REVIEW

Androgens and ovarian function: translation from basic discovery research to clinical impact

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Abstract

In the last decade, it has been revealed that androgens play a direct and important role in regulating female reproductive function. Androgens mediate their actions via the androgen receptor (AR), and global and cell-specific Ar-knockout mouse models have confirmed that AR-mediated androgen actions play a role in regulating female fertility and follicle health, development and ovulation. This knowledge, along with the clinical data reporting a beneficial effect of androgens or androgen-modulating agents in augmenting in vitro fertilization (IVF) stimulation in women termed poor responders, has supported the adoption of this concept in many IVF clinics worldwide. On the other hand, substantial evidence from human and animal studies now supports the hypothesis that androgens in excess, acting via the AR, play a key role in the origins of polycystic ovary syndrome (PCOS). The identification of the target sites of these AR actions and the molecular mechanisms involved in underpinning the development of PCOS is essential to provide the knowledge required for the future development of novel, mechanism-based therapies for the treatment of PCOS. This review will summarize the basic scientific discoveries that have enhanced our knowledge of the roles of androgens in female reproductive function, discuss the impact these findings have had in the clinic and how a greater understanding of the role androgens play in female physiology may shape the future development of effective strategies to improve IVF outcomes in poor responders and the amelioration of symptoms in patients with PCOS.

Introduction

Androgens are sex steroid hormones that are well known to be crucial for male sexual and reproductive function. In females, androgens play an essential role in ovarian follicular development as testosterone is the obligatory estradiol (E2) precursor (Simpson et al. 2002). In descending order of serum concentrations, dehydroepiandrosterone sulfate (DHEAS), dehydroepiandrosterone (DHEA), androstenedione (A4), testosterone and dihydrotestosterone (DHT) are the major circulating androgens in women (Davison & Davis 2003).

Androgens mediate their actions primarily via the androgen receptor (AR), which is a member of the nuclear receptor superfamily (Quigley et al. 1995). Classically, only the bioactive androgens, testosterone and DHT, bind directly to the AR, while the pro-androgens, DHEA and A4 require conversion to testosterone or DHT to exert androgenic effects (Burger 2002). However, recent reports have described direct effect of DHEA in the brain and vascular endothelial cells (Liu et al. 2008, Soma et al. 2015).

Keywords
- androgen
- ovarian follicle
- POR
- PCOS
AR mRNA and protein are expressed at all levels of the female hypothalamic-pituitary-gonadal (HPG) axis, including the brain, ovarian stroma, ovarian follicles and corpora lutea (Walters & Handelsman 2018). AR expression is present in fetal (Wilson & McPhaul 1996) and in adult (Suzuki et al. 1994) ovaries and has been identified in the majority of ovarian follicular stages, but with differential temporal and special patterns of expression, indicating distinct roles for AR-mediated actions during different stages of follicular development. In general, AR expression is present as follicles enter the growing pool, AR immunostaining is observed in the oocyte, granulosa cells and theca cells of preantral follicles, but during antral follicular growth, AR expression progressively declines in the outer mural granulosa cells but remains intense in the cumulus cells (Szoltys & Slomczynska 2000, Lenie & Smitz 2009) (Fig. 1). Interestingly, both phosphorylated and non-phosphorylated forms of AR have been detected in primate ovarian granulosa cells of developing follicles, inferring that apart from de novo synthesis, phosphorylation of AR may also regulate receptor levels and function (McEwan et al. 2010). Moreover, ovarian AR expression across numerous mammalian species (mouse; Lenie & Smitz 2009, rat; Szoltys & Slomczynska 2000, sheep; Juengel et al. 2006, pig; Slomczynska & Tabarowski 2001, cow; Hampton et al. 2004, primate; Hild-Petito et al. 1991 and human; Horie et al. 1992, Nielsen et al. 2011) is evolutionarily conserved, supporting a universal role for AR-mediated androgen actions on the function of the ovary.

### Defining poor ovarian response (POR) patients and evidence to support the use of androgens or androgen-modulating agents in POR patients undergoing IVF

#### Characteristics of poor ovarian response

As women age, there is a progressive decline in the number of oocytes in the ovaries, and this correlates with an age-related decrease in fecundity. This reduction in fertility is greatest in women in their late 30s and early 40s, with menopause and natural reproductive sterility reached.

![Intensity of AR expression in granulosa cells](https://joe.bioscientifica.com)

**Figure 1**

Pattern of androgen receptor (AR) expression and the impact of androgen exposure on ovarian dynamics at different stages of follicle development. AR expression in granulosa cells is first detected as follicles enter the growing pool and remains detectable throughout follicle development. In general, the intensity of AR expression in granulosa cells follows a gradient pattern during follicle development. AR expression increases to the antral stage and then progressively declines in the outer mural granulosa cells of antral follicles, but remains intense in the cumulus cells surrounding the oocyte (pattern of AR immunostaining observed in several species including porcine (Slomczynska & Tabarowski 2001) and mice (Lenie & Smitz 2009)). Overall, androgens have a stimulatory role on follicle development, with their actions observed to optimize follicle growth, follicle health and ovulation. COX-2, cyclo-oxygenase; FSHR, follicle-stimulating hormone receptor; IGF1, insulin-like growth factor 1; IGF1R, insulin-like growth factor 1 receptor.
at an average age of 51 years. Along with this observed decline in the quantity of oocytes, the quality and the competence of the oocytes to result in a live birth also diminishes as women age (Homan et al. 2007). As a result, women aged 35–39 years of age have a 50% reduced chance of conceiving spontaneously when compared to 19-25 year old women (Taylor 2003). Inevitably, as more couples are delaying the start of their family until women are in their late 30s, the number of couples seeking infertility treatment options has increased. IVF is a key assisted reproductive technology (ART); however, a notable proportion of women undergoing IVF treatments fail to have an adequate ovarian response to the hormonal stimulation (Tarlatzis et al. 2003). Age is known to be the main correlated risk factor for poor ovarian response (POR) (Ferraretti et al. 2011). This is not only because of the diminished follicular pool in the ovaries of aged women (Gougeon 1996), but also as a result of the declined ovarian response to follicle-stimulating hormone (FSH) with advancing age (Goverde et al. 2005).

Numerous definitions have been applied to define POR in women undergoing IVF (Polyzos & Devroey 2011); however, the Bologna criteria was the first sound attempt to form an international accepted consensus on the definition of POR (Ferraretti et al. 2011). According to the Bologna criteria, a women is defined as having POR if at least two of the following features are present: advanced maternal age (>40 years of age) or any other risk factor for POR; a previous POR (<3 oocytes with a conventional ovarian stimulation protocol) and/or an abnormal ovarian reserve test (i.e. antral follicle count (AFC) ≤5–7 follicles or anti-Müllerian hormone (AMH) <0.5–1.1 ng/mL).

Clinical evidence indicating the need for optimized androgen levels during follicle development

FSH and luteinizing hormone (LH) secreted from the pituitary act on the ovary to stimulate the production of androgens in theca cells and estrogen synthesis in granulosa cells (Hillier et al. 1994) (Fig. 2). Circulating levels of pro-androgens (DHEAS, DHEA, A4) and the bioactive androgen testosterone have been reported to decline with age (Zumoff et al. 1995, Davison et al. 2005). This decline is reported to be steepest in the early reproductive years, with no significant effect of natural menopause on circulating androgen levels, unlike E2 which displays a sharp decline at this time (Davison et al. 2005). This decline in androgen levels with age has been proposed to potentially contribute or reflect the diminishing ability of the aging ovary to respond to FSH-based stimulation in IVF. As such, it has been suggested that the bioavailability of androgens within the ovary may increase follicular response. Several studies have reported on whether serum androgen levels can predict ovarian response or IVF outcomes. In normal cycling women, there is a positive correlation between free androgen index and total ovarian follicle count following ovarian hyperstimulation (Dickerson et al. 2010). Similarly, serum testosterone levels have been described to positively correlate with ovarian response (Barbieri et al. 2005, Sun et al. 2014, Xiao et al. 2016) and pregnancy outcomes (Frattarelli & Peterson 2004, Lu et al. 2014) in IVF, inferring that a lower level of testosterone may relate with a poorer ovarian response (Sun et al. 2014, Xiao et al. 2016) and a decreased success in achieving a pregnancy. However, studies often do not report a consistent correlation between serum androgen levels and IVF reproductive outcomes (Lu et al. 2014, Sun et al. 2014, Xiao et al. 2016). Additionally, in a more recent study using highly sensitive and specific liquid chromatography–mass spectrometry methods, androgen levels in either serum or follicular fluid from the dominant follicle did not predict IVF outcomes (Walters et al. 2019).

Clinical and animal studies supporting a beneficial role for androgens in follicle development

Clinical conditions where excessive levels of androgens are present in women, such as congenital adrenal hyperplasia (Lucis et al. 1966), testosterone-treated female-to-male transsexuals (Becerra-Fernandez et al. 2014) and PCOS (Dumesc et al. 2015) have revealed that elevated levels of androgens stimulate early follicle development but then lead to arrested antral follicle development, as all of these patient groups have been reported to exhibit polycystic-like ovaries. Confirmation of an influence of androgens from the early stages of follicle development comes from animal studies where the bioactive androgens testosterone and DHT in mice (Yang et al. 2010) and primates (Vendola et al. 1999) stimulate primordial follicle initiation. AR gene expression in several species is most abundant in the granulosa cells of healthy growing follicles (Walters & Handelsman 2018). Various androgens (testosterone, A4, DHEA and DHT) also significantly promote preantral to antral follicle growth in mice (Murray et al. 1998, Wang et al. 2001), sheep (Narkwichean et al. 2014) and primates (Vendola et al. 1998). These effects were confirmed to be due to direct AR-mediated actions as the observed stimulatory effects in mice were blocked by an AR antagonist bicalutamide (Murray et al. 1998).
There is also evidence supporting a synergistic interaction between androgens and FSH in the regulation of follicle growth, which has obvious significance in terms of hormonal ovarian stimulation regimes during IVF. Testosterone and DHT treatment in a mouse in vitro culture system enhances FSH-mediated preantral to antral follicle development in a dose-dependent manner (Sen et al. 2014, Laird et al. 2017), with effects reversed by an AR antagonist flutamide (Laird et al. 2017). Moreover, findings from rat (Hillier & De Zwart 1981) and primate (Harlow et al. 1986) in vitro granulosa cell cultures have demonstrated that androgens can modulate FSH-induced steroidogenesis. FSH receptor mRNA expression in primate primary follicles increased following treatment with testosterone (Weil et al. 1999), while treatment with DHT or testosterone increased FSH receptor protein expression in growing mouse follicles (Sen et al. 2014, Laird et al. 2017). Similarly, both AR mRNA expression in granulosa cells and androgen levels in follicular fluid collected from human small antral follicles showed a highly significant positive association with the expression of FSH receptor mRNA in granulosa cells. These findings suggest that follicular sensitivity to FSH stimulation may be augmented by androgen stimulation mediated via the AR. Indeed, responsiveness of follicles to FSH was enhanced when mouse preantral follicles were cultured in the presence of testosterone (Wang et al. 2001).

