REVIEW

Key signalling pathways underlying the aetiology of polycystic ovary syndrome

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Abstract

Polycystic ovary syndrome (PCOS) is a common endocrine condition characterised by a range of reproductive, endocrine, metabolic and psychological abnormalities. Reports estimate that around 10% of women of reproductive age are affected by PCOS, representing a significant prevalence worldwide, which poses a high economic health burden. As the origin of PCOS remains largely unknown, there is neither a cure nor mechanism-based treatments leaving patient management suboptimal and focused solely on symptomatic treatment. However, if the underlying mechanisms underpinning the development of PCOS were uncovered then this would pave the way for the development of new interventions for PCOS. Recently, there have been significant advances in our understanding of the underlying pathways likely involved in PCOS pathogenesis. Key insights include the potential involvement of androgens, insulin, anti-Müllerian hormone and transforming growth factor beta in the development of PCOS. This review will summarise the significant scientific discoveries on these factors that have enhanced our knowledge of the mechanisms involved in the development of PCOS and discuss the impact these insights may have in shaping the future development of effective strategies for women with PCOS.

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine condition affecting females of reproductive age, with women suffering from a wide range of ill-health symptoms including endocrine, reproductive, metabolic and psychological features (March et al. 2010, Dumesic et al. 2015, Bozdag et al. 2016, Skiba et al. 2018). Reproductive features include the development of polycystic ovaries, where aberrant follicle maturation leads to ovulatory dysfunction associated with reduced fertility (Dumesic et al. 2015). Furthermore, if a woman with PCOS does achieve pregnancy she has a greater risk of pregnancy complications, such as gestational diabetes, hypertensive disorders and premature delivery (Boyle & Teede 2016). Hormonal dysregulation is common with hyperandrogenism, and luteinising hormone (LH) hypersecretion is frequently observed in women with
PCOS (Dumesic et al. 2015). Metabolic dysfunction is also a key characteristic, as PCOS is strongly associated with obesity, metabolic syndrome, hyperinsulinaemia, insulin resistance (IR), dyslipidaemia, hepatic steatosis and an increased risk of developing type 2 diabetes and cardiovascular disease (Shorakae et al. 2014, Dumesic et al. 2015, Rubin et al. 2017, Glintborg et al. 2018). Additionally, PCOS is now recognised to be associated with psychosocial issues, with the prevalence of depression and anxiety reported to be higher in women affected by PCOS (Dumesic et al. 2015).

Three different diagnostic criteria have been developed to diagnose PCOS, which are the National Institutes of Health (NIH) (Zawadzki & Dunaif 1992), Rotterdam (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2004) and Androgen Excess and PCOS (AE-PCOS) criteria (Azziz et al. 2009). However, the Rotterdam criteria are now widely recognised as the optimal clinical diagnostic criteria to be used for the diagnosis of PCOS (Teede et al. 2018). This requires a woman to have two of three cardinal symptoms – hyperandrogenism (biochemical or clinical), oligoovulation or anovulation and polycystic ovarian morphology (PCOM) observed on ultrasound, after exclusion of all other differential diagnoses (Teede et al. 2018). Even though PCOS is a highly prevalent condition with major health and economic impacts, a cure for PCOS remains to be identified. This is due to the fact that the origins and underlying mechanisms that drive the development of PCOS remain unclear, and consequently, no drug(s) has been specifically approved for PCOS (Escobar-Morreale 2018). Currently, the medical management of women with PCOS remains suboptimal as it focuses only on the treatment of the symptoms rather than targeting the underlying mechanisms involved. Due to this lack of knowledge on the aetiology and pathophysiology of PCOS, pioneering discovery studies are essential in this field to ensure the development of safe and effective treatments for the prevention and/or amelioration of PCOS in women.

