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.

Key Words

- ▶ androgen
- ▶ ovarian follicle
- ▶ POR
- ▶ PCOS

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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).

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 spatial 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 (Szoltyś & 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; Szoltyś & 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

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 (Tarlatis *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 woman 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 (Dumesic *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 (Narkiewicz *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).

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

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 3 β -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^{Ex1}) (Shiina *et al.* 2006), exon 2 (ARKO^{Ex2}) (Hu *et al.* 2004) or exon 3 (ARKO^{Ex3}) (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 loose 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^{Ex2}) (Sen & Hammes 2010) and exon 3 (GCARKO^{Ex3}) (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.

Transgenic ARKO mouse model	Fertility	Estrous cycles	Serum steroids and hormones	Growing follicle populations	Oocyte and follicle health	Corpora lutea population and ovulation	Embryo development
Global							
ARKO ^{E1} <i>Shiina et al.</i> (2006)	↓ pups/litter	–	No change in FSH, LH, E2, testosterone or P4 at proestrus	Normal at 8 weeks Total follicle exhaustion by 40 weeks	↑ atretic follicles	↓ CL	–
ARKO ^{E2} <i>Yeh et al.</i> (2002), <i>Hu et al.</i> (2004)	↓ pups/litter	↑ estrous cycle length	–	Normal at 4 and 16 weeks	Cumulus cells dissociation ↑ follicular atresia after hyperstimulation	↓ CL ↓ superovulated oocytes	–
ARKO ^{E3} <i>Walters et al.</i> (2007, 2009), <i>Cheng et al.</i> (2013)	↓ pups/litter	↑ estrous cycle length, irregular estrous cycles	No change in FSH, LH, E2, testosterone at diestrus. ↓ LH, E2 and E1 at proestrus ↓ LH after OVX. Normal LH response to GnRH and OVX+E2	Normal at 10–12, 26 and 52 weeks at diestrus At proestrus ↓ preovulatory follicles	↑ unhealthy antral follicles	↓ CL ↓ naturally ovulated oocytes Normal superovulated ovulation rates	No change in fertilization or progression to two-cell stage
Ovary							
GCARKO ^{E2} <i>Sen & Hammes</i> (2010)	↓ pups/litter	↑ estrous cycle length at 6 months but not 2 months	–	Normal at 4 weeks. At 2 and 6 months ↑ preantral follicles, but ↓ antral follicles	↑ atretic follicles	↓ CL ↓ naturally ovulated oocytes. Normal superovulated ovulation rates at 2 months but ↓ at 6 months	–
GCARKO ^{E3} <i>Walters et al.</i> (2012a,b)	↓ in cumulative pups/month from 6 months	↑ estrous cycle length at 6 months but not 3 months	No change in FSH or LH at diestrus	↓ large preantral and small antral follicles at 3 months. No difference in follicle populations at 6 months	↑ unhealthy follicles and ZPR counts at 6 months	↓ cumulus expansion No change in CL populations	↓ rate of fertilization
TCARKO <i>Ma et al.</i> (2017)	Normal fertility	Normal estrous cycles	No change in FSH, LH, E2 or testosterone at diestrus. Normal LH response to GnRH	Normal	–	No change in CL populations	–
OoARKO <i>Sen & Hammes</i> (2010)	Normal fertility	Normal estrous cycles	–	Normal	↓ DHT-induced GVBD <i>in vitro</i>	No change in CL populations	–
Brain							
PitARKO <i>Wu et al.</i> (2014)	↓ pups/litter	Trend to ↑ time at estrus	↑ 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	Normal	↑ pyknotic granulosa cells in antral follicles	↓ CL	–
NeurARKO <i>Caldwell et al.</i> (2017), <i>Walters</i> <i>et al.</i> (2019)	Normal fertility	Trend to less regular estrous cycle patterns	↑ LH but no change in FSH at diestrus. No change in LH or FSH at proestrus. ↓ LH response to OVX+E2	Normal at diestrus ↓ large antral follicles at proestrus	↑ unhealthy large preantral follicles ↓ unhealthy small antral follicles	No change in CL populations	–

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 (α GSU)-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).

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

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.

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

Table 2 Summary of studies assessing the impact on IVF outcomes of DHEA pretreatment in poor responder patients.

	Sample size	Intervention			Number of follicles ≥ 17 mm on the day of hCG	Number of oocytes retrieved	Number of MII oocytes	Fertilized oocytes	Embryo implantation rate	Clinical pregnancy rate	Live birth rate
		Type	Route	Dose							
Casson <i>et al.</i> (2000)	5	Self-control	DHEA	Oral	80 mg/day	Duration 2 months	Improved [^] (T:18 $\pm$ 1.4, C:3.0 $\pm$ 2) $P < 0.001$	Improved [^] (T:13.5 $\pm$ 3.5, C:3.0 $\pm$ 2) $P < 0.01$	-	-	(T:1, C:0) @
Barad & Gleicher (2005)*	1	Case report	DHEA	Oral	75 mg/day	7 months	Improved [^] (T:18 $\pm$ 1.4, C:3.0 $\pm$ 2) $P < 0.001$	Improved [^] (T:13.5 $\pm$ 3.5, C:3.0 $\pm$ 2) $P < 0.01$	-	-	-
Barad & Gleicher (2006)#	25	Self-control	DHEA	Oral	75 mg/day	16 weeks	Improved (T:4.4 $\pm$ 0.5, C:3.4 $\pm$ 0.5) $P < 0.05$	Improved (T:3.0 $\pm$ 0.5, C:1.4 $\pm$ 0.3) $P < 0.001$	-	-	-
Barad <i>et al.</i> (2007)#	190 (C:101, T:89)	Case control	DHEA	Oral	75 mg/day	4 months	D declined (T:3.9 $\pm$ 0.4, C:5.8 $\pm$ 0.5) $P < 0.01$	-	No effect	Improved (T:28.1%, C:10.9%) $P < 0.01$	-
Sonnezeer <i>et al.</i> (2009)*	19	Self-control	DHEA	Oral	75 mg/day	≥ 90 days	Improved (T:4.9 $\pm$ 2.3, C:2.5 $\pm$ 1.6) $P < 0.05$	No effect	Improved (T:23.9%, C:0%) $P < 0.05$	Improved (T:44.8%, C:0%) $P < 0.01$	-
Wiser <i>et al.</i> (2010)\$	33 (C:16, T:17)	RCT	DHEA	Oral	75 mg/day	≥ 6 weeks	No effect	No effect	-	Non-significant different trend ($P = 0.07$)	Improved\$ (T:23.1%, C:4%) $P = 0.05$
Artini <i>et al.</i> (2012)*	24 (C:12, T:12)	RCT	DHEA	Oral	75 mg/day	3 months	No effect	No effect	-	No effect	-
Moawad & Shaeer (2012)*	133 (C:66, T:67)	RCT	DHEA	Oral	75 mg/day	≥ 12 weeks	Improved (T:5.9 $\pm$ 3.6, C:3.5 $\pm$ 2.9) $P < 0.001$	No effect	-	Improved (T:20.7%, C:17%) $P = 0.048$	-
Xu <i>et al.</i> (2014)*	386 (C:197, T:189)	Retrospective cohort	DHEA	Oral	75 mg/day	90 days	No effect	No effect	Improved (T:18.7%, C:10.1%) $P = 0.005$	Improved (T:30.4%, C:18.7%) $P = 0.025$	-
Yeung <i>et al.</i> (2014)	32 (C:16, T:16)	RCT (+Placebo)	DHEA	Oral	75 mg/day	12 weeks	Follicles ≥ 14 mm Improved (T:4.41 $\pm$ 2.02, C:3.92 $\pm$ 1.58) $P = 0.03$	No effect	-	No effect	No effect
Jirge <i>et al.</i> (2014)#	22 (C:22, T:20)	Self-control	DHEA	Oral	75 mg/day	≥ 60 days	Improved (T:5.9 $\pm$ 0.6, C:2.73 $\pm$ 0.2) $P < 0.001$	Improved (T:4.45 $\pm$ 0.4, C:2.09 $\pm$ 0.2) $P = 0.0001$	No effect	No effect	Improved (T:25%, C:0%) $P = 0.012$
Kara <i>et al.</i> (2014)	208 (C:104, T:104)	RCT	DHEA	Oral	75 mg/day	≥ 12 weeks	No effect	No effect	-	No effect	-
Zhang <i>et al.</i> (2014)	95 (C:53, T:42)	RCT	DHEA	Oral	75 mg/day	For 3 consecutive menstrual cycles	No effect	No effect	-	No effect	-

Vlahos <i>et al.</i> (2015)	161 (C:113, T:48)	Case-control prospective study	DHEA	Oral	75 mg/day	≥12 weeks	-	No effect	-	No effect	No effect
Tsui <i>et al.</i> (2015)*	10	Self-control	DHEA	Oral	90 mg/day	3 months	-	Improved (T:4.2 ± 1.2, C:2.4 ± 1.1) <i>P</i> < 0.01	Improved (T:3.8 ± 1.1, C:1.7 ± 0.5) <i>P</i> < 0.001	Non- significant different trend (<i>P</i> = 0.065)	-
Kotb <i>et al.</i> (2016)*	140 (C:70, T:70)	RCT	DHEA	Oral	75 mg/day	12 weeks	No effect	Improved (T: 6.9 ± 3.3, C: 5.8 ± 3.1) <i>P</i> = 0.03	Improved (T: 4.7 ± 2.2, C: 3.6 ± 2.3) <i>P</i> = 0.007	Improved (T: 32.8%, C:15.7%) <i>P</i> = 0.029	-
Narkwichan <i>et al.</i> (2017)	52 (C:25, T:27)	RCT (+Placebo)	DHEA	Oral	75 mg/day	≥12 weeks	-	No effect	Non-significant trend to improved (<i>P</i> = 0.052)	No effect	No effect
Hu <i>et al.</i> (2017)	103 (C:50, T:53)	Case-control prospective cohort	DHEA	Oral	75 mg/d	8 weeks	-	No effect	No effect	No effect	-
Lin <i>et al.</i> (2017)	72 (C:38, T:34)	Case-control prospective study	DHEA	Oral	90 mg/day	≥8 weeks	-	No effect	Improved (T: 75.9%, C:58.8%) <i>P</i> < 0.05	No effect	No effect
Chern <i>et al.</i> (2018)*	151 (C:84, T:67)	Retrospective cohort study	DHEA	Oral	90 mg/day	3 months	-	Improved (T:3.3 ± 2.5, C:2.0 ± 1.5) <i>P</i> < 0.001	Improved (T:1.9 ± 1.5, C:1.0 ± 1.1) <i>P</i> < 0.001	Improved (T:23.9%, C:7.1%) <i>P</i> = 0.004	Improved (T:16.4%, C:6%) <i>P</i> = 0.038
Li <i>et al.</i> (2018)	38 (C:19, T:19)	Case-control prospective cohort	DHEA	Oral	90 mg/day	≥8 weeks	-	No effect	No effect	No effect	No effect
Al-Turki (2018)*	62 (C:28, T:34)	Prospective case control	DHEA	Oral	50 mg/day	3 months	-	Improved (T:6.35 ± 2.41, C:3.98 ± 3.2) <i>P</i> < 0.002	Improved (T: 4.9 ± 1.8, C: 3.16 ± 2.5) <i>P</i> < 0.003	Improved € (T:6.1%, C:3.6%) <i>P</i> < 0.05	Improved € (T:53%, C:14%) <i>P</i> < 0.01

*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, Bosdou *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 doses 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.