Several studies also indicate that the mechanisms regulating oocyte maturation and ovulation in the late stages of follicle development can also be influenced by androgens. In vitro germinal vesicle breakdown (GVBD) in mouse (Gill et al. 2004) and pig (Li et al. 2008) oocytes is enhanced in the presence of testosterone, with effects lost after the addition of the AR blocker flutamide. However, the level of androgen exposure appears to be important, as mouse oocyte meiotic competence was reduced by elevated levels of testosterone and A4 (Romero & Smitz 2010). Preovulatory follicle and corpora lutea numbers

Figure 2
Androgen biosynthesis in rodent ovarian follicles and different modes of androgenic actions. Androgens synthesis and metabolism occur in the theca and granulosa cells of the ovarian follicle. In comparison to rodents, in primates, including humans, progesterone is not a source for androgen biosynthesis as CYP17A1 does not convert 17α-hydroxyprogesterone to androstenedione. Theca cells from sheep, humans and primates principally produce androstenedione, whereas rodents produce testosterone as precursors for estradiol production in the neighboring granulosa cells. Androgens can mediate their actions directly via the androgen receptor (AR), or indirectly by their conversion into estrogens and 3β-diol, both of which can interact with the estrogen receptor (ER). 3βHSD, 3-β-hydroxysteroid dehydrogenase; 17β-hydroxysteroid dehydrogenase; 3β-diol, 5α-androstan-3α,17β-diol; 3α-diol, 5α-androstan-3α,17β-diol.
were increased after pigs were treated with testosterone or DHT during the late follicular phase (Cárdenas & Pope 1994, Cardenas et al. 2002). This effect on ovulatory processes appears to be AR mediated as treatment with the AR antagonist cyproterone acetate, decreased ovulations in mice (Sen et al. 2014) and rats (Kumari et al. 1978). However, again androgen levels appear to be important as low but not high doses of DHT were found in mice to enhance the ovulatory response to superovulation (Sen et al. 2014). Similarly, in a mouse in vitro culture system, AR-mediated effects on follicle growth and antrum formation display a biphasic pattern, with low doses of androgens stimulating rapid follicle maturation but high doses inhibiting oocyte maturation and follicle growth (Lebbe et al. 2017).

Confirmation from AR-knockout (ARKO) mouse models of a role for androgens in follicle development

Pharmacological studies have proved to be extremely informative in elucidating the role of androgens in ovarian function; however, they have often led to confusion when defining the mechanism by which the actions are mediated. This is due to the fact that aromatizable androgens can be converted into estrogens, and the non-aromatizable androgen DHT can be reduced into 3β-diol, all of which have the potential to exert indirect actions via the estrogen receptor (ER) (Hillier et al. 1994, Steckelbroeck et al. 2004) (Fig. 2). Moreover, while AR antagonists are useful tools they too can lead to difficulty in deciphering the precise mechanism, similar to all steroid blockers, AR antagonists are often mixed partial agonists/antagonist rather than pure blockers. However, with the advent of Cre/LoxP technologies (Kuhn & Torres 2002) and the generation of global and cell-specific AR-knockout mouse models, key roles for androgen actions and detailed insights into the precise AR-mediated mechanisms involved in regulating female reproduction have been revealed (Table 1).

To date, three global androgen receptor-knockout (ARKO) mouse models have been generated with targeted deletions of exon 1 (ARKO<sup>Δx1</sup>) (Shiina et al. 2006), exon 2 (ARKO<sup>Δx2</sup>) (Hu et al. 2004) or exon 3 (ARKO<sup>Δx3</sup>) (Walters et al. 2007) of the Ar gene. Subfertility, observed as fewer pups per litter, and dysfunctional ovarian follicle development are observed in all three of the different global ARKO mouse models (Yeh et al. 2002, Hu et al. 2004, Shiina et al. 2006, Walters et al. 2007). Hence, these models have confirmed a role for AR-mediated actions in ovarian follicle development and female fertility. A loss of AR actions leads to elevated levels of follicular atresia (Yeh et al. 2002, Hu et al. 2004, Shiina et al. 2006, Walters et al. 2007) and an alteration in key regulators of follicle health, evident by the observed reduction in ovarian expression of FSH and IGF1 receptors (Hu et al. 2004). In particular AR actions appear to play a crucial role during the later stages of follicle development as in global ARKO females, fewer preovulatory follicles are present, oocytes lose contact with surrounding cumulus cells and corpora lutea numbers are reduced, confirming reduced ovulation rates (Hu et al. 2004, Shiina et al. 2006, Walters et al. 2007, Cheng et al. 2013). Expression of several important regulatory genes have also been identified as being disrupted during these late stages by the loss of AR signaling, including hyaluronan synthase 2 (Hu et al. 2004), tumour necrosis factor-α-stimulated gene 6 (Hu et al. 2004), KIT ligand (Shiina et al. 2006), bone morphogenetic protein 15 (Shiina et al. 2006) and growth differentiation factor 9 (Shiina et al. 2006).

The involvement of AR signaling in the different ovarian cell types has been revealed by characterization of granulosa cell-specific ARKO (GCARKO) (Sen & Hammes 2010, Walters et al. 2012b), theca cell-specific (TCARKO) (Ma et al. 2017) and oocyte-specific ARKO (OoARKO) (Sen & Hammes 2010) mouse models. Two different GCARKO models have been reported with targeted deletions of exon 2 (GCARKO<sup>Δx2</sup>) (Sen & Hammes 2010) and exon 3 (GCARKO<sup>Δx3</sup>) (Ma et al. 2017). Similar to global ARKO mice, GCARKO females mice exhibited subfertility (Sen & Hammes 2010, Walters et al. 2012b), and granulosa cell AR actions appear to play an important role in maintaining normal follicular growth and health as GCARKO ovaries display signs of defective follicle development reduced follicle health (Sen & Hammes 2010, Walters et al. 2012b). These results confirm that granulosa cells are a key site for androgenic actions regulating female reproductive function. On the other hand, AR signaling within theca cells and oocytes do not appear to significantly influence ovarian function as both TCARKO and OoARKO females display normal estrous cycle patterns and fertility (Sen & Hammes 2010, Ma et al. 2017). However, it should be noted that low levels of Ar mRNA expression were identified in both the TCARKO (Ma et al. 2017) and OoARKO (Sen & Hammes 2010) models, indicating that potentially the contribution of AR theca cell and oocyte actions to ovarian function may be underestimated in these mouse models.

Along with a direct role for AR actions within the ovary, it is now clear that AR signaling across the
Table 1  Summary of the reproductive phenotype observed in distinct global and cell-specific female androgen receptor-knockout (ARKO) mouse models.

<table>
<thead>
<tr>
<th>Transgenic ARKO mouse model</th>
<th>Fertility</th>
<th>Estrous cycles</th>
<th>Serum steroids and hormones</th>
<th>Growing follicle populations</th>
<th>Oocyte and follicle health</th>
<th>Corpora lutea population and ovulation</th>
<th>Embryo development</th>
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<tbody>
<tr>
<td><strong>Global</strong></td>
<td>↓ pups/litter</td>
<td>–</td>
<td>No change in FSH, LH, E2, testosterone or P4 at proestrus</td>
<td>Normal at 8 weeks Total follicle exhaustion by 40 weeks</td>
<td>↓ atretic follicles</td>
<td>↓ CL</td>
<td>–</td>
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<tr>
<td>ARKOk decoy</td>
<td>↓ pups/litter</td>
<td>↑ estrous cycle length</td>
<td>No change in FSH, LH, E2, testosterone at diestrus. ↓ LH after OVX. Normal LH response to GnRH and OVX+E2</td>
<td>Normal at 10–12, 26 and 52 weeks at diestrus Preovulatory follicles</td>
<td>↑ unhealthy antral follicles</td>
<td>↓ CL</td>
<td>↑ superovulated oocytes</td>
</tr>
<tr>
<td>Shiina et al. (2006)</td>
<td></td>
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<td></td>
<td></td>
<td>No change in FSH, LH, E2</td>
<td>–</td>
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<tr>
<td>ARKOk decoy</td>
<td>↓ pups/litter</td>
<td>↑ estrous cycle length, irregular estrous cycles</td>
<td>No change in FSH, LH, E2, testosterone at diestrus. ↓ LH after OVX. Normal LH response to GnRH and OVX+E2</td>
<td>Normal at 10–12, 26 and 52 weeks at diestrus Preovulatory follicles</td>
<td>↑ unhealthy antral follicles</td>
<td>↓ CL</td>
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<td>Yeh et al. (2002), Hu et al. (2004)</td>
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<td></td>
<td>No change in FSH, LH, E2</td>
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<tr>
<td>Walters et al. (2007, 2009), Cheng et al. (2013)</td>
<td>↓ pups/litter</td>
<td>↑ estrous cycle length at 6 months but not 2 months</td>
<td>No change in FSH or LH at diestrus</td>
<td>Normal at 6 months Health of preantral follicles</td>
<td>↑ unhealthy large follicles and ZPR counts at 6 months</td>
<td>↓ CL</td>
<td>–</td>
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<tr>
<td>Ovary</td>
<td>↓ pups/litter</td>
<td>↑ estrous cycle length at 6 months but not 3 months</td>
<td>No change in FSH or LH at diestrus</td>
<td>↓ large preantral and small antral follicles at 3 months. No difference in follicle populations at 6 months Normal</td>
<td>↑ unhealthy antral follicles</td>
<td>↓ CL</td>
<td>–</td>
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<tr>
<td>GCARKO decoy</td>
<td>↓ in cumulative pups/month from 6 months</td>
<td>↑ estrous cycle length at 6 months but not 3 months</td>
<td>No change in FSH or LH at diestrus</td>
<td>Normal at 6 months Health of preantral follicles</td>
<td>↑ unhealthy large follicles and ZPR counts at 6 months</td>
<td>↓ CL</td>
<td>–</td>
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<tr>
<td>Walters et al. (2012a,b)</td>
<td></td>
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<td></td>
<td>No change in CL populations</td>
<td>–</td>
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<tr>
<td>TCARKO</td>
<td>Normal fertility</td>
<td>Normal estrous cycles</td>
<td>No change in FSH, LH, E2 or testosterone at diestrus. Normal LH response to GnRH</td>
<td>Normal</td>
<td>–</td>
<td>No change in CL populations</td>
<td>–</td>
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<tr>
<td>Ma et al. (2017)</td>
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<td>No change in CL populations</td>
<td>–</td>
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<tr>
<td>OoARKO</td>
<td>Normal fertility</td>
<td>Normal estrous cycles</td>
<td>–</td>
<td>Normal</td>
<td>–</td>
<td>No change in CL populations</td>
<td>–</td>
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<tr>
<td>Sen &amp; Hammes (2010)</td>
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<td></td>
<td>No change in CL populations</td>
<td>–</td>
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<tr>
<td>Brain</td>
<td>↓ pups/litter</td>
<td>Trend to ↑ time at estrus</td>
<td>↑ FSH at all estrous cycle stages. ↓ LH but no change in E2 or testosterone at proestrus. Normal LH response to GnRH. ↓ LH and FSH after OVX and OVX+E2</td>
<td>Normal</td>
<td>↑ pyknotic granulosa cells in antral follicles</td>
<td>↓ CL</td>
<td>–</td>
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<tr>
<td>PitarKo</td>
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<td></td>
<td></td>
<td>No change in CL populations</td>
<td>–</td>
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<tr>
<td>Wu et al. (2014)</td>
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<td></td>
<td></td>
<td>No change in CL populations</td>
<td>–</td>
</tr>
<tr>
<td>NeurARKO</td>
<td>Normal fertility</td>
<td>Trend to less regular estrous cycle patterns</td>
<td>↑ LH but no change in FSH at diestrus. No change in LH or FSH at proestrus. ↓ LH response to OVX+E2</td>
<td>Normal at diestrus Large antral follicles at proestrus. ↓ LH response to OVX+E2</td>
<td>Large preantral follicles Unhealthy small antral follicles</td>
<td>↓ CL</td>
<td>–</td>
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<tr>
<td>Caldwell et al. (2017), Walters et al. (2019)</td>
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<td>No change in CL populations</td>
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DHT, dihydrotestosterone; E2, estradiol; FSH, follicle-stimulating hormone; GnRH, gonadotrophin-releasing hormone; GVBD, germinal vesicle breakdown; LH, luteinizing hormone; OVX, ovariectomy; P4, progesterone; ZPR, zona pellucida remnants.
HPG axis is required to maintain normal female reproductive function. Strong evidence to support this initially came from the observation that transplantation of ovaries from a global ARKO or control female into an ovariectomized control host, caused no change in estrous cycle or fertility in the host (assessed by percentage of females to undergo a successful pregnancy and the number of litters per female). On the other hand, transplantation of ovaries from a control female into an ovariectomized global ARKO female, led to disrupted estrous cycles and reduced fertility in the host (Walters et al. 2009).