Clinical observation studies have, and continue to provide, important information to guide research into the potential underlying pathophysiological mechanisms governing the development of PCOS. However, determining the key drivers of the aetiology and pathophysiology of PCOS is challenging due to the ethical and logistical constraints on clinical studies. On the other hand, fully controlled studies aimed at defining the mechanistic pathogenesis of PCOS are possible in well-designed studies using naturally occurring or experimentally induced animal models that mimic the multiple features observed in women with PCOS (Stener-Victorin et al. 2020). Findings from preclinical rodent, sheep and non-human primate PCOS animal models have significantly enhanced our understanding of PCOS aetiology and hence are a key tool in providing insights into the specific mechanisms underpinning the pathogenesis of PCOS (Walters et al. 2018a, Abbott et al. 2019, Cardoso & Padmanabhan 2019, Stener-Victorin et al. 2019, Stener-Victorin et al. 2020).

The study of potential causes and the pathophysiology of PCOS has evolved considerably since PCOS was first described. Initially, research focused on the reproductive phenotype, including anovulation and hyperandrogenism, and the symptoms it causes. In the 1980s, it was observed that women with PCOS were whole-body insulin-resistant and that they develop type 2 diabetes at a higher rate (Cotrozzi et al. 1983). This broadened the scope of research to tissues and hormones not directly associated with reproduction. In the late 1990s, it was found that treatment of pregnant animals with androgens can lead to a PCOS phenotype in female offspring (Dumesic et al. 1997, Abbott et al. 1998), implicating a fetal origin and androgens as a cause of PCOS. The finding that PCOS was heritable (Franks et al. 1997) turned the focus to genetic studies which identified a number of genetic loci associated with PCOS. However, it was clear that these loci could only account for a small proportion of the heritability and that likely rare coding and non-coding mutants were an additional cause (Dapas & Dunaif 2020). In the 1980s, anti-Müllerian hormone (AMH) was discovered to be produced by ovarian granulosa cells and this led to clinical studies to understand its role in fertility and these were naturally extended to PCOS (Fallat et al. 1997, Cook et al. 2002). Additional studies also linked another pathway of the transforming growth factor beta (TGFβ) superfamily, namely TGFβs, to PCOS (Raja-Khan et al. 2010, Hatzirodos et al. 2011). For this review, we thought it timely to consider the different signalling pathways of androgens, insulin, AMH and TGFβ in the aetiology of PCOS.

Androgens

Androgen biosynthesis and action

It is well known that circulating androgens are present in women including, in descending order of serum concentrations, DHEAS, DHEA, androstenedione, testosterone and dihydrotestosterone (DHT) (Davison et al. 2005). More recently, 11-oxygenated androgens have also been identified in women, and mirroring the classic androgens (testosterone and androstenedione), serum levels...
of 11-oxygenated androgens (11-oxygenated androgens 11β-hydroxyandrostenedione, 11-ketoandrostenedione, 11β-hydroxytestosterone and 11-ketotestosterone) are significantly higher in PCOS than in control subjects (O’Reilly et al. 2017, Turcu & Auchus 2017). PCOS ovaries produce more androgens because PCOM ovaries contain a large population of small antral follicles but the population is not static as the follicles show high rates of atresia, with constant replacement from newer developing follicle cohorts (Jarrett et al. 2020). These follicles have an atypical stroma and theca interna that produces androgens (Lizneva et al. 2016). In PCOS ovaries, the thecal cells of the theca interna are stimulated by elevated levels of LH and insulin (Wu et al. 2014) and are also more androgenic. Even when maintained and passaged in long-term cultures, they still continue to produce elevated basal and cAMP-stimulated progesterone, DHEA, 17α-hydroxyprogesterone, androstenedione and testosterone production on a per cell basis (Nelson et al. 1999) due to increased expression of steroidogenic enzymes HSD3B and CYP17A1 on a per cell basis (Nelson et al. 2001).

