regulate P450c17 and have shown to be potent suppressors of basal and LH-induced thecal androgen production (Glister et al., 2005; Glister et al., 2013). Several local-binding proteins, such as gremlin, chordin and follistatin, can reverse BMP proteins’ function (Glister et al., 2005).

In antral follicles, granulosa cells produce activin that suppresses androgen synthesis in pre-antral follicles (Vitt et al., 2000b), thereby ensuring an androgen-rich environment. Other members of the TGF-beta superfamily, the bone morphogenetic proteins (BMP-4, BMP-6, BMP-7), act via insulin-like peptide 3 to down-regulate P450c17 and have shown to be potent suppressors of basal and LH-induced thecal androgen production (Glister et al., 2005; Glister et al., 2013). Several local-binding proteins, such as gremlin, chordin and follistatin, can reverse BMP proteins’ function (Glister et al., 2005).

Androgen effects on stroma and vasculature

Androgenized female-to-male transsexuals exhibit diffuse hyperplasia of the ovarian stroma with excessive collagen accumulation (Ikeda et al., 2013). In vitro, testosterone increases type I collagen production by fibroblasts (Jenkins et al., 2007). In the ovary and the endometrium, sex steroids regulate the expression of vascular endothelial growth cells has recently been observed (Nielsen et al., 2011). Testosterone administration to rhesus monkeys up-regulates AR and FSH-R expression in granulosa cells, thereby robustly stimulating antral follicle growth (Vendola et al., 1998; Weil et al., 1998; Weil et al., 1999).

Ligand-activated AR amplifies FSH action by increasing cAMP-mediated post-receptor signaling (Hillier and Tetsuka, 1997). This synergistic effect between androgens and FSH has also been demonstrated using in vitro murine follicular culture models (Murray et al., 1998; Wang et al., 2001; Lenie and Smitz, 2009).

Thus, at the antral stage, androgens, through the AR, not only enhance their own action but also prime the follicle for later FSH action, which in turn modulates LH responsiveness. We postulate that androgens stimulate AMH secretion, although this has not been demonstrated yet. This scenario, depicted in Fig. 1, would explain how the growing follicle is ‘protected’ from premature recruitment by FSH until it reaches the maturation stage for selection for dominance (Visser et al., 2006).

**Figure 1** Working model for androgen action in the pre-antral follicle. Androgens are secreted by theca cells from the secondary stage on, and amplify their local effects by increasing their own receptor expression and activity. Androgens induce the expression of FSH-R and prime the follicle for further FSH-driven follicular growth and maturation. We positulate that androgens stimulate AMH secretion that in turn inhibits FSH-induced aromatase expression thereby maintaining a predominantly androgenic intrafollicular milieu. In this scenario the growing follicle is ‘protected’ from premature selection by FSH.
factor (VEGF) (Shweiki et al., 1993). This endothelial-specific mitogen plays a crucial role in the natural neo-angiogenesis that occurs in the female reproductive tract (Perrot-Apellaniz et al., 2000). Clear spatiotemporal expression patterns of VEGF mRNA in steroid-producing cells and VEGF-R mRNA in adjacent endothelial cells are observed during various processes, such as neovascularization of the ovarian follicle, angiogenesis of the corpus luteum, tissue repair and uterine implantation in mice (Shweiki et al., 1993). VEGF and its receptor have been localized to human pre-antral follicles and the surrounding stroma (Abir et al., 2010). In vitro studies demonstrate that ligand-activated AR induces VEGF expression in human fetal prostatic fibroblasts (Levine et al., 1998) and in human aortic endothelial cells (Cai et al., 2011). Androgens activate hypoxia-inducible factor 1 (HIF1) (Mabjeesh et al., 2003; Shafiqhi et al., 2012), a known transcriptional activator of VEGF (Ferrara, 2004), and this AR-mediated effect is enhanced in hypoxic conditions (Mitani et al., 2011; Park et al., 2012). In human vascular endothelial and smooth muscle cells, androgen has a proliferative effect (Nheu et al., 2011). The androgen precursor DHEA is known to stimulate endothelial proliferation and angiogenesis through extracellular signal-regulated kinase 1/2-mediated mechanisms (Liu et al., 2008).

These in vitro studies throw new light on the mechanisms of androgen-promoted vascular proliferation in steroid target tissues. To date, the androgen effects on ovarian vasculogenesis remain unexplored. In the light of the ovarian cortical hypervascularity observed in androgen-excess conditions such as PCOS (see below), it would be worth investigating if a similar mechanism occurs in the ovary in vivo. A working model for androgen-promoted angiogenesis in the ovary is shown in Fig. 2.