Sample size	Study type	Intervention			Number of follicles ≥ 17 mm on the day of hCG	Number of oocytes retrieved	Number of MII oocytes	Fertilized oocytes	Embryo implantation rate	Clinical pregnancy rate	Live birth rate
		Type	Route	Dose							
Massin <i>et al.</i> (2006)	53 (C:25, T:24)	RCT (+Placebo)	Transdermal	10 mg/day	15–20 days	No effect	No effect	No effect	–	No effect	No effect
Balasch <i>et al.</i> (2006) [#]	25	Self-controlled	Transdermal	20 µg/kg per day	5 days	–	Improved@ 5.8 ± 0.4	Improved@ 3.8 ± 0.4	Improved@ 16.6%	Improved@ 24%	Improved@ 20%
Fabregues <i>et al.</i> (2009) [*]	62 (C:31, T:31)	RCT	Transdermal	20 µg/kg per day	5 days	No effect	No effect	No effect	No effect	No effect	–
Sipe <i>et al.</i> (2010)	12 (C:6, T:6)	RCT (+Placebo)	Transdermal	2.5 mg/day	12 days	No effect	–	–	–	–	–
Kim <i>et al.</i> (2011) [*]	110 (C:55, T:55)	RCT	Transdermal	12.5 mg/day	21 days	Improved (T:4.1 ± 1.4, C:2.7 ± 1.0) $P < 0.001$	Improved (T:5.4 ± 1.9, C:3.8 ± 1.4) $P < 0.001$	Improved (T:4.3 ± 1.7, C:3.0 ± 1.2) $P < 0.001$	Improved (T:14.3%, C:7.2%) $P = 0.019$	Improved (T:30.9%, C:14.5%) $P = 0.041$	Non-significant different trend ($P = 0.057$)
Kim <i>et al.</i> (2014) ^{*,^}	60 (C:30, T:30)	RCT	Transdermal	12.5 mg/day	4 weeks [^]	–	Improved (T: 5.8 ± 1.9, C: 3.9 ± 1.3) $P < 0.001$	Improved (T: 4.6 ± 1.6, C: 3.1 ± 1.1) $P < 0.001$	–	Improved (T:36.7%, C:10%) $P = 0.03$	Improved (T:30%, C:6.7%) $P = 0.042$
Mitri <i>et al.</i> (2016) [*]	26 (C:13, T:13)	Case control Observational pilot study	Transdermal	25 mg/day	4.4 ± 1.2 days	Improved (T: 4.92 ± 2.7, C: 1.69 ± 0.8) $P < 0.001$	No effect	–	–	(T:30%, C:0%)@	–
Bosdou <i>et al.</i> (2016)	50 (C:24, T:26)	RCT	Transdermal	10 mg/day	21 days	No effect	No effect	No effect	–	No effect	No effect
Doan <i>et al.</i> (2017) [*]	110 (C:55, T:55)	Case-control prospective, descriptive study	Transdermal	12.5 mg/day	6th day to 2nd day of next cycle (~24 days) ^s	Improved (T:4.5 ± 1.7, C:2.6 ± 2.1) $P = 0.00$	Improved (T:4.5 ± 1.6, C:3.1 ± 1.1) $P = 0.00$	Improved (T:22.5, C:145) $P = 0.02$	Improved (T:14.1%, C:6.1%) $P = 0.04$	Improved (T:21.8%, C:7.7%) $P = 0.04$	–
Saharkhiz <i>et al.</i> (2018) [*]	48 (C:23, T:25)	RCT single-blinded (+Placebo)	Transdermal	25 mg/day	From 2nd day of cycle to hCG treatment day (~12 days) ^s	–	Improved (T:2.48 ± 1.6, C:1.2 ± 1.3) $P = 0.004$	Improved (T:1.6 ± 1.6, C:0.3 ± 0.6) $P = 0.001$	–	Improved (T:16%, C:0%) $P = 0.04$	–

^{*}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; ^scalculated 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, Ruvolo *et 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 (Haydardedeoglu *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 weak 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 – 3 β -hydroxysteroid dehydrogenase (3 β -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, Dumesic *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 progestin with low or antiandrogen activity and/or antiandrogens, have been used for treating PCOS symptoms.

	HA + OA + PCOM (Phenotype A)	HA + OA (Phenotype B)	HA + PCOM (Phenotype C)	OA + PCOM (Phenotype D)
Rotterdam*	✓	✓	✓	✓
AE-PCOS	✓	✓	✓	
NIH	✓	✓		

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).

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 (Venturoli *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 reproductive

and metabolic disturbances associated 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- α 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 antiprogesterins 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 prenatally 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

Clinical features of PCOS	Women with PCOS	PCOS Rodent Models	PCOS Sheep Models	PCOS Primates Models
Irregular cycles/ acyclicity	✓	✓	✓	✓
Oligo- or anovulation	✓	✓	✓	✓
Polycystic ovarian morphology	✓	✓	✓	✓
Hyperandrogenism	✓	✓	✓	✓
LH hypersecretion	✓	✓	✓	✓
Aberrant follicle dynamics	✓	✓	✓	✓
Increased adiposity	✓	✓	X	✓
Dyslipidaemia	✓	✓	▲	✓
Insulin resistance	✓	✓	✓	✓
Non-alcoholic fatty liver	✓	✓	✓	--

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.

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

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 steroidogenic 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

K A W, V R P, A A and D J H wrote paper and created figures.

References

- Abbott DH 2017 Neuronal androgen receptor: molecular gateway to polycystic ovary syndrome? *PNAS* **114** 4045–4047. (<https://doi.org/10.1073/pnas.1703436114>)
- Abbott DH, Nicol LE, Levine JE, Xu N, Goodarzi MO & Dumesic DA 2013 Nonhuman primate models of polycystic ovary syndrome. *Molecular and Cellular Endocrinology* **373** 21–28. (<https://doi.org/10.1016/j.mce.2013.01.013>)
- Abbott DH, Levine JE & Dumesic DA 2016 Translational insight into polycystic ovary syndrome (PCOS) from female monkeys with PCOS-like traits. *Current Pharmaceutical Design* **22** 5625–5633. (<https://doi.org/10.2174/1381612822666160715133437>)
- Abbott DH, Vepraskas SH, Horton TH, Terasawa E & Levine JE 2018 Accelerated episodic luteinizing hormone release accompanies blunted progesterone regulation in PCOS-like female rhesus monkeys (*Macaca mulatta*) exposed to testosterone during early-to-mid gestation. *Neuroendocrinology* **107** 133–146. (<https://doi.org/10.1159/000490570>)
- Al-Turki HA 2018 Dehydroepiandrosterone supplementation in women undergoing assisted reproductive technology with poor ovarian response. A prospective case-control study. *Journal of International Medical Research* **46** 143–149. (<https://doi.org/10.1177/0300060517720005>)
- Albalawi FS, Daghestani MH, Daghestani MH, Eldali A & Warsy AS 2018 rs4889 polymorphism in KISS1 gene, its effect on polycystic ovary syndrome development and anthropometric and hormonal parameters in Saudi women. *Journal of Biomedical Science* **25** 50. (<https://doi.org/10.1186/s12929-018-0452-2>)
- Artini PG, Simi G, Ruggiero M, Pinelli S, Di Berardino OM, Papini F, Papini S, Monteleone P & Cela V 2012 DHEA supplementation improves follicular microenvironment in poor responder patients. *Gynecological Endocrinology* **28** 669–673. (<https://doi.org/10.3109/09513590.2012.705386>)
- Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Futterweit W, Janssen OE, Legro RS, Norman RJ, Taylor AE, *et al.* 2006 Positions statement: Criteria for defining polycystic ovary syndrome as a predominantly hyperandrogenic syndrome: an androgen excess society guideline. *Journal of Clinical Endocrinology and Metabolism* **91** 4237–4245. (<https://doi.org/10.1210/jc.2006-0178>)
- Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Futterweit W, Janssen OE, Legro RS, Norman RJ, Taylor AE, *et al.* 2009 The androgen excess and PCOS Society criteria for the polycystic ovary syndrome: the complete task force report. *Fertility and Sterility* **91** 456–488. (<https://doi.org/10.1016/j.fertnstert.2008.06.035>)
- Balasch J, Fabregues E, Penarrubia J, Carmona F, Casamitjana R, Creus M, Manau D, Casals G & Vanrell JA 2006 Pretreatment with transdermal testosterone may improve ovarian response to gonadotrophins in poor-responder IVF patients with normal basal concentrations of FSH. *Human Reproduction* **21** 1884–1893. (<https://doi.org/10.1093/humrep/del052>)
- Banaszewska B, Wrotyńska-Barczyńska J, Spaczynski RZ, Pawelczyk L & Duleba AJ 2016 Effects of resveratrol on polycystic ovary syndrome: a double-blind, randomized, placebo-controlled trial. *Journal of Clinical Endocrinology and Metabolism* **101** 4322–4328. (<https://doi.org/10.1210/jc.2016-1858>)
- Barad DH & Gleicher N 2005 Increased oocyte production after treatment with dehydroepiandrosterone. *Fertility and Sterility* **84** 756. (<https://doi.org/10.1016/j.fertnstert.2005.02.049>)
- Barad D & Gleicher N 2006 Effect of dehydroepiandrosterone on oocyte and embryo yields, embryo grade and cell number in IVF. *Human Reproduction* **21** 2845–2849. (<https://doi.org/10.1093/humrep/del254>)
- Barad D, Brill H & Gleicher N 2007 Update on the use of dehydroepiandrosterone supplementation among women with diminished ovarian function. *Journal of Assisted Reproduction and Genetics* **24** 629–634. (<https://doi.org/10.1007/s10815-007-9178-x>)
- Barbieri RL, Sluss PM, Powers RD, Mcshane PM, Vitonis A, Ginsburg E & Cramer DC 2005 Association of body mass index, age, and cigarette smoking with serum testosterone levels in cycling women undergoing in vitro fertilization. *Fertility and Sterility* **83** 302–308. (<https://doi.org/10.1016/j.fertnstert.2004.07.956>)
- Barrenetxea G, Agirregoikoa JA, Jiménez MR, Lopez De Larrea AL, Ganzabal T & Carbonero K 2008 Ovarian response and pregnancy outcome in poor-responder women: a randomized controlled trial on the effect of luteinizing hormone supplementation on in vitro fertilization cycles. *Fertility and Sterility* **89** 546–553. (<https://doi.org/10.1016/j.fertnstert.2007.03.088>)
- Becerra-Fernandez A, Perez-Lopez G, Roman MM, Martin-Lazaro JF, Lucio Perez MJ, Asenjo Araque N, Rodriguez-Molina JM, Berrocal Sertucha MC & Aguilar Vilas MV 2014 Prevalence of hyperandrogenism and polycystic ovary syndrome in female to male transsexuals. *Endocrinología y Nutrición* **61** 351–358. (<https://doi.org/10.1016/j.endonu.2014.01.010>)