Androgens have been shown to play an essential indirect role in ovarian follicular development as androgens are obligatory precursors for oestradiol (Hillier et al. 1994, Ghayee & Auchus 2007). A direct role for androgens in regulating female reproductive function has also been confirmed as androgens mediate their actions via the androgen receptor (AR), and transgenic female mice with a loss of AR signalling are sub-fertile (Walters et al. 2007). Across the female hypothalamic–pituitary–gonadal (HPG) axis, AR mRNA and protein have been identified, including in the brain, ovarian stroma, ovarian follicles and corpora lutea (Walters & Handelsman 2018). Ovarian AR expression has been confirmed in numerous mammalian species and is evolutionarily conserved, supporting a universal role for AR-mediated androgen actions on the function of the ovary (Walters & Handelsman 2018). AR expression is present in fetal (Wilson & McPhaul 1996) and adult (Suzuki et al. 1994) ovaries, and within ovarian follicles, it is present at the majority of follicular stages. However, differential temporal and special patterns of expression are present across different follicular stages, indicating distinct roles for AR-mediated actions during the various stages of follicular development. Generally, AR expression is present as follicles enter the growing pool and is expressed in the oocyte, granulosa cells and theca cells of preantral follicles, but during antral follicular growth, AR expression progressively declines in the outer mural granulosa cells but remains intense in the cumulus cells (Szolty & Słomczynska 2000, Lenie & Smitz 2009). Classically, the bioactive androgens, testosterone and DHT, bind directly to the AR, while the pro-androgens, DHEA and androstenedione, require conversion to testosterone or DHT to exert androgenic effects (Burger 2002). However, direct effects of DHEA in the brain and vascular endothelial cells have been described (Liu et al. 2008, Soma et al. 2015).

Clinical symptoms associated with androgen excess

The most common features present in women suffering from PCOS are due to hyperandrogenism, which is present in ~60% of patients (Livadas et al. 2014, Teede et al. 2018). Serum levels of testosterone, androstenedione and DHEAS and the enzyme required to transform pro-androgens into bioactive androgens – 3β-hydroxysteroid dehydrogenase (3β-HSD) – have all been reported to be elevated in hyperandrogenic PCOS females (Keele et al. 2014, Palomba et al. 2014, O’Reilly et al. 2017). Furthermore, it has been reported that 11-oxygenated androgens were observed to be significantly elevated in women with PCOS and cumulatively constitute a greater proportion of total circulating androgens than classic androgens (O’Reilly et al. 2017, Yoshida et al. 2018). Hirsutism, acne and alopecia are all manifestations of androgen excess observed in PCOS patients (Azziz et al. 2004). Androgen excess is also reported to be positively correlated with the presence of several PCOS traits, including increased intra-abdominal fat mass, triglyceride levels, LH/follicle-stimulating hormone (FSH) ratio and IR but decreased high-density lipoprotein levels (Daan et al. 2015, Dumesic et al. 2016, Li et al. 2016, Couto Alves et al. 2017). Moreover, a potentially important role for 11-oxygenated androgens as biomarkers of metabolic risk has been proposed, as a number of 11-oxygenated steroids are described to exhibit a close correlation with hyperinsulinaemia (O’Reilly et al. 2017).

Evidence supporting a role for androgens in PCOS aetiology

Evidence indicating a causative role for androgen excess in the development of PCOS symptoms comes from the observation that congenital adrenal hyperplasia patients, who exhibit elevated androgen levels, display the appearance of PCOM and chronic anovulation (Grynberget al. 2010, Pignatelliet al. 2019), similar to features observed in women with PCOS. A variety of methods including treatment with androgens, oestrogens or anti-progestins, genetic manipulation and chronic exposure to light have all been reported as methods to generate animal models that closely replicate features of human PCOS.
(Stener-Victorin et al. 2020). However, the most instructive information on the potential mechanisms that underlie the origins of PCOS has come from preclinical PCOS animal models generated by exposure to androgen excess (Stener-Victorin et al. 2020). The findings that exposure to androgen excess in these PCOS animal models consistently replicates a wide range of PCOS symptoms observed in humans and provide strong evidence that hyperandrogenism is a major driver in PCOS pathogenesis. Hyperandrogenic-induced PCOS symptoms have been generated in female rodents (Walters et al. 2012), sheep (Cardoso & Padmanabhan 2019) and non-human primates (Abbott et al. 2019) by prenatal or postnatal injection or s.c. implants containing the proandrogen DHEA, the androgens testosterone or DHT or the aromatase inhibitor letrozole. Furthermore, naturally hyperandrogenic females found in non-human primates and in cattle have been described (Summers et al. 2014, Abbott et al. 2017, Abedal-Majed & Cupp 2019). Indeed, spontaneously hyperandrogenic non-human primate females exhibit PCOS-like diagnostic traits together with additional neuroendocrine, ovarian and metabolic traits that are commonly observed in women with PCOS (Abbott et al. 2017).

