REPRODUCTION
REVIEW

Role of androgens in normal and pathological ovarian function

K A Walters

Andrology Laboratory, ANZAC Research Institute, Concord Hospital, University of Sydney, Sydney, New South Wales 2139, Australia

Correspondence should be addressed to K A Walters; Email: kwalters@anzac.edu.au

Abstract

Androgens mediate their actions via the androgen receptor (AR), a member of the nuclear receptor superfamily. AR-mediated androgen action is essential in male reproductive development and function; however, only in the last decade has the suspected but unproven role for AR-mediated actions in female reproduction been firmly established. Deciphering the specific roles and precise pathways by which AR-mediated actions regulate ovarian function has been hindered by confusion on how to interpret results from pharmacological studies using androgens that can be converted into oestrogens, which exert actions via the oestrogen receptors. The generation and analysis of global and cell-specific female *Ar* knockout mouse models have deduced a role for AR-mediated actions in regulating ovarian function, maintaining female fertility, and have begun to unravel the mechanisms by which AR-mediated androgen actions regulate follicle health, development and ovulation. Furthermore, observational findings from human studies and animal models provide substantial evidence to support a role for AR-mediated effects not only in normal ovarian function but also in the development of the frequent ovarian pathological disorder, polycystic ovarian syndrome (PCOS). This review focuses on combining the findings from observational studies in humans, pharmacological studies and animal models to reveal the roles of AR-mediated actions in normal and pathological ovarian function. Together these findings will enable us to begin understanding the important roles of AR actions in the regulation of female fertility and ovarian ageing, as well as providing insights into the role of AR actions in the androgen-associated reproductive disorder PCOS.

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Introduction

The androgen receptor (AR) is encoded by a single copy X-chromosomal gene and is a member of the nuclear receptor superfamily (Lubahn *et al*. 1988, Quigley *et al*. 1995). The fundamental roles of AR-mediated actions in male reproductive function and many of its disorders are well understood (Quigley *et al*. 1995). Various clinical observational studies and *in vitro* pharmacological studies have provided evidence to support the direct involvement of AR-mediated actions in female reproduction (reviewed in Walters *et al*. (2008, 2010)). However, this has been confirmed only in the last decade using *Ar* knockout mouse models (ARKO) (Hu *et al*. 2004, Shiina *et al*. 2006, Walters *et al*. 2007, 2009, 2012*a*, Cheng *et al*. 2013).

Clinical evidence supporting a direct role for AR-mediated androgen actions in ovarian function comes from the findings that women exposed to androgen excess from endogenous (e.g. congenital adrenal hyperplasia (Lucis *et al*. 1966, Hague *et al*. 1990)) or exogenous (testosterone treatment in female-to-male transgender (Pache & Fauser 1993)) sources display polycystic ovaries, supporting a role for androgens in stimulating follicle development. In addition, hyperandrogenism is associated with the female reproductive pathological disorder, polycystic ovary syndrome (PCOS), a common condition causing anovulation and infertility, and associated with an increase in metabolic abnormalities including obesity, insulin resistance, dyslipidaemia, cardiovascular disease and type 2 diabetes (Franks 1995, Pasquali *et al*. 2010, Goodarzi *et al*. 2011). Further evidence of a direct role for AR-mediated actions in ovarian function comes from *in vitro* pharmacological studies, where various androgens, including testosterone, androstenedione (A_4) and dihydrotestosterone (DHT), have been reported to enhance follicle growth and development (Murray *et al*. 1998, Wang *et al*. 2001), with stimulatory effects blocked by a non-steroidal AR blocker (antagonist; bicalutamide) (Murray *et al*. 1998). However, conflicting pharmacological findings have been reported regarding apparent androgen effects on ovarian function with ambiguity arising from conversion of androgens into oestrogens, which exert indirect actions via the oestrogen receptor (ER) (Fig. 1A), and

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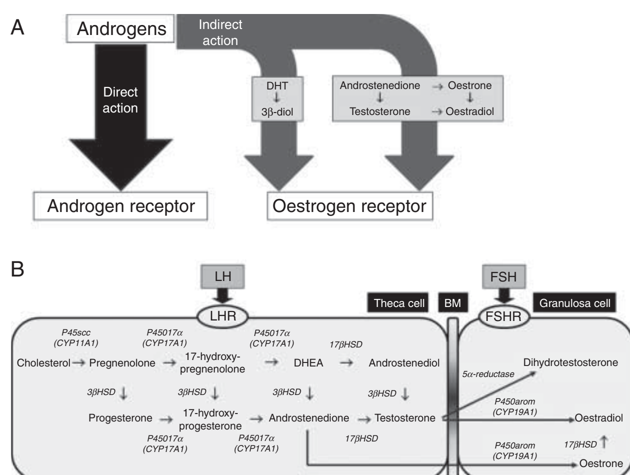

Figure 1 Mechanisms of androgen action and androgen biosynthesis. (A) Androgens can mediate their actions directly via the androgen receptor, or have indirect effects by their conversion into oestrogens and 3β-diol, which can activate the oestrogen receptor. DHT, dihydrotestosterone. (B) Androgen biosynthesis and metabolism. 3β-HSD, 3-β-hydroxysteroid dehydrogenase; 17β-HSD, 17β-hydroxysteroid dehydrogenase; 3β-diol, 5α-androstane-3β,17β-diol.

also because AR antagonists are often mixed partial agonists/antagonists rather than pure blockers.

The development of ARKO mouse models (Yeh *et al*. 2002, Hu *et al*. 2004, Shiina *et al*. 2006, Walters *et al*. 2007, 2012*a*, Sen & Hammes 2010, Wu *et al*. 2014) and PCOS models in rodents (Walters *et al*. 2012*b*), sheep (Padmanabhan & Veiga-Lopez 2013) and primates (Abbott *et al*. 2005) induced by excess androgen exposure provides unique mechanistic insights into the role for AR-mediated androgen actions in ovarian physiology function and the origins of PCOS. This review aims to provide a comprehensive integration of clinical observations with research findings from genetic and pharmacological studies based primarily on whole-animal models, to elucidate the specific roles of AR-mediated androgen actions in regulating normal and pathological ovarian function, and thereby female fertility.

Androgen biosynthesis and their actions via the AR

The major circulating steroids relevant to androgen effects in women, in descending order of serum concentrations, are DHEAS, DHEA, A_4, testosterone and DHT (Davison & Davis 2003). However, only testosterone and DHT (a pure, non-aromatisable androgen three to ten times more potent than testosterone) bind directly to the AR, while the others are androgen precursors that require conversion to testosterone and/or DHT to exert androgenic effects (Burger 2002). In females, bioactive androgens (testosterone, DHT) are predominantly formed by peripheral conversion (in liver, adipose tissue and skin) of androgen precursors (A_4, DHEA and DHEAS) that are secreted from the adrenal glands and the ovaries. DHEA and DHEAS originate predominantly from the adrenal glands (Abraham 1974), while testosterone, DHT and A_4 levels ultimately arise equally from the ovary and adrenals (Davison & Davis 2003), apart from mid-cycle when ovarian contribution of A_4 is twice that of the adrenal (Abraham 1974). Androgen synthesis and metabolism into other steroids involve a series of pathways and a range of enzymes (Fig. 1B). Androgen synthesis in the ovarian follicle and stroma favours the Δ^5-pathway, which involves the conversion of cholesterol into pregnenolone by the enzyme P450 side-chain cleavage (scc, CYP11A1), which is then metabolised to DHEA by P450c17 (CYP17A1) and then A_4 by 3β-hydroxysteroid dehydrogenase (3β-HSD), the immediate precursor of testosterone. 17β-HSD can then convert A_4 into testosterone, which itself can then be aromatised into oestradiol (E_2) by P450arom (CYP19) or reduced to DHT by 5α-reductase 1 (SRD5A1) and 2 (SRD5A2), the latter of which can be enzymatically reduced into 5α-androstanediols, reversibly into 3α-diol and irreversibly into 3β-diol (Longcope 1986, Miller & Auchus 2011). Within the ovary, steroidogenesis is compartmentalised in a cell-specific manner, with A_4 and testosterone being synthesised in the theca cells, before diffusing into the granulosa cells where they are converted into oestrone (E_1) or E_2 (Burger 2002, Ghayee & Auchus 2007). Androgen synthesis within the ovary is under the control of luteinizing hormone (LH), with LH acting via LH receptors on theca cells to stimulate the rate-limiting conversion of cholesterol into pregnenolone (Erickson *et al*. 1985, Longcope 1986).

Androgens mediate their action primarily via the AR, which resides in the cell cytoplasm in an inactive complex bound to chaperone heat shock proteins, until testosterone or DHT bind to the receptor, which dissociates heat shock protein. The androgen-activated AR can then stimulate transcription of target genes via a sequence of processes, including homodimerisation in an anti-parallel configuration, nuclear translocation, DNA binding, and complex formation with co-regulators and general transcription factors (Quigley *et al*. 1995). This classical response is termed genomic AR actions as it involves gene transcription. However, androgens are also reportedly capable of non-genomic AR actions, where androgenic actions occur within seconds or minutes after ligand binding, too quickly for nuclear translocation, and the androgenic response is insensitive to inhibitors of transcription or translocation (Foradori *et al*. 2008). The molecular basis of non-genomic AR effects, however, remains yet to be fully characterised.

Pattern of ovarian AR expression

AR is expressed throughout the hypothalamic–pituitary–gonadal (hpg) axis and the evolutionary conservation of

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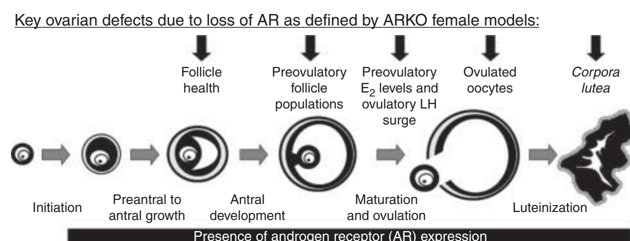

Figure 2 The pattern of AR expression during follicular development and ovarian defects due to the loss of AR signalling. AR expression is present throughout most stages of follicular development and loss of AR actions in ARKO female mouse models leads to several ovarian defects. E_2, oestradiol; LH, luteinizing hormone.

AR expression in all mammalian ovaries strongly supports a universal role for AR-mediated androgen actions in influencing ovarian follicle development. Furthermore, AR is expressed throughout most stages of follicular development (Fig. 2), and, at different stages of follicle development, distinct spatial and temporal patterns of AR expression are present, implying changes in the importance of AR-mediated actions at different follicular developmental stages.

AR expression in the developing ovary

AR is expressed in sheep (Juengel *et al*. 2006), pig (Burek *et al*. 2007) and human (Wilson & McPhaul 1996) foetal ovaries. In sheep, AR mRNA and protein is expressed in the stroma, surface epithelium and granulosa and theca cells (Juengel *et al*. 2006).

AR expression in primordial and primary follicles

AR expression has not been detected in primordial follicles of rat (protein: Szoltys & Slomczynska 2000), bovine (mRNA: Hampton *et al*. 2004), ovine (mRNA: Juengel *et al*. 2006), primate (protein: Hild-Petito *et al*. 1991) or human (mRNA: Rice *et al*. 2007; protein: Suzuki *et al*. 1994, Horie *et al*. 1992) ovaries. However, as follicles enter the growing pool, AR expression increases becoming detectable in rat (protein: Szoltys & Slomczynska 2000), bovine (mRNA: Hampton *et al*. 2004; protein: Salvetti *et al*. 2012), ovine (mRNA: Juengel *et al*. 2006), primate (protein: Hild-Petito *et al*. 1991) and human (mRNA: Rice *et al*. 2007; protein: Horie *et al*. 1992) primary follicles.

AR expression in preantral follicles

AR immunostaining is observed in the oocyte, granulosa cells and theca cells of rat preantral follicles (Szoltys & Slomczynska 2000, Lenie & Smitz 2009). Furthermore, granulosa and theca cells of bovine (mRNA: Hampton *et al*. 2004; protein: Salvetti *et al*. 2012), ovine (mRNA: Juengel *et al*. 2006) and porcine (mRNA: Slomczynska *et al*. 2001; protein: Slomczynska & Tabarowski 2001) preantral follicles also express AR. Primate preantral follicles express AR in granulosa cells, theca cells and oocytes (protein: Hild-Petito *et al*. 1991), with strongest expression in granulosa cells (mRNA: Weil *et al*. 1998; protein: Hillier *et al*. 1997). *AR* mRNA is also detectable in human preantral follicles (Rice *et al*. 2007).

AR expression in antral and preovulatory follicles

Strong AR immunostaining is observed in granulosa cells of early rat antral follicles (Szoltys & Slomczynska 2000). During rat antral follicular growth, AR expression progressively declines in the outer mural granulosa cells of later-stage antral follicles, but the cumulus cells surrounding the oocyte maintain strong AR-positive staining (Szoltys & Slomczynska 2000). This gradient of AR expression intensity was also confirmed in mouse antral follicles collected from an *in vitro* culture system. There the intensity of AR expression increased from the mural granulosa cells to the cumulus cells, and this gradient became more pronounced as antral follicles developed to the preovulatory developmental stage (Lenie & Smitz 2009). Furthermore, following an ovulatory stimulus, cumulus cells exhibit intense AR expression implying that regulation of AR protein expression may be regulated by oocyte-secreted factors (Lenie & Smitz 2009). AR expression is present in granulosa and theca cells of bovine (mRNA: Hampton *et al*. 2004; protein: Salvetti *et al*. 2012), ovine (mRNA: Juengel *et al*. 2006) and porcine (mRNA: Slomczynska *et al*. 2001; protein: Slomczynska & Tabarowski 2001) antral follicles, with staining most predominant in the granulosa cells. AR protein (Hild-Petito *et al*. 1991, Hillier *et al*. 1997) and mRNA (Weil *et al*. 1998) are expressed in mural granulosa cells and theca cells of primate antral and periovulatory follicles; however, the expression weakens in the granulosa cells as they progress from less mature follicles to periovulatory follicles (Hillier *et al*. 1997). AR immunostaining is also present in human granulosa and theca cells of antral and preovulatory follicles (Suzuki *et al*. 1994, Chadha *et al*. 1994), and AR mRNA and protein are detectable in granulosa cells extracted from human small and large antral follicles (Catteau-Jonard *et al*. 2008, Nielsen *et al*. 2011).

AR expression in corpora lutea

AR mRNA is present in primate *corpora lutea* (CL) (Duffy *et al*. 1999). During the early luteal phase of a cycle, developing and even regressing primate CL exhibit AR immunostaining (Hild-Petito *et al*. 1991). However, AR immunostaining is dramatically reduced in fully regressing CL in the early follicular phase of the following cycle (Hild-Petito *et al*. 1991). AR expression is also

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detected in ovine (mRNA: Juengel *et al.* 2006), porcine (mRNA: Slomczynska *et al.* 2001; protein: Slomczynska & Tabarowski 2001) and human (protein: Suzuki *et al.* 1994, Chadha *et al.* 1994) CL.

AR expression in ovarian stroma

Strong *AR* mRNA expression is present in ovine ovarian stroma, especially around small growing follicles (Juengel *et al.* 2006). During the follicular and luteal phases, stroma within primate ovaries exhibits AR protein (Hild-Petito *et al.* 1991) and mRNA (Weil *et al.* 1998), and human ovarian stromal cells also express AR protein (Suzuki *et al.* 1994).

Insights into the role of AR-mediated actions in ovarian function from clinical studies

The consistent expression of AR in human ovaries throughout follicular development strongly suggests that it plays a role in regulating ovarian function. Furthermore, increased tissue androgen exposure whether of endogenous androgens in women with congenital adrenal hyperplasia (Lucis *et al.* 1966), PCOS (Chang 2007) or exogenous testosterone treatment in female-to-male trans-sexuals (Pache & Fauser 1993, Becerra-Fernandez *et al.* 2014) is associated with a high prevalence of multi-follicular ovaries, supporting a role for androgens in stimulating and then arresting follicle development.

Population studies demonstrate a decrease in circulating blood levels of testosterone, DHEA and A_4 concentrations in women gradually between the ages of 20 and 45 years of age, without specific effects of the menopausal transition (Zumoff *et al.* 1995, Davison *et al.* 2005). This decline in pro-androgens and androgens over a woman's reproductive life may contribute to, or reflect, the diminishing ability of the ageing ovary to respond to follicle-stimulating hormone (FSH)-based stimulation for fertility treatments. Ovarian reserve declines steeply with age, and older women undergoing IVF yield fewer oocytes even after maximal FSH stimulation (Broekmans *et al.* 2009). In recent years, some IVF centres and individual women have initiated the use of androgen (DHEA or testosterone) pre-treatment for (mainly older) women who exhibit poor ovarian response to FSH stimulation. One recent study has claimed that one-third of all IVF centres worldwide use DHEA supplementation in such women (Gleicher & Barad 2011). This adjunct therapy originated following a 2000 report of a small, uncontrolled case study, claiming that rectifying the age-related decline in blood DHEA in five women produced an improved response to ovarian stimulation (Casson *et al.* 2000). A subsequent study reported the single case of a 43-year-old woman, whose yield of oocytes increased from one initially, to up to 17 oocytes in subsequent cycles after DHEA treatment (Barad & Gleicher 2005). Since then, studies using various means to increase androgen exposure (DHEA, testosterone, letrozole) have reported enhanced ovarian response to FSH stimulation (Fabregues *et al.* 2009), together with increased antral follicle, oocyte and embryo numbers, improved embryo quality and increased pregnancy and live births (Garcia-Velasco *et al.* 2005, Balasch *et al.* 2006, Wiser *et al.* 2010, Kim *et al.* 2011, Meldrum *et al.* 2013). However, other studies have failed to confirm these findings using DHEA (Yeung *et al.* 2014), testosterone (Massin *et al.* 2006, Sipe *et al.* 2010) or letrozole (Lossl *et al.* 2008, Ozmen *et al.* 2009). A meta-analysis confirmed that transdermal testosterone treatment increased pregnancy and live-birth rates, but there were insufficient data to support a beneficial role for DHEA or letrozole (Bosdou *et al.* 2012). Another meta-analysis confirmed the lack of sufficient evidence to confirm any beneficial effect of DHEA (Narkwichean *et al.* 2013). These inconclusive findings are mainly due to study design limitations, notably lack of placebo controls, small sample size, use of aromatisable androgens and inconsistent entry criteria.

