

Androgen actions in the ovary: balance is key

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Abstract

For many decades, elevated androgens in women have been associated with poor reproductive health. However, recent studies have shown that androgens play a crucial role in women's fertility. The following review provides an overall perspective about how androgens and androgen receptor-mediated actions regulate normal follicular development, as well as discuss emerging concepts, latest perceptions, and controversies regarding androgen actions and signaling in the ovary.

Key Words

- ▶ androgen
- ▶ androgen receptor
- ▶ female reproduction
- ▶ ovary

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Introduction

Recently, there has been a lot of interest towards the role of androgens in the regulation of follicular development and female fertility. In fact, over the years our understanding of the effects of androgens on follicular development and female fertility has undergone considerable change. Androgens have traditionally been considered detrimental to ovarian function and are often associated with infertility. However, development of different types of androgen receptor (AR) knockout mouse models, along with various *in vivo* and *in vitro* studies, as well as clinical reports, has established a new concept that sufficient androgen actions through the AR is necessary for normal follicle development and function. Consequently, it is now increasingly realized that, likely, a critical balance exists between the essentiality of androgens in normal follicular development and their detrimental effects in hyper-androgenic conditions that regulate female fertility. While androgen excess enhances follicular development and dysfunctional formation of antral follicles leading to polycystic ovary syndrome (PCOS), low androgen levels may be associated with abnormalities of follicular growth, low functional ovarian reserve (LFOR) and primary

ovarian insufficiency (POI), thereby negatively impacting female fertility. Many studies (Kimura *et al.* 2007, Walters *et al.* 2008, 2010, Gleicher *et al.* 2011, Sen & Hammes 2011, Lebbe & Woodruff 2013) in the past 5 years have addressed androgen actions in the ovary. In this review, we summarize what currently is known about the direct physiological actions of androgens in the regulation of normal follicular development, and provide the molecular and/or signaling basis of these androgen actions. We also highlight the ranging controversy regarding the use of androgens as a treatment option in LFOR/POI patients.

AR expression and regulation

The idea that androgens might regulate follicular development initially started with studies in the mid- and late 1990s looking at AR expression in the ovary. Studies across species reported that ARs are expressed in theca cells, granulosa cells (GCs), and the oocyte of the follicle and throughout most stages of follicular development (Horie *et al.* 1992, Chadha *et al.* 1994, Hirai *et al.* 1994, Suzuki *et al.* 1994, Tetsuka & Hillier 1996, Hillier *et al.* 1997, Szoltys &

Slomczynska 2000, Slomczynska & Tabarowski 2001, Gill *et al.* 2004, Hampton *et al.* 2004, Juengel *et al.* 2006). However, their expression patterns may differ between ovarian cell types (Tetsuka & Hillier 1996, Szoltys & Slomczynska 2000) and in most species, AR is abundant in the preantral/antral stages of follicular development but declines as follicles mature to the preovulatory stage (Horie *et al.* 1992, Chadha *et al.* 1994, Suzuki *et al.* 1994, Hillier *et al.* 1997, Weil *et al.* 1998, Duffy *et al.* 1999, Slomczynska *et al.* 2001, Cardenas & Pope 2002, Hampton *et al.* 2004, Juengel *et al.* 2006). In fact, AR is expressed in cell-specific regions of human ovarian follicles at all stages of follicular development beyond the primordial phase (Rice *et al.* 2007). Based on these AR expression pattern studies, it was suggested that androgens through an autocrine and/or paracrine effect might differentially regulate various stages of follicular development. What exactly regulates AR expression in the follicle is not yet clear. Studies in primates (Weil *et al.* 1998) demonstrate that androgens (testosterone) in a positive feedback loop increases AR expression in the theca and GCs of preantral follicles, while studies in rats have shown that AR expression is developmentally regulated (Tetsuka *et al.* 1995). The general sense is that, as follicles grow from the pre-antral to the antral/preovulatory phase, the AR expression decreases, and the dominant steroid receptor shifts from AR to estrogen receptor.

Stimulatory effects of androgens in follicular development

Looking at different aspects of follicular development following androgen stimulation in various species, *in vivo* and *in vitro* studies in the late 1990s and early 2000s first demonstrated direct androgen effects in folliculogenesis. In rodents, androgen treatment enhances pre-antral follicular development and improves ovulatory response by increasing GC proliferation and attenuating follicular atresia (Mori *et al.* 1977, Ware 1982, Wang *et al.* 2001, Sen *et al.* 2014). Moreover, *in vitro* culture of whole mouse follicles in presence of androgens significantly increases the diameter of immature follicles and enhances the development of preantral follicles (Murray *et al.* 1998, Wang *et al.* 2001, Sen *et al.* 2014). In large farm animals, *in vivo* administration of testosterone was shown to stimulate the transition of follicles from primary to secondary stage (Hampton *et al.* 2004, Yang & Fortune 2006) and to increase the number of ovulatory follicles and corpora lutea (Cardenas & Pope 1994, Cardenas *et al.* 2002, Hickey *et al.* 2004, 2005). Finally, administration