**New developments: implications for androgen excess and PCOS**

PCOS is the most frequent cause of oligoovulation and hyperandrogenaemia and affects 5–10% of women of reproductive age. PCOS is a heterogeneous condition, with a range of reproductive and long-term metabolic complications. A detailed description of this condition is beyond the scope of this review and is discussed elsewhere (Ehrmann et al., 1995; Fauser et al., 2012).

The etiology of PCOS is still unclear, but likely results from a genetic–environmental interaction. Animal studies are an important tool to study the effects of hyperandrogenaemia on the ovary and the metabolic system. Prenatally androgenized monkey (Abbott and Bacha, 2013) and sheep (Veiga-Lopez et al., 2011) exhibit adult reproductive and metabolic features that mimic PCOS in women. These important studies have focused the attention of the field on the role of prenatal/fetal androgen excess in the development of PCOS and metabolic syndrome later in life.

A similar reproductive–metabolic PCOS phenotype is obtained in rodents exposed to high doses of DHT before puberty (Manneras et al., 2007; van Houten et al., 2012). Findings through rodent PCOS models make extrapolation to the human situation more complex as, in contrast to women, rodents are poly-ovulators and lack adrenal androgen production. Also, the DHT doses employed are supraphysiologic compared with the PCOS situation.

With the physiological effects of androgens on early follicular development, stroma and ovarian vasculature in mind, we propose a ‘fresh’ look at this prevalent condition.

**Intrinsic deregulation of early follicular development**

The characteristic morphological feature of PCOS is the accumulation of small antral follicles in the ovarian cortex (Balen et al., 2003). There is growing consensus that follicular development in PCOS is deregulated from the very early, gonadotrophin-independent stage onward, resulting in follicular arrest and disruption of dominant follicle selection (Webber et al., 2003; Franks et al., 2008; Franks and Hardy, 2010). Hyperandrogenaemia is likely to be a key mediator in this process, but certainly not the only factor involved. Hyperinsulinaemia resulting from insulin resistance in PCOS is another prominent contributor (Nestler et al., 1998; Diamanti-Kandarakis and Dunai, 2012). Intra-ovarian androgen excess reflects an intrinsic abnormality in theca cell function (Ehrmann et al., 1995), resulting from an increase in steroidogenic enzyme activity (Nelson et al., 2001; Franks and Hardy, 2010). LH- and insulin excess jointly promote thecal Cyp17 activity and subsequent androgen production (Bergh et al., 1993; Dumesic and Richards, 2013).

The elevated AMH levels typically observed in women with PCOS are probably due to a combination of an increased antral follicle pool (Pigny et al., 2006; Hart et al., 2010) coupled with an elevated production of AMH by individual granulosa cells (Dunson et al., 2002; Nardo et al., 2009; Arabzadeh et al., 2010). Androgen-lowering treatments typically decrease AMH levels in PCOS (Piltonen et al., 2005; Amer et al., 2009; Falbo et al., 2010). AMH-induced aromatase inhibition (Grossman et al., 2008) results in an even greater androgenic milieu, which negatively influences the delicate balance between androgens and FSH. In PCOS, circulating FSH levels are thought to be insufficient to reach the increased
FSH threshold of the follicle required for selection (Hillier, 1994; Franks and Hardy, 2010). Therefore, restoring the androgen/FSH balance in PCOS by administration of low-dose exogenous FSH during controlled ovarian stimulation typically yields multiple oocytes. Although there are concerns regarding the effect of androgens on oocyte quality and embryonic developmental competence for review, see Qiao and Feng, 2011), there is little evidence that impaired oocyte function is a significant contributor to PCOS subfertility, as normal cumulative conception rates can be achieved with appropriate ovulation-restoring treatment (Fauser et al., 2012).

Stromal hyperplasia, rigidity, hypervascularity and inflammation

Polycystic ovaries have an increased ovarian stromal volume, and ultrasound measurements correlate with the degree of hyperandrogenism (Kyei-Mensah et al., 1998; Fulghesu et al., 2007). Histological assessment of polycystic ovaries reveals increased thickness of the cortical and medullar stroma and hypervascularity (Hughesdon, 1982). Microarray data in PCOS show differential expression of genes involved in extracellular matrix formation (Jansen et al., 2004). Proteomic research in PCOS identifies differential expression of several proteins and filaments involved in fibrogenesis (Ma et al., 2007). PCOS women have higher levels of basic fibroblast growth factor in serum and follicular fluid (Artini et al., 2006). This exaggerated fibroblast proliferation, coupled with an increased extracellular matrix deposition greatly increases the rigidity of the ovarian cortical tissue. An important question is whether this stromal hyperrigidity in PCOS alters the steroidogenic behavior of the antral follicles and contributes to hyperandrogenenaemia (Woodruff and Shea, 2011). Although non-permissive or ‘rigid’ culture conditions for mouse in vitro follicular culture are associated with decreased steroid production, this effect has only been studied in larger multi-layered follicles thus far (Xu et al., 2006).