In conclusion, clinical studies have provided evidence to support a role for AR-mediated androgen actions on ovarian function. However, despite several lines of evidence supporting a role for androgens in stimulating follicle development and improving ovarian FSH response, the available data from clinical studies remain unconvincing and decisive studies are eagerly awaited.

Insights into the role of AR-mediated actions in normal ovarian function from animal studies

Pharmacological studies

Primordial follicle initiation

Primordial follicle initiation is stimulated in mouse ovaries by testosterone and DHT (Yang *et al.* 2010), in sheep by DHEA (Narkwichean *et al.* 2014) and in primates by testosterone and DHT (Vendola *et al.* 1999*a*), implying that androgen treatment can promote initiation of primordial follicle growth. Interestingly, this is despite the lack of AR expression in primordial follicles, which indicates that androgen action must be mediated via indirect paracrine mechanisms such as upregulation of insulin-like growth factor 1 (IGF1) and/or its receptor (Vendola *et al.* 1999*a*).

Preantral-to-antral follicle development

AR-mediated actions are important in the early stages of follicular development. *In vitro* culture of preantral mouse follicles in the presence of anti-androgen antibodies or an AR antagonist (bicalutamide) significantly suppressed follicle growth and antral cavity development (Murray *et al.* 1998). In addition, treatment

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with DHT restored follicular growth and antral development in follicles with suppressed growth when cultured in a low FSH environment (Murray *et al*. 1998). Similarly, addition of testosterone, DHT, A_4, DHEA or DHEAS to an *in vitro* culture system enhances mouse preantral follicular development in a dose-dependent manner, with follicles undergoing rapid granulosa cell proliferation and amplified responsiveness to FSH (Wang *et al*. 2001). These stimulatory effects on follicle development appear not to be due to aromatisation because addition of an AR antagonist (flutamide) blocked the growth effects, and addition of oestrogens (E_1 or E_2) alone or the presence of an aromatase inhibitor (fadrozole) had no effect on growth (Wang *et al*. 2001). Similarly, testosterone, but not E_2, also stimulates the transition of bovine primary to secondary follicles, an effect blocked by an AR blocker (flutamide). These findings are most consistent with an effect mediated by a direct AR-mediated action, and not indirect effects of aromatisation of testosterone to E_2 with subsequent ER-mediated effects (Yang & Fortune 2006). Ten weeks of DHEA treatment in ewes increases the proportion of follicles observed at the antral stage (Narkwichean *et al*. 2014). Similarly, numbers of growing preantral and small antral follicles are significantly increased in primate ovaries after treatment with either an aromatisable (testosterone) or a non-aromatisable androgen (DHT) (Vendola *et al*. 1998), and follicle atresia is significantly decreased with follicles exhibiting fewer apoptotic granulosa cells (Vendola *et al*. 1998). These findings imply that androgens enhance preantral to antral stages of follicle growth and that these effects are mediated via the AR. On the other hand, supplementation of A_4 to an *in vitro* mouse preantral follicle culture system suppressed follicular growth and E_2 production (Almahbobi *et al*. 1995). However, it is not clear whether A_4 was acting as a substrate for conversion into an oestrogen, or an androgen such as DHT, which can be subsequently converted into 3β, 5α-androstanediol, both of which can mediate effects via the ERβ. In summary, overall, exogenous androgens appear to have a stimulatory effect on early stages of follicle development.

Preovulatory follicle development, oocyte maturation and ovulation

Administration of testosterone or DHT did not increase preovulatory follicle numbers in primate ovaries (Vendola *et al*. 1998). Yet, in pigs, treatment with testosterone or DHT during the late follicular phase increased the number of preovulatory follicles and CL (Cardenas & Pope 1994, Cardenas *et al*. 2002). In mice, DHT at a dose of 0.25 mg (but not 25 mg) (Sen *et al*. 2014) or 50 mg/kg (but not 100 or 200 mg/kg) (Ware 1982) improved the ovulatory response to superovulation, whereas treatment with a high dose of the progestational anti-androgen, cyproterone acetate, decreased ovulations (Ware 1982). Similarly, *in vivo* treatment of rats with a steroidal AR blocker (cyproterone acetate) leads to a decrease in fresh CL, indicating an inhibition of ovulation (Kumari *et al*. 1978). Administration of an anti-testosterone antibody also reduced ovulation rates in rat ovaries, whereas in the presence of anti-progesterone (anti-P_4) antibody (which blocks ovulation), ovulation was restored by the addition of testosterone or DHT (Mori *et al*. 1977). Conversely, immature female rats primed with pregnant mares serum gonadotrophin (PMSG) and treated with 1 mg/kg (but not 0.25, 0.5 or 2 mg/kg) DHT decreased ovulation rates. This effect appears to be a direct androgenic effect on the ovary as DHT treatment did not alter the surge of LH and FSH (Conway *et al*. 1990). Further investigations suggested that the reduced ovulation rate was due to decreased numbers of granulosa cells per follicle, thus reducing follicular steroidogenesis and leading to lowered circulating E_2 levels (Conway *et al*. 1990). Similarly, *in vivo* treatment of rats with doses >1 mg of DHT inhibited LH receptor induction in granulosa cells, resulting in absent ovulatory responses to human chorionic gonadotrophin (hCG) treatment (Farookhi 1985). Together, these data imply that optimal levels of androgens are required to maintain normal ovulatory function.

Androgens also play a role in oocyte maturation with testosterone capable of promoting *in vitro* germinal vesicle breakdown (GVBD) in murine (Gill *et al*. 2004) and porcine (Li *et al*. 2008) oocytes. Moreover, in the mouse, these effects appear to be transcription independent as the testosterone-mediated response is suppressed by the addition of AR antagonists (flutamide or hydroxyflutamide), but not the transcriptional inhibitor, actinomycin D (Gill *et al*. 2004). Conversely, in another study, testosterone inhibited mouse oocyte meiotic maturation and embryonic development in a dose-dependent manner (Anderiesz & Trounson 1995), indicating that testosterone maintains meiotic arrest. Androgen levels appear to be of real importance in the maintenance of oocyte maturation, as elevated levels of A_4 and testosterone reduced mouse oocyte meiotic competence (Romero & Smitz 2010). Taken together, these findings show that androgens play a role in the periovulatory stages of follicle development and emphasise that the regulation of oocyte maturation and ovulation are sensitive to the androgenic environment, and that a balance of androgen actions is required for optimal ovarian function. These opposing findings for the role of androgens in the periovulatory stage of follicle development and ovulatory response highlight the need for further mechanistic studies to better elucidate the underlying processes.

Genetic studies

There are many instances of conflicting findings from pharmacological studies investigating specific androgen

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effects on follicular development and health. This is mainly due to the conversion of testosterone or A_4 into oestrogens, or DHT into 3β-diol, all which can exert indirect steroidal effects by activating an ER (Fig. 1A). Furthermore, despite the availability of a variety of AR antagonists, this approach is also flawed as steroidal antagonists are usually not pure blockers but may be partial agonists especially when background steroid milieu is weak. Thus, another logical approach to determine the role of AR-mediated actions on ovarian function is provided by the study of female mice homozygous for an inactivated AR (ARKO). Data from these genetic models have complemented, extended and clarified the observations from previous pharmacological approaches and provided definitive proof of a role for AR-mediated actions in the regulation of ovarian function (summarised in Table 1).

Global ARKO

Female ARKO mice cannot be generated by natural breeding owing to obligate paternal sterility of hemizygous males bearing an inactive AR (the classical complete androgen insensitivity syndrome (CAIS), formerly known as testicular feminisation syndrome (tfm)) (Goldstein & Wilson 1972, Notini *et al*. 2005). In the early 1970s, the first mouse models allowing for analysis of female androgen insensitivity were created. These were the naturally occurring but rare $X^{Tfm}O$ female mice (Ohno *et al*. 1973), and the homozygous Ar^{Tfm}/Ar^{Tfm} female mice (Lyon & Glenister 1974). $X^{Tfm}O$ females displayed ovarian degeneration from ∼2 months of age, and although fertility was not quantitatively assessed the authors concluded that AR-mediated actions were essential for normal ovarian function (Ohno *et al*. 1973). Ar^{Tfm}/Ar^{Tfm} females did not display similar ovarian failure, with follicles still present in their ovaries at 6 months of age. However, they did exhibit a reduced reproductive lifespan, and their ovaries had fewer primordial follicles and increased follicle atresia, implying accelerated ovarian ageing (Lyon & Glenister 1974, 1980). Hence, presciently using these two models, AR-mediated androgen actions were found to be optimal rather than essential for ovarian function. Data on these two models developed in the 1970s were limited, presumably due to the complex production methods that were inefficient at providing sustainable lines of mice for analysis.

More recently, using a conditional gene-targeting approach, the Cre/loxP system (Kuhn & Torres 2002), global ARKO mice have been created. This approach generates mice that have a targeted deletion of exon(s) of the *Ar* gene. At present, three distinct female ARKO mouse models have been created with targeted deletions of exon 1 ($ARKO^{Ex1}$, generated using CMV-Cre) (Shiina *et al*. 2006), exon 2 ($ARKO^{Ex2}$, generated using β-Actin-Cre) (Hu *et al*. 2004) or exon 3 ($ARKO^{Ex3}$, generated using CMV-or Sox-Cre) (Walters *et al*. 2007, Cheng *et al*. 2013) of the *Ar* gene. Global ARKO males confirmed the complete abolition of classic genomic AR function as they each exhibit all features of the CAIS phenotype (Kato 2002, Yeh *et al*. 2002, De Gendt *et al*. 2004, Holdcraft & Braun 2004, Notini *et al*. 2005).

All of the global ARKO female mouse models are sub-fertile, exhibiting fewer pups/litter (Yeh *et al*. 2002, Hu *et al*. 2004, Shiina *et al*. 2006, Walters *et al*. 2007) (Table 1). $ARKO^{Ex2}$ and $ARKO^{Ex3}$ females exhibit abnormal oestrous cycles with the cycles being both longer and irregular (Hu *et al*. 2004, Walters *et al*. 2009), indicating that hpg function is defective in the absence of normal AR signalling. Further evidence supporting an extra-ovarian defect in gonadotrophin regulation comes from the findings that $ARKO^{Ex3}$ females exhibit a delay in their 1st litter (Walters *et al*. 2007), and that reduced naturally ovulated oocyte numbers can be overcome by gonadotrophin (pregnant mare serum gonadotrophin/ human chorionic gonadotrophin) hyperstimulation (Walters *et al*. 2007). Additionally, when $ARKO^{Ex3}$ or control ovaries are cross-transplanted into ovariectomised control hosts, the control hosts displayed normal oestrous cycles and fertility (percentage of females to produce a litter), whereas transplantation of control ovaries into ovariectomised $ARKO^{Ex3}$ hosts led to abnormal oestrous cycles and reduced fertility (Walters *et al*. 2009). These findings provide direct evidence of a role for extra-ovarian AR-mediated actions in maintaining female fertility. Recent findings have begun to unravel the precise role of AR signalling in neuroendocrine regulation, with $ARKO^{Ex3}$ females exhibiting a decreased, and often mistimed, ovulatory LH surge with corresponding reductions in follicular steroidogenesis reflected in decreased serum E_2 and E_1 levels at pro-oestrus (preovulatory stage) (Cheng *et al*. 2013). Consistent with the observed diminished preovulatory surge, *Kiss1* mRNA expression in the anteroventral periventricular nucleus is also reduced in $ARKO^{Ex3}$ females (Cheng *et al*. 2013), implying that AR actions play a role in regulating the control of the kisspeptin/ GnRH/LH cascade, which triggers ovulation.

Follicle populations within the ovaries of ARKO mice are normal for at least up to 16 weeks of age (Hu *et al*. 2004, Shiina *et al*. 2006, Walters *et al*. 2007). Older $ARKO^{Ex1}$ females exhibited an accelerated depletion of their follicle pool with a complete loss of follicles by 40 weeks of age (Shiina *et al*. 2006). However, growing follicles at all stages of development are still present in the ovaries of $ARKO^{Ex3}$ mice at 52 weeks of age (Walters *et al*. 2007). Reasons for this discrepancy may be the differences in the way the ARKO models were generated. $ARKO^{Ex1}$ and $ARKO^{Ex2}$ mouse models exhibit an almost complete loss of the AR protein, while on the other hand, the $ARKO^{Ex3}$ model retains a mutant AR protein that may still interact with co-regulators and other transcription factors. Therefore, the premature loss of follicles in the

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Table 1 *In vivo* effects of androgen deficiency defined by distinct global and cell-specific female ARKO mouse models.

ARKO mouse model	$X^{Tfm}O$ (Ohno *et al.* 1973)	Ar^{Tfm}/Ar^{Tfm} (Lyon & Glenister 1974)	$ARKO^{Ex1}$ (Shiina *et al.* 2006)	$ARKO^{Ex2}$ (Yeh *et al.* 2002, Hu *et al.* 2004)	$ARKO^{Ex3}$ (Walters *et al.* 2007, 2009, Cheng *et al.* 2013)	$GCARKO^{Ex2}$ (Sen & Hammes 2010)	$GCARKO^{Ex3}$ (Walters *et al.* 2012*a*)	$OoARKO^{Ex2}$ (Sen & Hammes 2010)	$PitARKO^{Ex2}$ (Wu *et al.* 2014)
Fertility	–	↓ Pups/litter and litters/month	↓ Pups/litter	↓ Pups/litter and litters/month	↓ Pups/litter	↓ Pups/litter and litters/female	↓ In cumulative pups/month from 6 months and ↓ litters in 6 months	Normal fertility	↓ Pups/litter
Oestrous cycles	–	–	–	↑ Oestrous cycle length	↑ Oestrous cycle length, irregular oestrous cycles	↑ Oestrous cycle length at 6 months but not 2 months	↑ Oestrous cycle length at 6 months but not 3 months	Normal oestrous cycles	Trend to ↑ time at oestrus
Serum steroids and hormones	–	–	No change in FSH, LH, E_2, testosterone or P_4 at pro-oestrus	–	No change in FSH, LH, E_2, testosterone at dioestrus. ↓ LH, E_2 and E_1 at pro-oestrus. ↓ LH after OVX. Normal LH response to GnRH and OVX+E_2	–	No change in FSH or LH at dioestrus	–	↓ FSH at all oestrous cycle stages. ↓ LH but no change in E_2 or testosterone at pro-oestrus. Normal LH response to GnRH. ↓ LH and FSH after OVX and OVX+E_2
Ovarian morphology	Ovaries show signs of deterioration	Ovaries normal in appearance	Ovaries and oviducts normal in appearance	Ovaries and oviducts normal in appearance	Ovaries and oviducts normal in appearance. ↓ ovary weight at 5–12 weeks	Ovaries and oviducts normal in appearance	Ovaries and oviducts normal in appearance	Ovaries normal in appearance	Ovaries normal in appearance
Follicle populations	↓ Primordial	↓ Primordial at 6 months	Growing follicle populations normal at 8 weeks. Total follicle exhaustion by 40 weeks. ↓ CL	Growing follicle populations normal at 4 and 16 weeks. ↓ CL	At diestrus growing follicles populations normal at 10–12, 26 and 52 weeks. ↓ CL. At pro-oestrus ↓ preovulatory follicles	Growing follicle populations normal at 4 weeks. At 2 and 6 months ↑ preantral follicles, but ↓ antral follicles and CL, followed by premature ovarian failure	↓ Large preantral and small antral follicles at 3 months. No difference in follicle populations at 6 months	Growing follicle populations and CL normal	At dioestrus no difference in follicle populations. ↓ CL
Follicle growth	–	–	–	–	No change in granulosa or theca cell proliferation rates	↓ Slower growth rates of preantral follicles *in vitro* culture	–	–	–

(continued)

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Table 1 *Continued.*

ARKO mouse model	$X^{Tfm}O$ (Ohno *et al.* 1973)	Ar^{Tfm}/Ar^{Tfm} (Lyon & Glenister 1974)	$ARKO^{Ex1}$ (Shiina *et al.* 2006)	$ARKO^{Ex2}$ (Yeh *et al.* 2002, Hu *et al.* 2004)	$ARKO^{Ex3}$ (Walters *et al.* 2007, 2009, Cheng *et al.* 2013)	$GCARKO^{Ex2}$ (Sen & Hammes 2010)	$GCARKO^{Ex3}$ (Walters *et al.* 2012*a*)	$OoARKO^{Ex2}$ (Sen & Hammes 2010)	$PitARKO^{Ex2}$ (Wu *et al.* 2014)
Oocyte and follicle health	↑ Oocyte deterioration	↑ Atretic follicles	↑ Atretic follicles	↓ Granulosa cell thickness in antral follicles. ↑ follicular atresia after hyper-stimulation. Dissociation of cumulus cells from oocyte in preovulatory follicles	↑ Unhealthy antral follicles. No dissociation of cumulus cells from oocyte in preovulatory follicles	↑ Atretic follicles	↑ Unhealthy follicles and ZPR counts at 6 months	↓ DHT-induced GVBD *in vitro*	↑ Pyknotic granulosa cells in antral follicles
Ovulation	–	–	–	↓ Superovulated oocytes	↓ Naturally ovulated oocytes. Superovulated ovulation rates normal	↓ Naturally ovulated oocytes. Superovulated ovulation rates normal at 2 months but ↓ a 6 months	↓ Cumulus expansion	–	–
Embryo development	–	–	–	–	No change in fertilisation or progression to 2-cell stage	–	↓ Rate of fertilisation	–	–
Luteinisation	Ovaries show signs of precocious luteinisation	–	–	Poor granulosa cell luteinisation	–	–	–	–	–
Ovarian gene expression	–	–	At pro-oestrus ↓ *Kitl*, *Bmp15*, *Gdf9*, *Hgf*, but no change in *Lhr*, *Fshr*, *Cyp11a1*, *Cyp17a1*, *Cyp19a1*, *Esr2*, *Ccnd2* or *Igf1*. No change in *Ptgs2* or *Pgr* at oestrus	↓ *Fshr* and *Igfr* at 10 days of age. After hyperstimulation ↓ *Pgr*, *Has2*, *Tsg6*, *p27*, *Cyp11a1* and ↑ *Cyp17a1*, but no change in *Cyp19a1*	No change in *Bax*, *Bcl2*, *Srd5a1*, *Srd5a2*, *Hsd3b1* and *Akr1c14* at dioestrus. At oestrus *Cyp19a1* ↓, but *Star*, *Cyp11a1* and *Cyp17a1* unchanged	–	No change in *Kitl*, *Igfr1* or *Fshr* at dioestrus	–	No change in *Star*, *Cyp17A1* or *Cyp19*

Tfm, testicular feminisation syndrome; *Fshr*, follicle-stimulating hormone receptor; *Lhr*, luteinizing hormone receptor; E_2, oestradiol; P_4, progesterone; E_1, oestrone; GnRH, gonadotrophin-releasing hormone; *OVX*, ovariectomy; CL, *corpora lutea*; ZPR, zona pellucida remnants; DHT, dihydrotestosterone; GVBD, germinal vesicle breakdown; *Kitl*, KIT ligand; *Bmp15*, bone morphogenetic protein 15; *Gdf9*, growth differentiation factor 9; *Hgf*, hepatocyte growth factor; *Cyp11a1*, cholesterol side-chain cleavage cytochrome P450; *Cyp17a1*, 17α-hydroxylase/C17-20 lyase cytochrome P450; *Cyp19a1*, aromatase cytochrome P450; *Ccnd2*, cyclin D2; *Igf1*, insulin-like growth factor 1; *Igf1r*, insulin-like growth factor 1 receptor; *Ptgs2*, cyclo-oxygenase 2; *Pgr*, progesterone receptor; *Has2*, hyaluronan synthase 2; *Tsg6*, tumour necrosis factor α-stimulate gene 6; *Bax*, Bcl2-associated X protein; *Bcl2*, B cell leukaemia/lymphoma 2; *Srd5a1*, 5α reductase type 1; *Srd5a2*, 5α reductase type II; *Hsd3b1*, 3β-hydroxysteroid dehydrogenase; *Akr1c14*, 3α-hydroxysteroid dehydrogenase; *Star*, StAR protein.