- Benrick A, Chancion B, Micallef P, Wu Y, Hadi L, Shelton JM, Stener-Victorin E & Wernstedt Asterholm I 2017 Adiponectin protects against development of metabolic disturbances in a PCOS mouse model. *PNAS* **114** E7187–E7196. (<https://doi.org/10.1073/pnas.1708854114>)
- Bercaire L, Nogueira SM, Lima PC, Alves VR, Donadio N, Dzik A & Cavagna M 2018 ANDRO-IVF: a novel protocol for poor responders to IVF controlled ovarian stimulation. *JBRA Assisted Reproduction* **22** 52–55. (<https://doi.org/10.5935/1518-0557.20180011>)
- Berkkanoglu M, Isikoglu M, Aydin D & Ozgur K 2007 Clinical effects of ovulation induction with recombinant follicle-stimulating hormone supplemented with recombinant luteinizing hormone or low-dose recombinant human chorionic gonadotropin in the midfollicular phase in microdose cycles in poor responders. *Fertility and Sterility* **88** 665–669. (<https://doi.org/10.1016/j.fertnstert.2006.11.150>)
- Bertoldo MJ, Caldwell ASL, Riepsamen AH, Lin D, Gonzalez MB, Robker RL, Ledger WL, Gilchrist RB, Handelsman DJ & Walters KA 2019 A hyperandrogenic environment causes intrinsic defects that are detrimental to follicular dynamics in a PCOS mouse model. *Endocrinology* **160** 699–715. (<https://doi.org/10.1210/en.2018-00966>)
- Bosdou JK, Venetis CA, Kolibianakis EM, Toulis KA, Goulis DG, Zepiridis L & Tarlatzis BC 2012 The use of androgens or androgen-modulating agents in poor responders undergoing in vitro fertilization: a systematic review and meta-analysis. *Human Reproduction Update* **18** 127–145. (<https://doi.org/10.1093/humupd/dmr051>)
- Bosdou JK, Venetis CA, Dafopoulos K, Zepiridis L, Chatzimeletiou K, Anifandis G, Mitsoli A, Makedos A, Messinis IE, Tarlatzis BC, *et al.* 2016 Transdermal testosterone pretreatment in poor responders undergoing ICSI: a randomized clinical trial. *Human Reproduction* **31** 977–985. (<https://doi.org/10.1093/humrep/dew028>)
- Boyle JA & Teede HJ 2016 PCOS: refining diagnostic features in PCOS to optimize health outcomes. *Nature Reviews: Endocrinology* **12** 630–631. (<https://doi.org/10.1038/nrendo.2016.157>)
- Bozdag G, Mumusoglu S, Zengin D, Karabulut E & Yildiz BO 2016 The prevalence and phenotypic features of polycystic ovary syndrome: a systematic review and meta-analysis. *Human Reproduction* **31** 2841–2855. (<https://doi.org/10.1093/humrep/dew218>)
- Brown RE, Wilkinson DA, Imran SA, Caraty A & Wilkinson M 2012 Hypothalamic kiss1 mRNA and kisspeptin immunoreactivity are reduced in a rat model of polycystic ovary syndrome (PCOS). *Brain Research* **1467** 1–9. (<https://doi.org/10.1016/j.brainres.2012.05.049>)
- Burger HG 2002 Androgen production in women. *Fertility and Sterility* **77** (Supplement 4) S3–S5. ([https://doi.org/10.1016/S0015-0282\(02\)02985-0](https://doi.org/10.1016/S0015-0282(02)02985-0))
- Calaf J, López E, Millet A, Alcañiz J, Fortuny A, Vidal O, Callejo J, Escobar-Jiménez F, Torres E, Espinós JJ, *et al.* 2007 Long-term efficacy and tolerability of flutamide combined with oral contraception in moderate to severe hirsutism: a 12-month, double-blind, parallel clinical trial. *Journal of Clinical Endocrinology and Metabolism* **92** 3446–3452. (<https://doi.org/10.1210/jc.2006-2798>)
- Caldwell AS, Middleton LJ, Jimenez M, Desai R, McMahon AC, Allan CM, Handelsman DJ & Walters KA 2014 Characterization of reproductive, metabolic, and endocrine features of polycystic ovary syndrome in female hyperandrogenic mouse models. *Endocrinology* **155** 3146–3159. (<https://doi.org/10.1210/en.2014-1196>)
- Caldwell AS, Eid S, Kay CR, Jimenez M, McMahon AC, Desai R, Allan CM, Smith JT, Handelsman DJ & Walters KA 2015 Haplosufficient genomic androgen receptor signaling is adequate to protect female mice from induction of polycystic ovary syndrome features by prenatal hyperandrogenization. *Endocrinology* **156** 1441–1452. (<https://doi.org/10.1210/en.2014-1887>)
- Caldwell ASL, Edwards MC, Desai R, Jimenez M, Gilchrist RB, Handelsman DJ & Walters KA 2017 Neuroendocrine androgen action is a key extraovarian mediator in the development of polycystic ovary syndrome. *PNAS* **114** E3334–E3343. (<https://doi.org/10.1073/pnas.1616467114>)
- Cárdenas H & Pope WF 1994 Administration of testosterone during the follicular phase increased the number of corpora lutea in gilts. *Journal of Animal Science* **72** 2930–2935. (<https://doi.org/10.2527/1994.72112930x>)
- Cardenas H, Herrick JR & Pope WF 2002 Increased ovulation rate in gilts treated with dihydrotestosterone. *Reproduction* **123** 527–533. (<https://doi.org/10.1530/rep.0.1230527>)
- Cardoso RC, Veiga-Lopez A, Moeller J, Beckett E, Pease A, Keller E, Madrigal V, Chazenbalk G, Dumesic D & Padmanabhan V 2016 Developmental programming: impact of gestational steroid and metabolic milieu on adiposity and insulin sensitivity in prenatal testosterone-treated female sheep. *Endocrinology* **157** 522–535. (<https://doi.org/10.1210/en.2015-1565>)
- Casson PR, Lindsay MS, Pisarska MD, Carson SA & Buster JE 2000 Dehydroepiandrosterone supplementation augments ovarian stimulation in poor responders: a case series. *Human Reproduction* **15** 2129–2132. (<https://doi.org/10.1093/humrep/15.10.2129>)
- Cernea M, Padmanabhan V, Goodman RL, Coolen LM & Lehman MN 2015 Prenatal testosterone treatment leads to changes in the morphology of KNDy neurons, their inputs, and projections to GnRH cells in female sheep. *Endocrinology* **156** 3277–3291. (<https://doi.org/10.1210/en.2014-1609>)
- Cheng XB, Jimenez M, Desai R, Middleton LJ, Joseph SR, Ning G, Allan CM, Smith JT, Handelsman DJ & Walters KA 2013 Characterizing the neuroendocrine and ovarian defects of androgen receptor-knockout female mice. *American Journal of Physiology: Endocrinology and Metabolism* **305** E717–E726. (<https://doi.org/10.1152/ajpendo.00263.2013>)
- Chern CU, Tsui KH, Vitale SG, Chen SN, Wang PH, Cianci A, Tsai HW, Wen ZH & Lin LT 2018 Dehydroepiandrosterone (DHEA) supplementation improves in vitro fertilization outcomes of poor ovarian responders, especially in women with low serum concentration of DHEA-S: a retrospective cohort study. *Reproductive Biology and Endocrinology* **16** 90. (<https://doi.org/10.1186/s12958-018-0409-z>)
- Conway G, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Franks S, Gambineri A, Kelestimir F, Macut D, Micic D, Pasquali R, *et al.* 2014 The polycystic ovary syndrome: a position statement from the European Society of Endocrinology. *European Journal of Endocrinology* **171** P1–29. (<https://doi.org/10.1530/EJE-14-0253>)
- Couto Alves A, Valcarcel B, Mäkinen VP, Morin-Papunen L, Sebert S, Kangas AJ, Soininen P, Das S, De Iorio M, Coin L, *et al.* 2017 Metabolic profiling of polycystic ovary syndrome reveals interactions with abdominal obesity. *International Journal of Obesity* **41** 1331–1340. (<https://doi.org/10.1038/ijo.2017.126>)
- Daan NM, Jaspers L, Koster MP, Broekmans FJ, De Rijke YB, Franco OH, Laven JS, Kavousi M & Fauser BC 2015 Androgen levels in women with various forms of ovarian dysfunction: associations with cardiometabolic features. *Human Reproduction* **30** 2376–2386. (<https://doi.org/10.1093/humrep/dev195>)
- Daghestani MH 2018 Evaluation of biochemical, endocrine, and metabolic biomarkers for the early diagnosis of polycystic ovary syndrome among non-obese Saudi women. *International Journal of Gynaecology and Obstetrics* **142** 162–169. (<https://doi.org/10.1002/ijgo.12527>)
- Davison SL & Davis SR 2003 Androgens in women. *Journal of Steroid Biochemistry and Molecular Biology* **85** 363–366. ([https://doi.org/10.1016/S0960-0760\(03\)00204-8](https://doi.org/10.1016/S0960-0760(03)00204-8))
- Davison SL, Bell R, Donath S, Montalto JG & Davis SR 2005 Androgen levels in adult females: changes with age, menopause, and oophorectomy. *Journal of Clinical Endocrinology and Metabolism* **90** 3847–3853. (<https://doi.org/10.1210/jc.2005-0212>)
- De Leo V, Lanzetta D, D'Antona D, La Marca A & Morgante G 1998 Hormonal effects of flutamide in young women with polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism* **83** 99–102. (<https://doi.org/10.1210/jcem.83.1.4500>)