Subsequently, transgenic mouse models exhibiting a loss of AR signaling in the pituitary (PitARKO) (Wu et al. 2014) or in the brain and pituitary (NeurARKO) (Caldwell et al. 2017, Walters et al. 2018b) have been generated and have confirmed a role for neuroendocrine AR actions in the regulation of the ovarian follicle dynamics. PitARKO females are subfertile, which appears to be due to the defects during late stages of follicle development as PitARKO ovaries display reduced antral follicle health and fewer corpora lutea (Wu et al. 2014). Surprisingly a loss of AR signaling in the neurons did not alter female fertility (Walters et al. 2018b). This inconsistency may be explained by the potential hypothyroidism in the PitARKO model due to this model being created by the use of gonadotrophin alpha subunit (αGSU)-Cre that is expressed in not only pituitary gonadotropes but also the thyrotropes. However, NeurARKO females also display altered follicle development with reduced preantral and antral follicle health and large antral follicle numbers at proestrus (Caldwell et al. 2017, Walters et al. 2018b). Moreover, NeurARKO females exhibited aberrant neuroendocrine control with females displaying elevated LH levels at diestrus, a compromised serum LH response to ovariectomy and E2 priming and reduced Kiss1 mRNA expression in the anteroventral periventricular nucleus, but elevated Kiss1 and neurokinin B mRNA expression in the arcuate nucleus at proestrus (Walters et al. 2018b).

Androgen administration

Dehydroepiandrosterone (DHEA)

Androgen pre-treatment therapy originated following a 2000 report of a small, uncontrolled investigation where administration of DHEA (80 mg/day) for 2 months prior to ovarian stimulation in five women with a previous history of POR was claimed to improve the ovarian response even after controlling for gonadotrophin dose (Casson et al. 2000). Subsequently, following DHEA treatment, a case study reported that a 43-year-old woman had an increase in her oocyte yield from initially 1 to up to 17 oocytes in subsequent cycles (Barad & Gleicher 2005), and in a larger self-controlled study, ovarian response and fertilization rate were reported to significantly improve (Barad & Gleicher 2006). These findings put forward the proposal that androgen supplementation may represent a novel way to enhance ovarian response.

Barad et al. followed up their observations in 2005 (Barad & Gleicher 2005) and 2006 (Barad & Gleicher 2006) with a much larger case-control study in 2007, which evaluated the efficacy of DHEA pre-treatment (75 mg/day for 4 months) in 190 patients (Barad et al. 2007). Analysis revealed a beneficial effect of DHEA treatment on ovarian response with an increased number of oocytes recovered and a significantly higher clinical pregnancy rate in DHEA-treated patients (Barad et al. 2007). Numerous investigations have now reported on the efficacy of DHEA pre-treatment in woman who exhibit POR (Table 2). In the majority of studies, a similar dose (75 mg/day) and treatment duration (~3 months) have been used, but the number of patients assessed (1–386 women) and the later stages of development. Based on this, it has been put forward that there is strong evidence to support the current, but still unproven, notion of administration of androgen pre-treatment to POR patients undergoing IVF, with the aim of enhancing their follicular response to IVF hyperstimulation. The use of androgen pre-treatments is now undertaken in IVF centers worldwide, with one study claiming that one-third of all IVF centers use DHEA supplementation (Gleicher & Barad 2011). At present three modes of treatment have been trialed clinically to increase androgen levels in POR IVF patients, including (i) systemic androgen administration (DHEA or testosterone), (ii) stimulation of endogenous androgen production in theca cells by LH or human chorionic gonadotrophin (hCG) and (iii) administration of an aromatase inhibitor (letrozole) to increase androgen levels by preventing the conversion of androgens into estrogens.