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ARKO^{Ex2} mouse model may be the consequence of secondary effects of the near-total loss of AR protein, which may disrupt other pathways beyond that of AR transcriptional activity, such as co-regulator interactions.

Direct intra-ovarian effects arising from absent AR action are also evident, with follicle development compromised in all ARKO model ovaries. Follicular atresia is significantly increased in all ARKO mouse models (Yeh *et al*. 2002, Hu *et al*. 2004, Shiina *et al*. 2006, Walters *et al*. 2007). Reduced follicle health, defined by the presence of degenerate oocytes and/or $>10\%$ pyknotic granulosa cells, and impaired antrum development were apparent in antral follicles (Walters *et al*. 2007, Cheng *et al*. 2013) in the ARKO^{Ex3} model, and granulosa cell thickness was significantly reduced in ARKO^{Ex2} antral follicles (Hu *et al*. 2004). Ovarian *Fsh* and *Igf1r* expression levels, both key regulators of follicle development, are significantly reduced in ARKO^{Ex2} mice (Hu *et al*. 2004), and ooctye/follicle diameter ratios are reduced in small antral follicles of ARKO^{Ex3} mice, indicating an altered pattern of follicle growth (Cheng *et al*. 2013). In addition, preovulatory follicle numbers, at the pro-oestrous stage, within the ovaries of ARKO^{Ex3} mice are significantly reduced (Cheng *et al*. 2013). Taken together, these finding imply that AR actions help maintain (or provide necessary support) late follicle development, and disruption of AR signalling leads to dysfunctional late follicle health and fewer follicles developing to the preovulatory stage.

Oocyte health and cumulus cell function are impaired during the final stages of follicle development with ARKO^{Ex2} oocytes in preovulatory follicles exhibiting a loss of cumulus cell contact during ovulation. On the other hand, in the ARKO^{Ex3} model, which retains non-functional AR protein, preovulatory follicles exhibit no disassociation of cumulus cells from their oocytes (Walters *et al*. 2008), and ARKO^{Ex3} embryo quality is unaffected with normal embryonic development to the blastocyst stage (Walters *et al*. 2007, Cheng *et al*. 2013). These findings imply that AR actions may influence oocyte and follicle health via mechanisms independent of direct DNA-binding-mediated transcription. Indeed, in the mouse, testosterone induces *in vitro* GVBD of mouse oocytes by transcription-independent mechanisms (Gill *et al*. 2004). AR signalling is essential for optimal ovulatory function, as the key finding from all ARKO female models was that they exhibited a significant reduction in the numbers of CL (Hu *et al*. 2004, Shiina *et al*. 2006, Walters *et al*. 2007, Cheng *et al*. 2013). The body of evidence from the ARKO models indicates that the observed reduction in ovulation rates in ARKO females is due to a combination of dysfunctional late oocyte/follicle heath, the development of fewer preovulatory follicles and reduced preovulatory E_2 and LH levels.

In conclusion, data from the three global ARKO mouse models have confirmed that both extra- and intra-ovarian AR-mediated actions play a role in maintaining normal ovarian function and female fertility. AR actions appear to have a positive role in follicle development, particularly during the later stages of follicle development where AR is involved in ovulation priming by regulating gonadotrophin secretion, and also maintaining antral follicle health and promoting preovulatory follicle development.

Global heterozygous ARKO$^{+/-}$

Limited data are available from two ARKO$^{+/-}$ female mouse models on the effect of AR haploinsufficiency on female fertility and ovarian function. For up to 6 months of age, ARKO$^{Ex2+/-}$ and ARKO$^{Ex3+/-}$ females display normal fertility (Hu *et al*. 2004, Walters *et al*. 2007). However, ARKO$^{Ex3+/-}$ females exhibit an age-dependent reduction in pups per litter evident from 6 months of age, indicating a significant *Ar* gene dosage effect on female fertility (Walters *et al*. 2007). ARKO$^{Ex3+/-}$ ovaries display normal growing follicle populations and follicular health, but a significant reduction in CL counts at 3 and 6 months of age (Walters *et al*. 2007). The number of oocytes collected after natural ovulation (Walters *et al*. 2007) and hyperstimulation (Hu *et al*. 2004, Walters *et al*. 2007) was not reduced in either ARKO$^{Ex2+/-}$ and ARKO$^{Ex3+/-}$ females, implying that the reduced CL numbers are due to defective CL formation and/or maintenance rather than reduced ovulation rates. Haploinsufficiency of the inactivated AR also has no effect on oocyte viability with ARKO$^{Ex3+/-}$ oocytes exhibiting normal fertilisation rates and progression to the two-cell stage (Walters *et al*. 2007). Interestingly, partial loss of AR action in granulosa cells, as observed in the heterozygous GCARKO$^{Ex2+/-}$ females, has no effect on fertility, oestrous cycles or ovarian morphology (Sen & Hammes 2010).

In summary, findings from the two ARKO$^{+/-}$ female mouse models have revealed a gene dosage effect of AR on female fertility, with a fully functional AR required for optimal female fertility.

Granulosa cell-specific ARKO

Two distinct granulosa cell-specific ARKO (GCARKO) mouse models have been reported in female mice (Sen & Hammes 2010, Walters *et al*. 2012*a*). These models aimed to achieve complete AR inactivation in granulosa cells but normal AR function in all other cells using the granulosa cell promoters *Amhr2* (Sen & Hammes 2010) and *Amh* (Walters *et al*. 2012*a*) to cause a deletion of exon 2 (GCARKO^{Ex2}) (Sen & Hammes 2010) and an in-frame deletion of exon 3 (GCARKO^{Ex3}) (Walters *et al*. 2012*a*). AR mRNA and protein were absent in granulosa cells from the ovaries of GCARKO^{Ex2} mice (Sen & Hammes 2010); however, nonspecific leakage expression of the Amhr2-Cre promoter has been detected in the uterus, oocyte and theca cells (Jorgez

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et al. 2004, Hernandez Gifford *et al*. 2009, Sen & Hammes 2010), inferring that while the phenotype of the GCARKO^{Ex2} mouse model may be primarily due to the loss of AR signalling within the granulosa cells, loss of AR action in non-granulosa cells of the reproductive tract may contribute to the phenotype. In the GCARKO^{Ex3} mouse model, PCR and lacZ staining analysis confirmed the excision of *Ar* exon 3 was located only in the granulosa cells, and not the oocyte, theca cells, uterus, brain or pituitary (Walters *et al*. 2012*a*). Strong AMH-Cre expression was observed in large preantral and antral follicles, consistent with AMH expression patterns, while expression was lower or undetectable in granulosa cells of primordial, primary and small preantral stages (Walters *et al*. 2012*a*). Thus, in the GCARKO^{Ex3} model, as not all granulosa cells exhibited the excised exon 3 of *Ar*, the observed findings may be an underestimation of the importance of granulosa cell AR actions on ovarian function, and thus female fertility.

Both GCARKO female mouse models are sub-fertile (Sen & Hammes 2010, Walters *et al*. 2012*a*). GCARKO^{Ex2} females exhibit a reduction in pups per litter and total litters from 2 months of age (Sen & Hammes 2010), while GCARKO^{Ex3} females display a reduction in total litters over 6 months and an age-dependent reduction in the total number of pups born, evident from 6 months of age (Walters *et al*. 2012*a*). Oestrous cycles in both GCARKO models were normal at 2 and 3 months of age but significantly longer by 6 months of age, implying that the loss of AR granulosa cell AR actions may alter hpg feedback signalling (Sen & Hammes 2010, Walters *et al*. 2012*a*).

Follicle dynamics are altered by the loss of granulosa cell AR function. Ovaries of GCARKO^{Ex2} mice exhibit an increase in preantral follicles, but a decrease in antral follicles and CL from 2 months of age, followed by premature ovarian failure (Sen & Hammes 2010). While ovaries of GCARKO^{Ex3} mice display a reduction in large preantral and small antral follicles at 3 months of age, but no difference in follicle populations at 6 months of age, or CL numbers at 3 and 6 months of age (Walters *et al*. 2012*a*). The observed reduction in the number of growing follicles at later stages of development supports the notion of AR having a stimulatory role in normal follicle development. The presence (GCARKO^{Ex2}) or absence (GCARKO^{Ex3}) of accelerated follicle depletion in the GCARKO models corresponds to their respective global ARKO models. Hence, the discrepancy in the findings appears to be due to the complete loss of AR protein in the GCARKO^{Ex2} model, compared with the maintenance of a mutant AR protein in the GCARKO^{Ex3} model. A role for AR in regulating granulosa cell survival and thus protecting the follicle from undergoing follicular atresia is supported by both GCARKO models, which displayed significant reductions in follicle health (Sen & Hammes 2010, Walters *et al*. 2012*a*). As shown in the global ARKO models, dysfunction ovulation was a key feature of the GCARKO^{Ex2} model, with females displaying reduced CL numbers and naturally ovulated oocyte numbers (Sen & Hammes 2010). However, in contrast, GCARKO^{Ex3} females had normal numbers of naturally ovulated oocytes but reduced cumulus expansion and oocyte/embryo viability, displayed by decreased fertilisation rates and progression to the two-cell stage (Walters *et al*. 2012*a*). Thus, granulosa cell AR actions appear to be important in numerous stages of the periovulatory phase.

In summary, the GCARKO female mouse models have demonstrated that within the ovary granulosa cells is an important site for AR actions, involved in maintaining normal follicle development and optimal female fertility. In particular, these data support a stimulatory role of granulosa cell AR-mediated androgen action on follicle development and a maintenance role during the periovulatory phase.

Oocyte cell-specific ARKO

One oocyte cell-specific ARKO (OoARKO) has been created by crossing the GDF9-Cre promoter-driven Cre line with the exon 2-floxed *Ar* (Sen & Hammes 2010). *Ar* mRNA analysis identified that *Ar* expression, while still present, was significantly reduced (approximately fourfold) in OoARKO denuded oocytes compared with control females (Sen & Hammes 2010). Hence, observed findings may undervalue the contribution of oocyte AR actions in ovarian function. OoARKO females were reported to have normal fertility, oestrous cycles, follicle populations and CL numbers at 2 months of age (Sen & Hammes 2010). However, oocyte maturation (GVBD) induced *in vitro* by a high concentration of the non-aromatisable androgen, DHT, was significantly reduced in OoARKO oocytes. These findings imply that while AR oocyte actions are not essential for overall ovarian function and female fertility, they may play an important role when intra-ovarian levels of androgens are elevated, such as in women with PCOS (Sen & Hammes 2010).

In conclusion, AR inactivation in the oocyte, as shown in the one OoARKO female mouse model, appears to have no major overall effect on female fertility; however, low *Ar* mRNA levels were still present and the presence of AR protein was not analysed, hence the effects of oocyte-specific AR actions may be underestimated in this mouse model.

Pituitary-specific ARKO

Recently, a pituitary-directed ARKO (PitARKO) has been created by crossing the α subunit of gonadotrophins (αGSU)-Cre promoter-driven Cre line with the exon 2-floxed *Ar* (Wu *et al*. 2014). As this pituitary glycoprotein alpha subunit is common to TSH as well as LH and FSH, targeting of the common alpha subunit would involve thyrotrophs as well as gonadotrophs and its inactivation produces hypothyroidism as well as

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gonadotrophin deficiency (Kendall *et al*. 1995). Deletion of *Ar* in those pituitary cells in this mouse model reduced *Ar* mRNA levels by 50%, as well as AR protein levels in PitARKO pituitaries compared with control, while AR expression was unaffected in the hypothalamus, ovary and liver (Wu *et al*. 2014). PitARKO females displayed normal oestrous cycles but were sub-fertile producing fewer pups per litter (Wu *et al*. 2014). Growing follicle populations were normal but antral follicle health was reduced and there were fewer CL (Wu *et al*. 2014), as observed in global (Hu *et al*. 2004, Shiina *et al*. 2006, Walters *et al*. 2007) and GCARKO^{Ex2} (Sen & Hammes 2010) mouse models, indicative of reduced ovulation rates. PitARKO females exhibited significantly reduced FSH levels at pro-oestrus (Wu *et al*. 2014) and, as observed in the global ARKO^{Ex3} model (Cheng *et al*. 2013), reduced ovulatory LH levels (Wu *et al*. 2014), confirming that AR signalling in the pituitary plays an important role in maintaining optimal ovulatory surge levels.

In summary, AR inactivation in some pituitary cells confirms a neuroendocrine role for AR-mediated actions in the regulation of female fertility, notably involving optimising ovulation, although the contribution of the concomitant hypothyroidism (Dittrich *et al*. 2011) to the features in this model remains to be evaluated.

Androgen–AR mediated molecular mechanisms controlling ovarian physiology

There is limited understanding of the underlying mechanisms of AR-mediated androgen regulation of follicular dynamics. However, testosterone and DHT promote primordial follicle activation in mouse neonatal ovaries via the phosphatidylinositol 3-kinase (PI3K)/Akt/Forkhead box 3a (Foxo3a) pathway (Yang *et al*. 2010). Additionally, testosterone and DHT also promote growth initiation of non-human primate primordial follicles, thought to be mediated by upregulation of IGF1R-mediated actions in primordial follicle oocytes (Vendola *et al*. 1999*a*). Several studies have indicated that androgens can synergise with FSH, with DHT enhancing FSH-mediated mouse preantral-to-antral follicular growth (Sen *et al*. 2014) and FSH-stimulated proliferation in porcine cumulus cells (Hickey *et al*. 2004), testosterone increasing FSH responsiveness in mouse preantral follicles (Wang *et al*. 2001) and A_4 increasing FSH-dependent E_2 secretion in bovine granulosa cells (Hamel *et al*. 2005). Pre-treatment with testosterone is reported to significantly reduce the duration of FSH ovarian stimulation and lower the total FSH dose administered to IVF patients, with the FSH-sparing effect of testosterone presumably due to improving ovarian sensitivity to FSH (Fabregues *et al*. 2009). Androgens appear to enhance FSH ovarian actions, as testosterone increases *FSHR* mRNA expression in primate primary follicles (Weil *et al*. 1999) and DHT increases FSHR expression in porcine preovulatory follicles (Cardenas *et al*. 2002). Interestingly, in mice, DHT and testosterone increase FSHR protein (but not mRNA) expression (Sen *et al*. 2014). This increase was blocked by the AR antagonist flutamide, a MEK inhibitor, and paxillin siRNA – with paxillin being a mediator between non-genomic and genomic transcription – thus, implying that the increase in FSHR protein expression was via non-genomic AR-driven transcription mechanisms (Sen *et al*. 2014).

Apart from the synergistic effects of androgens with FSH, DHT can enhance the proliferation of porcine granulosa cells by IGF1 alone or GDF9 in the presence of IGF1 (Hickey *et al*. 2004, 2005), with these effects reversed by the addition of an AR antagonist. Direct AR effects also promote the differentiation of rat granulosa cells, with testosterone acting via the AR increasing aromatase and *CYP11A1* mRNA and protein expression levels via an increase in liver receptor homologue 1 (NR5A2) (Wu *et al*. 2011), which is expressed in most steroidogenic tissues and regulates the expression of several steroid-metabolising enzymes. In addition, DHT, but not in the presence of the AR antagonist flutamide, reduces connexin43 (GJA1) expression in human granulosa cells *in vitro*, thus implying that AR-mediated actions may regulate follicle development by regulating gap junctional communication. AR-mediated actions are implicated in the regulation of ovulatory processes, with DHT inducing the expression of cyclo-oxygenase 2 and amphiregulin in rodent periovulatory granulosa cells (Yazawa *et al*. 2013). A loss of AR signalling leads to a reduction in expression of hyaluronan synthase 2 and tumour necrosis factor α-stimulated gene 6 in the ovaries of ARKO^{Ex2} mice, both of which are required for normal cumulus expansion (Hu *et al*. 2004). In addition, at the preovulatory stage (pro-oestrus), gene expression levels of KIT ligand, bone morphogenetic protein 15 and growth differentiation factor 9, all involved in the oocyte–granulosa cell regulatory loop, are reduced in the ovaries of ARKO^{Ex1} mice (Shiina *et al*. 2006).