of androgens in monkey (Abbott *et al.* 1998, Vendola *et al.* 1998) and ewes (Smith *et al.* 2009) demonstrated initiation of follicular recruitment, stimulation of early stages of follicular growth, and increase in the number of growing follicles. Importantly, in all these studies, it was demonstrated that the observed androgenic effects could be blocked by anti-androgens (Peluso *et al.* 1981, Murray *et al.* 1998, Yang & Fortune 2006). Despite all of these studies, the question still remained whether these reported androgen actions are the consequence of direct androgen actions via the AR or due to aromatization of androgens to estrogen.

AR knockout mouse models

Earlier, it was believed that androgens affect ovarian function either by acting as a precursor of steroidogenesis (specifically by aromatization to estrogen) or by indirect effects mediated by the hypothalamus–pituitary axis or metabolic tissues. The development of the global AR knockout (ARKO) female mouse models in the mid- and late 2000 established the importance of direct androgen action through ARs in normal female reproduction. The first *Ar*-null model (Lyon & Glenister 1974) carried homozygous mutation in the testicular feminization (*Tfm*) *Ar* gene on the X chromosome (*Ar^{tfm}/Ar^{tfm}*). *Ar^{tfm}/Ar^{tfm}* mice ovulated, mated, and maintained pregnancies, but demonstrated significantly reduced reproductive performance (Lyon & Glenister 1980). As the *Ar* gene is located on the X chromosome and males lacking functional *Ar* are infertile, homozygous female offspring lacking functional *Ar* were difficult to generate.

Later, however, using the Cre/LoxP system, three different global ARKO models were, nevertheless, generated by targeting deletion of exons 1 (*Ar₁^{-/-}*) (Shiina *et al.* 2006), exon 2 (*Ar₂^{-/-}*) (Hu *et al.* 2004), or exon 3 (*Ar₃^{-/-}*) (Walters *et al.* 2007) of the AR, leading to either the loss of AR protein (in *Ar₁^{-/-}* and *Ar₂^{-/-}*) or to a nonfunctional AR protein (in *Ar₃^{-/-}*). All of these *Ar*-null female mice were demonstrated to have normal ovarian and oviductal morphology but reduced fertility, with *Ar₁^{-/-}* and *Ar₂^{-/-}* exhibiting a mildly more severe phenotype than *Ar₃^{-/-}*. All three models are, however, distinctively sub-fertile, with smaller litter size, less corpora lutea, higher atretic follicle rates, and development of POI.

Given that ARs are expressed in all three components of the hypothalamus–pituitary–ovarian axis (Sullivan & Moenter 2004, Dart *et al.* 2013), two cell-specific (oocyte- and GC targeted) ARKO mice have been generated by crossing *Ar* (exon 2)-floxed mice with either growth-differentiation factor 9 (*GDF9*)-*Cre* or

anti-Müllerian hormone receptor type 2 (AMHR2)-*Cre* mice, respectively, to determine whether ovarian androgen activities are critical for normal female fertility (Sen & Hammes 2010). The oocyte-specific ARKO model has no reproductive phenotype, suggesting that, in the mice, androgen activity in oocytes is not necessary for normal female fertility. In contrast, the GC-specific ARKO model display reduced reproductive function in a very similar way to the global ARKO mice phenotype. The GC-specific ARKO mice are sub-fertile with longer estrus cycles and lower numbers of litters per female, have fewer ovulated oocytes with smaller litter sizes, and experience high rate of follicular atresia ultimately leading to early infertility. In addition, the ovaries from GC-specific ARKO mice contain more pre-antral and atretic follicles, with fewer antral follicles and corpora lutea. Finally, *in vitro* growth of follicles from GC-specific ARKO mice is slower than that of follicles from wild-type animals (Sen & Hammes 2010). These results suggest that, specifically in GCs, androgen activities regulate follicle progression from pre-antral stage into the antral stage. In absence of functional ARs in GCs, pre-antral follicles become atretic instead of developing into follicles that can be ovulated to produce corpora lutea (Fig. 1).