Use of androgens or androgen-modulating agents in POR patients undergoing IVF

For women who have responded poorly to ovarian stimulation, the challenge is how to increase their response in future cycles, as there is no evidence-based standard of care. The majority of findings from basic discovery research support a stimulatory role for androgens in early follicle growth (Fig. 1), maintenance of follicle health and priming of the follicle during the...
<table>
<thead>
<tr>
<th>Study type</th>
<th>Sample size</th>
<th>Study type</th>
<th>Intervention</th>
<th>Route</th>
<th>Fertilized oocytes retrieved</th>
<th>Number of oocytes retrieved</th>
<th>Number of MII oocytes</th>
<th>Fertilized oocytes rate</th>
<th>Embryo implantation rate</th>
<th>Clinical pregnancy rate</th>
<th>Embryo implantation rate</th>
<th>Clinical pregnancy rate</th>
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<th>Live birth rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casson et al. (2000)</td>
<td>5</td>
<td>Self-control</td>
<td>DHEA Oral</td>
<td>80 mg/day</td>
<td>Improved ( @ (T:2.2, C:1.0) )</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>(T:1, C:0)@</td>
<td></td>
</tr>
<tr>
<td>Barad &amp; Gleicher (2005)*</td>
<td>1</td>
<td>Case report</td>
<td>DHEA Oral</td>
<td>75 mg/day</td>
<td>Improved ( ^\wedge (T:1.8 \pm 1.4, C:3.0 \pm 2.2) P &lt; 0.001 )</td>
<td>Improved ( ^\wedge (T:1.35 \pm 3.5, C:3.0 \pm 2.2) P &lt; 0.01 )</td>
<td>–</td>
<td>Improved ( (T:3.0 \pm 0.5, C:1.4 \pm 0.3) P &lt; 0.001 )</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Barad &amp; Gleicher (2006)#</td>
<td>25</td>
<td>Self-control</td>
<td>DHEA Oral</td>
<td>75 mg/day</td>
<td>Improved ( (T:4.4 \pm 0.5, C:3.4 \pm 0.5) P &lt; 0.05 )</td>
<td>Improved ( (T:4.1 \pm 1.6, C:2.5 \pm 1.6) P &lt; 0.05 )</td>
<td>–</td>
<td>Improved ( (T:2.1 \pm 1.8) P &lt; 0.05 )</td>
<td>–</td>
<td>–</td>
<td>No effect</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Barad et al. (2007)*</td>
<td>190</td>
<td>Case control</td>
<td>DHEA Oral</td>
<td>75 mg/day</td>
<td>Improved ( (T:3 \pm 0.7, C:1.9 \pm 1.3) P &lt; 0.05 )</td>
<td>Improved ( (T:4.9 \pm 2.3, C:2.5 \pm 1.6) P &lt; 0.05 )</td>
<td>Improved ( (T:4.4 \pm 0.5, C:3.4 \pm 0.5) P &lt; 0.05 )</td>
<td>No effect</td>
<td>–</td>
<td>–</td>
<td>No effect</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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</tr>
<tr>
<td>Sonnezer et al. (2009)*</td>
<td>19</td>
<td>Self-control</td>
<td>DHEA Oral</td>
<td>75 mg/day</td>
<td>Improved ( (T:3 \pm 0.7, C:1.9 \pm 1.3) P &lt; 0.05 )</td>
<td>Improved ( (T:4.9 \pm 2.3, C:2.5 \pm 1.6) P &lt; 0.05 )</td>
<td>Improved ( (T:4.4 \pm 0.5, C:3.4 \pm 0.5) P &lt; 0.05 )</td>
<td>No effect</td>
<td>–</td>
<td>–</td>
<td>No effect</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Wiser et al. (2010)*</td>
<td>33</td>
<td>RCT</td>
<td>DHEA Oral</td>
<td>75 mg/day</td>
<td>≥90 days</td>
<td>Improved ( (T:3 \pm 0.7, C:1.9 \pm 1.3) P &lt; 0.05 )</td>
<td>Improved ( (T:4.9 \pm 2.3, C:2.5 \pm 1.6) P &lt; 0.05 )</td>
<td>Improved ( (T:4.4 \pm 0.5, C:3.4 \pm 0.5) P &lt; 0.05 )</td>
<td>No effect</td>
<td>–</td>
<td>–</td>
<td>No effect</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Artini et al. (2012)*</td>
<td>24</td>
<td>RCT</td>
<td>DHEA Oral</td>
<td>75 mg/day</td>
<td>Improved ( (T:3 \pm 0.7, C:1.9 \pm 1.3) P &lt; 0.05 )</td>
<td>Improved ( (T:4.9 \pm 2.3, C:2.5 \pm 1.6) P &lt; 0.05 )</td>
<td>Improved ( (T:4.4 \pm 0.5, C:3.4 \pm 0.5) P &lt; 0.05 )</td>
<td>No effect</td>
<td>–</td>
<td>–</td>
<td>No effect</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Moawad &amp; Shaeer (2012)*</td>
<td>133</td>
<td>RCT</td>
<td>DHEA Oral</td>
<td>75 mg/day</td>
<td>≥12 weeks</td>
<td>Improved ( (T:3 \pm 0.7, C:1.9 \pm 1.3) P &lt; 0.05 )</td>
<td>Improved ( (T:4.9 \pm 2.3, C:2.5 \pm 1.6) P &lt; 0.05 )</td>
<td>Improved ( (T:4.4 \pm 0.5, C:3.4 \pm 0.5) P &lt; 0.05 )</td>
<td>No effect</td>
<td>–</td>
<td>–</td>
<td>No effect</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Xu et al. (2014)*</td>
<td>386</td>
<td>Self-control cohort</td>
<td>DHEA Oral</td>
<td>75 mg/day</td>
<td>≥6 weeks</td>
<td>Improved ( (T:3 \pm 0.7, C:1.9 \pm 1.3) P &lt; 0.05 )</td>
<td>Improved ( (T:4.9 \pm 2.3, C:2.5 \pm 1.6) P &lt; 0.05 )</td>
<td>Improved ( (T:4.4 \pm 0.5, C:3.4 \pm 0.5) P &lt; 0.05 )</td>
<td>No effect</td>
<td>–</td>
<td>–</td>
<td>No effect</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Yeung et al. (2014)</td>
<td>32</td>
<td>RCT (+Placebo)</td>
<td>DHEA Oral</td>
<td>75 mg/day</td>
<td>≥60 days</td>
<td>Improved ( (T:3 \pm 0.7, C:1.9 \pm 1.3) P &lt; 0.05 )</td>
<td>Improved ( (T:4.9 \pm 2.3, C:2.5 \pm 1.6) P &lt; 0.05 )</td>
<td>Improved ( (T:4.4 \pm 0.5, C:3.4 \pm 0.5) P &lt; 0.05 )</td>
<td>No effect</td>
<td>–</td>
<td>–</td>
<td>No effect</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Jirge et al. (2014)*</td>
<td>22</td>
<td>Self-control</td>
<td>DHEA Oral</td>
<td>75 mg/day</td>
<td>≥60 days</td>
<td>Improved ( (T:3 \pm 0.7, C:1.9 \pm 1.3) P &lt; 0.05 )</td>
<td>Improved ( (T:4.9 \pm 2.3, C:2.5 \pm 1.6) P &lt; 0.05 )</td>
<td>Improved ( (T:4.4 \pm 0.5, C:3.4 \pm 0.5) P &lt; 0.05 )</td>
<td>No effect</td>
<td>–</td>
<td>–</td>
<td>No effect</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Kara et al. (2014)</td>
<td>208</td>
<td>RCT</td>
<td>DHEA Oral</td>
<td>75 mg/day</td>
<td>≥12 weeks</td>
<td>Improved ( (T:3 \pm 0.7, C:1.9 \pm 1.3) P &lt; 0.05 )</td>
<td>Improved ( (T:4.9 \pm 2.3, C:2.5 \pm 1.6) P &lt; 0.05 )</td>
<td>Improved ( (T:4.4 \pm 0.5, C:3.4 \pm 0.5) P &lt; 0.05 )</td>
<td>No effect</td>
<td>–</td>
<td>–</td>
<td>No effect</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Zhang et al. (2014)</td>
<td>95</td>
<td>RCT</td>
<td>DHEA Oral</td>
<td>75 mg/day</td>
<td>≥12 weeks</td>
<td>Improved ( (T:3 \pm 0.7, C:1.9 \pm 1.3) P &lt; 0.05 )</td>
<td>Improved ( (T:4.9 \pm 2.3, C:2.5 \pm 1.6) P &lt; 0.05 )</td>
<td>Improved ( (T:4.4 \pm 0.5, C:3.4 \pm 0.5) P &lt; 0.05 )</td>
<td>No effect</td>
<td>–</td>
<td>–</td>
<td>No effect</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Table 2: Summary of studies assessing the impact on IVF outcomes of DHEA pretreatment in poor responder patients.
<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
<th>Study Design</th>
<th>Treatment</th>
<th>Dose/Route</th>
<th>Duration</th>
<th>Outcome</th>
<th>Control</th>
<th>Treatment</th>
<th>P-value 1</th>
<th>Control</th>
<th>Treatment</th>
<th>P-value 2</th>
<th>Control</th>
<th>Treatment</th>
<th>P-value 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vlahos et al. (2015)</td>
<td>161</td>
<td>Case-control prospective study</td>
<td>DHEA Oral</td>
<td>75 mg/day</td>
<td>≥12 weeks</td>
<td>No effect</td>
<td>No effect</td>
<td>–</td>
<td>–</td>
<td>No effect</td>
<td>No effect</td>
<td>–</td>
<td>No effect</td>
<td>No effect</td>
<td></td>
</tr>
<tr>
<td>Tsui et al. (2015)*</td>
<td>10</td>
<td>Self-control</td>
<td>DHEA Oral</td>
<td>90 mg/day</td>
<td>3 months</td>
<td>Improved (T:4.2 ± 1.2, C:2.4 ± 1.1)</td>
<td>P &lt; 0.01</td>
<td>–</td>
<td>Improved (T:3.8 ± 1.1, C:1.7 ± 0.5)</td>
<td>P &gt; 0.05</td>
<td>–</td>
<td>Improved (T:9.3 ± 2.2, C:5.8 ± 3.1)</td>
<td>P &gt; 0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kotb et al. (2016)*</td>
<td>140</td>
<td>RCT</td>
<td>DHEA Oral</td>
<td>75 mg/day</td>
<td>12 weeks</td>
<td>No effect</td>
<td>No effect</td>
<td>–</td>
<td>No effect</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Nakawichean et al. (2017)</td>
<td>52</td>
<td>RCT (+Placebo)</td>
<td>DHEA Oral</td>
<td>75 mg/day</td>
<td>≥12 weeks</td>
<td>No effect</td>
<td>No effect</td>
<td>–</td>
<td>–</td>
<td>No effect</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Hu et al. (2017)</td>
<td>103</td>
<td>Case-control prospective cohort study</td>
<td>DHEA Oral</td>
<td>75 mg/d</td>
<td>8 weeks</td>
<td>No effect</td>
<td>No effect</td>
<td>–</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td></td>
</tr>
<tr>
<td>Lin et al. (2017)</td>
<td>72</td>
<td>Case-control prospective cohort study</td>
<td>DHEA Oral</td>
<td>90 mg/day</td>
<td>≥8 weeks</td>
<td>No effect</td>
<td>No effect</td>
<td>–</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td></td>
</tr>
<tr>
<td>Chern et al. (2018)*</td>
<td>151</td>
<td>Retrospective cohort study</td>
<td>DHEA Oral</td>
<td>90 mg/day</td>
<td>3 months</td>
<td>Improved (T:3.3 ± 2.5, C:2.0 ± 1.5)</td>
<td>P &lt; 0.001</td>
<td>No effect</td>
<td>Improved (T:1.9 ± 1.5, C:1.0 ± 1.1)</td>
<td>P &lt; 0.01</td>
<td>No effect</td>
<td>Improved (T:6.3 ± 2.4, C:3.9 ± 3.2)</td>
<td>P &lt; 0.002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Li et al. (2018)</td>
<td>38</td>
<td>Case-control prospective cohort study</td>
<td>DHEA Oral</td>
<td>90 mg/day</td>
<td>≥8 weeks</td>
<td>No effect</td>
<td>No effect</td>
<td>–</td>
<td>No effect</td>
<td>No effect</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Al-Turki (2018)*</td>
<td>62</td>
<td>Prospective case control</td>
<td>DHEA Oral</td>
<td>50 mg/day</td>
<td>3 months</td>
<td>Improved (T:4.9 ± 1.8, C:3.16 ± 2.5)</td>
<td>P &lt; 0.003</td>
<td>–</td>
<td>Improved (T:6.3 ± 2.7, C:3.9 ± 3.2)</td>
<td>P &lt; 0.002</td>
<td>–</td>
<td>Improved (T:6.1 ± 1.9, C:3.6 ± 2.1)</td>
<td>P &lt; 0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Results are reported as mean ± s.d.; †results are reported as mean ± s.e. ‡: No statistical analysis was reported; €C refers to average of 1–3 cycles and T refers to average of 8–9 cycles results; ‡live birth rate in this study was improved when cumulative results of two consecutive cycles were pooled and analyzed; §proportions were calculated based on the numbers reported in the paper.

C, control; DHEA, dehydroepiandrosterone; RCT, randomized controlled trial; T, treatment.
type of studies undertaken varies. Studies have reported that DHEA administration has improved antral follicle numbers, oocyte retrieval numbers, fertilization rates, embryo implantation rates, embryo quality, clinical pregnancy rates and live birth rates in some women (observations from studies summarized in Table 2). However, there are also a noteworthy number of studies, including well-designed placebo-controlled randomized control trials (RCTs), which have reported no consistent improvement in any endpoints assessed after exposure of women to DHEA prior to ovarian stimulation (Artini et al. 2012, Yeung et al. 2014, Kara et al. 2014, Zhang et al. 2014, Vlahos et al. 2015, Hu et al. 2017, Narkwichean et al. 2017, Li et al. 2018).

In an attempt to determine in POR patients if the use of DHEA pre-treatment increases the likelihood of improved ovarian response and ultimately a live birth, several groups have published systematic reviews and meta-analysis on the current literature. In the most recent systematic reviews and meta-analysis reporting on data from 5 to 21 studies, there is evidence to support the use of pre-IVF DHEA treatment in patients with POR, based on the finding that in all these studies, DHEA supplementation was associated with a significant increase in clinical pregnancy rate (Ji et al. 2016, Zhang et al. 2016, Hu et al. 2018, Schwarze et al. 2018) In addition, in some pooled analysis studies DHEA treatment was also significantly associated with an increase in antral follicle count (Zhang et al. 2016), the number of retrieved oocytes (Ji et al. 2016), endometrial thickness (Hu et al. 2018), implantation rate (Zhang et al. 2016) and live birth rates (Zhang et al. 2016, Hu et al. 2018).

Testosterone
Reports have also assessed the efficacy of administering the bioactive androgen, testosterone, in POR patients to improve ovarian response and IVF outcomes (Table 3). Although four RCTs (two with placebo controls) assessing the impact of transdermal testosterone administration for 5–20 days in POR patients showed no significant effect of testosterone pre-treatment (Massin et al. 2006, Fabregues et al. 2009, Sipe et al. 2010, Boudou et al. 2016), since then numerous studies, including a placebo-controlled RCT, have reported on a range of beneficial effects of pre-IVF testosterone pre-treatment (Balasch et al. 2006, Kim et al. 2011, 2014, Mitri et al. 2016, Doan et al. 2017, Saharkhiz et al. 2018). These beneficial effects include improved antral follicle numbers, oocyte retrieval numbers, fertilization rates, embryo implantation rates, embryo quality, clinical pregnancy rates and live birth rates in some women (observations from studies summarized in Table 3).