Androgens indirectly protect the follicle from atresia by acting as the essential substrate for the follicle survival factor E_2 and augmenting the FSH responsiveness of granulosa cells in a developmental stage-dependent manner (Harlow *et al*. 1988). However, androgens can also directly influence follicle atresia with testosterone and DHT, inducing oocyte degeneration, granulosa cell pyknosis and somatic cell atresia in hypophysectomised rats (Hillier & Ross 1979, Azzolin & Saiduddin 1983), and inappropriate exposure of testosterone to immature murine oocytes leading to a reduced capacity for them to mature and undergo normal embryonic development (Anderiesz & Trounson 1995). A loss of AR signalling, as shown in ARKO mouse models, also increased levels of follicular atresia, revealing that a balance in AR actions is necessary for optimal follicle health (Hu *et al*. 2004,

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Shiina *et al*. 2006, Walters *et al*. 2007, 2012*a*, Sen & Hammes 2010, Cheng *et al*. 2013). Furthermore, recently, an androgenic mechanism for attenuating follicular atresia has been revealed with testosterone and DHT, but not E_2, increasing granulosa cell expression of microRNA-125b (Sen *et al*. 2014), which suppresses proapoptotic protein (BAK1, BMX, BMF and TRP53) expression (Shi *et al*. 2007, Sen *et al*. 2014). This upregulation is mediated via non-genomic androgen-induced matrix metalloproteinase (MMP)-mediated transactivation of membrane-bound epidermal growth factor receptor (EGFR), triggering MAPK3/1 signalling in the cytoplasm as required for genomic *Ar* transcriptional effects. In addition, the scaffold protein paxillin, required for AR nuclear localisation in granulosa cells (Sen *et al*. 2012), has been identified an essential mediator of non-genomic and genomic AR signalling, which in turn regulates miR-125b expression in granulosa cells (Sen *et al*. 2014).

In summary, while only a few target genes, so far, have been shown to be regulated by AR activation in the ovary, it is clear that AR-mediated activity plays an important role in the augmentation of several key regulators of follicle growth and health, through all stages of follicle development.

Human implications in the role of AR-mediated actions in normal ovarian function from clinical and animal studies

A large number of clinical, pharmacological and ARKO studies imply that AR signalling enhances follicle development by stimulating growth, maintaining health and increasing the response of the ovary to FSH with obvious implications for women undergoing assisted reproductive techniques (ARTs). Recent studies have highlighted potential beneficial effects on antral follicle, oocyte and embryo numbers, embryo quality, and pregnancy and live birth rates, of treating women who have had a poor ovarian response with pre-treatments that elevate androgen exposure. In addition, the findings that AR expression remains intense in the cumulus cells of preovulatory follicles (Szoltys & Slomczynska 2000, Lenie & Smitz 2009), and loss of AR signalling in granulosa cells impaired cumulus expansion and reduced oocyte/embryo viability (Walters *et al*. 2012*a*), imply a key role for AR actions in late stages of oocyte maturation and warrant investigations into the use of androgens in *in vitro* maturation (IVM) culture systems.

When depletion of the follicular pool falls below a critical threshold, menopause ensues. Thus, the functional lifespan of the ovary and hence the age of menopause are largely dictated by the rate of follicle atresia (Richardson 1993). As all global (Hu *et al*. 2004, Shiina *et al*. 2006, Walters *et al*. 2007, Cheng *et al*. 2013) and granulosa cell-specific (Sen & Hammes 2010, Walters *et al*. 2012*a*) ovaries of ARKO mice display increased follicular atresia, it is likely that AR signalling influences the rate of follicle atresia. Moreover, ARKO^{Ex1} females exhibit an accelerated depletion of their follicle pool and complete ovarian failure by 40 weeks of age (Shiina *et al*. 2006). Premature ovarian failure (POF) is characterised in women by the premature depletion of follicles before the age of 40 years and has been linked with abnormalities involving X-linked genes (Persani *et al*. 2010). AR is located on the X chromosome and alterations in the polymorphic CAG repeat located on exon 1 (Xq11-12) of the *Ar* gene have been suggested as a possible susceptibility factor for the development of POF (Sugawa *et al*. 2008, Laisk *et al*. 2010); however, further evidence is required to support this association. To date, no evidence has been published describing the prevalence of inactivating *AR* mutations in women with POF (Persani *et al*. 2010). Furthermore, the fact that the complete loss of follicles was not observed in the ARKO^{Ex3} mouse model (Walters *et al*. 2007), which retains a non-functional protein, implies that genomic AR activation does not play an essential role in follicle depletion.

Although females homozygous for an inactivated AR cannot occur naturally in women, the finding that ARKO$^{Ex3+/-}$ female mice exhibit an age-dependent reduction in fertility entails a gene dosage effect of *Ar* on determining female fecundity (Walters *et al*. 2007). This may have implications for the reproductive performance of mothers of males with CAIS who are obligate heterozygotes of CAIS *AR* mutations, as it predicts that these women may exhibit a reduced reproductive lifespan and/or protection against hyperandrogenic disorders such as PCOS. Clinical studies examining these predictions would be of great interest.

Insights into the role of AR-mediated actions in PCOS from clinical studies

PCOS is a common endocrine condition affecting 5–10% of women of reproductive age (Franks 1995). It is characterised by reproductive, endocrine and metabolic features including anovulation, infertility, hyperandrogenism, obesity, hyperinsulinism and an increased risk of type 2 diabetes and cardiovascular disease (Fauser *et al*. 2012). This review will outline the evidence supporting a role for AR actions in the development of traits of PCOS, with the main focus being on reproductive and endocrine features, which lead to the dysfunctional ovarian function observed in many patients with PCOS. Reduced fertility is a key feature of PCOS, caused by arrested follicular maturation, dysfunctional ovulation and an increased risk of gestational diabetes, pre-eclampsia, pre-term birth and miscarriage (Tandulwadkar *et al*. 2014). Patterns of reproductive hormone release are also altered, with

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PCOS women exhibiting LH hypersecretion and hyperandrogenism. Highlighting the phenotypic heterogeneity of the condition, there are at present three clinical definitions used to classify PCOS in women. In 1990, the National Institute of Child Health and Human Development Conference recommended that the diagnostic criteria, in the order of importance, should be defined as hyperandrogenism, menstrual dysfunction and the exclusion of other known factors (Zawadski & Dunaif 1992). Subsequently, the 2003 Rotterdam consensus criteria were formulated to require for diagnosis two out of the following three criteria: oligo-ovulation or anovulation, hyperandrogenism and polycystic ovaries (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2004). A diagnosis of polycystic ovaries is based on morphological criteria obtained from ultrasonographic data, where ovaries are observed to be enlarged and exhibit more than 12 follicles per ovary with a diameter of 2–10 mm, a thickened outer tunica albuginea, and an increased density and area of stroma (Takahashi *et al*. 1994, Franks 1995, Chang 2007). This definition for PCOS was followed in 2006 by the Androgen Excess PCOS Society's recommended diagnostic criteria of the presence of hyperandrogenism, and/or oligo-ovulation and polycystic ovaries, and the exclusion of other related disorders (Azziz *et al*. 2006, 2009). Unity has still not been reached on the diagnostic criteria for PCOS, with recent recommendations that metabolic disorders be added when defining the PCOS phenotype (Conway *et al*. 2014). Nevertheless, the most consistent feature of PCOS is hyperandrogenism, affecting at least 60% of women with PCOS (Abbott *et al*. 2005, Livadas *et al*. 2014), highlighting the importance of androgenic actions in the disorder.

Despite substantial research, the origins and underlying mechanisms of PCOS remain unclear. However, numerous lines of clinical evidence implement AR-mediated actions as playing an important, if not essential, role in the development and maintenance of PCOS. Serum levels of various androgens (testosterone, A_4 and DHEAS) and the enzyme 3β-HSD (required in the pathway to convert pro-androgens into bioactive androgens) are consistently elevated in PCOS patients compared with controls (Keefe *et al*. 2014, Palomba *et al*. 2014). High levels of androgen exposure caused by endogenous adrenal androgen hypersecretion in congenital adrenal hyperplasia (Lucis *et al*. 1966, Hague *et al*. 1990) or exogenous testosterone treatment in female-to-male trans-sexuals (Futterweit & Deligdisch 1986, Spinder *et al*. 1989, Pache & Fauser 1993) induces the ovarian PCOS traits of enlarged, multi-cystic ovaries and theca interstitial hyperplasia, implicating androgens in the development of the PCOS ovarian phenotype. Furthermore, elevated androgen secretion *in vitro* by human theca interna cells excised from PCOS ovaries has been reported, with theca cells producing 20 times more A_4 than those from normal ovaries (Gilling-Smith *et al*. 1994), with this feature persisting over the entire period of long-term culture (Nelson *et al*. 1999). Consistent with a direct role for AR-mediated androgen actions in the etiopathogenesis of PCOS, treatment with the AR antagonist flutamide restores menstrual regularity and ovulation in some PCOS patients (Rittmaster 1999, Paradisi *et al*. 2013). Furthermore, a role for AR-mediated androgen actions in the neuroendocrine dysfunction observed in PCOS women is supported by the finding that blockade of androgen actions by flutamide restored the reduced sensitivity of the GnRH pulse generator to feedback inhibition by E_2 and P_4 (Eagleson *et al*. 2000).

An increase in AR activity at the level of the ovary, hypothalamus or potentially the adipocyte or skeletal muscle has been proposed as a possible mechanism involved in PCOS pathogenesis (Baculescu 2013). Some studies have linked alterations in the length of CAG repeats within exon 1 of the *AR* with changes in the activity of AR (Chamberlain *et al*. 1994, Choong *et al*. 1996, Simanainen *et al*. 2011), and several studies have reported an association between variations in the length of CAG repeats and the prevalence of PCOS (reviewed in Baculescu (2013)). However, the incidence of PCOS has been associated with both short (Mifsud *et al*. 2000, Schuring *et al*. 2012) and long (Hickey *et al*. 2002) CAG repeat lengths in different sub-populations, and other studies show no difference in CAG repeat length between PCOS patients and controls (Kim *et al*. 2008, Skrgatic *et al*. 2012). Inconsistencies in these findings may be explained by variability in diagnostic criteria and/or differences in ethnic backgrounds as well as chance findings where no relationship exists. Hence, a role for alterations in AR-mediated transcriptional activity, due to the CAG repeat polymorphism, in the prevalence of PCOS is yet to be confirmed.

A foetal origin of PCOS has been proposed previously (Xita & Tsatsoulis 2006). Observations supporting this hypothesis include the findings that women with foetal androgen excess disorders such as congenital 21-hydroxylase deficiency and congenital adrenal virilising tumours develop PCOS features during their adult life (Hague *et al*. 1990, Barnes *et al*. 1994). Additionally, umbilical vein testosterone level in female foetuses of PCOS mothers is elevated to levels present in males (Barry *et al*. 2010), and pregnant PCOS women exhibit elevated serum levels of androgens (A_4, testosterone and DHEAS), and increased 3β-HSD1 but decreased placental aromatase enzyme activity, compared with pregnant non-PCOS women (Sir-Petermann *et al*. 2002, Maliqueo *et al*. 2013). These findings imply that female offspring of PCOS mothers are exposed to elevated levels of androgens. However, a large prospective cohort study did not find any relationship between maternal or umbilical cord androgen levels and the incidence of PCOS in adolescence (Hickey *et al*. 2009).

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In summary, several findings from clinical observations and studies are consistent with, but cannot prove, a role for AR-mediated action in the aetiology of PCOS.

Insights into the role of AR-mediated actions in PCOS from androgen-induced PCOS animal studies

The origins and underlying mechanisms of PCOS remain unclear, and decisive clinical studies are limited by ethical and logistical constraints. Therefore, since the 1960s, increased androgen exposure has been used to induce characteristics of the human condition of PCOS in rodents, sheep and non-human primates (Table 2), and these models have been employed to study the origins and pathology of this disorder (reviewed in Walters *et al*. (2012*b*), Abbott *et al*. (2013) and Padmanabhan & Veiga-Lopez (2013)).

Androgen-induced rodent PCOS models

Testosterone

Prenatal. Testosterone treatment of rats during late gestation (days 16–19) induces the reproductive PCOS features of irregular oestrous cycles and an ovarian phenotype of increased preantral and antral follicle numbers, and decreased CL populations, indicative of oligo-ovulations, but the classic polycystic ovary phenotype is not present (Wu *et al*. 2010*a*). Treated rats also exhibited the endocrine PCOS-like traits of elevated testosterone and LH serum levels, and an increase in the frequency of LH pulse secretion (Foecking *et al*. 2005, Wu *et al*. 2010*a*). Both prenatal aromatisable (testosterone) and non-aromatisable (DHT) androgen exposure abolished an E_2 benzoate-induced LH surge, implying that direct prenatal AR activation is involved in altering the GnRH/LH neurosecretory system (Foecking *et al*. 2005). On the other hand, in other studies, late gestational treatment of mice with testosterone (Keisler *et al*. 1991) or rats with TP (Swanson & Werff ten Bosch 1964, 1965, Fels & Bosch 1971, Huffman & Hendricks 1981, Slob *et al*. 1983, Tyndall *et al*. 2012) had no effect on cyclicity or ovarian function, inferred by the presence of follicles at various stages and CL. Variations in the observed phenotypes induced by prenatal treatment with testosterone or TP may be due to the degree of transplacental transfer of the administered steroid into the foetus (Fels & Bosch 1971). The timing of the prenatal androgen exposure may also play a role, as a previous study has identified that androgen exposure on gestational days 16–19 increases the number of preantral and antral follicles observed, while testosterone treatment on day 20 of gestation only had no effect on follicle populations (Ramezani *et al*. 2014). In addition, prenatal treatment with testosterone on days 16–19 of gestation in rats induced some metabolic PCOS features, including increased body weight and body fat, and elevated serum cholesterol and triglyceride levels, but no change in insulin sensitivity (Demissie *et al*. 2008).

Postnatal. Postnatal treatment of rats with TP during the first 6 days of life led to oligo- or anovulation, acyclicity, polycystic ovaries with atretic follicles and increased production of oestrogens (E_1 and E_2) and androgens (testosterone and A_4) (Weisz & Lloyd 1965, McDonald & Doughty 1972, Ota *et al*. 1983, Pinilla *et al*. 1993, Kamijo *et al*. 1994). Similarly, mice treated in early postnatal life with TP or testosterone exhibited anovulation and the presence of polyfollicular ovaries (Edwards 1971, Kamijo *et al*. 1994, Tyndall *et al*. 2012). However, early postnatal treatment with TP did not cause the PCOS characteristics of LH hypersecretion (Pinilla *et al*. 1993, Tyndall *et al*. 2012). TP treatment for 35 consecutive days in pre-pubertal rats (∼3 weeks of age) resulted in polycystic ovaries, anovulation and reduced follicle health (Beloosesky *et al*. 2004). Females also exhibited the metabolic PCOS characteristics of insulin resistance, indicating that androgens can induce insulin resistance (Beloosesky *et al*. 2004). Conversely, in another study using rats, TP treatment for 15–25 days did not induce a PCOS-like phenotype with females displaying morphologically normal ovaries (Tyndall *et al*. 2012). These findings highlight that androgenic effects involved in the development of the PCOS phenotype may only induce PCOS traits during specific time windows.

DHEA

Postnatal. Postnatal treatment of DHEA for 20 consecutive days in mice (Familiari *et al*. 1985, Sander *et al*. 2006, Lai *et al*. 2014) and rats (Ward *et al*. 1978, Lee *et al*. 1991, Anderson *et al*. 1992) induced some reproductive PCOS traits including acyclicity, polycystic ovaries and anovulation. Hyperandrogenism was present in this model (Familiari *et al*. 1985, Lee *et al*. 1991, Henmi *et al*. 2001, Lai *et al*. 2014), but conflict remains as to whether the feature of LH hypersecretion is present or not (Ward *et al*. 1978, Lee *et al*. 1991, Henmi *et al*. 2001). DHEA treatment for 20 days in mice did not affect body weight, glucose tolerance or cholesterol levels but did induce the metabolic PCOS traits of enlarged adipocytes, elevated serum fasting insulin levels and insulin resistance (Sander *et al*. 2006, Lai *et al*. 2014). In contrast to these studies, long-term (∼13 weeks) exposure to DHEA induced no PCOS features in mature mice, indicating that the reported effects from short-term DHEA treatment in rodent models may be transient and may also indicate that DHEA is unlikely to cause/maintain PCOS features in women (Caldwell *et al*. 2014).

Dihydrotestosterone

Prenatal. Late gestational exposure of rats (days 16–19 of gestation) and mice (days 16–18 of gestation) to DHT did not induce the classic polycystic ovarian morphology in

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Table 2 Summary of the presence of human PCOS traits in adult rodent, sheep and primate PCOS models after treatment prenatally or postnatally with testosterone or DHT.

	Rodents				Sheep			Primates		
Human diagnostic traits of PCOS	Prenatal testosterone (rat, GD 16–19[a,b] and16–20[c])	Prenatal DHT (mouse, GD 16–18)	Postnatal testosterone (rat, exposure from 3 weeks of age for 35 days)	Postnatal DHT (mouse, 3–16 weeks of age)	Prenatal testosterone (GD 30–90)	Prenatal testosterone (GD 62–102)	Prenatal DHT (GD 30–90)	Prenatal testosterone (GD 40–80 for 15–40 days)	Prenatal testosterone (GD 94–140 for 15–25 days)	Prenatal DHT (GD 40–45 for 55–70 days)
Irregular cycles/ acyclicity	Yes[a]/No[c]	Yes[d,e,f,g]	Yes[h]	Yes[d,i]	Yes[j]	–	Yes[k]	Yes[l,m]	Yes[m]	Yes[n]
Oligo- or anovulation	Yes[a]/No[c]	Yes[d,f]	Yes[h]	Yes[d,i]	Yes[j]	–	Yes[k]	Yes[m]	Yes[m]	–
Hyperandrogenism (↑ serum testosterone)	Yes[a]	Yes[e,f]/No[d]	–	No[d]	–	No[o]	–	Yes[p,m]/No[l]	Yes[m]	–
LH hypersecretion	Yes[a]	Yes[e,f]/No[d]	–	No[d,i]	Yes[q]	–	Yes[r]	Yes[l,m]	No[m]	–
Polycystic ovaries	No[a]	Yes[f]/No[d]	Yes[h]	Yes[d,i]	Yes[s]	–	No[s,t]	Yes[m]	Yes[m,n]	–
↑ Ovary weight	No[c]	No[d,f]	–	No[d,i]	Yes[s]/No[t]	–	No[s,t]	Yes[m]	Yes[m]	–
↑ Numbers of preantral and/or antral follicles	Yes[a]/No[c]	Yes[f]/No[d]	–	No[d]	Yes[s,t]	–	Yes[k]/No[s,t]	–	–	–
↓ Follicle health	–	Yes[d,f]	Yes[h]	Yes[d,i]	Yes[s]	–	No[t]	–	–	–
↑ Body fat	Yes[b]	No[d,g]	–	Yes[d,i]	–	No[o]	–	Yes[u]/No[v]	Yes[m]	–
Adipocyte hypertrophy	–	Yes[d,g]	–	Yes[d,i]	–	–	–	No[v]	–	–
Dyslipidaemia	Yes[b]	No[d]	–	Yes[d]	–	No[o]	–	Yes[m]	–	–
Insulin resistance	No[b]	No[d,g]	Yes[h]	No[d]	–	No[o]	–	Yes[m]	No[m]	–
Presence of steatosis	Yes[b]	Yes[d]	–	Yes[d]	–	Yes[o]	–	–	–	–

Yes, PCOS trait present; No, PCOS trait not present; Yes/No, conflicting findings; –, not determined in publication(s); GD, gestational day of treatment.
[a]Wu *et al.* (2010*a*). [b]Demissie *et al.* (2008). [c]Slob *et al.* (1983). [d]Caldwell *et al.* (2014). [e]Sullivan & Moenter (2004). [f]Moore *et al.* (2013). [g]Roland *et al.* (2010). [h]Belooseskv *et al.* (2004). [i]van Houten *et al.* (2012). [j]Manikkam *et al.* (2006). [k]Steckler *et al.* (2007). [l]Dumesic *et al.* (1997). [m]Abbott *et al.* (2005). [n]Abbott *et al.* (2013). [o]Hogg *et al.* (2011). [p]Eisner *et al.* (2002). [q]Sarma *et al.* (2005). [r]Veiga-Lopez *et al.* (2009). [s]West *et al.* (2001). [t]Smith *et al.* (2009). [u]Eisner *et al.* (2003). [v]Keller *et al.* (2014).