- De Roo C, Lierman S, Tillemans K, Peynshaert K, Braeckmans K, Caenen M, Lambalk CB, Weyers S, T'Sjoen G, Cornelissen R, *et al.* 2017 Ovarian tissue cryopreservation in female-to-male transgender people: insights into ovarian histology and physiology after prolonged androgen treatment. *Reproductive Biomedicine Online* **34** 557–566. (<https://doi.org/10.1016/j.rbmo.2017.03.008>)
- Diamanti-Kandaraki E, Mitrakou A, Raptis S, Tolis G & Duleba AJ 1998 The effect of a pure antiandrogen receptor blocker, flutamide, on the lipid profile in the polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism* **83** 2699–2705. (<https://doi.org/10.1210/jcem.83.8.5041>)
- Dickerson EH, Cho LW, Maguiness SD, Killick SL, Robinson J & Atkin SL 2010 Insulin resistance and free androgen index correlate with the outcome of controlled ovarian hyperstimulation in non-PCOS women undergoing IVF. *Human Reproduction* **25** 504–509. (<https://doi.org/10.1093/humrep/dep393>)
- Diri H, Karaburgu S, Acmaz B, Unluhizarci K, Tanriverdi F, Karaca Z & Kelestimur F 2016 Comparison of spironolactone and spironolactone plus metformin in the treatment of polycystic ovary syndrome. *Gynecological Endocrinology* **32** 42–45. (<https://doi.org/10.3109/09513590.2015.1080679>)
- Doan HT, Quan LH & Nguyen TT 2017 The effectiveness of transdermal testosterone gel 1% (AndroGel) for poor responders undergoing in vitro fertilization. *Gynecological Endocrinology* **33** 977–979. (<https://doi.org/10.1080/09513590.2017.1332586>)
- Dumesic DA, Oberfield SE, Stener-Victorin E, Marshall JC, Laven JS & Legro RS 2015 Scientific statement on the diagnostic criteria, epidemiology, pathophysiology, and molecular genetics of polycystic ovary syndrome. *Endocrine Reviews* **36** 487–525. (<https://doi.org/10.1210/er.2015-1018>)
- Dumesic DA, Akopians AL, Madrigal VK, Ramirez E, Margolis DJ, Sarma MK, Thomas AM, Grogan TR, Haykal R, Schooler TA, *et al.* 2016 Hyperandrogenism accompanies increased intra-abdominal fat storage in normal weight polycystic ovary syndrome women. *Journal of Clinical Endocrinology and Metabolism* **101** 4178–4188. (<https://doi.org/10.1210/jc.2016-2586>)
- Eagleson CA, Gingrich MB, Pastor CL, Arora TK, Burt CM, Evans WS & Marshall JC 2000 Polycystic ovarian syndrome: evidence that flutamide restores sensitivity of the gonadotropin-releasing hormone pulse generator to inhibition by estradiol and progesterone. *Journal of Clinical Endocrinology and Metabolism* **85** 4047–4052. (<https://doi.org/10.1210/jcem.85.11.6992>)
- Ebrahimi M, Akbari-Asbagh F & Ghalandar-Attar M 2017 Letrozole+ GnRH antagonist stimulation protocol in poor ovarian responders undergoing intracytoplasmic sperm injection cycles: an RCT. *International Journal of Reproductive BioMedicine* **15** 101–108. (<https://doi.org/10.29252/ijrm.15.2.101>)
- Ergenoglu M, Yildirim N, Yildirim AG, Yeniel O, Erbas O, Yavasoglu A, Taskiran D & Karadadas N 2015 Effects of resveratrol on ovarian morphology, plasma anti-Müllerian hormone, IGF-1 levels, and oxidative stress parameters in a rat model of polycystic ovary syndrome. *Reproductive Sciences* **22** 942–947. (<https://doi.org/10.1177/1933719115570900>)
- Escobar-Morreale HF 2018 Polycystic ovary syndrome: definition, aetiology, diagnosis and treatment. *Nature Reviews: Endocrinology* **14** 270–284. (<https://doi.org/10.1038/nrendo.2018.24>)
- Fabregues F, Penarrubia J, Creus M, Manau D, Casals G, Carmona F & Balasch J 2009 Transdermal testosterone may improve ovarian response to gonadotrophins in low-responder IVF patients: a randomized, clinical trial. *Human Reproduction* **24** 349–359. (<https://doi.org/10.1093/humrep/den428>)
- Ferraretti AP, Gianaroli L, Magli MC, D'Angelo A, Farfalli V & Montanaro N 2004 Exogenous luteinizing hormone in controlled ovarian hyperstimulation for assisted reproduction techniques. *Fertility and Sterility* **82** 1521–1526. (<https://doi.org/10.1016/j.fertnstert.2004.06.041>)
- Ferraretti AP, La Marca A, Fauser BC, Tarlatzis B, Nargund G, Gianaroli L & ESHRE working group on Poor Ovarian Response Definition 2011 ESHRE consensus on the definition of 'poor response' to ovarian stimulation for in vitro fertilization: the Bologna criteria. *Human Reproduction* **26** 1616–1624. (<https://doi.org/10.1093/humrep/der092>)
- Ferraretti AP, Gianaroli L, Motrenko T, Feliciani E, Tabanelli C & Magli MC 2014 LH pretreatment as a novel strategy for poor responders. *BioMed Research International* **2014** 926172. (<https://doi.org/10.1155/2014/926172>)
- Fraser GL, Ramael S, Hoveyda HR, Gheyle L & Combalbert J 2016 The NK3 receptor antagonist ESN364 suppresses sex hormones in men and women. *Journal of Clinical Endocrinology and Metabolism* **101** 417–426. (<https://doi.org/10.1210/jc.2015-3621>)
- Frattarelli JL & Peterson EH 2004 Effect of androgen levels on in vitro fertilization cycles. *Fertility and Sterility* **81** 1713–1714. (<https://doi.org/10.1016/j.fertnstert.2003.11.032>)
- Futterweit W & Deligdisch L 1986 Histopathological effects of exogenously administered testosterone in 19 female to male transsexuals. *Journal of Clinical Endocrinology and Metabolism* **62** 16–21. (<https://doi.org/10.1210/jcem-62-1-16>)
- Gambineri A, Patton L, Vaccina A, Cacciari M, Morselli-Labate AM, Cavazza C, Pagotto U & Pasquali R 2006 Treatment with flutamide, metformin, and their combination added to a hypocaloric diet in overweight-obese women with polycystic ovary syndrome: a randomized, 12-month, placebo-controlled study. *Journal of Clinical Endocrinology and Metabolism* **91** 3970–3980. (<https://doi.org/10.1210/jc.2005-2250>)
- Garcia-Velasco JA, Moreno L, Pacheco A, Guillen A, Duque L, Requena A & Pellicer A 2005 The aromatase inhibitor letrozole increases the concentration of intraovarian androgens and improves in vitro fertilization outcome in low responder patients: a pilot study. *Fertility and Sterility* **84** 82–87. (<https://doi.org/10.1016/j.fertnstert.2005.01.117>)
- George JT, Kakkar R, Marshall J, Scott ML, Finkelman RD, Ho TW, Veldhuis J, Skorupskaitė K, Anderson RA, McIntosh S, *et al.* 2016 Neurokinin B receptor antagonism in women with polycystic ovary syndrome: a randomized, placebo-controlled trial. *Journal of Clinical Endocrinology and Metabolism* **101** 4313–4321. (<https://doi.org/10.1210/jc.2016-1202>)
- Gill A, Jamnongjit M & Hammes SR 2004 Androgens promote maturation and signaling in mouse oocytes independent of transcription: a release of inhibition model for mammalian oocyte meiosis. *Molecular Endocrinology* **18** 97–104. (<https://doi.org/10.1210/me.2003-0326>)
- Gleicher N & Barad DH 2011 Dehydroepiandrosterone (DHEA) supplementation in diminished ovarian reserve (DOR). *Reproductive Biology and Endocrinology* **9** 67. (<https://doi.org/10.1186/1477-7827-9-67>)
- Gonzalez-Comadran M, Duran M, Sola I, Fabregues F, Carreras R & Checa MA 2012 Effects of transdermal testosterone in poor responders undergoing IVF: systematic review and meta-analysis. *Reproductive Biomedicine Online* **25** 450–459. (<https://doi.org/10.1016/j.rbmo.2012.07.011>)
- Goswami SK, Das T, Chattopadhyay R, Sawhney V, Kumar J, Chaudhury K, Chakravarty BN & Kabir SN 2004 A randomized single-blind controlled trial of letrozole as a low-cost IVF protocol in women with poor ovarian response: a preliminary report. *Human Reproduction* **19** 2031–2035. (<https://doi.org/10.1093/humrep/deh359>)
- Gougeon A 1996 Regulation of ovarian follicular development in primates: facts and hypotheses. *Endocrine Reviews* **17** 121–155. (<https://doi.org/10.1210/edrv-17-2-121>)
- Goverde AJ, McDonnell J, Schats R, Vermeiden JP, Homburg R & Lambalk CB 2005 Ovarian response to standard gonadotrophin stimulation for IVF is decreased not only in older but also in younger women in couples with idiopathic and male subfertility. *Human Reproduction* **20** 1573–1577. (<https://doi.org/10.1093/humrep/deh827>)