An additional GC-specific ARKO has been generated recently by crossing AR (exon 3)-floxed mice with the AMH-*Cre* mice (GC-ARKO) (Walters *et al.* 2012). Similar to the first GC-specific ARKO (Sen & Hammes 2010), this GC-ARKO female mouse model is subfertile, with longer estrous cycles, more atretic follicles, and fewer litters and

pups. In addition, these animals show decreased cumulus cell expansion and lower rates of fertilization, confirming in normal female fertility the importance of selective AR-mediated activities in GCs. GC-ARKO mice, however, exhibit less potent ovarian phenotypes, with no depletion of antral follicles and no reduction in corpora lutea. The main difference between these two GC-specific ARKO models is that in the first generated GC-specific ARKO mouse model GC-AR expression is completely lost, while the second (GC-ARKO) model retains a nonfunctional AR protein. Whether this truncated AR protein is still capable of inducing non-genomic/membrane-initiated signaling and thereby still can influence follicular development in the ovary remains to be determined.

The picture now evolving, based on all these studies for two decades, is that androgens are essential drivers of early follicular stage development, and are not just precursors of steroidogenesis in the later stages of folliculogenesis. It is now generally accepted that androgens primarily affect pre-antral follicles, and that their activities are important for pre-antral follicle growth and prevention of follicular atresia (Fig. 1). Though still lacking substantial evidence, a perception has recently arisen that androgens, in addition, may also be involved in activation of primordial follicles (Fig. 1). However, only few studies have so far suggested this concept (Abbott *et al.* 2005, Smith *et al.* 2009, Magamage *et al.* 2011), and in global and GC-specific ARKO mouse models, the number of primordial follicles was normal. Therefore, it is possible that androgens may not be essential for primordial

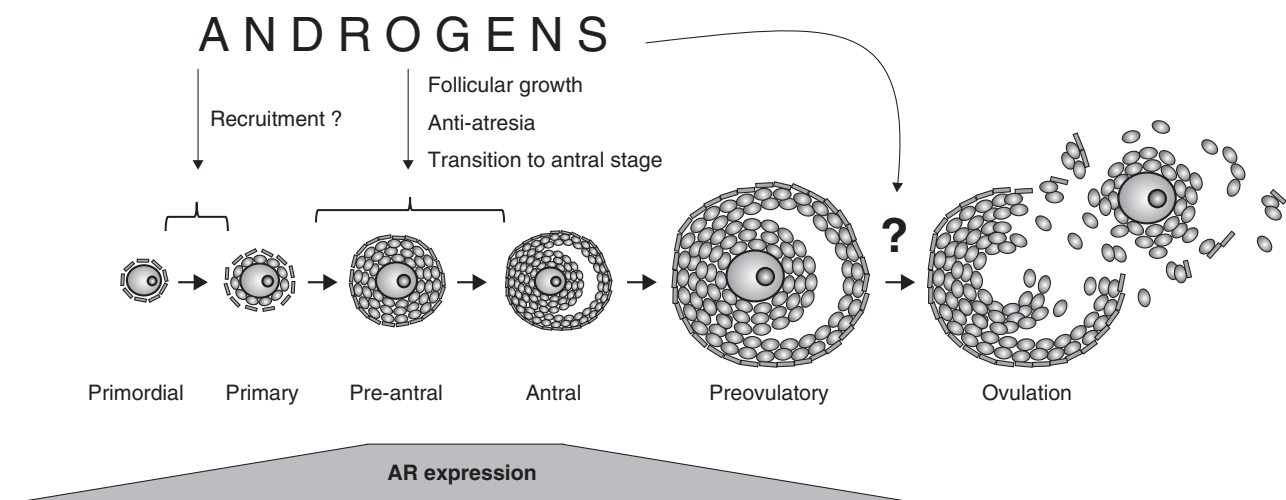


Figure 1

Physiological actions of androgens in follicular development. Androgens through androgen receptors directly regulate pre-antral follicle growth, prevent follicular atresia, and are involved in antral follicle formation.

Androgens have also been proposed to play a role in primordial follicle recruitment as well as in the ovulation process. However, further studies are needed to establish these proposed androgen actions.

follicle recruitment but may promote primordial follicle activation. Whether and how androgens effect primordial follicle recruitment and whether this is a primary or secondary response to androgens, are thus still an open-ended question and need further investigation.

Intracellular mechanisms of androgen actions in the ovary

Although our knowledge about AR expression and physiological effects of androgens during follicular development has greatly increased, our understanding about the underlying mechanism(s) of androgen-AR interactions regulating specific aspects of follicular physiology is still limited.