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adult life but did lead to irregular oestrous cycles, altered follicular development, and reduced follicular health and ovulation rates, indicated by decreased CL numbers (Sullivan & Moenter 2004, Wu *et al*. 2010*a*, Moore *et al*. 2013, Yan *et al*. 2013, Caldwell *et al*. 2014). Prenatal exposure to DHT also replicated the PCOS traits of androgen and LH hypersecretion, with rodents exhibiting increased testosterone and LH serum levels (Sullivan & Moenter 2004, Wu *et al*. 2010*a*, Moore *et al*. 2013) and an increase in the frequency of LH pulse secretion (Wu *et al*. 2010*a*), in some but not all (Yan *et al*. 2013, Caldwell *et al*. 2014) rodent models. Importantly, the disruption in cycling and LH secretion appears to be mediated via the AR as treatment with flutamide restores oestrous cyclicity and GABAergic drive to GnRH neurons in female treated prenatally with DHT (Sullivan & Moenter 2004). In addition, treatment of mice with DHT on days 16–18 of gestation did not alter body weight and/or fat mass, but it did induce some metabolic dysfunctions, including impaired glucose tolerance but normal insulin sensitivity and increased adipocyte size indicating altered adipocyte function (Roland *et al*. 2010, Caldwell *et al*. 2014). Additionally, in rats, prenatal exposure of DHT on days 16–19 of gestation induced an increase in body weight, hyperinsulinaemia and insulin resistance (Yan *et al*. 2013).

Postnatal. Early postnatal treatment (day 1 or 5) of rats with DHT propionate (DHTP, DHT ester with prolonged depot duration of action relative to DHT) had no effect upon cyclicity or ovarian morphology (McDonald & Doughty 1972). In contrast, long-term treatment (>11 weeks) of DHT from ∼3 weeks of age induces numerous reproductive and metabolic features of PCOS in rats and mice (Manneras *et al*. 2007, van Houten *et al*. 2012, Caldwell *et al*. 2014). Rats and mice displayed irregular oestrous cycles, oligo-ovulation and polycystic ovaries containing large atretic follicles with a thickened theca interna cell layer and a thin granulosa cell layer (Manneras *et al*. 2007, van Houten *et al*. 2012, Caldwell *et al*. 2014). DHT and 3α- and 3β-diol serum levels were elevated and P_4 was significantly decreased, confirming the reduced ovulations rates, but LH and testosterone levels were unaltered (Manneras *et al*. 2007, Caldwell *et al*. 2014). Numerous metabolic traits of human PCOS are observed in DHT-treated rats and mice, including increased body weight, body fat, enlarged adipocytes, elevated leptin and cholesterol levels and increased presence of steatosis (Manneras *et al*. 2007, Johansson *et al*. 2010, Yanes *et al*. 2011, van Houten *et al*. 2012). DHT-treated rats displayed insulin resistance, while mice did not (Caldwell *et al*. 2014), but they did exhibit glucose intolerance (van Houten *et al*. 2012).

Overall, these findings imply that androgens play a role in the pathogenesis of PCOS, but animal models that use testosterone or DHEA to induce PCOS features are not ideal when trying to define AR-regulated mechanisms, as steroid effects may be induced by AR and/or ER, because testosterone or DHEA can be converted into steroids with the potential to exert ER-mediated effects. DHT is a non-aromatisable pure androgen that activates only AR signalling, thus making it the preferred androgen to decipher the true role of AR-mediated actions in the development of PCOS. Prenatal exposure with DHT induced irregular reproductive cycles and reduced ovulations, directly implicating abnormal AR signalling in the mechanisms leading to disrupted regulation of the hpg axis in PCOS patients. Postnatal DHT treatment from 3 weeks of age replicated a breadth of human PCOS traits including anovulation, acyclicity, polycystic ovaries, obesity, adipocyte hypertrophy and dyslipidaemia. Collectively, these findings strongly support a direct role for AR-mediated actions in the development of reproductive, endocrine and metabolic PCOS features.

Androgen-induced sheep PCOS models

Testosterone

Prenatal. Excess prenatal exposure to testosterone or TP leads to irregular cycling and oligo- or anovulation in adult ewes (Clarke *et al*. 1976), with the severity of disruption higher in females treated earlier in gestation (days 30–80), than in those exposed later (Clarke *et al*. 1977, Savabieasfahani *et al*. 2005). Prenatal treatment with testosterone or TP in ewes between the days of 30–90 of gestation induces the PCOS ovarian characteristics of increased ovarian weight (West *et al*. 2001, Forsdike *et al*. 2007), polycystic ovaries (West *et al*. 2001, Forsdike *et al*. 2007), increased follicular recruitment (Clarke *et al*. 1977, West *et al*. 2001, Smith *et al*. 2009) and increased presence of large antral follicles (Manikkam *et al*. 2006, Steckler *et al*. 2007). However, other studies failed to observe an increase in ovarian weight (Smith *et al*. 2009, Hogg *et al*. 2012) and numbers of growing follicles (preantral and/or antral) (Hogg *et al*. 2012). The endocrine PCOS feature of LH excess has also been observed in some (Sarma *et al*. 2005, Savabieasfahani *et al*. 2005) but not all (West *et al*. 2001, Hogg *et al*. 2012) female ewes prenatally exposed to testosterone or TP. Moreover, while prenatal testosterone treatment was found to selectively increase granulosa cell AR expression in antral follicles (Ortega *et al*. 2009), suggesting increased AR activity; the predominant PCOS feature of hyperandrogenism has not been observed, with serum testosterone levels similar to that of control females (Hogg *et al*. 2012).

Female sheep prenatally exposed to testosterone or TP on days 30–90 of gestation have been reported to exhibit some metabolic characteristics of PCOS, including reduced insulin sensitivity (Recabarren *et al*. 2005, Padmanabhan *et al*. 2010), elevated plasma free fatty acids (Veiga-Lopez *et al*. 2013), hypertension (King *et al*. 2007) and hepatic steatosis (Hogg *et al*. 2011). However, conflicting findings are present with one study finding that prenatal exposure to excess testosterone can increase, rather than decrease, insulin sensitivity

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(Veiga-Lopez *et al*. 2013), while another showed no change in plasma free fatty acid, triglyceride or cholesterol levels (Hogg *et al*. 2011). Additionally, the PCOS characteristic of obesity is not a feature of prenatal testosterone- or TP-induced PCOS sheep models with studies showing no change in body weight (Steckler *et al*. 2009, Hogg *et al*. 2011).

Dihydrotestosterone

Prenatal. Few studies are available on the ovine PCOS model, where ewes have been treated prenatally with DHT, rather than testosterone or TP. The key PCOS characteristics of cycle irregularity and ovulation disruption (Steckler *et al*. 2007) have been reported in ewes prenatally exposed to excess DHT on days 30–90 of gestation, but features such as the classic polycystic ovary appearance (Smith *et al*. 2009) and increased ovarian weight (West *et al*. 2001) are not displayed. Interestingly, while prenatal testosterone exposure increased the number of large antral follicles and follicular persistence in one study, prenatal DHT exposure only increased the number of small growing follicles, but not the number of large antral follicles (Steckler *et al*. 2007). Furthermore, in another study, while prenatal testosterone was found to increase follicle recruitment, prenatal DHT did not do so (Smith *et al*. 2009). These findings imply that both androgenic and oestrogenic mechanisms are involved in the altered follicular dynamics within PCOS ovaries. In addition, there is disagreement on whether prenatal exposure to DHT can induce the endocrine PCOS feature of LH hypersecretion, with studies reporting both a significant increase (Veiga-Lopez *et al*. 2009) and no change in LH levels (West *et al*. 2001). At present, there are limited data available on whether prenatal DHT treatment can induce metabolic disturbances associated with PCOS. However, at 11 weeks of age, females treated prenatally with DHT exhibited a body weight similar to control females, but reduced insulin sensitivity (Padmanabhan *et al*. 2010).

In summary, prenatal exposure with testosterone and TP in sheep can mimic several features of the reproductive and metabolic phenotypes of human PCOS, with prenatally testosterone-treated ewes exhibiting acyclicity, disrupted ovulations, polycystic ovaries, insulin resistance, hypertension and hepatic steatosis. While there are some inconsistencies in the findings between models, on the whole these findings suggest that excess levels of androgens, via either direct or indirect actions, are involved in the manifestation of both the PCOS ovarian phenotype and altered metabolic function. Less data are available on the effects of prenatal DHT treatment in ewes. However, it has been reported to cause the PCOS features of irregular cycles, LH hypersecretion and the stimulation of early-stage follicle growth, indicating that direct AR actions play a role in the altered endocrine patterns of follicle development observed in PCOS.

Androgen-induced primate PCOS models

Testosterone

Prenatal. Adult female rhesus monkeys exposed to excess TP during early–mid-gestation (treated for 15–40 days between days 40–80 of gestation) or late gestation (treated for 15–25 days between days 94–140 of gestation) have been reported to fulfil the diagnostic criteria for PCOS in women by exhibiting irregular cycles, polycystic ovaries and/or hyperandrogenism (Dumesic *et al*. 1997, Eisner *et al*. 2002, Abbott *et al*. 2005, 2013). The endocrine PCOS characteristic of LH hypersecretion is present in females exposed to TP during early–mid-gestation, but not in those exposed during late gestation (Dumesic *et al*. 1997, 2002, Abbott *et al*. 2005). Furthermore, many PCOS metabolic traits are also present in female monkeys exposed to TP during early–mid-gestation, including increased abdominal adiposity, hyperlipidaemia, hyperglycaemia, impaired pancreatic β-cell function, insulin resistance and type 2 diabetes (Eisner *et al*. 2003, Abbott *et al*. 2005, 2009, 2013, Keller *et al*. 2014). Females androgenised during late gestation displayed fewer metabolic traits, with only impaired glucose tolerance and increased abdominal fat reported (Abbott *et al*. 2005).

Postnatal. Treatment of rhesus monkeys with testosterone within 24 h of birth induced no obvious defects in menstrual cyclicity or ovarian morphology (Treloar *et al*. 1972). Similarly, testosterone treatment for 50 days after birth had no effect on ovulation in female marmoset monkeys (Abbott & Hearn 1978). Testosterone treatment in pre-pubertal female rhesus monkeys also had no effect on ovulation rates or ovarian morphology, but did lead to an increase in LH pulse frequency in early adulthood (McGee *et al*. 2012). Exposure of an adult female rhesus monkey to high doses of testosterone for 3–10 days causes enhanced ovarian follicle recruitment and induced ovarian phenotype of numerous small-to-medium-sized antral follicles without increasing levels of follicular atresia (Vendola *et al*. 1998, 1999*a*). However, exposure to testosterone for a longer period of time (13–16 months) caused no difference in follicle populations (Faiman *et al*. 1988). Additionally, while pre-pubertal testosterone treatment led to increased body weight by early adulthood, there was no change in overall per cent body fat, insulin sensitivity or glucose tolerance (McGee *et al*. 2012). Similarly, chronic testosterone exposure in adult female rhesus monkeys for up to 4 and a half years did not cause any insulin resistance or glucose intolerance (Billiar *et al*. 1987).

Dihydrotestosterone

Prenatal. DHT exposure on days 40–45 of gestation (early gestation) for 55–70 consecutive days caused adolescent onset of irregular menstrual cycles (Abbott *et al*. 2013); however, other reproductive and metabolic PCOS traits have not been assessed at present.

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Postnatal. As observed for testosterone, exposure of adult female rhesus monkeys to high doses of DHT for 3–10 days stimulated ovarian follicle recruitment and increased numbers of growing preantral and small antral follicles, implying that these actions are mediated directly via the AR (Vendola *et al*. 1998, 1999*a*).

In summary, the PCOS phenotype is closely mimicked by in uteri exposure of rhesus monkeys to excess levels of testosterone, with females exhibiting a wide range of reproductive, hormonal and metabolic PCOS traits. Little data are available on the phenotype of females exposed prenatally to DHT; therefore, it is difficult to decipher the specific role of direct AR-mediated mechanisms. However, postnatal treatment with both testosterone and DHT stimulates follicle recruitment and follicle development, and provides evidence to support a role for AR signalling the development of the ovarian phenotype of increased numbers of growing follicles.

In conclusion, rodent, sheep and primate animal PCOS models induced by androgen excess have advanced our understanding of the pathogenesis of PCOS and strongly support a role for androgen and AR-mediated actions in the aetiology of this disorder. The comparison of aromatisable (testosterone, TP) and non-aromatisable (DHT) androgens in the induction of PCOS animal models has allowed for an insight into the PCOS traits, which are programmed via AR- vs ER-mediated actions. The majority of studies allowing for comparison of testosterone and DHT have been conducted in rodents and demonstrate that non-aromatisable androgen DHT can induce the key diagnostic criteria of irregular cycles, disrupted ovulations and hyperandrogenism, as well as many metabolic PCOS characteristics. However, when interpreting findings, it should be noted that DHT can be converted into 3β-diol, which has the potential to elicit ER-mediated effects. Furthermore, it is clear that both androgenic and oestrogenic mechanisms are involved in the programming of the PCOS phenotype as in the prenatally androgenised sheep model testosterone, but DHT does not induce polycystic ovaries and abnormal antral follicle morphology and does not increase follicle activation (West *et al*. 2001, Smith *et al*. 2009). In saying this, further support for AR-mediated actions playing a predominant role in the manifestation of PCOS traits comes from the findings that exposure of rats to E_2 valerate can induce anovulation and polycystic ovaries, but it fails to provoke the breadth of features induced by androgens including LH hypersecretion, hyperandrogenism, obesity and alterations in glucose and insulin sensitivity (Brawer *et al*. 1978, Stener-Victorin *et al*. 2005). Furthermore, differences in the timing of the elevated androgen exposure have recently been put forward as a key determinant in the development of various characteristics of PCOS.

Androgen–AR-mediated molecular mechanisms controlling ovarian traits of PCOS

The ovarian phenotype of PCOS is characterised by polycystic ovaries, disordered follicle development, ovarian enlargement, thickened outer tunica albuginea and increased density and area of stroma (Franks 1995, Chang 2007, Franks *et al*. 2008). However, the underlying molecular mechanism leading to these altered ovarian features remains unclear. IGFs are survival factors shown to stimulate follicle development (Walters *et al*. 2006). Elevated IGF activity is proposed as a mechanism leading to increased activation and accelerated growth of early-stage follicles within PCOS ovaries. IGFR1 mRNA and protein expressions are elevated in anovulatory polycystic ovaries (Stubbs *et al*. 2013), and testosterone or DHT increases *IGF1* and *IGF1R* mRNA in primate ovaries (Vendola *et al*. 1999*a*,*b*), suggesting that hyperandrogenism, a key feature of PCOS, may induce accelerated follicle growth via upregulation of IGF actions. Similarly, hyperandrogenism in PCOS may also promote primordial follicle activation through the PI3K/Akt/Foxo3a pathway, as testosterone stimulates activation of primordial follicles by inducing Foxo3a phosphorylation and activating PI3K/Akt signalling via non-genomic mechanisms (Yang *et al*. 2010). AR-mediated androgen actions are also implicated in the follicular arrest observed in PCOS ovaries. Androgen excess is reported to reduce Cx43 expression in human granulosa cells, with the suppression blocked by addition of flutamide. Thus, it has been proposed that hyperandrogenism in PCOS patients may impair communication between granulosa cells leading to follicle development dysfunction and ovulatory dysfunction (Wu *et al*. 2010*b*). Additionally, GDF9 expression, an oocyte-derived growth factor, which is essential for ovarian follicular development (Dong *et al*. 1996), is reduced in the oocytes of women with PCOS (Teixeira Filho *et al*. 2002). Treatment of mouse ovaries with testosterone also reduces GDF9 expression (Yang *et al*. 2010), suggesting that elevated androgen levels may downregulate GDF9 expression and cause follicular arrest. Furthermore, recent studies have implicated the actions of chemerin and its receptor, chemokine-like receptor (CMKLR1), in the antral follicle growth arrest observed in polycystic ovaries. In a DHT-treated rat PCOS model, chemerin and CMKLR1 expressions are elevated, and *in vitro*, chemerin treatment suppressed basal, FSH- and GDF9-stimulated follicle growth, FSH-induced follicular steroidogenesis and induced granulosa cell apoptosis (Wang *et al*. 2012, Kim *et al*. 2013).