- Hague WM, Adams J, Rodda C, Brook CGD, De Bruyn R, Grant DB & Jacobs HS 1990 The prevalence of polycystic ovaries in patients WITH congenital adrenal hyperplasia and their close relatives. *Clinical Endocrinology* **33** 501–510. (<https://doi.org/10.1111/j.1365-2265.1990.tb03887.x>)
- Hampton JH, Manikkam M, Lubahn DB, Smith MF & Garverick HA 2004 Androgen receptor mRNA expression in the bovine ovary. *Domestic Animal Endocrinology* **27** 81–88. (<https://doi.org/10.1016/j.domaniend.2004.01.005>)
- Harlow CR, Hillier SG & Hodges JK 1986 Androgen modulation of follicle-stimulating hormone-induced granulosa cell steroidogenesis in the primate ovary. *Endocrinology* **119** 1403–1405. (<https://doi.org/10.1210/endo-119-3-1403>)
- Haydardedeoglu B, Isik AZ & Bulgan Kilicdag E 2015 The combination of dehydroepiandrosterone, transdermal testosterone, and growth hormone as an adjuvant therapy in assisted reproductive technology cycles in patients aged below 40 years with diminished ovarian reserve. *Turkish Journal of Obstetrics and Gynecology* **12** 60–65. (<https://doi.org/10.4274/tjod.32656>)
- Hild-Petito S, West NB, Brenner RM & Stouffer RL 1991 Localization of androgen receptor in the follicle and corpus luteum of the primate ovary during the menstrual cycle. *Biology of Reproduction* **44** 561–568. (<https://doi.org/10.1095/biolreprod44.3.561>)
- Hillier SG & De Zwart FA 1981 Evidence that granulosa cell aromatase induction/activation by follicle-stimulating hormone is an androgen receptor-regulated process in-vitro. *Endocrinology* **109** 1303–1305. (<https://doi.org/10.1210/endo-109-4-1303>)
- Hillier SG, Whitelaw PF & Smyth CD 1994 Follicular oestrogen synthesis: the ‘two-cell, two-gonadotrophin’ model revisited. *Molecular and Cellular Endocrinology* **100** 51–54. ([https://doi.org/10.1016/0303-7207\(94\)90278-X](https://doi.org/10.1016/0303-7207(94)90278-X))
- Homan GF, Davies M & Norman R 2007 The impact of lifestyle factors on reproductive performance in the general population and those undergoing infertility treatment: a review. *Human Reproduction Update* **13** 209–223. (<https://doi.org/10.1093/humupd/dml056>)
- Horie K, Takakura K, Fujiwara H, Suginami H, Liao S & Mori T 1992 Immunohistochemical localization of androgen receptor in the human ovary throughout the menstrual cycle in relation to oestrogen and progesterone receptor expression. *Human Reproduction* **7** 184–190. (<https://doi.org/10.1093/oxfordjournals.humrep.a137614>)
- Hu YC, Wang PH, Yeh S, Wang RS, Xie C, Xu Q, Zhou X, Chao HT, Tsai MY & Chang C 2004 Subfertility and defective folliculogenesis in female mice lacking androgen receptor. *PNAS* **101** 11209–11214. (<https://doi.org/10.1073/pnas.0404372101>)
- Hu Q, Hong L, Nie M, Wang Q, Fang Y, Dai Y, Zhai Y, Wang S, Yin C & Yang X 2017 The effect of dehydroepiandrosterone supplementation on ovarian response is associated with androgen receptor in diminished ovarian reserve women. *Journal of Ovarian Research* **10** 32–32. (<https://doi.org/10.1186/s13048-017-0326-3>)
- Huang X & Harlan RE 1993 Absence of androgen receptors in LHRH immunoreactive neurons. *Brain Research* **624** 309–311. ([https://doi.org/10.1016/0006-8993\(93\)90094-4](https://doi.org/10.1016/0006-8993(93)90094-4))
- Humaidan P, Bungum L, Bungum M & Andersen CY 2002 Ovarian response and pregnancy outcome related to mid-follicular LH levels in women undergoing assisted reproduction with GnRH agonist down-regulation and recombinant FSH stimulation. *Human Reproduction* **17** 2016–2021. (<https://doi.org/10.1093/humrep/17.8.2016>)
- Jeve YB & Bhandari HM 2016 Effective treatment protocol for poor ovarian response: a systematic review and meta-analysis. *Journal of Human Reproductive Sciences* **9** 70–81. (<https://doi.org/10.4103/0974-1208.183515>)
- Ji J, Ju X, Wang J, Wu H & Fan H 2016 Effectiveness of dehydroepiandrosterone in poor ovarian responders undergoing in vitro fertilization: a meta-analysis of randomized controlled trials. *International Journal of Clinical and Experimental Medicine* **9** 20835–20845.
- Jirge PR, Chougule SM, Gavali VG & Bhomkar DA 2014 Impact of dehydroepiandrosterone on clinical outcome in poor responders: a pilot study in women undergoing in vitro fertilization, using Bologna criteria. *Journal of Human Reproductive Sciences* **7** 175–180. (<https://doi.org/10.4103/0974-1208.142477>)
- Jovanovic VP, Kort DH, Guarnaccia MM, Sauer MV & Lobo RA 2011 Does the addition of clomiphene citrate or letrozole to gonadotropin treatment enhance the oocyte yield in poor responders undergoing IVF? *Journal of Assisted Reproduction and Genetics* **28** 1067–1072. (<https://doi.org/10.1007/s10815-011-9643-4>)
- Juengel JL, Heath DA, Quirke LD & McNatty KP 2006 Oestrogen receptor alpha and beta, androgen receptor and progesterone receptor mRNA and protein localisation within the developing ovary and in small growing follicles of sheep. *Reproduction* **131** 81–92. (<https://doi.org/10.1530/rep.1.00704>)
- Kamath MS, Maheshwari A, Bhattacharya S, Lor KY & Gibreel A 2017 Oral medications including clomiphene citrate or aromatase inhibitors with gonadotropins for controlled ovarian stimulation in women undergoing in vitro fertilisation. *Cochrane Database of Systematic Reviews* **11** CD008528. (<https://doi.org/10.1002/14651858.CD008528.pub3>)
- Kara M, Aydin T, Aran T, Turktekin N & Ozdemir B 2014 Does dehydroepiandrosterone supplementation really affect IVF-ICSI outcome in women with poor ovarian reserve? *European Journal of Obstetrics, Gynecology, and Reproductive Biology* **173** 63–65. (<https://doi.org/10.1016/j.ejogrb.2013.11.008>)
- Katulski K, Podfigurna A, Czyzyk A, Meczekalski B & Genazzani AD 2018 Kisspeptin and LH pulsatile temporal coupling in PCOS patients. *Endocrine* **61** 149–157. (<https://doi.org/10.1007/s12020-018-1609-1>)
- Kauffman AS, Thackray VG, Ryan GE, Tolson KP, Glidewell-Kenney CA, Semaan SJ, Poling MC, Iwata N, Breen KM, Duleba AJ, *et al.* 2015 A novel letrozole model recapitulates both the reproductive and metabolic phenotypes of polycystic ovary syndrome in female mice. *Biology of Reproduction* **93** 69. (<https://doi.org/10.1095/biolreprod.115.131631>)
- Keefe CC, Goldman MM, Zhang K, Clarke N, Reitz RE & Welt CK 2014 Simultaneous measurement of thirteen steroid hormones in women with polycystic ovary syndrome and control women using liquid chromatography-tandem mass spectrometry. *PLoS ONE* **9** e93805. (<https://doi.org/10.1371/journal.pone.0093805>)
- Keller E, Chazenbalk GD, Aguilera P, Madrigal V, Grogan T, Elashoff D, Dumesic DA & Abbott DH 2014 Impaired preadipocyte differentiation into adipocytes in subcutaneous abdominal adipose of PCOS-like female rhesus monkeys. *Endocrinology* **155** 2696–2703. (<https://doi.org/10.1210/en.2014-1050>)
- Kim CH, Howles CM & Lee HA 2011 The effect of transdermal testosterone gel pretreatment on controlled ovarian stimulation and IVF outcome in low responders. *Fertility and Sterility* **95** 679–683. (<https://doi.org/10.1016/j.fertnstert.2010.07.1077>)
- Kim CH, Ahn JW, Moon JW, Kim SH, Chae HD & Kang BM 2014 Ovarian features after 2 weeks, 3 weeks and 4 weeks transdermal testosterone gel treatment and their associated effect on IVF outcomes in poor responders. *Development and Reproduction* **18** 145–152. (<https://doi.org/10.12717/DR.2014.18.3.145>)
- Kotb MM, Hassan AM & Awadallah AM 2016 Does dehydroepiandrosterone improve pregnancy rate in women undergoing IVF/ICSI with expected poor ovarian response according to the Bologna criteria? A randomized controlled trial. *European Journal of Obstetrics, Gynecology, and Reproductive Biology* **200** 11–15. (<https://doi.org/10.1016/j.ejogrb.2016.02.009>)
- Kuhn R & Torres RM 2002 Cre/loxP recombination system and gene targeting. *Methods in Molecular Biology* **180** 175–204. (<https://doi.org/10.1385/1-59259-178-7:175>)

- Kumari GL, Datta JK & Roy S 1978 Evidence for a role of androgens in the growth and maturation of ovarian follicles in rats. *Hormone Research* **9** 112–120. (<https://doi.org/10.1159/000178903>)
- Laird M, Thomson K, Fenwick M, Mora J, Franks S & Hardy K 2017 Androgen stimulates growth of mouse preantral follicles in vitro: interaction with follicle-stimulating hormone and with growth factors of the TGFbeta superfamily. *Endocrinology* **158** 920–935. (<https://doi.org/10.1210/en.2016-1538>)
- Lakryc EM, Motta EL, Soares JM, Haidar MA, de Lima GR & Baracat EC 2003 The benefits of finasteride for hirsute women with polycystic ovary syndrome or idiopathic hirsutism. *Gynecological Endocrinology* **17** 57–63. (<https://doi.org/10.1080/713603187>)
- Lebbe M, Taylor AE, Visser JA, Kirkman-Brown JC, Woodruff TK & Arlt W 2017 The steroid metabolome in the isolated ovarian follicle and its response to androgen exposure and antagonism. *Endocrinology* **158** 1474–1485. (<https://doi.org/10.1210/en.2016-1851>)
- Lee KH, Kim CH, Suk HJ, Lee YJ, Kwon SK, Kim SH, Chae HD & Kang BM 2014 The effect of aromatase inhibitor letrozole incorporated in gonadotrophin-releasing hormone antagonist multiple dose protocol in poor responders undergoing in vitro fertilization. *Obstetrics and Gynecology Science* **57** 216–222. (<https://doi.org/10.5468/ogs.2014.57.3.216>)
- Lee BH, Indran IR, Tan HM, Li Y, Zhang Z, Li J & Yong EL 2016 A dietary medium-chain fatty acid, decanoic acid, inhibits recruitment of Nur77 to the HSD3B2 promoter in vitro and reverses endocrine and metabolic abnormalities in a rat model of polycystic ovary syndrome. *Endocrinology* **157** 382–394. (<https://doi.org/10.1210/en.2015-1733>)
- Legro RS, Arslanian SA, Ehrmann DA, Hoeger KM, Murad MH, Pasquali R, Welt CK & Endocrine Society 2013 Diagnosis and treatment of polycystic ovary syndrome: an Endocrine Society clinical practice guideline. *Journal of Clinical Endocrinology and Metabolism* **98** 4565–4592. (<https://doi.org/10.1210/jc.2013-2350>)
- Lenie S & Smits J 2009 Functional AR signaling is evident in an in vitro mouse follicle culture bioassay that encompasses most stages of folliculogenesis. *Biology of Reproduction* **80** 685–695. (<https://doi.org/10.1095/biolreprod.107.067280>)
- Li M, Ai JS, Xu BZ, Xiong B, Yin S, Lin SL, Hou Y, Chen DY, Schatten H & Sun QY 2008 Testosterone potentially triggers meiotic resumption by activation of intra-oocyte SRC and MAPK in porcine oocytes. *Biology of Reproduction* **79** 897–905. (<https://doi.org/10.1095/biolreprod.108.069245>)
- Li H, Xu X, Wang X, Liao X, Li L, Yang G & Gao L 2016 Free androgen index and Irisin in polycystic ovary syndrome. *Journal of Endocrinological Investigation* **39** 549–556. (<https://doi.org/10.1007/s40618-015-0403-7>)
- Li CJ, Chen SN, Lin LT, Chern CU, Wang PH, Wen ZH & Tsui KH 2018 Dehydroepiandrosterone ameliorates abnormal mitochondrial dynamics and mitophagy of cumulus cells in poor ovarian responders. *Journal of Clinical Medicine* **7** E293. (<https://doi.org/10.3390/jcm7100293>)
- Lin LT, Wang PH, Wen ZH, Li CJ, Chen SN, Tsai EM, Cheng JT & Tsui KH 2017 The application of dehydroepiandrosterone on improving mitochondrial function and reducing apoptosis of cumulus cells in poor ovarian responders. *International Journal of Medical Sciences* **14** 585–594. (<https://doi.org/10.7150/ijms.18706>)
- Liu D, Iruthayanathan M, Homan LL, Wang Y, Yang L, Wang Y & Dillon JS 2008 Dehydroepiandrosterone stimulates endothelial proliferation and angiogenesis through extracellular signal-regulated kinase 1/2-mediated mechanisms. *Endocrinology* **149** 889–898. (<https://doi.org/10.1210/en.2007-1125>)
- Liu Y, Hu L, Fan L & Wang F 2018 Efficacy of dehydroepiandrosterone (DHEA) supplementation for in vitro fertilization and embryo transfer cycles: a systematic review and meta-analysis. *Gynecological Endocrinology* **34** 178–183. (<https://doi.org/10.1080/09513590.2017.1391202>)
- Livadas S, Pappas C, Karachalios A, Marinakis E, Tolia N, Drakou M, Kaldrymides P, Panidis D & Diamanti-Kandarakis E 2014 Prevalence and impact of hyperandrogenemia in 1,218 women with polycystic ovary syndrome. *Endocrine* **47** 631–638. (<https://doi.org/10.1007/s12020-014-0200-7>)
- Lu Q, Shen H, Li Y, Zhang C, Wang C, Chen X, Liang R & Wei L 2014 Low testosterone levels in women with diminished ovarian reserve impair embryo implantation rate: a retrospective case-control study. *Journal of Assisted Reproduction and Genetics* **31** 485–491. (<https://doi.org/10.1007/s10815-014-0186-3>)
- Lucis OJ, Hobkirk R, Hollenberg CH, Macdonald SA & Blahey P 1966 Polycystic ovaries associated with congenital adrenal hyperplasia. *Canadian Medical Association Journal* **94** 1–7.
- Luo S, Li S, Li X, Qin L & Jin S 2014 Effect of pretreatment with transdermal testosterone on poor ovarian responders undergoing IVF/ICSI: a meta-analysis. *Experimental and Therapeutic Medicine* **8** 187–194. (<https://doi.org/10.3892/etm.2014.1683>)
- Ma Y, Andrisse S, Chen Y, Childress S, Xue P, Wang Z, Jones D, Ko C, Divall S & Wu S 2017 Androgen receptor in the ovary theca cells plays a critical role in androgen-induced reproductive dysfunction. *Endocrinology* **158** 98–108. (<https://doi.org/10.1210/en.2016-1608>)
- Manneras-Holm L, Leonhardt H, Kullberg J, Jennische E, Oden A, Holm G, Hellstrom M, Lonn L, Olivecrona G, Stener-Victorin E, *et al.* 2011 Adipose tissue has aberrant morphology and function in PCOS: enlarged adipocytes and low serum adiponectin, but not circulating sex steroids, are strongly associated with insulin resistance. *Journal of Clinical Endocrinology and Metabolism* **96** E304–E311. (<https://doi.org/10.1210/jc.2010-1290>)
- March WA, Moore VM, Willson KJ, Phillips DI, Norman RJ & Davies MJ 2010 The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. *Human Reproduction* **25** 544–551. (<https://doi.org/10.1093/humrep/dep399>)
- Marti N, Bouchoucha N, Sauter KS & Fluck CE 2017 Resveratrol inhibits androgen production of human adrenocortical H295R cells by lowering CYP17 and CYP21 expression and activities. *PLoS ONE* **12** e0174224. (<https://doi.org/10.1371/journal.pone.0174224>)
- Massin N, Cedrin-Durnerin I, Coussieu C, Galey-Fontaine J, Wolf JP & Hugues JN 2006 Effects of transdermal testosterone application on the ovarian response to FSH in poor responders undergoing assisted reproduction technique – a prospective, randomized, double-blind study. *Human Reproduction* **21** 1204–1211. (<https://doi.org/10.1093/humrep/dei481>)
- McEwan IJ, McGuinness D, Hay CW, Millar RP, Saunders PT & Fraser HM 2010 Identification of androgen receptor phosphorylation in the primate ovary in vivo. *Reproduction* **140** 93–104. (<https://doi.org/10.1530/REP-10-0140>)
- Mendoza N, Simoncini T & Genazzani AD 2014 Hormonal contraceptive choice for women with PCOS: a systematic review of randomized trials and observational studies. *Gynecological Endocrinology* **30** 850–860. (<https://doi.org/10.3109/09513590.2014.943725>)
- Mitri F, Behan LA, Murphy CA, Hershko-Klement A, Casper RF & Bentov Y 2016 Microdose flare protocol with interrupted follicle stimulating hormone and added androgen for poor responders – an observational pilot study. *Fertility and Sterility* **105** 100.e1–105.e1. (<https://doi.org/10.1016/j.fertnstert.2015.09.038>)
- Mitwally MF & Casper RF 2002 Aromatase inhibition improves ovarian response to follicle-stimulating hormone in poor responders. *Fertility and Sterility* **77** 776–780. ([https://doi.org/10.1016/S0015-0282\(01\)03280-0](https://doi.org/10.1016/S0015-0282(01)03280-0))
- Moawad A & Shaeer M 2012 Long-term androgen priming by use of dehydroepiandrosterone (DHEA) improves IVF outcome in poor-responder patients. A randomized controlled study. *Middle East Fertility Society Journal* **17** 268–274. (<https://doi.org/10.1016/j.mefs.2012.11.002>)
- Moggetti P, Tosi F, Tosti A, Negri C, Misciali C, Perrone F, Caputo M, Muggeo M & Castello R 2000 Comparison of spironolactone,