AR signaling

Androgen functions are mediated via either 'nuclear/genomic' or 'extra-nuclear/non-genomic' actions of ARs (Fig. 2). Recently (Sen *et al.* 2010, 2012, 2014), it has been found that androgens can promote Erk signaling via matrix-metalloproteinases (MMPs)-mediated trans-activation of

the epidermal growth factor receptor (EGFR), a mechanism that is conserved from *Xenopus* (Rasar *et al.* 2006) to mice (Carbajal *et al.* 2011) and humans (Evaul & Hammes 2008). These observations have given rise to the interesting concept that, outside the nucleus, androgen actions are very similar to those of growth factors. Intriguingly, a multi-domain adaptor protein, called paxillin (PXN), traditionally thought to regulate cytoskeletal remodeling and focal adhesion functions, is an essential mediator of androgen-induced Erk activation (Sen *et al.* 2010, 2012, 2014). Furthermore, PXN serves as a liaison between extranuclear signaling and nuclear transcription in response to androgens (Sen *et al.* 2012). Infact, PXN is necessary for nuclear localization of AR.

A recent study (Sen *et al.* 2014) has reported that the physiological effects of androgens may involve synergistic interaction between the nuclear and extranuclear signaling of ARs. Using mice GCs, a human GC tumor cell line (KGN), and primary human GCs isolated during oocyte retrieval from women undergoing *in vitro* fertilization, it was demonstrated that androgens, through PXN-regulated genomic as well as non-genomic AR actions, induce the expression of a micro-RNA (*miR-125b*) that decreases pro-apoptotic proteins, thereby contributing to

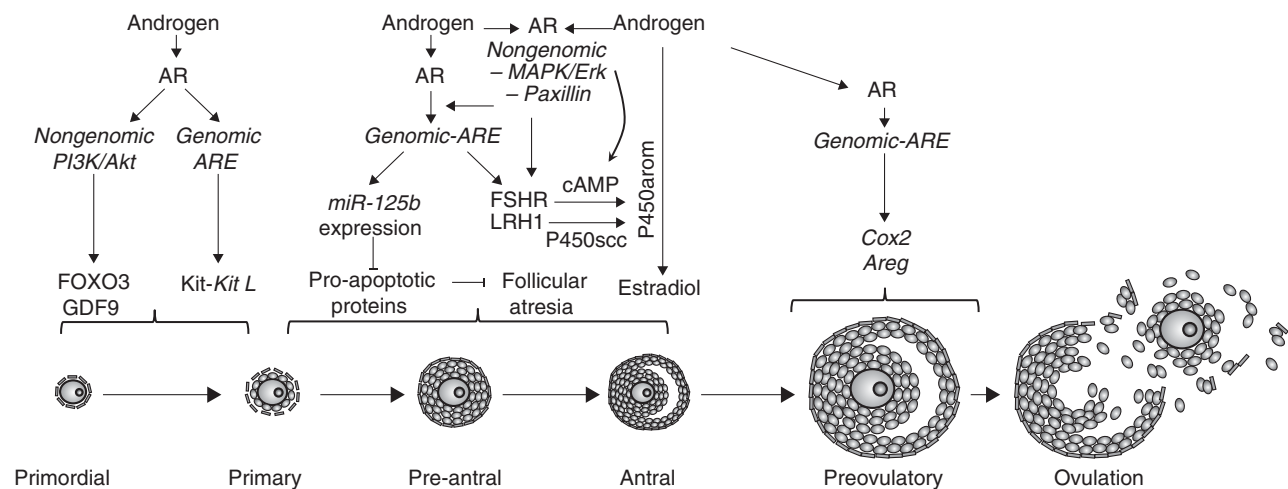


Figure 2

Underlying mechanisms of androgen actions in follicular development. Physiological functions of androgens are mediated through androgen response element (ARE)-dependent genomic actions and/or via membrane-initiated non-genomic signaling. During primordial follicle recruitment, androgens induce expression of KIT ligand. Also, it is hypothesized that the androgen receptor (AR)-induced PI3K/AKT pathway through modulation of FOXO3 and GDF9 may be involved in primordial follicle recruitment. In the pre-antral stage of follicular development, androgens through a synergistic interaction between the nuclear (ARE-dependent genomic actions) and extranuclear signaling (AR-induced Erk signaling via MMP-mediated trans-activation of the EGFR), regulated by a common adaptor protein called paxillin, induce the expression of a micro-RNA (*miR-125b*) in granulosa cells, which contribute to follicular survival by inhibiting

pro-apoptotic protein levels and preventing follicular atresia. Androgens also increase FSHR and intracellular cAMP levels that enhance the sensitivity of pre-antral follicles toward FSH actions. Moreover, androgens stimulate the expression of key steroidogenic enzymes, aromatase (P450arom) and P450 side chain cleavage enzyme (P450sc) in a mechanism mediated by ARE-dependent induction of an orphan nuclear receptor, liver receptor homolog 1 (LRH1). In addition, androgens serve as a precursor of estradiol synthesis. All these androgen-AR actions together promote pre-antral follicle growth and transition to antral stage. In peri-ovulatory GCs, androgens through ARE-dependent genomic actions can induce the expression of *Cox2* and *Areg* genes and thereby can directly influence the ovulatory process.

androgen-induced follicular survival (Sen *et al.* 2014). Based on these observations, it has been proposed (Fig. 2) that under normal conditions in the ovary, androgens may maintain a certain level of *miR-125b* expression, essential for preservation of a balance between follicular survival and atresia.