Reasons suggested for the absence of any benefit of testosterone treatment in some studies have included differences in the timing and duration of androgen treatment. In contrast to studies which used DHEA pre-treatment, studies which assessed the efficacy of testosterone pre-treatment vary widely in the dose used (2.5–25 mg/day) and the duration of exposure (4–5 days – 4 weeks), making comparison between studies difficult. Kim et al. conducted a pilot controlled study with the aim to investigate the effect of transdermal testosterone gel in POR patients exposed for 2, 3 or 4 weeks prior to their IVF cycle (Kim et al. 2014). Interestingly, while testosterone pre-treatment for 2 weeks failed to show any significant effects, patients treated for 3 and 4 weeks exhibited a significantly improved ovarian response to hyperstimulation, but only patients exposed for 4 weeks displayed a significant increase in clinical pregnancy and live birth rates (Kim et al. 2014). Systematic reviews and meta-analysis have reported that POR patients may benefit from the use of testosterone pre-treatment transdermal. This is based on the evidence that testosterone pre-treatment significantly increased the number of oocytes retrieved (Bosdou et al. 2012, Luo et al. 2014, Noventa et al. 2019) and clinical pregnancy and live birth rates (Bosdou et al. 2012, Gonzalez-Comadran et al. 2012, Luo et al. 2014, Jeve & Bhandari 2016, Noventa et al. 2019).

Use of androgen-modulating agents

Aromatase inhibitors
Letrozole, an aromatase inhibitor, has also been trialed as a potential way to improve IVF outcomes in poor responders as inhibition of aromatase activity would subsequently increase levels of androgens by blocking their aromatization to estrogens. In a preliminary self-controlled report on 12 patients with unexplained infertility and previous poor response treated with letrozole (2.5 mg/day), it was concluded that letrozole may be of potential benefit for improving ovarian response to FSH in poor responders (Mitwally & Casper 2002). Similarly, in a case-controlled study Garcia-Velasco et al. reported a significantly higher numbers of oocytes retrieved and implantation rates in letrozole-treated patients (Garcia-Velasco et al. 2005). Several further studies have reported on the positive effect of letrozole in lowering the total does of FSH used (Mitwally & Casper 2002, Goswami et al. 2004, Ozmen et al. 2009, Yarali et al. 2009, Mohsen & El Din 2013, Lee et al. 2014), but the key IVF outcomes of number of oocyte retrieved, fertilization rates and pregnancy rates have not consistently been found to
Table 3: Summary of studies assessing the impact on IVF outcomes of testosterone pretreatment in poor responder patients.

<table>
<thead>
<tr>
<th>Study Type</th>
<th>Sample size</th>
<th>Type</th>
<th>Route</th>
<th>Dose</th>
<th>Duration</th>
<th>17 mm follicles ≥ day of hCG</th>
<th>Number of oocytes retrieved</th>
<th>Number of MII oocytes</th>
<th>Fertilized oocytes</th>
<th>Embryo implantation rate</th>
<th>Clinical pregnancy rate</th>
<th>Live birth rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>RCT (+Placebo)</td>
<td>53 (C:25, T:24)</td>
<td>Testosterone (gel 1%)</td>
<td>Transdermal</td>
<td>10 mg/day</td>
<td>15-20 days</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>–</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>RCT</td>
<td>25</td>
<td>Self-controlled</td>
<td>Testosterone (patch)</td>
<td>Transdermal</td>
<td>20 μg/kg per day</td>
<td>5 days</td>
<td>–</td>
<td>Improved (T: 5.8 ± 0.4)</td>
<td>–</td>
<td>Improved (T: 16.6%)</td>
<td>–</td>
<td>Improved (T: 20%)</td>
</tr>
<tr>
<td>Fabregues et al. (2009)*</td>
<td>62 (C:31, T:31)</td>
<td>Testosterone (patch)</td>
<td>Transdermal</td>
<td>20 μg/kg per day</td>
<td>5 days</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
<td>–</td>
<td>No effect</td>
<td>–</td>
</tr>
<tr>
<td>Sipe et al. (2010)</td>
<td>12 (C:6, T:6)</td>
<td>Testosterone (gel 1%)</td>
<td>Transdermal</td>
<td>2.5 mg/day</td>
<td>12 days</td>
<td>No effect</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Kim et al. (2011)*</td>
<td>110</td>
<td>RCT (+Placebo)</td>
<td>Testosterone (gel 1%)</td>
<td>Transdermal</td>
<td>12.5 mg/day</td>
<td>21 days</td>
<td>Improved (T:4.1 ± 1.4, C: 2.7 ± 1.0)</td>
<td>P &lt; 0.001</td>
<td>Improved (T:3.8 ± 1.4)</td>
<td>P &lt; 0.001</td>
<td>Improved (T: 3.2 ± 1.2)</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Kim et al. (2014)**</td>
<td>60 (C:30, T:30)</td>
<td>Testosterone (gel 1%)</td>
<td>Transdermal</td>
<td>12.5 mg/day</td>
<td>4 weeks</td>
<td>–</td>
<td>Improved (T:4.9 ± 1.6, C: 3.1 ± 1.1)</td>
<td>P &lt; 0.001</td>
<td>Improved (T:3.1 ± 1.2)</td>
<td>P &lt; 0.001</td>
<td>Improved (T:14.3%, C:14.5%)</td>
<td>P &lt; 0.019</td>
</tr>
<tr>
<td>Mitr et al. (2016)*</td>
<td>26 (C:13, T:13)</td>
<td>Case control Observational pilot study</td>
<td>Testosterone (gel 1%)</td>
<td>Transdermal</td>
<td>25 mg/day</td>
<td>4.4 ± 1.2 days</td>
<td>Improved (T: 4.92 ± 2.7, C: 1.69 ± 0.8)</td>
<td>P &lt; 0.001</td>
<td>Improved (T:3.5 ± 1.3)</td>
<td>P &lt; 0.001</td>
<td>Improved (T:36.7%, C:6.7%)</td>
<td>P &lt; 0.042</td>
</tr>
<tr>
<td>Bosdou et al. (2017)*</td>
<td>50 (C:24, T:26)</td>
<td>Testosterone (gel 2%)</td>
<td>Transdermal</td>
<td>10 mg/day</td>
<td>21 days</td>
<td>No effect</td>
<td>No effect</td>
<td>Improved (T: 3.2 ± 1.2)</td>
<td>–</td>
<td>Improved (T:14.1%, C:6.1%)</td>
<td>P &lt; 0.004</td>
<td>Improved (T:16.2%, C:7.7%)</td>
</tr>
<tr>
<td>Doan et al. (2017)*</td>
<td>110</td>
<td>Case-control prospective, descriptive study</td>
<td>Testosterone (gel 1%)</td>
<td>Transdermal</td>
<td>12.5 mg/day</td>
<td>6th day to 2nd day of next cycle (~24 days)</td>
<td>Improved (T:4.5 ± 1.7, C:3.1 ± 1.1)</td>
<td>P &lt; 0.001</td>
<td>Improved (T:3.8 ± 1.6, C:1.2 ± 1.3)</td>
<td>P = 0.001</td>
<td>Improved (T:16%, C:0%)</td>
<td>P = 0.04</td>
</tr>
<tr>
<td>Saharkhz et al. (2018)*</td>
<td>48 (C:23, T:25)</td>
<td>Testosterone (gel)</td>
<td>Transdermal</td>
<td>25 mg/day</td>
<td>From 2nd day of cycle to HCG treatment day (~12 days)</td>
<td>Improved (T:4.5 ± 2.6 ± 2.1)</td>
<td>–</td>
<td>Improved (T:2.48 ± 1.6, C:1.2 ± 1.3)</td>
<td>–</td>
<td>Improved (T:16%, C:0%)</td>
<td>P = 0.04</td>
<td></td>
</tr>
</tbody>
</table>

*Results are reported as mean ± S.D., *results are reported as mean ± S.E.M., @No statistical analysis was reported; ^data published also reported on 2 and 3 weeks but only 4 weeks results are present in this table; calculated based on the average menstrual cycle duration of 28 days.

C, control; RCT, randomized controlled trial; T, treatment.
be significantly improved by the supplementation of poor responders with letrozole (Goswami et al. 2004, Schoolcraft et al. 2008, Ozmen et al. 2009, Yarali et al. 2009, Jovanovic et al. 2011, Mohsen & El Din 2013, Lee et al. 2014, Ebrahimi et al. 2017). Furthermore, analysis of pooled data from several studies also fail to show a significant association with increased clinical pregnancy rates (Bosdou et al. 2012, Jeve & Bhandari 2016, Kamath et al. 2017). Hence, at present, the incorporation of letrozole does not appear to be an effective way to manage poor responders, as overall, the findings are inconclusive and provide little evidence to support its use.

Recombinant luteinizing hormone (rLH)/recombinant human chorionic gonadotrophin (rhCG)

Based on the two-cell two-gonadotrophin hypothesis (Fig. 2), androgen production in the theca cells is stimulated by the presence of LH (Hillier et al. 1994), inferring that LH activity is crucial for maintaining adequate levels of intraovarian androgens during ovarian follicle development. It has also been reported that ovarian response and pregnancy outcome are related to LH levels in women undergoing assisted reproduction, with women receiving pituitary downregulation with gonadotrophin-releasing hormone (GnRH) agonists displaying a compromised pregnancy outcome (Westergaard et al. 2000, Humaidan et al. 2002). Hence, the use of rLH/rhCG to mimic endogenous LH has been put forward as a potential strategy to manage poor responders.

In two randomized controlled studies published by Ferraretti et al. in 2004 and 2014, administration of rLH treatment was reported to significantly improve live birth rates in poor responders (Ferraretti et al. 2004, 2014). However, in another three controlled studies published on the use of rLH or rhCG in poor responders, no statistically significant beneficial effects were reported on the number of oocytes retrieved or pregnancy rates (Berkkanoglu et al. 2007, Ruvolet al. 2007, Barrenetxea et al. 2008). Unfortunately, all reported studies lack a placebo, and systematic review and meta-analysis of the limited available evidence have concluded that there are insufficient data to draw firm conclusions and support a beneficial effect of rLH or rhCG in poor responders (Bosdou et al. 2012, Jeve & Bhandari 2016).

Androgen or androgen-modulating agent combination therapies

In a few cases, modified strategies combining androgens or androgen-modulating agents have also been trialed in POR patients. A significant improvement in the number of oocytes retrieved and clinical pregnancy rates was reported in a study that used a combination therapy of DHEA for 12 weeks, transdermal testosterone for 4 weeks and growth hormone later in the luteal phase (Haydardeedoglu et al. 2015). Additionally, in another treatment strategy, called ANDRO-IVF, combining pre-treatment with transdermal testosterone, letrozole and hCG, it was reported that POR patients exhibited a significant increase in the number of oocytes retrieved and fertilization rates (Bercaire et al. 2018). However, it is noteworthy that neither study had a placebo control.