- flutamide, and finasteride efficacy in the treatment of hirsutism: a randomized, double blind, placebo-controlled trial. *Journal of Clinical Endocrinology and Metabolism* **85** 89–94. (<https://doi.org/10.1210/jcem.85.1.6245>)
- Mohsen IA & El Din RE 2013 Minimal stimulation protocol using letrozole versus microdose flare up GnRH agonist protocol in women with poor ovarian response undergoing ICSI. *Gynecological Endocrinology* **29** 105–108. (<https://doi.org/10.3109/09513590.2012.730569>)
- Moore AM, Prescott M & Campbell RE 2013 Estradiol negative and positive feedback in a prenatal androgen-induced mouse model of polycystic ovarian syndrome. *Endocrinology* **154** 796–806. (<https://doi.org/10.1210/en.2012-1954>)
- Moore AM, Prescott M, Marshall CJ, Yip SH & Campbell RE 2015 Enhancement of a robust arcuate GABAergic input to gonadotropin-releasing hormone neurons in a model of polycystic ovarian syndrome. *PNAS* **112** 596–601. (<https://doi.org/10.1073/pnas.1415038112>)
- Moran LJ, Norman RJ & Teede HJ 2015 Metabolic risk in PCOS: phenotype and adiposity impact. *Trends in Endocrinology and Metabolism* **26** 136–143. (<https://doi.org/10.1016/j.tem.2014.12.003>)
- Mumme K & Stonehouse W 2015 Effects of medium-chain triglycerides on weight loss and body composition: a meta-analysis of randomized controlled trials. *Journal of the Academy of Nutrition and Dietetics* **115** 249–263. (<https://doi.org/10.1016/j.jand.2014.10.022>)
- Murray AA, Gosden RG, Allison V & Spears N 1998 Effect of androgens on the development of mouse follicles growing in vitro. *Journal of Reproduction and Fertility* **113** 27–33. (<https://doi.org/10.1530/jrf.0.1130027>)
- Nagels HE, Rishworth JR, Siristatidis CS & Kroon B 2015 Androgens (dehydroepiandrosterone or testosterone) for women undergoing assisted reproduction. *Cochrane Database of Systematic Reviews* CD009749. (<https://doi.org/10.1002/14651858.CD009749.pub2>)
- Nakhjavani M, Hamidi S, Esteghamati A, Abbasi M, Nosrati-Jahromi S & Pasalar P 2009 Short term effects of spironolactone on blood lipid profile: a 3-month study on a cohort of young women with hirsutism. *British Journal of Clinical Pharmacology* **68** 634–637. (<https://doi.org/10.1111/j.1365-2125.2009.03483.x>)
- Narkwichan A, Jayaprakasan K, Maalouf WE, Hernandez-Medrano JH, Pincott-Allen C & Campbell BK 2014 Effects of dehydroepiandrosterone on in vivo ovine follicular development. *Human Reproduction* **29** 146–154. (<https://doi.org/10.1093/humrep/det408>)
- Narkwichan A, Maalouf W, Baumgarten M, Polanski L, Raine-Fenning N, Campbell B & Jayaprakasan K 2017 Efficacy of dehydroepiandrosterone (DHEA) to overcome the effect of ovarian ageing (DITTO): a proof of principle double blinded randomized placebo controlled trial. *European Journal of Obstetrics, Gynecology, and Reproductive Biology* **218** 39–48. (<https://doi.org/10.1016/j.ejogrb.2017.09.006>)
- Navarro VM, Gottsch ML, Chavkin C, Okamura H, Clifton DK & Steiner RA 2009 Regulation of gonadotropin-releasing hormone secretion by kisspeptin/dynorphin/neurokinin B neurons in the arcuate nucleus of the mouse. *Journal of Neuroscience* **29** 11859–11866. (<https://doi.org/10.1523/JNEUROSCI.1569-09.2009>)
- Nelson VL, Legro RS, Strauss JF III & McAllister JM 1999 Augmented androgen production is a stable steroidogenic phenotype of propagated theca cells from polycystic ovaries. *Molecular Endocrinology* **13** 946–957. (<https://doi.org/10.1210/mend.13.6.0311>)
- Nielsen ME, Rasmussen IA, Kristensen SG, Christensen ST, Mollgard K, Wreford Andersen E, Byskov AG & Yding Andersen C 2011 In human granulosa cells from small antral follicles, androgen receptor mRNA and androgen levels in follicular fluid correlate with FSH receptor mRNA. *Molecular Human Reproduction* **17** 63–70. (<https://doi.org/10.1093/molehr/gaq073>)
- Nohara K, Laque A, Allard C, Munzberg H & Mauvais-Jarvis F 2014 Central mechanisms of adiposity in adult female mice with androgen excess. *Obesity* **22** 1477–1484. (<https://doi.org/10.1002/oby.20719>)
- Noventa M, Vitagliano A, Andrisani A, Blagancje M, Vigano P, Papaio E, Scioscia M, Cavallin F, Ambrosini G & Cozzolino M 2019 Testosterone therapy for women with poor ovarian response undergoing IVF: a meta-analysis of randomized controlled trials. *Journal of Assisted Reproduction and Genetics* **36** 673–683. (<https://doi.org/10.1007/s10815-018-1383-2>)
- O'Reilly MW, Kempegowda P, Jenkinson C, Taylor AE, Quanson JL, Storbeck KH & Arlt W 2017 11-Oxygenated C19 steroids are the predominant androgens in polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism* **102** 840–848. (<https://doi.org/10.1210/jc.2016-3285>)
- Osuka S, Iwase A, Nakahara T, Kondo M, Saito A, Bayasula, Nakamura T, Takikawa S, Goto M, Kotani T & Kikkawa F 2017 Kisspeptin in the hypothalamus of 2 rat models of polycystic ovary syndrome. *Endocrinology* **158** 367–377. (<https://doi.org/10.1210/en.2016-1333>)
- Ozmen B, Sonmezer M, Atabekoglu CS & Olmus H 2009 Use of aromatase inhibitors in poor-responder patients receiving GnRH antagonist protocols. *Reproductive Biomedicine Online* **19** 478–485. (<https://doi.org/10.1016/j.rbmo.2009.05.007>)
- Padmanabhan V & Veiga-Lopez A 2013 Sheep models of polycystic ovary syndrome phenotype. *Molecular and Cellular Endocrinology* **373** 8–20. (<https://doi.org/10.1016/j.mce.2012.10.005>)
- Padmanabhan V, Veiga-Lopez A, Herkimer C, Abi Salloum B, Moeller J, Beckett E & Sreedharan R 2015 Developmental programming: prenatal and postnatal androgen antagonist and insulin sensitizer interventions prevent advancement of puberty and improve LH surge dynamics in prenatal testosterone-treated sheep. *Endocrinology* **156** 2678–2692. (<https://doi.org/10.1210/en.2015-1235>)
- Palomba S, Falbo A, Chiossi G, Muscogiuri G, Fornaciari E, Orio F, Tolino A, Colao A, La Sala GB & Zullo F 2014 Lipid profile in nonobese pregnant women with polycystic ovary syndrome: a prospective controlled clinical study. *Steroids* **88** 36–43. (<https://doi.org/10.1016/j.steroids.2014.06.005>)
- Paradisi R, Fabbri R, Battaglia C & Venturoli S 2013 Ovulatory effects of flutamide in the polycystic ovary syndrome. *Gynecological Endocrinology* **29** 391–395. (<https://doi.org/10.3109/09513590.2012.754876>)
- Polyzos NP & Devroey P 2011 A systematic review of randomized trials for the treatment of poor ovarian responders: is there any light at the end of the tunnel? *Fertility and Sterility* **96** 1058–61.e7. (<https://doi.org/10.1016/j.fertnstert.2011.09.048>)
- Polyzos NP, Davis SR, Drakopoulos P, Humaidan P, De Geyter C, Vega AG, Martinez F, Evangelou E, Van De Vijver A, Smits J, *et al.* 2018 Testosterone for poor ovarian responders: lessons from ovarian physiology. *Reproductive Sciences* **25** 980–982. (<https://doi.org/10.1177/1933719116660849>)
- Quigley CA, De Bellis A, Marschke KB, El-Awady MK, Wilson EM & French FS 1995 Androgen receptor defects: historical, clinical, and molecular perspectives. *Endocrine Reviews* **16** 271–321. (<https://doi.org/10.1210/edrv-16-3-271>)
- Roland AV, Nunemaker CS, Keller SR & Moenter SM 2010 Prenatal androgen exposure programs metabolic dysfunction in female mice. *Journal of Endocrinology* **207** 213–223. (<https://doi.org/10.1677/JOE-10-0217>)
- Romero S & Smits J 2010 Exposing cultured mouse ovarian follicles under increased gonadotropin tonus to aromatizable androgens influences the steroid balance and reduces oocyte meiotic capacity. *Endocrine* **38** 243–253. (<https://doi.org/10.1007/s12020-010-9380-y>)
- Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2004 Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Human Reproduction* **19** 41–47. (<https://doi.org/10.1093/humrep/deh098>)