Androgens and follicle-stimulating hormone receptor

In different species, it has been for a long time reported that androgens demonstrate positive correlations with follicle-stimulating hormone receptor (FSHR) levels during follicular development. A recent study in human GCs from small antral follicles has reported a positive correlation between mRNA levels of *AR* and *FSHR* and androgen levels in follicular fluid (Nielsen *et al.* 2011). Furthermore, androgens were found to induce *FSHR* mRNA expression during pre-antral to antral follicle progression in several animal models, such as primates (Vendola *et al.* 1998, Weil *et al.* 1999), gilts (Cardenas *et al.* 2002), and bovine (Luo & Wiltbank 2006). Whether this induction of *FSHR* gene expression by androgens is mediated directly through AR-androgen response elements (ARE) genomic actions or is an indirect effect of androgens, themselves, is not yet clear. In contrast, a recent study has shown that androgens increase FSHR protein levels in a transcription-independent (non-genomic) fashion (Sen *et al.* 2014) through a PXN-dependent pathway. Despite these differences, all the studies uniformly suggest that androgen stimulation enhances follicular sensitivity toward FSH actions by increasing FSHR levels, which, potentially, contributes to follicle growth (Fig. 2). As specific AR antagonists block testosterone and dihydrotestosterone (DHT) stimulation of FSHR, this androgen-induced FSHR stimulation is specifically regulated through the AR (Luo & Wiltbank 2006, Sen *et al.* 2014).

AR-induced increase in FSHR levels, in turn, indirectly modulates downstream physiological actions of the FSHR. For example, it is well established that during follicular development, FSH stimulates P450 aromatase (P450arom) expression (Hillier 1994), a key enzyme in the biosynthesis of 17 β -estradiol (E₂) from androgens (Vegetti & Alagna 2006). Therefore, androgens can indirectly modulate P450arom activity in two distinct ways: as an enhancer, by increasing FSHR expression and augmenting FSH functions; and also as a substrate of E₂ synthesis (Tetsuka & Hillier 1997).

Various studies support a direct synergism between androgens and FSH in the ovary. In mice GCs, FSH alone

promoted follicular growth; however, addition of androgens significantly increased FSH-mediated follicular diameter (Wang *et al.* 2001, Sen *et al.* 2014). In addition, synergistic actions of testosterone and FSH increased P450arom expression and conversion of androgens to estrogens in the rat (Hillier & De Zwart 1981, Fitzpatrick & Richards 1991, Tetsuka *et al.* 1995), bovine (Luo & Wiltbank 2006), and primate GCs (Weil *et al.* 1999) as well as in porcine (Slomczynska *et al.* 2003) and mice small follicles (Wang *et al.* 2001). In primate GCs, P450arom mRNA was selectively expressed in AR and FSHR positive follicles (Weil *et al.* 1999); and in rat GCs, the stimulatory effect of testosterone on P450arom in response to FSH was inhibited by the non-steroidal anti-androgen SCH16423 (Hillier & De Zwart 1981). Importantly, as mentioned previously, the expression of AR gradually decreases as follicles mature, whereas expression of P450arom is increased as follicular differentiation progresses (Tetsuka & Hillier 1997, Weil *et al.* 1998). GCs from large preovulatory follicles, therefore, demonstrate decreased responses to FSH after androgen supplementation (Harlow *et al.* 1988, Hillier *et al.* 1988). Taken together, these results indicate that FSH-mediated P450arom induction in the ovary may be an AR-dependent and a developmental-stage regulated process (Hillier 1994).

The synergistic activity of androgen on FSH-induced actions may also lie at intracellular cyclic AMP (cAMP) level, a major intracellular mediator of FSHR signaling necessary for follicular cell proliferation and differentiation (Dorrington *et al.* 1983, Richards *et al.* 1983, Hillier 1987). Studies in rat GCs showed that testosterone stimulation of FSH-induced steroidogenesis was associated with increased cAMP accumulation in culture medium (Dorrington *et al.* 1983). This stimulatory effect of testosterone was again blocked by the nonsteroidal anti-androgen (SCH16423; Hillier & de Zwart 1982). Whether these androgen actions on cAMP levels are just a secondary effect of increased FSHR or are due to direct androgen effects on cAMP level is still unclear. Some studies proposed that androgen/AR signaling may potentiate FSH-induced steroidogenesis during GC differentiation by suppressing cAMP catabolism (Hillier & de Zwart 1982, Knecht *et al.* 1983, Hsueh *et al.* 1984).