Current implications and future directions from basic research on the clinical use of androgens or androgen-modulating agents in POR patients

In summary, at present, further studies are required to confirm the efficacy of adjacent administration of androgens or androgen-modulating agents in poor responder cases. There is accumulating evidence from basic discovery research, clinical trials, meta-analysis and systematic reviews (including a Cochran review; Nagels et al. 2015) to support the use of androgen pre-treatment, in particular transdermal testosterone, in POR patients. However, conflicting results have been reported on the efficacy of these strategies. Reasons for these differential effects include study design flaws such as lack of placebo, small sample size, inconsistent inclusion criteria and ambiguous use of aromatizable androgens. Testosterone is a bioactive androgen that can bind directly to the AR, while DHEA is considered an inactive androgen precursor which has a very week affinity for the AR. Hence, for DHEA pre-treatment to elicit a physiological response, the correct enzyme machinery must be in place to convert this androgen precursor into one of the active androgens, testosterone or DHT. Following this logic, it could be proposed that the use of testosterone, rather than DHEA, would be of more benefit in the clinic.

The molecular mechanism(s) that mediate the proposed beneficial effects of androgen pre-treatment on IVF outcomes remain undefined. However, animal studies have clearly shown that androgens increase FSH receptor expression, and synergize with FSH to stimulate follicle growth and follicle responsiveness to FSH (Weil et al. 1999, Wang et al. 2001, Sen et al. 2014). In support of this synergistic interaction, DHEA supplementation was reported to significantly increase protein and mRNA...
FSH receptor expression in preovulatory granulosa cells collected from POR patients (Hu et al. 2017). Another potential mechanism by which androgen pre-treatment may mediate a beneficial effect is by maintaining follicle health. Androgens have been demonstrated to influence follicle atresia as systemic treatment with testosterone or DHT reduced follicle atresia in primate growing follicles (Vendola et al. 1998). On the other hand, a loss of androgen actions, as in ARKO models, leads to elevated levels of follicular atresia, evident by the presence of pyknotic granulosa cells and degenerate oocytes (Hu et al. 2004, Shiina et al. 2006, Walters et al. 2007). Clinical evidence supporting this mechanism come from the findings from one study that compared to normal ovarian responders, POR patients supplemented with DHEA were reported to produce a greater number of top-quality embryos on day 3 and increased fertilization rates, and their cumulus cells displayed reduced DNA damage and expression of apoptosis-related genes (Lin et al. 2017). Similarly in another study, cumulus cells collected before and after DHEA treatment in women with POR displayed an increase in the ratio of BCL2 (anti-apoptotic) to BAX (pro-apoptotic) (Tsui et al. 2014). Moreover, in the same study, genes involved in extracellular matrix formation were upregulated in cumulus cells, indicating that DHEA treatment may improve oocyte-cumulus cell process during maturation (Tsui et al. 2014).

In spite of the abundant reports of beneficial effects, numerous reviews on this subject have reiterated that at present caution should still be taken when interpreting results as the majority of studies lack placebo controls, have small sample sizes and display major heterogeneity between studies in terms of dose and duration of androgen/androgen-modulating agent treatment. Hence, further RCTs with rigorous methodology and inclusion criteria are urgently needed. At present one such trial, called the Testosterone TRANsdermal Gel for Poor Ovarian Responders Trial (T-TRANSPORT; NCT02418572; Polyzos et al. 2018) is currently underway with an estimated completion date of June 2019. This trial has aimed to undertake a double-blind placebo-control trial in five IVF centers, in at least four countries, with a large sample size (400 women) to assess effectively the impact of transdermal testosterone (5.5 mg/day) for ~65 days with fixed stimulation protocols (Trial details available at https://clinicaltrials.gov/ct2/show/NCT02418572). While we await the outcome from studies such as this with great interest, and despite indications of promising beneficial effects of increasing androgen exposure prior to an IVF cycle, at present androgen pre-treatments continue to be widely used in IVF centers around the world without convincing evidence or defined molecular mechanisms to support their continued use.

### Defining polycystic ovary syndrome (PCOS) and evidence to support a role for androgen-driven mechanisms in the development of PCOS

**Characteristics and diagnosis of PCOS**

PCOS has a significant prevalence worldwide, with reports of up to 20% of women of reproductive age being affected by the condition, which poses a high economic health burden (March et al. 2010, Dumesic et al. 2015, Bozdag et al. 2016, Skiba et al. 2018). PCOS women suffer from a wide range of ill-health traits, which include endocrine, reproductive, metabolic and psychological features. Polycystic ovaries classically exhibit aberrant follicle maturation which leads to ovulatory dysfunction and is associated with reduced fertility (Dumesic et al. 2015). If pregnancy is achieved, PCOS patients have a greater risk of pregnancy complications, such as gestational diabetes, hypertensive disorders and premature delivery (Boyle & Teede 2016). Other key traits of PCOS include hormonal disturbances with hyperandrogenism and LH hypersecretion frequently observed in PCOS women (Dumesic et al. 2015). PCOS also has a substantial metabolic impact as it is strongly associated with obesity, metabolic syndrome, hyperinsulinemia, insulin resistance, dyslipidemia, hepatic steatosis and an increased risk of developing type 2 diabetes and cardiovascular disease (Shorakae et al. 2014, Dumesic et al. 2015, Moran et al. 2015). Moreover, PCOS is associated with psychosocial issues with the prevalence of depression and anxiety reported to be higher in PCOS women (Dumesic et al. 2015).

Three different diagnostic criteria for the diagnosis of PCOS have been used including the National Institutes of Health (NIH) (Zawadzki & Da 1992), Rotterdam (Rotterdam 2004) and Androgen Excess and PCOS (AE-PCOS) criteria (Azziz et al. 2006, 2009). In 2018, the international evidence-based guidelines for the assessment and management of PCOS endorsed the most widely used criteria, the Rotterdam diagnostic criteria, which states that a women must present two out of the following three PCOS features for a diagnosis: clinical and/or biochemical androgen excess, oligo-anovulation or anovulation and polycystic ovary morphology (PCOM).
on ultrasound (Rotterdam 2004, Teede et al. 2018) (Fig. 3). Unfortunately, despite PCOS being a highly prevalent condition with major health and economic impacts, a cure for PCOS is yet to be identified. This is because the origins and underlying mechanisms driving PCOS remain unclear, and consequently, no drug has been specifically approved for the indication of PCOS (Escobar-Morreale 2018). Due to this, the current medical management of women with PCOS remains suboptimal as it focuses only on treatment of the symptoms rather than the underlying mechanisms.

**Clinical evidence supporting a role for androgens in driving the development of PCOS**

**Correlation between androgen levels and the observation of PCOS traits**

Androgen excess is a key trait in the majority (~60%) of women with PCOS, and the most frequent common feature, as hyperandrogenism is a diagnostic criteria in three out of the four PCOS phenotypes (A-D) endorsed by the 2018 international evidence-based guidelines (Livadas et al. 2014, Teede et al. 2018). Interestingly, the presence of elevated androgen levels observed in congenital adrenal hyperplasia patients (Lucis et al. 1966, Hague et al. 1990) and female-to-male transsexuals treated with testosterone (Futterweit & Deligdisch 1986, Spinder et al. 1989, De Roo et al. 2017) are reported to cause the appearance of polycystic ovarian morphology (PCOM), similar to that observed in women with PCOS, with enlarged ovaries that exhibit multiple cysts and theca interstitial hyperplasia. Serum levels of testosterone, A4 and DHEAS, and the enzyme required to transform pro-androgens to bioactive androgens – 3β-hydroxysteroid dehydrogenase (3β-HSD) – have all been reported to be elevated in hyperandrogenic PCOS women (Keefe et al. 2014, Palomba et al. 2014, O’Reilly et al. 2017). In addition, recently, it has been reported that 11-oxygenated androgens, including the potent androgen 11-ketotestosterone (11KT), represent the majority of circulating androgens in women with PCOS, and there is a close correlation of a number of 11-oxygenated steroids with hyperinsulinemia, highlighting a potential important role for these steroids as biomarkers of metabolic risk (O’Reilly et al. 2017). Furthermore, human theca interna cells excised from ovaries of PCOS women display elevated levels of androgen secretion which persists over long term *in vitro*, inferring that PCOS ovaries are a site of endogenous excess androgen production (Nelson et al. 1999). Observational studies have also reported that androgen excess is positively correlated with several PCOS characteristics, including increased intra-abdominal fat mass, triglyceride levels, LH/FSH ratio and insulin resistance, but decreased high-density lipoprotein levels (Daan et al. 2015, Dumesc et al. 2016, Li et al. 2016, Couto Alves et al. 2017). Taken together, these findings provide substantial clinical evidence pointing toward an important role for androgenic actions in the development and progression of PCOS.

**Pharmacological targeting of androgen excess effects in patients with PCOS**

No universal treatment for PCOS is currently available and therefore treatments are based on an individual patient and symptom basis. Hirsutism, acne and alopecia are all manifestations of androgen excess observed in PCOS patients. In women not attempting to conceive, drugs with anti-androgenic properties, including the oral contraceptive pill containing progesterin with low or antiandrogenic activity and/or antiandrogens, have been used for treating PCOS symptoms.

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**Figure 3**

Combinations of clinical observations used to diagnose PCOS according to the three different PCOS diagnostic criteria (Rotterdam, AE-PCOS, NIH) and the four different PCOS phenotypes (A-D). ✓ presence of clinical observations. *Rotterdam is the recommended diagnostic criterion which is endorsed by the 2018 international evidence-based guidelines for the assessment and management of PCOS (Teede et al. 2018). HA, hyperandrogenism (clinical: acne, alopecia (Ludwig visual score), and hirsutism (Ferriman–Gallwey score ≥4–6) and/or biochemical: calculated free testosterone, free androgen index or calculated bioavailable testosterone using liquid chromatography-mass spectrometry (LCMS) and extraction/chromatography immunoassays). OA, oligo- or anovulation (≥21 or >35 days cycles or <8 cycles per year 3 years post menarche to perimenopause). PCOM, polycystic ovarian morphology (≥20 follicles/ovary and/or an ovarian volume ≥10 mL on either ovary using endovaginal ultrasound transducer with a frequency ≥8 mHz; and/or ovarian volume ≥10 mL using older technology or transabdominal ultrasound).