Zaidi et al. (1995) used color Doppler ultrasound to demonstrate significantly increased blood flow velocity in polycystic ovaries, with blood vessels running in an almost linear configuration in the ovarian stroma. This vascular increase is mainly observed in the cortical area (Delgado-Rosas et al., 2009). Circulating VEGF levels are typically higher in PCOS and are a reliable marker for ovarian stromal blood flow (Agrawal et al., 1998). Laparoscopic ovarian drilling reduces Doppler indices of ovarian stromal blood flow (Parsanezhad et al., 2003), with concomitant reduction in circulating androgen (Kaaijk et al., 2000) and VEGF levels (El Behery et al., 2011). The increased blood supply to the PCOS cortex further fuels the connective tissue proliferation and, importantly, provides the enclosed follicles with inappropriate amounts of oxygen, nutrients, growth factors and hormones. The profoundly altered follicular microenvironment in PCOS is an important disruptor of the normal follicle dynamics.

PCOS is considered to be a low-grade inflammatory condition, as illustrated by elevated C-reactive protein (CRP) levels, inflammatory cytokines (such as IL-6) and hyperleucocytosis (Diamanti-Kandarakis et al., 2006). Further studies are required to determine the contribution of these inflammatory cells, and their secreted cytokines, chemokines and growth factors in the pathogenesis of PCOS. In physiological conditions, stromal cells differentiate as the follicle matures, and it has been postulated that ovarian macrophages are attracted toward activated follicles and stay associated with this follicle throughout its development (Tingen et al., 2011). In benign prostate hypertrophy, an androgen-dependent recruitment and infiltration of macrophages is well described and contributes to the stromal hyperplasia (Izumi et al., 2013). It is worth investigating if a similar epithelial–stromal interaction occurs in PCOS.

The dialog between the follicular and stromal compartment is an essential, and somewhat forgotten, concept that one has to keep in mind when unraveling the complex pathogenesis of PCOS. The described mutual interactions are visualized in Fig. 3.

Implications for clinical translation: androgen treatment in subfertility

Low ovarian reserve is characterized by an impaired quantity and quality of the ovarian follicles, resulting in a diminished fertility potential, with advancing age as an important determinant. In the last decade, the idea has emerged that androgen administration to the improperly growing follicles in this condition would result in a PCOS-like phenotype, thereby improving the oocyte yield during ovarian hyperstimulation. A number of controlled studies suggest that adjuvant DHEA and testosterone treatment can enhance fertility in women with low ovarian reserve (Casson et al., 2000; Barad and Gleicher, 2005; Barad and Gleicher, 2006; Wiser et al., 2010; Gleicher et al., 2010b; Gleicher and Barad, 2011; Sunkara and Coomarasamy, 2011). In a randomized, open-label trial in 33 women with low ovarian reserve, DHEA (75 mg/day) was associated with a higher oocyte yield and a significantly increased birth rate (Wiser et al., 2012). In benign prostate hypertrophy, an androgen-dependent recruitment and infiltration of macrophages is well described and contributes to the stromal hyperplasia (Izumi et al., 2013). It is worth investigating if a similar epithelial–stromal interaction occurs in PCOS.

Figure 3 Working model for excess androgen action on follicle and stroma in PCOS. In PCOS, the disturbed balance between androgens, AMH and FSH leads to antral follicular arrest. Circulating FSH-levels are insufficient to reach the increased FSH-threshold of the follicles and selection for dominance does not occur. The hypervascular, rigid and inflammatory cortex negatively impacts the follicular dynamics. Exaggerated blood supply, partly mediated by local androgen overproduction, fuels the whole process. The blue ovals indicate follicles, red circles blood vessels, yellow squares inflammatory cells and green bowed lines stand for connective tissue.

---

832 Lebbe and Woodruff

Downloaded from https://academic.oup.com/molehr/article-abstract/19/12/828/1077154 by guest on 15 March 2019
et al., 2010). Decreased functional ovarian reserve not only results in longer time to conceive, but also comes with an increased risk for spontaneous miscarriage, aneuploidy and birth defects (Duncan et al., 2012). While mainstream thinking in the field incriminates degenerative changes that affect oocyte health (Navot et al., 1991), some researchers are opposed to the current dogma and speculate that primordial oocytes do not age, and that it is the follicular microenvironment that is prone to an age-related reduction in the quality (Gleicher and Barad, 2011; Gleicher et al., 2011). This hypothesis is based on limited clinical data suggesting that DHEA supplementation reduces age-related increases in aneuploidy (Gleicher et al., 2010a) and miscarriage (Gleicher et al., 2009). DHEA has the advantage of being a prohormone that is metabolized in target cells depending on the steroidogenic enzymes expressed, and therefore does not lead to supraphysiological androgen levels in the circulation. Despite the still weak clinical evidence and lack of rigorous randomized trials of sufficient size, it is estimated that approximately one-quarter of all IVF centers today use DHEA supplementation in women with low ovarian reserve (Sunkara et al., 2012).