Androgens, insulin-like growth factor, and GDF9 signaling

Androgens, acting via the AR, may regulate the expression and action of key ovarian growth factors during different stages of follicle growth. *In vitro* follicle culture studies in rats suggest that GDF9 promotes pre-antral to early

antral follicle transition by upregulating CYP17 expression (Vitt *et al.* 2000) and follicular androgen biosynthesis (Orisaka *et al.* 2009). In the same model system, specific AR antagonist suppressed the observed GDF9-induced pre-antral follicle growth (Orisaka *et al.* 2009).

In primate ovaries, *in vivo* treatment of both testosterone and DHT significantly enhanced insulin-like growth factor 1 (*IGF1*) and IGF1 receptor (*IGF1R*) mRNAs (Vendola *et al.* 1999a,b). More recently, DHT has been shown to enhance IGF1-stimulated porcine GC proliferation, by potentiating the mitogenic effects of GDF9 (Hickey *et al.* 2004), secreted by denuded oocytes (Hickey *et al.* 2005). These DHT effects were blocked by the presence of an AR antagonist (Hickey *et al.* 2004, 2005). A study in cultured rat pre-antral follicles, in addition, found that insulin-like 3 (INSL3), a theca cell-derived growth factor, increased testosterone production and promoted growth, an action mediated by GDF9 (Xue *et al.* 2014). Flutamide inhibited these effects of INSL3 on pre-antral follicular growth, suggesting that androgens are involved in GDF9- and INSL3-induced pre-antral follicular growth. All these observations indicate that an intra-ovarian growth factor system plays an essential role in ovarian follicular development, regulated by androgen/AR actions.

AR-induced genes in follicular development

Only a small number of direct AR-induced genes have unfortunately so far been identified in the ovary. They include the following:

Kit ligand (*Kitl*) *Kitl*, which is localized to oocytes and its receptor, *Kit*, localized to GCs, are among the first ligand-receptor systems to be identified in ovarian follicle (Motro & Bernstein 1993, Thomas & Vanderhyden 2006). *Kitl* was identified using genome-wide microarray analysis of RNA from *Ar1*^{-/-} mice ovaries (Shiina *et al.* 2006). In primary wild-type mice GCs, DHT induced the expression of *Kitl* (Wu *et al.* 2013), whereas flutamide attenuated the induction (Shiina *et al.* 2006). In addition, androgen-induced transactivation of *KITL* promoter was observed by a luciferase reporter assay in a tumor GC KGN cell line (Shiina *et al.* 2006). Thus, *KITL* represents a direct downstream target of androgen signaling (Fig. 2).

MicroRNA-125b (*miR-125b*) As mentioned earlier, in primary mice GCs, androgens through extranuclear AR signaling and nuclear AR actions synergistically induce the expression of *miR-125b* (Sen *et al.* 2014). The promoter

region of *miR-125b* contains AREs, and a ChIP assay confirmed that ARs bind to *miR-125b* promoter, following DHT stimulation.

Cyclooxygenase-2 (*Cox2* (*Ptgs*)) and amphiregulin (*Areg*) A recent study has demonstrated that during the ovulation process the androgen-AR pathway plays a role in the last stage of folliculogenesis (Yazawa *et al.* 2013). In peri-ovulatory GCs, *Cox2* and *Areg* genes were shown to contain AREs and can be induced by the DHT-AR pathway (Yazawa *et al.* 2013). These findings suggested that androgens, produced by the luteinizing hormone (LH) surge, are likely to act through the AR, and may be involved in the ovulatory process by directly regulating the expression of *Cox2* and *Areg* genes and their actions.

Cyclin-dependent kinase inhibitor-1/p21 p21 is known to play a role in GC luteinization (Robker & Richards 1998), and is downregulated in *Ar2*^{-/-} mice ovaries (Hu *et al.* 2004). The promoter region of p21 contains an ARE and, therefore, the AR likely regulates its expression. The marked reduction in p21 expression in the Ar-null ovary during the peri-ovulatory stage may disturb the process of terminating proliferation and explain the increased cell apoptosis and follicular atresia in these *Ar2*^{-/-} mice (Hu *et al.* 2004, Walters *et al.* 2010).