Perturbation of the epigenome by disrupted DNA methylation is another mechanism that is proposed as a possible origin for the development of PCOS. Epigenetic alterations have been identified in a prenatally androgenised primate PCOS model, implying that an increased predisposition to PCOS may be induced by

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excess foetal androgen exposure altering the epigenome (Xu *et al*. 2011). In particular, the most significantly differentially methylated genes identified were involved in transforming growth factor beta (TGFβ) signalling, which has been implicated in the aetiology of PCOS (Raja-Khan *et al*. 2014). It is clear that PCOS ovaries exhibit gene dysregulation (Diao *et al*. 2004). A possible role for altered miRNA levels as a mode of action for altered gene expression involved in the pathophysiology of PCOS has been suggested (Sorensen *et al*. 2014). Recent data has implicated (or proposes) androgens in the alteration of post-transcription regulation of gene expression, as dysregulation of ovarian miRNA expression has been identified in a DHT-induced rat PCOS model (Hossain *et al*. 2013). However, our understanding of a potential role for miRNAs in PCOS is limited.

In summary, the mechanisms underlying the development of the PCOS ovarian phenotype still remain poorly understood. In recent years, *in vitro* studies and *in vivo* animal models have identified several intra-ovarian pathways, which are responsive to elevated androgen exposure, providing various possible pathways for AR-mediated androgen actions in fulfilling a key role in the development of the polycystic ovarian phenotype.

Human implications for PCOS from clinical and animal studies of AR-mediated actions

Despite PCOS being one of the most common endocrine conditions in women, and various proposed hypotheses, the aetiology of PCOS remains unknown. As a result, curative treatments are lacking and optimal management via manipulation of causative pathways, rather than symptomatic measures, remain yet to be identified. Elevation of androgen exposure in humans (Hague *et al*. 1990, Pache & Fauser 1993) and animal PCOS models (Table 2) causes the development of polycystic ovaries, suggesting that androgen excess *per se* can initiate ovarian features of PCOS. Furthermore, while none of the animal PCOS models created to date display all of the human characteristics of PCOS, elevated androgen exposure in a range of mammalian species (mouse, rat, sheep and primate) can induce most of the spectra of reproductive, endocrine and metabolic features of human PCOS (Table 2), strongly supporting a role for androgens in the pathogenesis of PCOS. Future analysis should focus on a better parsing of the roles of androgenic (mediated via AR) vs oestrogenic (mediated via ER) mechanisms in the features of PCOS, using AR antagonists and comparison of experimental models of mature animals created by administration of aromatisable vs non-aromatisable androgens or inducing aromatase inhibition. One such study has been published, reporting that postnatal treatment of prenatally testosterone-exposed females with the androgen antagonist flutamide increased the total LH surge response to E_2 positive feedback challenge. These findings support the use of androgen antagonists to improve ovulation rates in PCOS patients (Abi *et al*. 2012). In addition, the most significant findings on specific genes discovered from GWAS or similar hypothesis-free studies involved in the development of PCOS, and the functionality of specific genes in the PCOS environment, may be revealed by the use of gene knock in/out mouse models in combination with a mouse PCOS model. These novel approaches will have great promise to enhance our understanding of the mechanisms underlying the development of PCOS and, in the future, may lead to the development of novel and evidence-based treatments for this disorder based on its pathogenic mechanisms.

Conclusions

There is now substantial evidence supporting an important role for direct AR-mediated androgen actions in follicle growth and health, ovarian function, and the development and/or maintenance of human PCOS. AR-mediated actions play an important role in optimising follicle development and ovulation. Studies have revealed that AR can act on ovarian follicle development from initiation of primordial follicles right through to ovulatory processes, and its actions are important in stimulating follicle growth and the maintenance of follicle/oocyte health and embryo viability. Granulosa cells have been identified as a crucial site for AR-mediated actions, however, it is clear that AR-regulated neuroendocrine control also plays a role in the regulation of normal ovarian function. It is now apparent that optimal levels of androgenic actions are required for normal ovarian function, with reduced levels, as observed in the ARKO models, leading to sub-fertility, while androgen excess has a negative effect on ovulation rates and is the most consistent feature of PCOS. Numerous androgen-induced PCOS animal models have confirmed that elevated androgen exposure can replicate many PCOS features. In particular, these animal models have identified that elevated androgen levels can dysregulate the intra-ovarian expression of key factors and processes involved in normal follicle development.

In conclusion, by combining clinical observations with judiciously selected and well-designed animal models, informative information on the specific AR-mediated mechanisms regulating female fertility and the development of PCOS is being revealed, with the hope that, in the future, elucidation of the role of androgens in female reproduction will translate into treatment that will assist in improving ovarian response and ART outcomes, and allow for the development of novel and evidence-based treatments for PCOS.

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Declaration of interest

The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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References

Abbott DH & Hearn JP 1978 The effects of neonatal exposure to testosterone on the development of behaviour in female marmoset monkeys. *Ciba Foundation Symposium* **14–16** 299–327.

Abbott DH, Barnett DK, Bruns CM & Dumesic DA 2005 Androgen excess fetal programming of female reproduction: a developmental aetiology for polycystic ovary syndrome? *Human Reproduction Update* **11** 357–374. (doi:10.1093/humupd/dmi013)

Abbott DH, Tarantal AF & Dumesic DA 2009 Fetal, infant, adolescent and adult phenotypes of polycystic ovary syndrome in prenatally androgenized female rhesus monkeys. *American Journal of Primatology* **71** 776–784. (doi:10.1002/ajp.20679)

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. (doi:10.1016/j.mce.2013.01.013)

Abi SB, Herkimer C, Lee JS, Veiga-Lopez A & Padmanabhan V 2012 Developmental programming: prenatal and postnatal contribution of androgens and insulin in the reprogramming of estradiol positive feedback disruptions in prenatal testosterone-treated sheep. *Endocrinology* **153** 2813–2822. (doi:10.1210/en.2011-2074)

Abraham GE 1974 Ovarian and adrenal contribution to peripheral androgens during the menstrual cycle. *Journal of Clinical Endocrinology and Metabolism* **39** 340–346. (doi:10.1210/jcem-39-2-340)

Almahbobi G, Nagodavithane A & Trounson AO 1995 Effects of epidermal growth factor, transforming growth factor α and androstenedione on follicular growth and aromatization in culture. *Human Reproduction* **10** 2767–2772.

Anderiesz C & Trounson AO 1995 The effect of testosterone on the maturation and developmental capacity of murine oocytes *in vitro*. *Human Reproduction* **10** 2377–2381. (doi:10.1093/oxfordjournals.humrep.a136302)

Anderson E, Lee MT & Lee GY 1992 Cystogenesis of the ovarian antral follicle of the rat: ultrastructural changes and hormonal profile following the administration of dehydroepiandrosterone. *Anatomical Record* **234** 359–382. (doi:10.1002/ar.1092340307)

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. (doi: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. (doi:10.1016/j.fertnstert.2008.06.035)

Azzolin GC & Saiduddin S 1983 Effect of androgens on the ovarian morphology of the hypophysectomized rat. *Proceedings of the Society for Experimental Biology and Medicine* **172** 70–73. (doi:10.3181/00379727-172-41528)

Baculescu N 2013 The role of androgen receptor activity mediated by the CAG repeat polymorphism in the pathogenesis of PCOS. *Journal of Medicine and Life* **6** 18–25.

Balasch J, Fabregues F, 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. (doi:10.1093/humrep/del052)

Barad DH & Gleicher N 2005 Increased oocyte production after treatment with dehydroepiandrosterone. *Fertility and Sterility* **84** 756. (doi:10.1016/j.fertnstert.2005.02.049)

Barnes RB, Rosenfield RL, Ehrmann DA, Cara JF, Cuttler L, Levitsky LL & Rosenthal IM 1994 Ovarian hyperandrogynism as a result of congenital adrenal virilizing disorders: evidence for perinatal masculinization of neuroendocrine function in women. *Journal of Clinical Endocrinology and Metabolism* **79** 1328–1333. (doi:10.1210/jcem.79.5.7962325)

Barry JA, Kay AR, Navaratnarajah R, Iqbal S, Bamfo JE, David AL, Hines M & Hardiman PJ 2010 Umbilical vein testosterone in female infants born to mothers with polycystic ovary syndrome is elevated to male levels. *Journal of Obstetrics and Gynaecology* **30** 444–446. (doi:10.3109/01443615.2010.485254)

Becerra-Fernandez A, Perez-Lopez G, Roman MM, Martin-Lazaro JF, Lucio Perez MJ, Asenjo AN, 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. (doi:10.1016/j.endonu.2014.01.010)

Beloosesky R, Gold R, Almog B, Sasson R, Dantes A, Land-Bracha A, Hirsh L, Itskovitz-Eldor J, Lessing JB, Homburg R *et al.* 2004 Induction of polycystic ovary by testosterone in immature female rats: modulation of apoptosis and attenuation of glucose/insulin ratio. *International Journal of Molecular Medicine* **14** 207–215.

Billiar RB, Richardson D, Schwartz R, Posner B & Little B 1987 Effect of chronically elevated androgen or estrogen on the glucose tolerance test and insulin response in female rhesus monkeys. *American Journal of Obstetrics and Gynecology* **157** 1297–1302. (doi:10.1016/S0002-9378(87)80319-8)

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. (doi:10.1093/humupd/dmr051)

Brawer JR, Naftolin F, Martin J & Sonnenschein C 1978 Effects of a single injection of estradiol valerate on the hypothalamic arcuate nucleus and on reproductive function in the female rat. *Endocrinology* **103** 501–512. (doi:10.1210/endo-103-2-501)

Broekmans FJ, Soules MR & Fauser BC 2009 Ovarian aging: mechanisms and clinical consequences. *Endocrine Reviews* **30** 465–493. (doi:10.1210/er.2009-0006)

Burek M, Duda M, Knapczyk K, Koziorowski M & Slomczynska M 2007 Tissue-specific distribution of the androgen receptor (AR) in the porcine fetus. *Acta Histochemica* **109** 358–365. (doi:10.1016/j.acthis.2007.03.003)

Burger HG 2002 Androgen production in women. *Fertility and Sterility* **77** (Suppl 4) S3–S5. (doi:10.1016/S0015-0282(02)02985-0)

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. (doi:10.1210/en.2014-1196)

Cardenas 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.

Cardenas H, Herrick JR & Pope WF 2002 Increased ovulation rate in gilts treated with dihydrotestosterone. *Reproduction* **123** 527–533. (doi:10.1530/rep.0.1230527)

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. (doi:10.1093/humrep/15.10.2129)

Catteau-Jonard S, Jamin SP, Leclerc A, Gonzales J, Dewailly D & di Clemente N 2008 Anti-Mullerian hormone, its receptor, FSH receptor, and androgen receptor genes are overexpressed by granulosa cells from stimulated follicles in women with polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism* **93** 4456–4461. (doi:10.1210/jc.2008-1231)

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Chadha S, Pache TD, Huikeshoven JM, Brinkmann AO & van der Kwast TH 1994 Androgen receptor expression in human ovarian and uterine tissue of long-term androgen-treated transsexual women. *Human Pathology* **25** 1198–1204. (doi:10.1016/0046-8177(94)90037-X)

Chamberlain NL, Driver ED & Miesfeld RL 1994 The length and location of CAG trinucleotide repeats in the androgen receptor N-terminal domain affect transactivation function. *Nucleic Acids Research* **22** 3181–3186. (doi:10.1093/nar/22.15.3181)

Chang RJ 2007 The reproductive phenotype in polycystic ovary syndrome. *Nature Clinical Practice. Endocrinology & Metabolism* **3** 688–695. (doi:10.1038/ncpendmet0637)

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. (doi:10.1152/ajpendo.00263.2013)

Choong CS, Kemppainen JA, Zhou ZX & Wilson EM 1996 Reduced androgen receptor gene expression with first exon CAG repeat expansion. *Molecular Endocrinology* **10** 1527–1535. (doi:10.1210/mend.10.12.8961263)

Clarke IJ, Scaramuzzi RJ & Short RV 1976 Sexual differentiation of the brain: endocrine and behavioural responses of androgenized ewes to oestrogen. *Journal of Endocrinology* **71** 175–176. (doi:10.1677/joe.0.0710175)

Clarke IJ, Scaramuzzi RJ & Short RV 1977 Ovulation in prenatally androgenized ewes. *Journal of Endocrinology* **73** 385–389. (doi:10.1677/joe.0.0730385)

Conway BA, Mahesh VB & Mills TM 1990 Effect of dihydrotestosterone on the growth and function of ovarian follicles in intact immature female rats primed with PMSG. *Journal of Reproduction and Fertility* **90** 267–277. (doi:10.1530/jrf.0.0900267)

Conway GS, Dewailly D, Diamanti-Kandarakis E, Escobar MH, Franks S, Gambineri A, Kelestimur F, Macut D, Micic D, Pasquali R *et al.* 2014 The polycystic ovary syndrome: an endocrinological perspective from the European Society of Endocrinology. *European Journal of Endocrinology* **171** P1–29. (doi:10.1530/EJE-14-0253)

Davison SL & Davis SR 2003 Androgens in women. *Journal of Steroid Biochemistry and Molecular Biology* **85** 363–366. (doi: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. (doi:10.1210/jc.2005-0212)

De Gendt K, Swinnen JV, Saunders PT, Schoonjans L, Dewerchin M, Devos A, Tan K, Atanassova N, Claessens F, Lecureuil C *et al.* 2004 A Sertoli cell-selective knockout of the androgen receptor causes spermatogenic arrest in meiosis. *PNAS* **101** 1327–1332. (doi:10.1073/pnas.0308114100)

Demissie M, Lazic M, Foecking EM, Aird F, Dunaif A & Levine JE 2008 Transient prenatal androgen exposure produces metabolic syndrome in adult female rats. *American Journal of Physiology. Endocrinology and Metabolism* **295** E262–E268. (doi:10.1152/ajpendo.90208.2008)

Diao FY, Xu M, Hu Y, Li J, Xu Z, Lin M, Wang L, Zhou Y, Zhou Z, Liu J *et al.* 2004 The molecular characteristics of polycystic ovary syndrome (PCOS) ovary defined by human ovary cDNA microarray. *Journal of Molecular Endocrinology* **33** 59–72. (doi:10.1677/jme.0.0330059)

Dittrich R, Beckmann MW, Oppelt PG, Hoffmann I, Lotz L, Kuwert T & Mueller A 2011 Thyroid hormone receptors and reproduction. *Journal of Reproductive Immunology* **90** 58–66. (doi:10.1016/j.jri.2011.02.009)

Dong J, Albertini DF, Nishimori K, Kumar TR, Lu N & Matzuk MM 1996 Growth differentiation factor-9 is required during early ovarian folliculogenesis. *Nature* **383** 531–535. (doi:10.1038/383531a0)

Duffy DM, Abdelgadir SE, Stott KR, Resko JA, Stouffer RL & Zelinsk-Wooten MB 1999 Androgen receptor mRNA expression in the rhesus monkey ovary. *Endocrine* **11** 23–30. (doi:10.1385/ENDO:11:1:23)

Dumesic DA, Abbott DH, Eisner JR & Goy RW 1997 Prenatal exposure of female rhesus monkeys to testosterone propionate increases serum luteinizing hormone levels in adulthood. *Fertility and Sterility* **67** 155–163. (doi:10.1016/S0015-0282(97)81873-0)

Dumesic DA, Schramm RD, Peterson E, Paprocki AM, Zhou R & Abbott DH 2002 Impaired developmental competence of oocytes in adult prenatally androgenized female rhesus monkeys undergoing gonadotropin stimulation for *in vitro* fertilization. *Journal of Clinical Endocrinology and Metabolism* **87** 1111–1119. (doi:10.1210/jcem.87.3.8287)

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. (doi:10.1210/jcem.85.11.6992)

Edwards DA 1971 Neonatal administration of androstenedione, testosterone or testosterone propionate: effects on ovulation, sexual receptivity and aggressive behavior in female mice. *Physiology & Behavior* **6** 223–228. (doi:10.1016/0031-9384(71)90030-8)

Eisner JR, Barnett MA, Dumesic DA & Abbott DH 2002 Ovarian hyperandrogenism in adult female rhesus monkeys exposed to prenatal androgen excess. *Fertility and Sterility* **77** 167–172. (doi:10.1016/S0015-0282(01)02947-8)

Eisner JR, Dumesic DA, Kemnitz JW, Colman RJ & Abbott DH 2003 Increased adiposity in female rhesus monkeys exposed to androgen excess during early gestation. *Obesity Research* **11** 279–286. (doi:10.1038/oby.2003.42)

Erickson GF, Magoffin DA, Dyer CA & Hofeditz C 1985 The ovarian androgen producing cells: a review of structure/function relationships. *Endocrine Reviews* **6** 371–399. (doi:10.1210/edrv-6-3-371)

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. (doi:10.1093/humrep/den428)

Faiman C, Reyes FI, Dent DW, Fuller GB, Hobson WC & Thliveris JA 1988 Effects of long-term testosterone exposure on ovarian function and morphology in the rhesus monkey. *Anatomical Record* **222** 245–251. (doi:10.1002/ar.1092220305)

Familiari G, Toscano V & Motta PM 1985 Morphological studies of polycystic mouse ovaries induced by dehydroepiandrosterone. *Cell and Tissue Research* **240** 519–528. (doi:10.1007/BF00216340)

Farookhi R 1985 Effects of aromatizable and nonaromatizable androgen treatments on luteinizing hormone receptors and ovulation induction in immature rats. *Biology of Reproduction* **33** 363–369. (doi:10.1095/biolreprod33.2.363)

Fauser BC, Tarlatzis BC, Rebar RW, Legro RS, Balen AH, Lobo R, Carmina E, Chang J, Yildiz BO, Laven JS *et al.* 2012 Consensus on women's health aspects of polycystic ovary syndrome (PCOS): the Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. *Fertility and Sterility* **97** 28–38. (doi:10.1016/j.fertnstert.2011.09.024)

Fels E & Bosch LR 1971 Effect of prenatal administration of testosterone on ovarian function in rats. *American Journal of Obstetrics and Gynecology* **111** 964–969.