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[Rotterdam 2004, Teede et al. 2018] (Fig. 3). Observational
Administration of the oral contraceptive pill inhibits the midcycle LH surge by suppressing GnRH secretion from the hypothalamus and gonadotrophin secretion from the pituitary. In addition to the primary role of providing contraception, the suppression of gonadotrophin secretion in turn also reduces ovarian steroidogenesis, and hence, leads to a reduction in circulating androgen levels (Zimmerman et al. 2014). Furthermore, oral contraceptive pill treatment causes an estrogen dose-dependent increase in sex hormone-binding globulin (SHBG) from the liver, thereby further reducing the availability of active androgens (Zimmerman et al. 2014). These anti-androgenic qualities make the oral contraceptive pill an effective mainstay pharmacological therapy for treating the symptoms of menstrual irregularity, hirsutism, acne and androgenic alopecia in patients with PCOS not wishing to conceive (Yildiz 2008, Legro et al. 2013, Conway et al. 2014, Mendoza et al. 2014). In addition, recent evidence on third-generation combined oral contraceptive pills that contain an anti-androgenic compound has suggested that they may have beneficial effects on metabolic phenotypes of PCOS, with improved lipid profiles and adipokine secretion (Legro et al. 2013).

Spironolactone and cyproterone acetate are both steroidal AR blockers that compete with the bioactive androgens testosterone and DHT for the AR ligand-binding domain. Numerous studies have reported that the treatment of PCOS patients with either AR blocker is an effective way to significantly decrease hirsutism and acne (Conway et al. 2014, Escobar-Morreale 2018). Furthermore, spironolactone therapy in women with PCOS has been associated with a significant improvement in metabolic phenotype (Zulian et al. 2005). However this is not the case in all studies, with either no effect observed (Diri et al. 2016) or an adverse effect reported on serum lipoprotein levels (Nakhjavani et al. 2009).

Flutamide is a synthetic nonsteroidal antiandrogen that is a competitive antagonist of the AR. Flutamide has been reported to exert a beneficial effect in women with PCOS, by decreasing the degree of hirsutism and acne observed (Ventuoli et al. 1999, Moghetti et al. 2000, Calaf et al. 2007). Treatment of PCOS patients with flutamide restores menstrual regularity and ovulation (De Leo et al. 1998, Paradisi et al. 2013) and ameliorates the impaired sensitivity of the GnRH pulse generator to feedback inhibition by E2 and progesterone (Eagleson et al. 2000). Interestingly, when flutamide was used in combination with a hypocaloric diet in overweight-obese PCOS women, it was reported to not only improve hirsutism but also have beneficial effects on other reproductively and metabolically disturbed women with PCOS, including menstrual pattern, glucose-stimulated glucose levels, insulin sensitivity and low-density lipoprotein and cholesterol levels (Gambineri et al. 2006). Moreover, treatment of obese and lean PCOS women with flutamide improves lipid profile independent of changes in weight, with treatment associated with a significant decrease in low-density lipoproteins (LDL) to high-density lipoproteins (HDL) ratio, total cholesterol, LDL and triglycerides (Diamanti-Kandarakis et al. 1998).

Finasteride, a 5-alpha reductase inhibitor, has been described to be effective for the treatment of the hirsutism in patients with PCOS (Lakryc et al. 2003, Tartagni et al. 2014). Moreover, in a double-blind randomized study, hyperandrogenic anovulatory PCOS women who previously did not respond to stimulation with gonadotrophin, were found to display improved ovulation rates after the addition of finasteride during ovarian stimulation (Tartagni et al. 2010).

Collectively findings from the use of anti-androgenic drugs in PCOS patients either alone or in combination have shown that targeted suppression of androgen excess, and thus, androgenic actions, has a positive impact with improvements observed in several features of PCOS. As androgens mediate their actions via the AR, these findings provided support for a link between androgen excess and the development of a wide range of PCOS traits. However, importantly these treatments are not curative, and such pharmacological treatments are only an option for PCOS women not desiring a pregnancy as antiandrogens should only be used with concomitant contraception to avoid masculinization of a female fetus in the event of an unplanned pregnancy. Furthermore, there is evidence to support that antiandrogens have unacceptable hepatotoxicity (Conway et al. 2014), which outweighs their benefits in use for non-lethal disorders, such as PCOS. Hence, while systemic androgen blockade is a logical approach for treating PCOS, a more targeted pharmacological approach is needed, but this requires an in-depth knowledge of the physiological mechanisms underpinning its evolution.

**Evidence from hyperandrogenized preclinical animal PCOS models supporting a role for androgens in driving the development of PCOS**

Fully controlled studies into the fundamental biological mechanisms underlying the development of PCOS are not possible in humans as clinical studies are limited by ethical and logistical constraints. Therefore, preclinical PCOS...
animal models that mimic PCOS-like traits are essential in providing insights into the specific mechanisms underpinning the pathogenesis of PCOS. Animal models for the study of PCOS have been developed using a variety of methods, from treatment with androgens, estrogens and antiprogestins to genetic manipulation and chronic exposure to light (Walters et al. 2012a, 2018a, Walters 2015). However, the most insightful information on the underlying mechanisms involved in the origins of PCOS have come from preclinical PCOS animal models generated by inducing hyperandrogenism (Walters et al. 2018a). Androgen excess-induced PCOS traits have been generated in female rodents, sheep and rhesus macaque monkeys by prenatal or postnatal injection or subcutaneous implants containing the pro-androgen DHEA, androgens testosterone or DHT or the aromatase inhibitor letrozole (Walters et al. 2012a, Abbott et al. 2013, Padmanabhan & Veiga-Lopez 2013) (Fig. 4). Collectively, these PCOS animal models provide strong evidence that hyperandrogenism is a major driver in PCOS pathogenesis as they consistently replicate a wide range of PCOS traits observed in humans.

Two rodent PCOS models that have been reported to closely mimic human key endocrine, reproductive and metabolic PCOS traits are the models generated by prenatal or early postnatal exposure to DHT. The prenatal DHT-induced PCOS mouse model exhibits the clinical endocrine and reproductive PCOS features of hyperandrogenism, LH hypersecretion, irregular cycles, reduced fertility and ovulatory dysfunction (Sullivan & Moenter 2004, Moore et al. 2013). While this model has been reported to not have a strong metabolic PCOS phenotype and may be most representative of the lean PCOS phenotype, it has been reported that adipocyte hypertrophy and impaired glucose tolerance are still present, inferring that metabolic function is still aberrant (Roland et al. 2010, Caldwell et al. 2014). Early postnatal DHT exposure induces a robust mouse PCOS model with a wide range of PCOS traits including irregular cycles, ovulatory dysfunction, PCOM, adiposity, adipocyte hypertrophy, dyslipidemia, hepatic steatosis and altered glucose and insulin homeostasis (Caldwell et al. 2014, Bertoldo et al. 2019). In sheep and nonhuman primate PCOS models, prenatal exposure to testosterone generates the closest simulation to the clinical features of PCOS, with both models also displaying a breadth of endocrine, reproductive and metabolic PCOS traits, including irregular cycles, oligo- or anovulation, PCOM, LH hypersecretion, lipid abnormalities and insulin resistance (Padmanabhan & Veiga-Lopez 2013, Abbott et al. 2016).

Further evidence to support androgens as key drivers in the etiology of PCOS come from the observations that treatment of PCOS animal models with AR antagonists prevents/or rescues the development of some PCOS traits. Ovulatory dysfunction is present in the prena tally androgenized PCOS sheep model, but co-treatment with the AR antagonist flutamide restores LH surges (Padmanabhan et al. 2015). In a mouse PCOS model, androgen excess leads to irregular cycles and a disruption within the arcuate nucleus of the hypothalamus of neuronal networks involved in mediating progesterone-sensitive GABAergic input to GnRH neurons (Sullivan & Moenter 2004, Moore et al. 2015). However, treatment of adult prenatally androgenized female mice with flutamide ameliorated the disrupted estrous cycling and altered GABAergic drive to GnRH neurons (Sullivan & Moenter 2004). Metabolic dysfunction associated with PCOS has also been speculated to be, at least in part, regulated by androgen-mediated mechanisms impacting on specific hypothalamic circuitry important in energy balance and metabolism. Prenatally androgenized sheep exhibit alterations in agouti-related peptide (AgRP) neurons (known to be involved in regulating energy homeostasis and glucose-insulin metabolism), but these changes are blocked by prenatal co-treatment with flutamide (Sheppard et al. 2011). Collectively, these intervention studies provide evidence that androgen actions mediated via the AR significantly contribute to the development of PCOS traits.

**Use of ARKO mouse models to decipher key target sites involved in the pathogenesis of PCOS**

With the large body of evidence from observational clinical studies and the generation and characterization of androgen excess-induced PCOS animal models supporting a role for hyperandrogenism in the developmental origins of PCOS, recent research has been focusing on identifying the site of these actions and the mechanisms involved (Fig. 5). As direct androgen actions mediate their actions via the AR, several recent studies have incorporated the use of ARKO mouse models as a tool to unravel the role of androgens in the development of PCOS. PCOS could not be induced in homozygous global ARKO mice by exposure of female mice to androgen excess (Caldwell et al. 2015, 2017), providing strong evidence of a requirement for a functional AR in the pathogenesis of experimental PCOS. It has been proposed that androgen excess acting via the AR at various locations throughout the body, including the...
hypothalamus, ovary, skeletal muscle or adipocyte cells, are involved in the origins of PCOS.

The contribution and importance of intra- versus extra-ovarian mechanisms in the pathogenesis of PCOS has been a key question in understanding the processes involved in the development of PCOS. Recent studies combining PCOS mouse models and global and cell-specific ARKO mouse models have made significant advances in our understanding of the likely mechanisms involved. Induction of PCOS traits by androgen excess in control mice and ovariectomized control mice with transplanted ARKO ovaries (i.e. only the ovaries have non-functional AR signaling) leads to the development of PCOS traits of disrupted cycles. In contrast, when PCOS was induced by excess androgen exposure in ovariectomized global ARKO mice with transplanted control ovaries (i.e. only ovaries have functional AR signaling), normal cyclicity was retained (Caldwell et al. 2017), inferring that extra-ovarian mechanism are the major mediators in the development of PCOS. To tease out the key sites of these AR actions, several studies have induced PCOS in mice with a loss of AR function only in the brain, granulosa cells or theca cells. Findings from these studies have pinpointed the brain as a key site at the core of PCOS pathogenesis, as silencing of AR actions in the brain protected female mice from developing the majority of reproductive and metabolic traits of PCOS.

<table>
<thead>
<tr>
<th>Clinical features of PCOS</th>
<th>Women with PCOS</th>
<th>PCOS Rodent Models</th>
<th>PCOS Sheep Models</th>
<th>PCOS Primates Models</th>
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<td>✓</td>
<td>✓</td>
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<tr>
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<td>✓</td>
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<td>✓</td>
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</table>

Figure 4
Presence of clinical PCOS traits in hyperandrogenic animal models. Prenatal and/or postnatal treatment of testosterone or DHT in rodents (mice and rats), sheep and nonhuman primates is an effective way to replicate most endocrine, reproductive and metabolic features of human PCOS, hence providing valuable preclinical animal models for basic discovery research. ✓, presence of PCOS phenotype. X, absence of PCOS phenotype. ▲, non-significant tendency for increased cholesterol, triglycerides, high-density lipoprotein (HDL) and low-density lipoprotein (LDL). --, not reported.