Liver receptor homolog (LRH1) The orphan nuclear hormone receptor LRH1 is expressed exclusively in GCs at all stages of follicular development (Falender *et al.* 2003). Interestingly, LRH1 has the ability to activate the transcription of number of steroidogenic enzymes, such as P450arom and cholesterol side-chain cleavage enzyme (P450csc; Sirianni *et al.* 2002). A recent study has demonstrated that testosterone stimulated P450arom and P450csc expression in rat GCs in a mechanism mediated by LRH1 expression (Wu *et al.* 2011). ARE was identified in *LRH1* promoter that responded selectively to testosterone by AR binding. A subsequent study demonstrated that testosterone stimulated the expression of aryl hydrocarbon receptor, which then formed a complex with the AR on the *LHR1* promoter (Wu *et al.* 2013).

These findings indicate that androgens not only synergize with FSH in the stimulation of P450arom and P450csc activity but also directly regulate their expression and, therefore, play a crucial positive stimulatory role in the differentiation process of GCs.

Future direction

The development of *Ar*-knockout female mouse models, along with various *in vivo* and *in vitro* studies, has established the importance of androgens and AR-mediated actions in normal female fertility. Despite all of these studies, our understanding of how androgens regulate follicular development is still limited.

As mentioned previously, further studies are needed to elucidate how androgens may regulate primordial follicle recruitment. Another area that has received little attention to date is membrane-initiated AR signaling, its interaction with other steroids and growth factors and specific aspects of ovarian physiology regulated by AR signaling. For example, it is hypothesized (Yang *et al.* 2010, Lebbe & Woodruff 2013) that the AR-induced PI3K/AKT pathway through modulation of FOXO3 and GDF9 may be involved in primordial follicle recruitment. However, further studies are needed to establish this fact. Moreover, other than the recent report that androgens may regulate FSHR levels in a transcription-independent fashion (Sen *et al.* 2014), nothing is known about the non-genomic effects of androgens and the hormone's underlying mechanism in the ovary. Many over the years have also proposed the idea that androgens may regulate AMH expression and/or levels (Lebbe & Woodruff 2013). This idea comes from correlation studies showing a positive relationship between androgens and AMH levels in follicular fluid. However, direct evidence of androgen-induced AMH expression and its underlying mechanism in GCs are still lacking. One of the biggest limitations for our understanding of how androgens regulate follicular development lies in the fact that very few *AR*-dependent genes with importance in ovarian physiology have yet been identified. With focus towards differentiating extra-ovarian and intra-ovarian effects, and the mechanism involved, another important question that needs to be addressed is the effects of androgen on the ovulation process. Addressing these questions would not only help to gain a better understanding of how androgen signaling contributes to pathophysiological conditions, such as LFOR, POI, and PCOS but, once these clinical conditions are better understood, allow for targeted therapeutic strategies to manipulate AR-mediated signaling and improve the prognosis for these common causes of infertility

Clinical perspectives

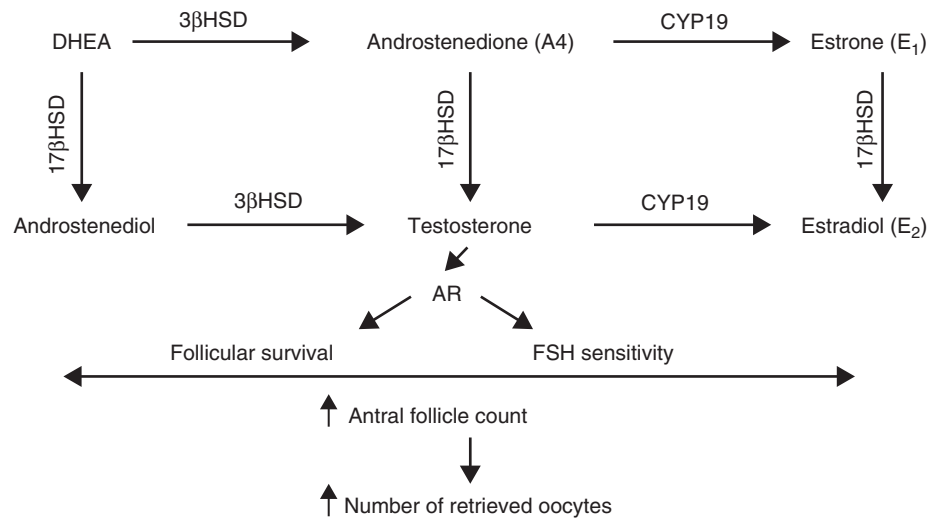
As evident from the above-mentioned scientific data, with respect to androgen effects on female fertility, 'Goldilocks' concept is the key. Too much is bad but too little is also bad

– it needs to be just right. In fact, there is evidence that androgen levels in women with LFOR are low, irrespective of age or premature ovarian aging (Gleicher *et al.* 2013a). It is hypothesized that this decrease in androgen level in women with LFOR is likely at least partially due to a relative adrenal insufficiency in androgen production. While androgen excess and PCOS have received a lot of focus, clinical perspectives with respect to low levels of androgens and its effect on female fertility have got little attention till date. Thus, in this section, we primarily focus on the 'too little' side of androgen levels/actions in the ovary.