Keeping in mind the crucial role of androgens in ovarian biology, we postulate that androgen deficiency has a negative impact on fertility. Female androgen excess is clearly recognized as detrimental for fertility, and the same causal relationship should be considered for androgen deficiency. Severe androgen deficiency is encountered in primary adrenal insufficiency (Addison’s disease) due to the pathological loss in adrenal DHEA synthesis (Lebbe and Ark, 2012). In a survey reporting on 269 Norwegian women with primary adrenal insufficiency, fertility was significantly reduced; the standardized incidence ratio for childbirth was 0.97 in the women before being diagnosed with adrenal failure, but dropped to 0.69 after the diagnosis had been established. This remained significantly reduced at 0.72 when excluding all women with premature ovarian failure (Erichsen et al., 2010). No studies thus far have looked at the effect of androgen-replacement therapy on fertility in adrenal insufficiency patients. The guidelines published by the Endocrine Society in 2006 recommend against making a diagnosis of androgen deficiency in women with normal adrenal function because of the lack of a clearly defined clinical syndrome, and of normative data on testosterone levels across women’s lifespan that can be used to define the disorder (Wierman et al., 2006). Given recent publications and developments, it may be time to revisit this guidance, considering the development of mass-spectrometry-based highly sensitive testosterone assays, the availability of satisfactory forms of androgen administration and emerging data on short-term safety data on androgen replacement in women (for review, see Marie Lebbe, 2012).

**Summary**

During folliculogenesis, the maturing follicle undergoes dramatic shifts in its steroidogenic capacity and endocrine responsiveness. In the preantral stage, the follicle is an androgen-secreting and paracrine-signaling unit. Gonadotrophin receptor expression and responsiveness converts the follicle to a predominantly estradiol-secreting endocrine organ, with androgens as the essential substrate.

Androgens exert their effect via genomic and possibly non-genomic ways, and amplify their local effects via an autocrine stimulatory loop involving the AR. AR expression is highest in the small antral follicles, where the trophic effects of androgen are maximal and synergistic with FSH and AMH. Androgens stimulate extracellular matrix and possibly new blood vessel formation, especially in the low oxygen and low androgen milieu of the ovarian cortex.

Androgen excess in PCOS not only disturbs the delicate balance between androgens, AMH and FSH, but also crucially contributes to ovarian tissue remodeling; stromal hyperplasia and rigidity, hypervascularity and inflammation. This joint follicular–stromal deregulation is a key mechanism in the pathogenesis of PCOS. Future research is required to gain molecular insight into the central negative role of androgen excess in these processes, aiming to offer new therapeutic opportunities for restoring fertility in women with PCOS.

Further studies are required to determine which subgroup of subfertile women could benefit from the positive effects of androgens on preantral follicle development.

**Acknowledgements**

The authors wish to thank Dr Stacey C. Tobin for editorial support.

**Authors’ roles**

M.L. conceived the article and wrote the first draft of this review. T.K.W. then commented on the manuscript and both authors approved the final version for publication.

**Funding**

This work was supported by Medical Research Council UK (Research Fellowship RRAK 16242 to M.L.) and by Project 1 of the U54HD076188 Center for Reproductive Health after Disease from the National Institutes of Health, Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), Specialized Cooperative Centers Program in Reproduction and Infertility Research (SCCPR) to T.K.W.

**Conflict of interest**

None declared.

**References**


Barad D, Gleicher N. Effect of dehydroepiandrosterone on oocyte and embryo yields, embryo grade and cell number in IVF. Hum Reprod 2006; 21:2845 – 2849.
Delgado-Rosas F, Gaytan M, Morales C, Gomez R, Gaytan F. Superficial ovarian cortex vascularization is inversely related to the follicle residue in normal cycling ovaries and is increased in polycystic ovary syndrome. Hum Reprod 2009; 24:1142 – 1151.
Role of androgens in ovarian biology


Gistrator C, Richards SL, Knight PG. Bone morphogenetic proteins (BMP)-4, -6, and -7 potently suppress basal and luteinizing hormone-induced androgen production by bovine theca interna cells in primary culture: could ovarian hyperandrogenic dysfunction be caused by a defect in theca BMP signaling? Endocrinology 2005;146:1883–1892.


Hillier SG, Tetsuka M. Role of androgen receptors and androgen-binding proteins in nongenomic androgen actions. Mol Cell Endocrinol 2002b;189:79–90.


Labrie F. DHEA, an important source of sex steroids in men and even more in women. Progr Brain Res 2010;182:97–148.


Downloaded from https://academic.oup.com/molehr/article-abstract/19/12/828/1077154 by guest on 15 March 2019