- Ruvolo G, Bosco L, Pane A, Morici G, Cittadini E & Roccheri MC 2007 Lower apoptosis rate in human cumulus cells after administration of recombinant luteinizing hormone to women undergoing ovarian stimulation for in vitro fertilization procedures. *Fertility and Sterility* **87** 542–546. (<https://doi.org/10.1016/j.fertnstert.2006.06.059>)
- Saharkhiz N, Zadmodares S, Salehpour S, Hosseini S, Nazari L & Tehrani HG 2018 The effect of testosterone gel on fertility outcomes in women with a poor response in in vitro fertilization cycles: a pilot randomized clinical trial. *Journal of Research in Medical Sciences* **23** 3–3. (https://doi.org/10.4103/jrms.JRMS_864_17)
- Sarma HN, Manikkam M, Herkimer C, Dell'Orco J, Welch KB, Foster DL & Padmanabhan V 2005 Fetal programming: excess prenatal testosterone reduces postnatal luteinizing hormone, but not follicle-stimulating hormone responsiveness, to estradiol negative feedback in the female. *Endocrinology* **146** 4281–4291. (<https://doi.org/10.1210/en.2005-0322>)
- Schoolcraft WB, Surrey ES, Minjarez DA, Stevens JM & Gardner DK 2008 Management of poor responders: can outcomes be improved with a novel gonadotropin-releasing hormone antagonist/letrozole protocol? *Fertility and Sterility* **89** 151–156. (<https://doi.org/10.1016/j.fertnstert.2007.02.013>)
- Schwarze JE, Canales J, Crosby J, Ortega-Hrepich C, Villa S & Pommer R 2018 DHEA use to improve likelihood of IVF/ICSI success in patients with diminished ovarian reserve: a systematic review and meta-analysis. *JBRA Assisted Reproduction* **22** 369–374. (<https://doi.org/10.5935/1518-0557.20180046>)
- Sen A & Hammes SR 2010 Granulosa cell-specific androgen receptors are critical regulators of ovarian development and function. *Molecular Endocrinology* **24** 1393–1403. (<https://doi.org/10.1210/me.2010-0006>)
- Sen A, Prizant H, Light A, Biswas A, Hayes E, Lee HJ, Barad D, Gleicher N & Hammes SR 2014 Androgens regulate ovarian follicular development by increasing follicle stimulating hormone receptor and microRNA-125b expression. *PNAS* **111** 3008–3013. (<https://doi.org/10.1073/pnas.1318978111>)
- Sheppard KM, Padmanabhan V, Coolen LM & Lehman MN 2011 Prenatal programming by testosterone of hypothalamic metabolic control neurones in the ewe. *Journal of Neuroendocrinology* **23** 401–411. (<https://doi.org/10.1111/j.1365-2826.2011.02126.x>)
- Shina H, Matsumoto T, Sato T, Igarashi K, Miyamoto J, Takemasa S, Sakari M, Takada I, Nakamura T, Metzger D, *et al.* 2006 Premature ovarian failure in androgen receptor-deficient mice. *PNAS* **103** 224–229. (<https://doi.org/10.1073/pnas.0506736102>)
- Shorakae S, Boyle J & Teede H 2014 Polycystic ovary syndrome: a common hormonal condition with major metabolic sequelae that physicians should know about. *Internal Medicine Journal* **44** 720–726. (<https://doi.org/10.1111/imj.12495>)
- Simpson ER, Clyne C, Rubin G, Boon WC, Robertson K, Britt K, Speed C & Jones M 2002 Aromatase – a brief overview. *Annual Review of Physiology* **64** 93–127. (<https://doi.org/10.1146/annurev.physiol.64.081601.142703>)
- Singh A, Bora P & Krishna A 2017 Systemic adiponectin treatment reverses polycystic ovary syndrome-like features in an animal model. *Reproduction, Fertility, and Development* **30** 571–584. (<https://doi.org/10.1071/RD17255>)
- Sipe CS, Thomas MR, Stegmann BJ & Van Voorhis BJ 2010 Effects of exogenous testosterone supplementation in gonadotrophin stimulated cycles. *Human Reproduction* **25** 690–696. (<https://doi.org/10.1093/humrep/dep442>)
- Skiba MA, Islam RM, Bell RJ & Davis SR 2018 Understanding variation in prevalence estimates of polycystic ovary syndrome: a systematic review and meta-analysis. *Human Reproduction Update* **24** 694–709. (<https://doi.org/10.1093/humupd/dmy022>)
- Skorupskaitė K, George JT & Anderson RA 2014 The kisspeptin-GnRH pathway in human reproductive health and disease. *Human Reproduction Update* **20** 485–500. (<https://doi.org/10.1093/humupd/dmu009>)
- Slomczynska M & Tabarowski Z 2001 Localization of androgen receptor and cytochrome P450 aromatase in the follicle and corpus luteum of the porcine ovary. *Animal Reproduction Science* **65** 127–134. ([https://doi.org/10.1016/S0378-4320\(00\)00225-6](https://doi.org/10.1016/S0378-4320(00)00225-6))
- Smith JT 2013 Sex steroid regulation of kisspeptin circuits. *Advances in Experimental Medicine and Biology* **784** 275–295. (https://doi.org/10.1007/978-1-4614-6199-9_13)
- Soma KK, Rendon NM, Boonstra R, Albers HE & Demas GE 2015 DHEA effects on brain and behavior: insights from comparative studies of aggression. *Journal of Steroid Biochemistry and Molecular Biology* **145** 261–272. (<https://doi.org/10.1016/j.jsbmb.2014.05.011>)
- Sonmezer M, Ozmen B, Cil AP, Ozkavukcu S, Tasci T, Olmus H & Atabekoglu CS 2009 Dehydroepiandrosterone supplementation improves ovarian response and cycle outcome in poor responders. *Reproductive Biomedicine Online* **19** 508–513. (<https://doi.org/10.1016/j.rbmo.2009.06.006>)
- Spinder T, Spijkstra JJ, Van Den Tweel JG, Burger CW, Van Kessel H, Hompes PGA & Gooren LJG 1989 The effects of long term testosterone administration on pulsatile luteinizing hormone secretion and on ovarian histology in eugonadal female to male transsexual subjects. *Journal of Clinical Endocrinology and Metabolism* **69** 151–157. (<https://doi.org/10.1210/jcem-69-1-151>)
- Steckelbroeck S, Jin Y, Gopishetty S, Oyesanmi B & Penning TM 2004 Human cytosolic 3alpha-hydroxysteroid dehydrogenases of the aldoketo reductase superfamily display significant 3beta-hydroxysteroid dehydrogenase activity: implications for steroid hormone metabolism and action. *Journal of Biological Chemistry* **279** 10784–10795. (<https://doi.org/10.1074/jbc.M313308200>)
- Sullivan SD & Moenter SM 2004 Prenatal androgens alter GABAergic drive to gonadotropin-releasing hormone neurons: implications for a common fertility disorder. *PNAS* **101** 7129–7134. (<https://doi.org/10.1073/pnas.0308058101>)
- Sun B, Wang F, Sun J, Yu W & Sun Y 2014 Basal serum testosterone levels correlate with ovarian response but do not predict pregnancy outcome in non-PCOS women undergoing IVF. *Journal of Assisted Reproduction and Genetics* **31** 829–835. (<https://doi.org/10.1007/s10815-014-0246-8>)
- Suzuki T, Sasano H, Kimura N, Tamura M, Fukaya T, Yajima A & Nagura H 1994 Immunohistochemical distribution of progesterone, androgen and oestrogen receptors in the human ovary during the menstrual cycle: relationship to expression of steroidogenic enzymes. *Human Reproduction* **9** 1589–1595. (<https://doi.org/10.1093/oxfordjournals.humrep.a138757>)
- Szolyti M & Slomczynska M 2000 Changes in distribution of androgen receptor during maturation of rat ovarian follicles. *Experimental and Clinical Endocrinology and Diabetes* **108** 228–234. (<https://doi.org/10.1055/s-2000-7747>)
- Tarlatzis BC, Zepiridis L, Grimbizis G & Bontis J 2003 Clinical management of low ovarian response to stimulation for IVF: a systematic review. *Human Reproduction Update* **9** 61–76. (<https://doi.org/10.1093/humupd/dmg007>)
- Tartagni M, Cicinelli E, De Pergola G, Lavopa C, Di Naro E, De Salvia MA & Loverro G 2010 Effect of finasteride on ovulation induction in nonresponder (hyperandrogenic) polycystic ovary syndrome (PCOS) women. *Fertility and Sterility* **94** 247–249. (<https://doi.org/10.1016/j.fertnstert.2009.01.150>)
- Tartagni MV, Alrasheed H, Damiani GR, Montagnani M, De Salvia MA, De Pergola G, Tartagni M & Loverro G 2014 Intermittent low-dose Finasteride Administration is effective for treatment of hirsutism in adolescent girls: a pilot study. *Journal of Pediatric and Adolescent Gynecology* **27** 161–165. (<https://doi.org/10.1016/j.jpbg.2013.09.010>)
- Taylor A 2003 ABC of subfertility: extent of the problem. *BMJ* **327** 434–436. (<https://doi.org/10.1136/bmj.327.7412.434>)
- Teede HJ, Misso ML, Costello MF, Dokras A, Laven J, Moran L, Piltonen T, Norman RJ & International PCOS Network 2018 Recommendations from the international evidence-based guideline for the assessment