In recent years, the use of androgens, specifically DHEA supplementation in women with LFOR, has gained increasing popularity around the world. The possibility that DHEA supplementation may beneficially affect women with LFOR was first suggested by Casson *et al.* (2000) who in a small cohort of women reported improved oocyte yields with short-term supplementation via DHEA. Thereafter, Gleicher and Barad in a series of studies with LFOR patients reported that DHEA supplementation not only improves oocyte yield but also positively affected egg and embryo quality, as well as *in vitro* fertilization (IVF) pregnancy rates (Barad & Gleicher 2005, 2006, Gleicher & Barad 2006, 2011). In recent years, many other research groups have also reported the positive effects of DHEA (Mamas & Mamas 2009, Sonmezer *et al.* 2009, Wisner *et al.* 2010, Sunkara & Coomarasamy 2011, Hyman *et al.* 2013, Yilmaz *et al.* 2013) and testosterone supplementation (Gonzalez-Comadran *et al.* 2012) on female fertility.

However, opinions are still divided on the issue of androgen treatment in LFOR patients. Initially, two main questions were raised against the idea of androgen supplementation: i) Lack of scientific evidence regarding the positive effects of androgens on ovarian physiology and ii) lack of enough rigorous randomized trials of sufficient size.

The studies mentioned earlier now quite well established the positive physiological effects of androgen supplementation on follicular development. We, recently, also started to understand the underlying cellular and molecular mechanisms of androgen supplementation at various stages of follicular development (Fig. 2). Moreover, as noted in preceding sections, it is now well recognized that androgens play a major role in follicular development and female fertility. Based on these studies, it is believed that DHEA gets metabolized to testosterone, and that the positive effects of DHEA supplementation are mediated through the AR (Fig. 3). However, not all women respond equally well to DHEA supplementation, a frequent argument against DHEA supplementation. Non-responding women apparently do not adequately

**Figure 3**

Proposed mode of DHEA actions in low functional ovarian reserve (LFOR) patients. Based on the studies in various animal models, it is hypothesized that when LFOR patients are supplemented with DHEA, the latter gets metabolized to testosterone. Thereafter, testosterone acting through the

convert DHEA to testosterone, a phenomenon apparently associated with advanced female age and so-called low mutations in the fragile X mental retardation 1 (*FMR1*) gene, characterized by trinucleotide repeat $CGG_{n<26}$ (Gleicher *et al.* 2013b). Currently, a clinical trial is underway to investigate the effects of transdermal testosterone in this nonresponding patient population. Whether simply increasing serum testosterone levels is always sufficient to improve fertility in women with LFOR is still unclear. Some women may exhibit an inherent problem with respect to AR signaling. In fact, AR polymorphism and/or role of AR-spliced variants with respect to AR functions in ovarian physiology have not been studied in great details.

A counter point that has been raised against the lack of rigorous randomized trials of sufficient size involving androgen supplementation in LFOR patients is the strategic difficulty in conducting such clinical trials. It has been argued that women with LFOR, perceiving time pressure, refuse randomization to placebo. Such clinical trials, from the start, therefore, face difficulties with patient recruitment. In the U.S., another problem is that, Congress legislatively prohibits funding of all IVF-related research, and there is lack of interest by the pharmaceutical industry to support such research. As a consequence, so far, therefore, only two small, greatly underpowered clinical trials of DHEA have been published in women with LFOR (Wiser *et al.* 2010, Yeung *et al.* 2014).

Despite these concerns, it is estimated that approximately one-quarter of all IVF clinics today use DHEA

androgen receptor (AR) promotes pre-antral follicular growth and survival, as well as increases the sensitivity of pre-antral-follicles toward FSH actions. All of these together increase the number of antral follicle count that ultimately leads to more number of retrieved oocytes during IVF treatment.

supplementation in women with LFOR (Sunkara *et al.* 2012). Regarding androgen supplementation in LFOR patients, it can, therefore, be stated that consensus has so far not been reached. As with any new emerging concept, time will be the best judge, as more and more studies from different clinical centers are published on this issue from around the world.

Declaration of interest

H P and A S have nothing to disclose. N G received in the past research and travel funds from various pharmaceutical and medical device companies, unrelated to the topics discussed in this study. He received travel funds and speaker honoraria from Sun Pharmaceutical Industries Ltd (India) to lecture on the utilization of DHEA in women with infertility. He holds a number of U.S. patents, claiming benefits on female fertility in women with LFOR from supplementation with androgens, including DHEA, and receives license fees from Fertility Nutraceuticals LLC, a supplement manufacturer. He is also a shareholder in this company.

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