Figure 5
Schematic diagram of proposed site-specific androgen receptor (AR)-mediated actions contributing to the induction of PCOS traits. Currently, as the origins of PCOS remains unknown, mechanism-based treatments are not feasible, and management relies on the treatment of symptoms only. Here we illustrate the proposed sites of AR-mediated actions involved in generating PCOS traits. This knowledge serves as a guide for future basic discovery research by highlighting target sites to focus on unraveling the defective mechanisms causing the development of PCOS traits. Identification of these underlying altered mechanisms involved in the pathogenesis of PCOS would allow development of new targeted, evidence-based interventions for PCOS.
PCOS traits, including ovulatory dysfunction, increased adiposity, dyslipidemia and pronounced adipocyte hypertrophy and hepatic steatosis (Abbott 2017, Caldwell et al. 2017). In comparison to the brain-specific ARKO mouse, like controls, female mice with a loss of function only in the granulosa cells still displayed the majority of PCOS characteristics with only a full protection against an increase in granulosa cell degeneration in antral follicles observed (Caldwell et al. 2017). In addition, only a partial prevention of the development of acyclicity, dysfunctional ovulation and infertility were observed in a hyperandrogenic PCOS mouse model with an inactivation of AR signaling only in the theca cells (Ma et al. 2017). Collectively, these results provide strong evidence that while ovarian AR actions may contribute to the development of reproductive features in PCOS, the brain is the prominent site and neuroendocrine androgen-driven molecular mechanisms are the key mediators in the developmental origins of PCOS traits.

Current implications and future directions from basic research on the development of novel PCOS treatments targeting androgen-driven mechanisms

Substantial evidence supports a role for androgen excess mediating its actions via the AR in the origin of PCOS, but systemic treatment with present generations of antiandrogens is not a viable option due to unacceptable liver toxicity that preclude their use for non-lethal chronic disorders. Therefore, recent research has been aimed at trying to identify more targeted ways by which pharmacological strategies may be able to suppress excess androgenic effects in women with PCOS.

One such approach would be to target AR-driven neuroendocrine pathways. A specific loss of AR signaling in the brain protects hyperandrogenized PCOS mice against the development of PCOS traits, pinpointing the brain as a key site involved in experimental PCOS pathogenesis (Caldwell et al. 2017). An increase in LH-to-FSH ratio and LH pulse frequency are frequently observed in PCOS patients (Dumesic et al. 2015) and also in rat (Wu et al. 2010), mouse (Moore et al. 2013, 2015), sheep (Sarma et al. 2005) and primate (Abbott et al. 2018) PCOS models. The activity of GnRH neurons, which regulate gonadotrophin secretion, is highly dependent upon homeostatic feedback and gonadal steroid hormone signaling in the brain. However, GnRH neurons do not express AR (Huang & Harlan 1993). Instead, AR is expressed within intermediary neuronal networks that lie upstream of the GnRH neurons, allowing an indirect pathway for AR-mediated actions. One such neuronal network that expresses AR is the kisspeptin-neurokinin B (NKB)- dynorphin ‘KNDy’ system within the arcuate nucleus which plays a pivotal role in the regulation of GnRH secretion (Navarro et al. 2009, Smith 2013, Skorupskaite et al. 2014). AR-mediated signaling plays a role in regulating the KNDy system (Walters et al. 2018b) and rodent and sheep hyperandrogenized PCOS animal models display changes in KNDy expression and circuitry (Brown et al. 2012, Cernea et al. 2015, Kauffman et al. 2015, Osuka et al. 2017). These findings imply that the KNDy system may be an attractive therapeutic target to modulate AR-driven neuroendocrine activity in PCOS patients. Indeed, increased kisspeptin has been reported in several populations of PCOS patients (Albalawi et al. 2018, Katulski et al. 2018, Umayal et al. 2019). Of note, not all populations of women with PCOS exhibit changes in kisspeptin levels (Daghestani 2018, Katulski et al. 2018), inferring alterations in the KNDy system may be related to differences in PCOS phenotype or severity. Recent clinical studies also provide support for modulation of the KNDy system as a potential new target for the development of treatment for PCOS. Treatment of women with a NK3R antagonist decreases ovarian hormone levels (Fraser et al. 2016), while more specifically, treatment of PCOS patients with a NKB receptor antagonist reduced LH pulse frequency and LH and testosterone concentrations, which are characteristics of PCOS (George et al. 2016).

Moreover, the finding that a specific loss of AR signaling in the brain protects hyperandrogenized PCOS mice against the development of key metabolic PCOS traits, including increased body weight and visceral fat, dyslipidemia and pronounced adipocyte hypertrophy and hepatic steatosis, indicates that some aspects of metabolic dysfunction observed in PCOS patients may be mediated via AR-regulated central mechanisms (Caldwell et al. 2017). In fact, there is evidence to suggest that an androgen–brain–adipocyte axis may play a role in the etiology of PCOS-associated metabolic dysfunction. The adipokine leptin, which plays an important role in regulating energy homeostasis, has reduced impact on energy expenditure in a mouse model of androgen excess. This coincides with a decrease in sympathetic outflow to brown adipose tissue (BAT) (Nohara et al. 2014). Proopiomelanocortin (POMC) and neuropeptide Y/agouti-related peptide (NPY/AgRP) neurons are well-known targets of leptin (Villanueva & Myers 2008) and have been reported to be influenced by androgen excess. POMC mRNA and fiber projections...
are reduced in androgenized female mice (Nohara et al. 2014), while in the ewe prenatal androgen excess leads to increased NPY/AgRP cell number and fiber projections (Sheppard et al. 2011). In addition, treatment with the AR antagonist flutamide blocks the observed changes in NPY/AgRP neurons in androgenized sheep (Sheppard et al. 2011) and also improves lipid profiles in women with PCOS, independent of weight change, glucose metabolism and insulin sensitivity (Diamanti-Kandarakis et al. 1998). Collectively, these results are supportive of a central role for androgens in mediating PCOS-related metabolic dysregulation and support further research in determining the precise mechanisms involved.

AR-driven pathways within adipose tissue may also be important to investigate in the development of new strategies to treat PCOS. Studies have shown intra-abdominal fat mass is significantly increased in women with PCOS and that this increase is positively correlated with serum androgen levels (Dumesic et al. 2016). Hyperandrogenized rodent, sheep and primate PCOS models exhibit changes in adipocyte morphology and/or function (Keller et al. 2014, Cardoso et al. 2016, Caldwell et al. 2017). Interestingly, in the ewe model, observed alterations in adipocyte morphology are present before insulin insensitivity is evident, inferring that defective adipocyte function may precede the onset of metabolic dysfunction. Hence, research identifying the mechanisms underpinning these alterations may provide targets for the development of interventions aimed at treating PCOS from an early stage of its development and potentially blocking its progression. A potential androgen excess AR-driven defect within adipocyte cells involved in generating the PCOS phenotype may be the ability of adipocytes to produce adequate levels of the adipokines. At present, understanding the precise role the adipokine adiponectin plays in the development of PCOS is of particular interest. Adiponectin is important in glucose and lipid metabolism and has been observed to be decreased in women with PCOS (Manneras-Holm et al. 2011) and several PCOS mouse models (Benrick et al. 2017, Caldwell et al. 2017, Singh et al. 2017). Transplantation of BAT from control healthy rats into hyperandrogenic PCOS female rats enhances their BAT activity, increases serum adiponectin levels and ameliorates several key PCOS traits including irregular cycles and insulin resistance (Yuan et al. 2016). This study also revealed that exogenous adiponectin administration recapitulated the beneficial effects from BAT transplantation (Yuan et al. 2016), which was also confirmed in another hyperandrogenized mouse model of PCOS (Singh et al. 2017). Moreover, the findings that transgenic mice that overexpress adiponectin are protected from the induction of androgen excess metabolic PCOS traits, while mice that lack adiponectin display exaggerated or comparable PCOS features to the classic hyperandrogenized PCOS mouse model, highlights that modulation of adipokines, such as adiponectin, may provide a promising novel therapeutic strategy for the management of PCOS (Benrick et al. 2017). However, an understanding of the downstream mechanisms influenced by such factors is required. In addition while adipocyte AR actions appear to be important, the findings that altered glucose homeostasis in conjunction with hepatic steatosis but not insulin resistance has been observed in a DHT-induced mouse model of PCOS (Caldwell et al. 2017), suggests that AR actions within the hepatocyte, and potentially the muscle, may also play a role in the development of PCOS and warrant future investigation.

Less site-specific targeted approaches aimed at reducing androgen excess have also been put forward from preclinical research, with some directly translated into clinical trials. Dietary medium-chain fatty acid (decanoic acid) reduces androgen biosynthesis in vitro by modulating the steroidalogenic enzyme HSD3B2, implying it may be a novel way to reduce androgen excess. In support of this, administration of decanoic acid to a rat PCOS model is reported to restore cyclicity and reduce testosterone and fasting insulin levels (Lee et al. 2016). Decanoic acid can be attained via dietary supplementation with medium-chain triglyceride oil, which has been shown to reduce body weight and total body fat in healthy adults (Mumme & Stonehouse 2015), inferring this may be a useful therapy in tackling obesity which is often displayed in women with PCOS. Similarly, in vitro (Marti et al. 2017) and in vivo (Ergenoglu et al. 2015) studies have revealed that resveratrol, a polyphenol found in several plant species, inhibits androgen production by lowering CYP17 and CYP21 expression and activity and is effective in the treatment of some ovarian PCOS features observed in a rat PCOS model. A following clinical trial reported that PCOS patients treated with resveratrol exhibited a decrease in total testosterone and fasting insulin levels and an increase in Insulin Sensitivity Index (Banaszewska et al. 2016).

Conclusion

Results from clinical and animal studies have provided valuable congruent insight on the role of androgenic actions in regulating female reproduction and the
mechanisms underpinning the development of PCOS. Discovery research has demonstrated that the correct balance in androgenic actions optimizes ovarian follicle development and female fertility. While clinical studies indicate that the use of androgen pre-treatment prior to an IVF cycle has a promising beneficial impact on IVF outcomes. Research using PCOS animal models has significantly advanced our understanding of the pathogenesis of PCOS, and the value of this knowledge is clearly highlighted by recent publications reporting on the evaluation of potential new treatments for PCOS that have been directly translated from preclinical studies. By continuing to combine results from clinical observations and basic discovery research, the precise androgenic mechanisms regulating ovarian function and the development of PCOS will be identified. This will open-up new avenues on how in the future we may achieve the development of evidence-based interventions that enhance ovarian response in POR patients and ameliorate symptoms in women with PCOS.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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Author contribution statement

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