- and management of polycystic ovary syndrome. *Human Reproduction* **33** 1602–1618. (<https://doi.org/10.1093/humrep/dey256>)
- Tsui KH, Lin LT, Horng HC, Chang R, Huang BS, Cheng JT & Wang PH 2014 Gene expression of cumulus cells in women with poor ovarian response after dehydroepiandrosterone supplementation. *Taiwanese Journal of Obstetrics and Gynecology* **53** 559–565. (<https://doi.org/10.1016/j.tjog.2014.09.003>)
- Tsui KH, Lin LT, Chang R, Huang BS, Cheng JT & Wang PH 2015 Effects of dehydroepiandrosterone supplementation on women with poor ovarian response: a preliminary report and review. *Taiwanese Journal of Obstetrics and Gynecology* **54** 131–136. (<https://doi.org/10.1016/j.tjog.2014.07.007>)
- Umayal B, Jayakody SN, Chandrasekharan NV, Wijesundera WS & Wijeyaratne CN 2019 Polycystic ovary syndrome (PCOS) and kisspeptin – a Sri Lankan study. *Journal of Postgraduate Medicine* **65** 18–23. (https://doi.org/10.4103/jpgm.JPGM_683_17)
- Vendola KA, Zhou J, Adesanya OO, Weil SJ & Bondy CA 1998 Androgens stimulate early stages of follicular growth in the primate ovary. *Journal of Clinical Investigation* **101** 2622–2629. (<https://doi.org/10.1172/JCI2081>)
- Vendola K, Zhou J, Wang J, Famuyiwa OA, Bievre M & Bondy CA 1999 Androgens promote oocyte insulin-like growth factor I expression and initiation of follicle development in the primate ovary. *Biology of Reproduction* **61** 353–357. (<https://doi.org/10.1095/biolreprod61.2.353>)
- Venturoli S, Marescalchi O, Colombo FM, Macrelli S, Ravaioli B, Bagnoli A, Paradisi R & Flamigni C 1999 A prospective randomized trial comparing low dose flutamide, finasteride, ketoconazole, and cyproterone acetate-estrogen regimens in the treatment of hirsutism. *Journal of Clinical Endocrinology and Metabolism* **84** 1304–1310. (<https://doi.org/10.1210/jcem.84.4.5591>)
- Villanueva EC & Myers MG 2008 Leptin receptor signaling and the regulation of mammalian physiology. *International Journal of Obesity* **32** (Supplement 7) S8–S12. (<https://doi.org/10.1038/ijo.2008.232>)
- Vlahos N, Papalouka M, Triantafyllidou O, Vlachos A, Vakas P, Grimbizis G, Creatsas G & Zikopoulos K 2015 Dehydroepiandrosterone administration before IVF in poor responders: a prospective cohort study. *Reproductive Biomedicine Online* **30** 191–196. (<https://doi.org/10.1016/j.rbmo.2014.10.005>)
- Walters KA 2015 Role of androgens in normal and pathological ovarian function. *Reproduction* **149** R193–R218. (<https://doi.org/10.1530/REP-14-0517>)
- Walters KA & Handelsman DJ 2018 Role of androgens in the ovary. *Molecular and Cellular Endocrinology* **465** 36–47. (<https://doi.org/10.1016/j.mce.2017.06.026>)
- Walters KA, Allan CM, Jimenez M, Lim PR, Davey RA, Zajac JD, Illingworth P & Handelsman DJ 2007 Female mice haploinsufficient for an inactivated androgen receptor (AR) exhibit age-dependent defects that resemble the AR null phenotype of dysfunctional late follicle development, ovulation, and fertility. *Endocrinology* **148** 3674–3684. (<https://doi.org/10.1210/en.2007-0248>)
- Walters KA, Mctavish KJ, Seneviratne MG, Jimenez M, McMahon AC, Allan CM, Salamonsen LA & Handelsman DJ 2009 Subfertile female androgen receptor knockout mice exhibit defects in neuroendocrine signaling, intraovarian function, and uterine development but not uterine function. *Endocrinology* **150** 3274–3282. (<https://doi.org/10.1210/en.2008-1750>)
- Walters KA, Allan CM & Handelsman DJ 2012a Rodent models for human polycystic ovary syndrome. *Biology of Reproduction* **86** 1–12. (<https://doi.org/10.1095/biolreprod.111.097808>)
- Walters KA, Middleton LJ, Joseph SR, Hazra R, Jimenez M, Simanainen U, Allan CM & Handelsman DJ 2012b Targeted loss of androgen receptor signaling in murine granulosa cells of preantral and antral follicles causes female subfertility. *Biology of Reproduction* **87** 151. (<https://doi.org/10.1095/biolreprod.112.102012>)
- Walters KA, Bertoldo MJ & Handelsman DJ 2018a Evidence from animal models on the pathogenesis of PCOS. *Best Practice and Research: Clinical Endocrinology and Metabolism* **32** 271–281. (<https://doi.org/10.1016/j.beem.2018.03.008>)
- Walters KA, Edwards MC, Tesic D, Caldwell ASL, Jimenez M, Smith JT & Handelsman DJ 2018b The role of central androgen receptor actions in regulating the hypothalamic-pituitary-ovarian axis. *Neuroendocrinology* **106** 389–400. (<https://doi.org/10.1159/000487762>)
- Walters KA, Eid S, Edwards MC, Thuis-Watson R, Desai R, Bowman M, Marren AJ & Handelsman DJ 2019 Steroid profiles by liquid chromatography-mass spectrometry of matched serum and single dominant ovarian follicular fluid from women undergoing IVF. *Reproductive Biomedicine Online* **38** 30–37. (<https://doi.org/10.1016/j.rbmo.2018.10.006>)
- Wang H, Andoh K, Hagiwara H, Xiaowei L, Kikuchi N, Abe Y, Yamada K, Fatima R & Mizunuma H 2001 Effect of adrenal and ovarian androgens on type 4 follicles unresponsive to FSH in immature mice. *Endocrinology* **142** 4930–4936. (<https://doi.org/10.1210/endo.142.11.8482>)
- Weil S, Vendola K, Zhou J & Bondy CA 1999 Androgen and follicle-stimulating hormone interactions in primate ovarian follicle development. *Journal of Clinical Endocrinology and Metabolism* **84** 2951–2956. (<https://doi.org/10.1210/jcem.84.8.5929>)
- Westergaard LG, Laursen SB & Andersen CY 2000 Increased risk of early pregnancy loss by profound suppression of luteinizing hormone during ovarian stimulation in normogonadotrophic women undergoing assisted reproduction. *Human Reproduction* **15** 1003–1008. (<https://doi.org/10.1093/humrep/15.5.1003>)
- Wilson CM & McPhaul MJ 1996 A and B forms of the androgen receptor are expressed in a variety of human tissues. *Molecular and Cellular Endocrinology* **120** 51–57. ([https://doi.org/10.1016/0303-7207\(96\)03819-1](https://doi.org/10.1016/0303-7207(96)03819-1))
- Wiser A, Gonen O, Ghetler Y, Shavit T, Berkovitz A & Shulman A 2010 Addition of dehydroepiandrosterone (DHEA) for poor-responder patients before and during IVF treatment improves the pregnancy rate: a randomized prospective study. *Human Reproduction* **25** 2496–2500. (<https://doi.org/10.1093/humrep/deq220>)
- Wu XY, Li ZL, Wu CY, Liu YM, Lin H, Wang SH & Xiao WF 2010 Endocrine traits of polycystic ovary syndrome in prenatally androgenized female Sprague-Dawley rats. *Endocrine Journal* **57** 201–209. (<https://doi.org/10.1507/endocrj.K09E-205>)
- Wu S, Chen Y, Fajobi T, Divall SA, Chang C, Yeh S & Wolfe A 2014 Conditional knockout of the androgen receptor in gonadotropes reveals crucial roles for androgen in gonadotropin synthesis and surge in female mice. *Molecular Endocrinology* **28** 1670–1681. (<https://doi.org/10.1210/me.2014-1154>)
- Xiao S, Li Y, Long L, Luo C & Mai Q 2016 Basal serum testosterone levels correlate with ovarian reserve and ovarian response in cycling women undergoing in vitro fertilization. *Gynecological Endocrinology* **32** 51–54. (<https://doi.org/10.3109/09513590.2015.1076784>)
- Xu B, Li Z, Yue J, Jin L, Li Y, Ai J, Zhang H & Zhu G 2014 Effect of dehydroepiandrosterone administration in patients with poor ovarian response according to the Bologna criteria. *PLoS ONE* **9** e99858. (<https://doi.org/10.1371/journal.pone.0099858>)
- Yang JL, Zhang CP, Li L, Huang L, Ji SY, Lu CL, Fan CH, Cai H, Ren Y, Hu ZY, *et al.* 2010 Testosterone induces redistribution of forkhead box-3a and down-regulation of growth and differentiation factor 9 messenger ribonucleic acid expression at early stage of mouse folliculogenesis. *Endocrinology* **151** 774–782. (<https://doi.org/10.1210/en.2009-0751>)
- Yarali H, Esinler I, Polat M, Bozdogan G & Tiras B 2009 Antagonist/letrozole protocol in poor ovarian responders for intracytoplasmic sperm injection: a comparative study with the microdose flare-up protocol. *Fertility and Sterility* **92** 231–235. (<https://doi.org/10.1016/j.fertnstert.2008.04.057>)

- Yeh S, Tsai MY, Xu Q, Mu XM, Lardy H, Huang KE, Lin H, Yeh SD, Altuwaijri S, Zhou X, *et al.* 2002 Generation and characterization of androgen receptor knockout (ARKO) mice: an in vivo model for the study of androgen functions in selective tissues. *PNAS* **99** 13498–13503. (<https://doi.org/10.1073/pnas.212474399>)
- Yeung TW, Chai J, Li RH, Lee VC, Ho PC & Ng EH 2014 A randomized, controlled, pilot trial on the effect of dehydroepiandrosterone on ovarian response markers, ovarian response, and in vitro fertilization outcomes in poor responders. *Fertility and Sterility* **102** 108.e1–115.e1. (<https://doi.org/10.1016/j.fertnstert.2014.03.044>)
- Yildiz BO 2008 Oral contraceptives in polycystic ovary syndrome: risk-benefit assessment. *Seminars in Reproductive Medicine* **26** 111–120. (<https://doi.org/10.1055/s-2007-992931>)
- Yuan X, Hu T, Zhao H, Huang Y, Ye R, Lin J, Zhang C, Zhang H, Wei G, Zhou H, *et al.* 2016 Brown adipose tissue transplantation ameliorates polycystic ovary syndrome. *PNAS* **113** 2708–2713. (<https://doi.org/10.1073/pnas.1523236113>)
- Zawadzki JK & Dunaif A 1992 Diagnostic criteria for polycystic ovary syndrome: towards a rational approach. In *Polycystic Ovary Syndrome*. Eds A Dunaif, HR Givens, FP Haseltine & GR Merriam. Boston, MA, USA: Blackwell Scientific Publications.
- Zhang HH, Xu PY, Wu J, Zou WW, Xu XM, Cao XY & Wei LZ 2014 Dehydroepiandrosterone improves follicular fluid bone morphogenetic protein-15 and accumulated embryo score of infertility patients with diminished ovarian reserve undergoing in vitro fertilization: a randomized controlled trial. *Journal of Ovarian Research* **7** 93. (<https://doi.org/10.1186/s13048-014-0093-3>)
- Zhang M, Niu W, Wang Y, Xu J, Bao X, Wang L, Du L & Sun Y 2016 Dehydroepiandrosterone treatment in women with poor ovarian response undergoing IVF or ICSI: a systematic review and meta-analysis. *Journal of Assisted Reproduction and Genetics* **33** 981–991. (<https://doi.org/10.1007/s10815-016-0713-5>)
- Zimmerman Y, Eijkemans MJ, Coelingh Bennink HJ, Blankenstein MA & Fauser BC 2014 The effect of combined oral contraception on testosterone levels in healthy women: a systematic review and meta-analysis. *Human Reproduction Update* **20** 76–105. (<https://doi.org/10.1093/humupd/dmt038>)
- Zulian E, Sartorato P, Benedini S, Baro G, Armanini D, Mantero F & Scaroni C 2005 Spironolactone in the treatment of polycystic ovary syndrome: effects on clinical features, insulin sensitivity and lipid profile. *Journal of Endocrinological Investigation* **28** 49–53. (<https://doi.org/10.1007/BF03345529>)
- Zumoff B, Strain GW, Miller LK & Rosner W 1995 Twenty-four-hour mean plasma testosterone concentration declines with age in normal premenopausal women. *Journal of Clinical Endocrinology and Metabolism* **80** 1429–1430. (<https://doi.org/10.1210/jcem.80.4.7714119>)

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