Foecking EM, Szabo M, Schwartz NB & Levine JE 2005 Neuroendocrine consequences of prenatal androgen exposure in the female rat: absence of luteinizing hormone surges, suppression of progesterone receptor gene expression, and acceleration of the gonadotropin-releasing hormone pulse generator. *Biology of Reproduction* **72** 1475–1483. (doi:10.1095/biolreprod.105.039800)

Foradori CD, Weiser MJ & Handa RJ 2008 Non-genomic actions of androgens. *Frontiers in Neuroendocrinology* **29** 169–181. (doi:10.1016/j.yfrne.2007.10.005)

Forsdike RA, Hardy K, Bull L, Stark J, Webber LJ, Stubbs S, Robinson JE & Franks S 2007 Disordered follicle development in ovaries of prenatally androgenized ewes. *Journal of Endocrinology* **192** 421–428. (doi:10.1677/joe.1.07097)

Franks S 1995 Polycystic ovary syndrome. *New England Journal of Medicine* **333** 853–861. (doi:10.1056/NEJM199509283331307)

Franks S, Stark J & Hardy K 2008 Follicle dynamics and anovulation in polycystic ovary syndrome. *Human Reproduction Update* **14** 367–378. (doi:10.1093/humupd/dmn015)

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. (doi:10.1210/jcem-62-1-16)

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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. (doi:10.1016/j.fertnstert.2005.01.117)

Ghayee HK & Auchus RJ 2007 Basic concepts and recent developments in human steroid hormone biosynthesis. *Reviews in Endocrine & Metabolic Disorders* **8** 289–300. (doi:10.1007/s11154-007-9052-2)

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. (doi:10.1210/me.2003-0326)

Gilling-Smith C, Willis DS, Beard RW & Franks S 1994 Hypersecretion of androstenedione by isolated thecal cells from polycystic ovaries. *Journal of Clinical Endocrinology and Metabolism* **79** 1158–1165. (doi:10.1210/jcem.79.4.7962289)

Gleicher N & Barad DH 2011 Dehydroepiandrosterone (DHEA) supplementation in diminished ovarian reserve (DOR). *Reproductive Biology and Endocrinology* **9** 67. (doi:10.1186/1477-7827-9-67)

Goldstein JL & Wilson JD 1972 Studies on the pathogenesis of the pseudohermaphroditism in the mouse with testicular feminization. *Journal of Clinical Investigation* **51** 1647–1658. (doi:10.1172/JCI106966)

Goodarzi MO, Dumesic DA, Chazenbalk G & Azziz R 2011 Polycystic ovary syndrome: etiology, pathogenesis and diagnosis. *Nature Reviews. Endocrinology* **7** 219–231. (doi:10.1038/nrendo.2010.217)

Hague WM, Adams J, Rodda C, Brook CG, 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. (doi:10.1111/j.1365-2265.1990.tb03887.x)

Hamel M, Vanselow J, Nicola ES & Price CA 2005 Androstenedione increases cytochrome P450 aromatase messenger ribonucleic acid transcripts in nonluteinizing bovine granulosa cells. *Molecular Reproduction and Development* **70** 175–183. (doi:10.1002/mrd.20194)

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. (doi:10.1016/j.domaniend.2004.01.005)

Harlow CR, Shaw HJ, Hillier SG & Hodges JK 1988 Factors influencing follicle-stimulating hormone-responsive steroidogenesis in marmoset granulosa cells: effects of androgens and the stage of follicular maturity. *Endocrinology* **122** 2780–2787. (doi:10.1210/endo-122-6-2780)

Henmi H, Endo T, Nagasawa K, Hayashi T, Chida M, Akutagawa N, Iwasaki M, Kitajima Y, Kiya T, Nishikawa A *et al.* 2001 Lysyl oxidase and MMP-2 expression in dehydroepiandrosterone-induced polycystic ovary in rats. *Biology of Reproduction* **64** 157–162. (doi:10.1095/biolreprod64.1.157)

Hernandez Gifford JA, Hunzicker-Dunn ME & Nilson JH 2009 Conditional deletion of β-catenin mediated by Amhr2cre in mice causes female infertility. *Biology of Reproduction* **80** 1282–1292. (doi:10.1095/biolreprod.108.072280)

Hickey T, Chandy A & Norman RJ 2002 The androgen receptor CAG repeat polymorphism and X-chromosome inactivation in Australian Caucasian women with infertility related to polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism* **87** 161–165. (doi:10.1210/jcem.87.1.8137)

Hickey TE, Marrocco DL, Gilchrist RB, Norman RJ & Armstrong DT 2004 Interactions between androgen and growth factors in granulosa cell subtypes of porcine antral follicles. *Biology of Reproduction* **71** 45–52. (doi:10.1095/biolreprod.103.026484)

Hickey TE, Marrocco DL, Amato F, Ritter LJ, Norman RJ, Gilchrist RB & Armstrong DT 2005 Androgens augment the mitogenic effects of oocyte-secreted factors and growth differentiation factor 9 on porcine granulosa cells. *Biology of Reproduction* **73** 825–832. (doi:10.1095/biolreprod.104.039362)

Hickey M, Sloboda DM, Atkinson HC, Doherty DA, Franks S, Norman RJ, Newnham JP & Hart R 2009 The relationship between maternal and umbilical cord androgen levels and polycystic ovary syndrome in adolescence: a prospective cohort study. *Journal of Clinical Endocrinology and Metabolism* **94** 3714–3720. (doi:10.1210/jc.2009-0544)

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. (doi:10.1095/biolreprod44.3.561)

Hillier SG & Ross GT 1979 Effects of exogenous testosterone on ovarian weight, follicular morphology and intraovarian progesterone concentration in estrogen-primed hypophysectomized immature female rats. *Biology of Reproduction* **20** 261–268. (doi:10.1095/biolreprod20.2.261)

Hillier SG, Tetsuka M & Fraser HM 1997 Location and developmental regulation of androgen receptor in primate ovary. *Human Reproduction* **12** 107–111. (doi:10.1093/humrep/12.1.107)

Hogg K, Wood C, McNeilly AS & Duncan WC 2011 The *in utero* programming effect of increased maternal androgens and a direct fetal intervention on liver and metabolic function in adult sheep. *PLoS ONE* **6** e24877. (doi:10.1371/journal.pone.0024877)

Hogg K, Young JM, Oliver EM, Souza CJ, McNeilly AS & Duncan WC 2012 Enhanced thecal androgen production is prenatally programmed in an ovine model of polycystic ovary syndrome. *Endocrinology* **153** 450–461. (doi:10.1210/en.2011-1607)

Holdcraft RW & Braun RE 2004 Androgen receptor function is required in Sertoli cells for the terminal differentiation of haploid spermatids. *Development* **131** 459–467. (doi:10.1242/dev.00957)

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.

Hossain MM, Cao M, Wang Q, Kim JY, Schellander K, Tesfaye D & Tsang BK 2013 Altered expression of miRNAs in a dihydrotestosterone-induced rat PCOS model. *Journal of Ovarian Research* **6** 36. (doi:10.1186/1757-2215-6-36)

van Houten EL, Kramer P, McLuskey A, Karels B, Themmen AP & Visser JA 2012 Reproductive and metabolic phenotype of a mouse model of PCOS. *Endocrinology* **153** 2861–2869. (doi:10.1210/en.2011-1754)

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. (doi:10.1073/pnas.0404372101)

Huffman L & Hendricks SE 1981 Prenatally injected testosterone propionate and sexual behavior of female rats. *Physiology & Behavior* **26** 773–778. (doi:10.1016/0031-9384(81)90097-4)

Johansson J, Feng Y, Shao R, Lonn M, Billig H & Stener-Victorin E 2010 Intense electroacupuncture normalizes insulin sensitivity, increases muscle GLUT4 content, and improves lipid profile in a rat model of polycystic ovary syndrome. *American Journal of Physiology. Endocrinology and Metabolism* **299** E551–E559. (doi:10.1152/ajpendo.00323.2010)

Jorgez CJ, Klysik M, Jamin SP, Behringer RR & Matzuk MM 2004 Granulosa cell-specific inactivation of follistatin causes female fertility defects. *Molecular Endocrinology* **18** 953–967. (doi:10.1210/me.2003-0301)

Juengel JL, Heath DA, Quirke LD & McNatty KP 2006 Oestrogen receptor α and β, androgen receptor and progesterone receptor mRNA and protein localisation within the developing ovary and in small growing follicles of sheep. *Reproduction* **131** 81–92. (doi:10.1530/rep.1.00704)

Kamijo T, Mizunuma H, Yamada K & Ibuki Y 1994 *In vitro* fertilization of androgen sterilized mice. *Life Sciences* **55** 527–531. (doi:10.1016/0024-3205(94)00745-4)

Kato S 2002 Androgen receptor structure and function from knock-out mouse. *Clinical Pediatric Endocrinology* **11** 1–7. (doi:10.1297/cpe.11.1)

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. (doi:10.1371/journal.pone.0093805)

Keisler LW, Vom Saal FS, Keisler DH & Walker SE 1991 Hormonal manipulation of the prenatal environment alters reproductive morphology and increases longevity in autoimmune NZB/W mice. *Biology of Reproduction* **44** 707–716. (doi:10.1095/biolreprod44.4.707)

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. (doi:10.1210/en.2014-1050)

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Kendall SK, Samuelson LC, Saunders TL, Wood RI & Camper SA 1995 Targeted disruption of the pituitary glycoprotein hormone α-subunit produces hypogonadal and hypothyroid mice. *Genes and Development* **9** 2007–2019. (doi:10.1101/gad.9.16.2007)

Kim JJ, Choung SH, Choi YM, Yoon SH, Kim SH & Moon SY 2008 Androgen receptor gene CAG repeat polymorphism in women with polycystic ovary syndrome. *Fertility and Sterility* **90** 2318–2323. (doi:10.1016/j.fertnstert.2007.10.030)

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. (doi:10.1016/j.fertnstert.2010.07.1077)

Kim JY, Xue K, Cao M, Wang Q, Liu JY, Leader A, Han JY & Tsang BK 2013 Chemerin suppresses ovarian follicular development and its potential involvement in follicular arrest in rats treated chronically with dihydrotestosterone. *Endocrinology* **154** 2912–2923. (doi:10.1210/en.2013-1001)

King AJ, Olivier NB, Mohankumar PS, Lee JS, Padmanabhan V & Fink GD 2007 Hypertension caused by prenatal testosterone excess in female sheep. *American Journal of Physiology. Endocrinology and Metabolism* **292** E1837–E1841. (doi:10.1152/ajpendo.00668.2006)

Kuhn R & Torres RM 2002 Cre/loxP recombination system and gene targeting. *Methods in Molecular Biology* **180** 175–204.

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. (doi:10.1159/000178903)

Lai H, Jia X, Yu Q, Zhang C, Qiao J, Guan Y & Kang J 2014 High-fat diet induces significant metabolic disorders in a mouse model of polycystic ovary syndrome. *Biology of Reproduction* **91** 127. (doi:10.1095/biolreprod.114.120063)

Laisk T, Haller-Kikkatalo K, Laanpere M, Jakovlev U, Peters M, Karro H & Salumets A 2010 Androgen receptor epigenetic variations influence early follicular phase gonadotropin levels. *Acta Obstetricia et Gynecologica Scandinavica* **89** 1557–1563. (doi:10.3109/00016349.2010.526182)

Lee MT, Anderson E & Lee GY 1991 Changes in ovarian morphology and serum hormones in the rat after treatment with dehydroepiandrosterone. *Anatomical Record* **231** 185–192. (doi:10.1002/ar.1092310206)

Lenie S & Smitz 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. (doi: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. (doi:10.1095/biolreprod.108.069245)

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. (doi:10.1007/s12020-014-0200-7)

Longcope C 1986 Adrenal and gonadal androgen secretion in normal females. *Journal of Clinical Endocrinology and Metabolism* **15** 213–228. (doi:10.1016/S0300-595X(86)80021-4)

Lossl K, Andersen CY, Loft A, Freiesleben NL, Bangsboll S & Andersen AN 2008 Short-term androgen priming by use of aromatase inhibitor and hCG before controlled ovarian stimulation for IVF. A randomized controlled trial. *Human Reproduction* **23** 1820–1829. (doi:10.1093/humrep/den131)

Lubahn DB, Joseph DR, Sullivan PM, Willard HF, French FS & Wilson EM 1988 Cloning of human androgen receptor complementary DNA and localization to the X chromosome. *Science* **240** 327–330. (doi:10.1126/science.3353727)

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.

Lyon MF & Glenister PH 1974 Evidence from Tfm-O that androgen is inessential for reproduction in female mice. *Nature* **247** 366–367. (doi:10.1038/247366a0)

Lyon MF & Glenister PH 1980 Reduced reproductive performance in androgen-resistant Tfm/Tfm female mice. *Proceedings of the Royal Society of London. Series B, Biological Sciences* **208** 1–12. (doi:10.1098/rspb.1980.0040)

Maliqueo M, Lara HE, Sanchez F, Echiburu B, Crisosto N & Sir-Petermann T 2013 Placental steroidogenesis in pregnant women with polycystic ovary syndrome. *European Journal of Obstetrics, Gynecology, and Reproductive Biology* **166** 151–155. (doi:10.1016/j.ejogrb.2012.10.015)

Manikkam M, Steckler TL, Welch KB, Inskeep EK & Padmanabhan V 2006 Fetal programming: prenatal testosterone treatment leads to follicular persistence/luteal defects; partial restoration of ovarian function by cyclic progesterone treatment. *Endocrinology* **147** 1997–2007. (doi:10.1210/en.2005-1338)

Manneras L, Cajander S, Holmang A, Seleskovic Z, Lystig T, Lonn M & Stener-Victorin E 2007 A new rat model exhibiting both ovarian and metabolic characteristics of polycystic ovary syndrome. *Endocrinology* **148** 3781–3791. (doi:10.1210/en.2007-0168)

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. (doi:10.1093/humrep/dei481)

McDonald PG & Doughty C 1972 Comparison of the effect of neonatal administration of testosterone and dihydrotestosterone in the female rat. *Journal of Reproduction and Fertility* **30** 55–62. (doi:10.1530/jrf.0.0300055)

McGee WK, Bishop CV, Bahar A, Pohl CR, Chang RJ, Marshall JC, Pau FK, Stouffer RL & Cameron JL 2012 Elevated androgens during puberty in female rhesus monkeys lead to increased neuronal drive to the reproductive axis: a possible component of polycystic ovary syndrome. *Human Reproduction* **27** 531–540. (doi:10.1093/humrep/der393)

Meldrum DR, Chang RJ, Giudice LC, Balasch J & Barbieri RL 2013 Role of decreased androgens in the ovarian response to stimulation in older women. *Fertility and Sterility* **99** 5–11. (doi:10.1016/j.fertnstert.2012.10.011)

Mifsud A, Ramirez S & Yong EL 2000 Androgen receptor gene CAG trinucleotide repeats in anovulatory infertility and polycystic ovaries. *Journal of Clinical Endocrinology and Metabolism* **85** 3484–3488. (doi:10.1210/jcem.85.9.6832)

Miller WL & Auchus RJ 2011 The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. *Endocrine Reviews* **32** 81–151. (doi:10.1210/er.2010-0013)

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. (doi:10.1210/en.2012-1954)

Mori T, Suzuki A, Nishimura T & Kambegawa A 1977 Evidence for androgen participation in induced ovulation in immature rats. *Endocrinology* **101** 623–626. (doi:10.1210/endo-101-2-623)

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. (doi:10.1530/jrf.0.1130027)

Narkwichean A, Maalouf W, Campbell BK & Jayaprakasan K 2013 Efficacy of dehydroepiandrosterone to improve ovarian response in women with diminished ovarian reserve: a meta-analysis. *Reproductive Biology and Endocrinology* **11** 44. (doi:10.1186/1477-7827-11-44)

Narkwichean 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. (doi:10.1093/humrep/det408)

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. (doi:10.1210/mend.13.6.0311)

Nielsen ME, Rasmussen IA, Kristensen SG, Christensen ST, Mollgard K, Wreford AE, Byskov AG & Yding AC 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. (doi:10.1093/molehr/gaq073)

Notini AJ, Davey RA, McManus JF, Bate KL & Zajac JD 2005 Genomic actions of the androgen receptor are required for normal male sexual differentiation in a mouse model. *Journal of Molecular Endocrinology* **35** 547–555. (doi:10.1677/jme.1.01884)

Ohno S, Christian L & Attardi B 1973 Role of testosterone in normal female function. *Nature: New Biology* **243** 119–120. (doi:10.1038/243119a0)

Ortega HH, Salvetti NR & Padmanabhan V 2009 Developmental programming: prenatal androgen excess disrupts ovarian steroid receptor balance. *Reproduction* **137** 865–877. (doi:10.1530/REP-08-0491)

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Ota H, Fukushima M & Maki M 1983 Endocrinological and histological aspects of the process of polycystic ovary formation in the rat treated with testosterone propionate. *Tohoku Journal of Experimental Medicine* **140** 121–131. (doi:10.1620/tjem.140.121)

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. (doi:10.1016/j.rbmo.2009.05.007)

Pache TD & Fauser BC 1993 Polycystic ovaries in female-to-male transsexuals. *Clinical Endocrinology* **39** 702–703.

Padmanabhan V & Veiga-Lopez A 2013 Sheep models of polycystic ovary syndrome phenotype. *Molecular and Cellular Endocrinology* **373** 8–20. (doi:10.1016/j.mce.2012.10.005)

Padmanabhan V, Veiga-Lopez A, Abbott DH, Recabarren SE & Herkimer C 2010 Developmental programming: impact of prenatal testosterone excess and postnatal weight gain on insulin sensitivity index and transfer of traits to offspring of overweight females. *Endocrinology* **151** 595–605. (doi:10.1210/en.2009-1015)

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 non-obese pregnant women with polycystic ovary syndrome: a prospective controlled clinical study. *Steroids* **88C** 36–43. (doi: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. (doi:10.3109/09513590.2012.754876)

Pasquali R, Stener-Victorin E, Yildiz BO, Duleba AJ, Hoeger K, Mason H, Homburg R, Hickey T, Franks S, Tapanainen J *et al.* 2010 PCOS Forum: research in polycystic ovary syndrome today and tomorrow. *Clinical Endocrinology* **74** 424–433. (doi:10.1111/j.1365-2265.2010.03956.x)

Persani L, Rossetti R & Cacciatore C 2010 Genes involved in human premature ovarian failure. *Journal of Molecular Endocrinology* **45** 257–279. (doi:10.1677/JME-10-0070)

Pinilla L, Trimino E, Garnelo P, Bellido C, Aguilar R, Gaytan F & Aguilar E 1993 Changes in pituitary secretion during the early postnatal period and anovulatory syndrome induced by neonatal oestrogen or androgen in rats. *Journal of Reproduction and Fertility* **97** 13–20. (doi:10.1530/jrf.0.0970013)

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. (doi:10.1210/edrv-16-3-271)

Raja-Khan N, Urbanek M, Rodgers RJ & Legro RS 2014 The role of TGF-β in polycystic ovary syndrome. *Reproductive Sciences* **21** 20–31. (doi:10.1177/1933719113485294)

Ramezani TF, Noroozzadeh M, Zahediasl S, Piryaei A, Hashemi S & Azizi F 2014 The time of prenatal androgen exposure affects development of polycystic ovary syndrome-like phenotype in adulthood in female rats. *International Journal of Endocrinology and Metabolism* **12** e16502. (doi:10.5812/ijem.16502)

Recabarren SE, Padmanabhan V, Codner E, Lobos A, Duran C, Vidal M, Foster DL & Sir-Petermann T 2005 Postnatal developmental consequences of altered insulin sensitivity in female sheep treated prenatally with testosterone. *American Journal of Physiology. Endocrinology and Metabolism* **289** E801–E806. (doi:10.1152/ajpendo.00107.2005)

Rice S, Ojha K, Whitehead S & Mason H 2007 Stage-specific expression of androgen receptor, follicle-stimulating hormone receptor, and anti-Mullerian hormone type II receptor in single, isolated, human preantral follicles: relevance to polycystic ovaries. *Journal of Clinical Endocrinology and Metabolism* **92** 1034–1040. (doi:10.1210/jc.2006-1697)

Richardson SJ 1993 The biological basis of the menopause. *Baillière's Clinical Endocrinology and Metabolism* **7** 1–16.

Rittmaster RS 1999 Antiandrogen treatment of polycystic ovary syndrome. *Endocrinology and Metabolism Clinics of North America* **28** 409–421. (doi:10.1016/S0889-8529(05)70077-3)

Roland AV, Nunemaker CS, Keller SR & Moenter SM 2010 Prenatal androgen exposure programs metabolic dysfunction in female mice. *Journal of Endocrinology* **207** 213–223. (doi:10.1677/JOE-10-0217)

Romero S & Smitz 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. (doi: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. (doi:10.1093/humrep/deh098)

Salvetti NR, Alfaro NS, Velazquez MM, Amweg AN, Matiller V, Diaz PU & Ortega HH 2012 Alteration in localization of steroid hormone receptors and coregulatory proteins in follicles from cows with induced ovarian follicular cysts. *Reproduction* **144** 723–735. (doi:10.1530/REP-12-0188)

Sander V, Luchetti CG, Solano ME, Elia E, Di GG, Gonzalez C & Motta AB 2006 Role of the *N,N'*-dimethylbiguanide metformin in the treatment of female prepuberal BALB/c mice hyperandrogenized with dehydroepiandrosterone. *Reproduction* **131** 591–602. (doi:10.1530/rep.1.00941)

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. (doi:10.1210/en.2005-0322)

Savabieasfahani M, Lee JS, Herkimer C, Sharma TP, Foster DL & Padmanabhan V 2005 Fetal programming: testosterone exposure of the female sheep during midgestation disrupts the dynamics of its adult gonadotropin secretion during the periovulatory period. *Biology of Reproduction* **72** 221–229. (doi:10.1095/biolreprod.104.031070)

Schuring AN, Welp A, Gromoll J, Zitzmann M, Sonntag B, Nieschlag E, Greb RR & Kiesel L 2012 Role of the CAG repeat polymorphism of the androgen receptor gene in polycystic ovary syndrome (PCOS). *Experimental and Clinical Endocrinology & Diabetes* **120** 73–79. (doi:10.1055/s-0031-1291343)

Sen A & Hammes SR 2010 Granulosa cell-specific androgen receptors are critical regulators of ovarian development and function. *Molecular Endocrinology* **24** 1393–1403. (doi:10.1210/me.2010-0006)

Sen A, De Castro I, Defranco DB, Deng FM, Melamed J, Kapur P, Raj GV, Rossi R & Hammes SR 2012 Paxillin mediates extranuclear and intranuclear signaling in prostate cancer proliferation. *Journal of Clinical Investigation* **122** 2469–2481. (doi:10.1172/JCI62044)

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. (doi:10.1073/pnas.1318978111)

Shi XB, Xue L, Yang J, Ma AH, Zhao J, Xu M, Tepper CG, Evans CP, Kung HJ & deVere White RW 2007 An androgen-regulated miRNA suppresses Bak1 expression and induces androgen-independent growth of prostate cancer cells. *PNAS* **104** 19983–19988. (doi:10.1073/pnas.0706641104)

Shiina 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. (doi:10.1073/pnas.0506736102)

Simanainen U, Brogley M, Gao YR, Jimenez M, Harwood DT, Handelsman DJ & Robins DM 2011 Length of the human androgen receptor glutamine tract determines androgen sensitivity *in vivo*. *Molecular and Cellular Endocrinology* **342** 81–86. (doi:10.1016/j.mce.2011.05.011)

Sipe CS, Thomas MR, Stegmann BJ & Van Voorhis BJ 2010 Effects of exogenous testosterone supplementation in gonadotrophin stimulated cycles. *Human Reproduction* **25** 690–696. (doi:10.1093/humrep/dep442)

Sir-Petermann T, Maliqueo M, Angel B, Lara HE, Perez-Bravo F & Recabarren SE 2002 Maternal serum androgens in pregnant women with polycystic ovarian syndrome: possible implications in prenatal androgenization. *Human Reproduction* **17** 2573–2579. (doi:10.1093/humrep/17.10.2573)

Skrgatic L, Baldani DP, Cerne JZ, Ferk P & Gersak K 2012 CAG repeat polymorphism in androgen receptor gene is not directly associated with polycystic ovary syndrome but influences serum testosterone levels. *Journal of Steroid Biochemistry and Molecular Biology* **128** 107–112. (doi:10.1016/j.jsbmb.2011.11.006)

Slob AK, den Hamer R, Woutersen PJ & van der Werff ten Bosch JJ 1983 Prenatal testosterone propionate and postnatal ovarian activity in the rat. *Acta Endocrinologica* **103** 420–427. (doi:10.1530/acta.0.1030420)

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. (doi:10.1016/S0378-4320(00)00225-6)

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Slomczynska M, Duda M & Sl zak K 2001 The expression of androgen receptor, cytochrome P450 aromatase and FSH receptor mRNA in the porcine ovary. *Folia Histochemica et Cytobiologica* **39** 9–13.

Smith P, Steckler TL, Veiga-Lopez A & Padmanabhan V 2009 Developmental programming: differential effects of prenatal testosterone and dihydrotestosterone on follicular recruitment, depletion of follicular reserve, and ovarian morphology in sheep. *Biology of Reproduction* **80** 726–736. (doi:10.1095/biolreprod.108.072801)

Sorensen AE, Wissing ML, Salo S, Englund AL & Dalgaard LT 2014 MicroRNAs related to polycystic ovary syndrome (PCOS). *Genes* **5** 684–708. (doi:10.3390/genes5030684)

Spinder T, Spijkstra JJ, van den Tweel JG, Burger CW, van Kessel H, Hompes PG & Gooren LJ 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. (doi:10.1210/jcem-69-1-151)

Steckler T, Manikkam M, Inskeep EK & Padmanabhan V 2007 Developmental programming: follicular persistence in prenatal testosterone-treated sheep is not programmed by androgenic actions of testosterone. *Endocrinology* **148** 3532–3540. (doi:10.1210/en.2007-0339)

Steckler TL, Herkimer C, Dumesic DA & Padmanabhan V 2009 Developmental programming: excess weight gain amplifies the effects of prenatal testosterone excess on reproductive cyclicity – implication for polycystic ovary syndrome. *Endocrinology* **150** 1456–1465. (doi:10.1210/en.2008-1256)

Stener-Victorin E, Ploj K, Larsson BM & Holmang A 2005 Rats with steroid-induced polycystic ovaries develop hypertension and increased sympathetic nervous system activity. *Reproductive Biology and Endocrinology* **3** 44. (doi:10.1186/1477-7827-3-44)

Stubbs SA, Webber LJ, Stark J, Rice S, Margara R, Lavery S, Trew GH, Hardy K & Franks S 2013 Role of Insulin-like growth factors in initiation of follicle growth in normal and polycystic human ovaries. *Journal of Clinical Endocrinology and Metabolism* **98** 3298–3305. (doi:10.1210/jc.2013-1378)

Sugawa F, Wada Y, Maruyama T, Uchida H, Ishizuka B & Ogata T 2008 Premature ovarian failure and androgen receptor gene CAG repeat lengths weighted by X chromosome inactivation patterns. *Fertility and Sterility* **91** 649–652. (doi:10.1016/j.fertnstert.2007.11.085)

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. (doi:10.1073/pnas.0308058101)

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.

Swanson HE & Werff ten Bosch JJ 1964 The "early-androgen" syndrome; differences in response to pre-natal and post-natal administration of various doses of testosterone propionate in female and male rats. *Acta Endocrinologica* **47** 37–50.

Swanson HE & Werff ten Bosch JJ 1965 The "early-androgen" syndrome; effects of pre-natal testosterone propionate. *Acta Endocrinologica* **50** 379–390.

Szoltys M & Slomczynska M 2000 Changes in distribution of androgen receptor during maturation of rat ovarian follicles. *Experimental and Clinical Endocrinology & Diabetes* **108** 228–234. (doi:10.1055/s-2000-7747)

Takahashi K, Eda Y, Abu-Musa A, Okada S, Yoshino K & Kitao M 1994 Transvaginal ultrasound imaging, histopathology and endocrinopathy in patients with polycystic ovarian syndrome. *Human Reproduction* **9** 1231–1236.

Tandulwadkar SR, Lodha PA & Mangeshikar NT 2014 Obstetric complications in women with IVF conceived pregnancies and polycystic ovarian syndrome. *Journal of Human Reproductive Sciences* **7** 13–18. (doi:10.4103/0974-1208.130802)

Teixeira Filho FL, Baracat EC, Lee TH, Suh CS, Matsui M, Chang RJ, Shimasaki S & Erickson GF 2002 Aberrant expression of growth differentiation factor-9 in oocytes of women with polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism* **87** 1337–1344. (doi:10.1210/jcem.87.3.8316)

Treloar OL, Wolf RC & Meyer RK 1972 Failure of a single neonatal dose of testosterone to alter ovarian function in the Rhesus monkey. *Endocrinology* **90** 281–284. (doi:10.1210/endo-90-1-281)

Tyndall V, Broyde M, Sharpe R, Welsh M, Drake AJ & McNeilly AS 2012 Effect of androgen treatment during foetal and/or neonatal life on ovarian function in prepubertal and adult rats. *Reproduction* **143** 21–33. (doi:10.1530/REP-11-0239)

Veiga-Lopez A, Astapova OI, Aizenberg EF, Lee JS & Padmanabhan V 2009 Developmental programming: contribution of prenatal androgen and estrogen to estradiol feedback systems and periovulatory hormonal dynamics in sheep. *Biology of Reproduction* **80** 718–725. (doi:10.1095/biolreprod.108.074781)

Veiga-Lopez A, Moeller J, Patel D, Ye W, Pease A, Kinns J & Padmanabhan V 2013 Developmental programming: impact of prenatal testosterone excess on insulin sensitivity, adiposity, and free fatty acid profile in postpubertal female sheep. *Endocrinology* **154** 1731–1742. (doi:10.1210/en.2012-2145)

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. (doi:10.1172/JCI2081)

Vendola K, Zhou J, Wang J, Famuyiwa OA, Bievre M & Bondy CA 1999*a* Androgens promote oocyte insulin-like growth factor I expression and initiation of follicle development in the primate ovary. *Biology of Reproduction* **61** 353–357. (doi:10.1095/biolreprod61.2.353)

Vendola K, Zhou J, Wang J & Bondy CA 1999*b* Androgens promote insulin-like growth factor-I and insulin-like growth factor-I receptor gene expression in the primate ovary. *Human Reproduction* **14** 2328–2332. (doi:10.1093/humrep/14.9.2328)

Walters KA, Binnie JP, Campbell BK, Armstrong DG & Telfer EE 2006 The effects of IGF-I on bovine follicle development and IGFBP-2 expression are dose and stage dependent. *Reproduction* **131** 515–523. (doi:10.1530/rep.1.00682)

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. (doi:10.1210/en.2007-0248)

Walters KA, Allan CM & Handelsman DJ 2008 Androgen actions and the ovary. *Biology of Reproduction* **78** 380–389. (doi:10.1095/biolreprod.107.064089)

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. (doi:10.1210/en.2008-1750)

Walters KA, Simanainen U & Handelsman DJ 2010 Molecular insights into androgen actions in male and female reproductive function from androgen receptor knockout models. *Human Reproduction Update* **16** 543–558. (doi:10.1093/humupd/dmq003)

Walters KA, Middleton LJ, Joseph SR, Hazra R, Jimenez M, Simanainen U, Allan CM & Handelsman DJ 2012*a* Targeted loss of androgen receptor signaling in murine granulosa cells of preantral and antral follicles causes female subfertility. *Biology of Reproduction* **87** 151. (doi:10.1095/biolreprod.112.102012)

Walters KA, Allan CM & Handelsman DJ 2012*b* Rodent models for human polycystic ovary syndrome. *Biology of Reproduction* **86** 149. 1–149,12. (doi:10.1095/biolreprod.111.097808)

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. (doi:10.1210/endo.142.11.8482)

Wang Q, Kim JY, Xue K, Liu JY, Leader A & Tsang BK 2012 Chemerin, a novel regulator of follicular steroidogenesis and its potential involvement in polycystic ovarian syndrome. *Endocrinology* **153** 5600–5611. (doi:10.1210/en.2012-1424)

Ward RC, Costoff A & Mahesh VB 1978 The induction of polycystic ovaries in mature cycling rats by the administration of dehydroepiandrosterone (DHA). *Biology of Reproduction* **18** 614–623. (doi:10.1095/biolreprod18.4.614)

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Ware VC 1982 The role of androgens in follicular development in the ovary. I. A quantitative analysis of oocyte ovulation. *Journal of Experimental Zoology* **222** 155–167. (doi:10.1002/jez.1402220207)

Weil SJ, Vendola K, Zhou J, Adesanya OO, Wang J, Okafor J & Bondy CA 1998 Androgen receptor gene expression in the primate ovary: cellular localization, regulation, and functional correlations. *Journal of Clinical Endocrinology and Metabolism* **83** 2479–2485. (doi:10.1210/jcem.83.7.4917)

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. (doi:10.1210/jcem.84.8.5929)

Weisz J & Lloyd CW 1965 Estrogen and androgen production *in vitro* from 7-3-H-progesterone by normal and polycystic rat ovaries. *Endocrinology* **77** 735–744. (doi:10.1210/endo-77-4-735)

West C, Foster DL, Evans NP, Robinson J & Padmanabhan V 2001 Intra-follicular activin availability is altered in prenatally-androgenized lambs. *Molecular and Cellular Endocrinology* **185** 51–59. (doi:10.1016/S0303-7207(01)00632-3)

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. (doi: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. (doi:10.1093/humrep/deq220)

Wu XY, Li ZL, Wu CY, Liu YM, Lin H, Wang SH & Xiao WF 2010*a* Endocrine traits of polycystic ovary syndrome in prenatally androgenized female Sprague–Dawley rats. *Endocrine Journal* **57** 201–209. (doi:10.1507/endocrj.K09E-205)

Wu CH, Yang JG, Yang JJ, Lin YM, Tsai HD, Lin CY & Kuo PL 2010*b* Androgen excess down-regulates connexin43 in a human granulosa cell line. *Fertility and Sterility* **94** 2938–2941. (doi:10.1016/j.fertnstert.2010.06.077)

Wu YG, Bennett J, Talla D & Stocco C 2011 Testosterone, not 5α-dihydrotestosterone, stimulates LRH-1 leading to FSH-independent expression of Cyp19 and P450scc in granulosa cells. *Molecular Endocrinology* **25** 656–668. (doi:10.1210/me.2010-0367)

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. (doi:10.1210/me.2014-1154)

Xita N & Tsatsoulis A 2006 Review: fetal programming of polycystic ovary syndrome by androgen excess: evidence from experimental, clinical, and genetic association studies. *Journal of Clinical Endocrinology and Metabolism* **91** 1660–1666. (doi:10.1210/jc.2005-2757)

Xu N, Kwon S, Abbott DH, Geller DH, Dumesic DA, Azziz R, Guo X & Goodarzi MO 2011 Epigenetic mechanism underlying the development of polycystic ovary syndrome (PCOS)-like phenotypes in prenatally androgenized rhesus monkeys. *PLoS ONE* **6** e27286. (doi:10.1371/journal.pone.0027286)

Yan X, Dai X, Wang J, Zhao N, Cui Y & Liu J 2013 Prenatal androgen excess programs metabolic derangements in pubertal female rats. *Journal of Endocrinology* **217** 119–129. (doi:10.1530/JOE-12-0577)

Yanes LL, Romero DG, Moulana M, Lima R, Davis DD, Zhang H, Lockhart R, Racusen LC & Reckelhoff JF 2011 Cardiovascular-renal and metabolic characterization of a rat model of polycystic ovary syndrome. *Gender Medicine* **8** 103–115. (doi:10.1016/j.genm.2010.11.013)

Yang MY & Fortune JE 2006 Testosterone stimulates the primary to secondary follicle transition in bovine follicles *in vitro*. *Biology of Reproduction* **75** 924–932. (doi:10.1095/biolreprod.106.051813)

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. (doi:10.1210/en.2009-0751)

Yazawa T, Kawabe S, Kanno M, Mizutani T, Imamichi Y, Ju Y, Matsumura T, Yamazaki Y, Usami Y, Kuribayashi M *et al.* 2013 Androgen/androgen receptor pathway regulates expression of the genes for cyclooxygenase-2 and amphiregulin in periovulatory granulosa cells. *Molecular and Cellular Endocrinology* **369** 42–51. (doi:10.1016/j.mce.2013.02.004)

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. (doi: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** 108–115. (doi:10.1016/j.fertnstert.2014.03.044)

Zawadski JK & Dunaif A 1992 Diagnostic criteria for polycystic ovary syndrome: towards a rational approach. In *Polycystic Ovary Syndrome*, pp 377–384. Eds A Dunaif, JR Givens, FP Haseltine& GR Merriam. Boston: Blackwell Scientific Publications.

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.

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