As there is now strong evidence from observational clinical studies and the generation and characterisation of androgen excess-induced PCOS animal models supporting a role for hyperandrogenism in the developmental origins of PCOS, recent research has started to focus on unravelling the mechanism of androgenic actions driving the development of PCOS phenotypes. As androgens mediate their actions directly via the AR, several studies have utilised interventions that block AR actions or transgenic mouse models that have a loss of AR signalling (Fig. 1) as tools to provide insight into androgenic actions involved in the development of PCOS. Blockade of AR signalling with the AR antagonist flutamide has a beneficial effect on PCOS-associated reproductive and metabolic abnormalities in murine (Sullivan & Moenter 2004, Ryan et al. 2018, Silva et al. 2018) and ovine (Sheppard et al. 2011, Padmanabhan et al. 2015, Cardoso et al. 2016, Lu et al. 2016, Landers et al. 2020) hyperandrogenised PCOS models. Additionally, in utero exposure to excess levels of...
DHT replicates reproductive features of human PCOS in adult WT (wild type) mice but not global ARKO (androgen receptor knock out) mice, inferring that androgen excess during prenatal life acts directly via the AR to induce PCOS traits in adult life (Caldwell et al. 2015, 2017).

While there is cumulating evidence supporting a key role for AR signalling in the development of PCOS (Walters 2015, Walters et al. 2018c), testosterone, a major circulating androgen known to be elevated in the majority of women with PCOS (Davisson & Davis 2003, Handelsman et al. 2017), can be aromatised into oestradiol (Hillier et al. 1994, Ghayee & Auchus 2007) and act via the oestrogen receptor (ER). Hence, this raises the question of whether along with direct AR signalling pathways, indirect androgen actions mediated via oestrogenic pathways may also be involved in the development of PCOS symptoms. Data from the sheep PCOS model suggests that the developmental programming of PCOS could occur via both androgenic and oestrogenic pathways as prenatal treatment with testosterone increases not only the maternal but also the fetal concentrations of androgens and oestradiol (Veiga-Lopez et al. 2011, Abi Salloum et al. 2015). Comparisons of the differential effects of prenatal testosterone (aromatisable androgen) and DHT (non-aromatisable androgen) on ovarian phenotype in sheep revealed androgenic programming mediated the observed increase in follicular recruitment but alterations in post-pubertal follicular growth appeared to be facilitated through oestrogenic programming (Smith et al. 2009).

A recent publication further explored the potential pathways of androgenic actions in PCOS by comparing the capacity of DHT and testosterone to induce PCOS symptoms in control WT and ARKO mice. Findings revealed that both androgens induced the reproductive PCOS-like features of acyclicity and anovulation in control females, while in ARKO mice only testosterone treatment induced irregular cycles and ovulatory disruption. These data infer that hyperandrogenism acting directly via the AR, and likely also indirectly via the ER, is involved in mediating the development of PCOS reproductive symptoms (Aflatounian et al. 2020). Support for a role for oestrogenic actions in driving PCOS reproductive features comes from the findings that exposure of female rats to elevated levels of oestradiol valerate induces several reproductive PCOS-like symptoms (Brawer et al. 1986, McCarthy & Brawer 1990), but metabolic features associated with human PCOS were lacking in this model (Stener-Victorin et al. 2000). In addition, the knowledge that the first-line pharmacological treatment options to restore ovulation in PCOS patients are clomiphene citrate (an ER modulator) and letrozole (an inhibitor of oestradiol synthesis) (Legro 2016, Teede et al. 2018, Wang et al. 2019a) indicates ER-mediated signalling pathways may play a role in the development of PCOS. On the other hand, DHT, but not testosterone, induced adverse PCOS-associated metabolic traits in WT mice and neither androgen-induced adverse metabolic features in ARKO mice. These data further highlight an important role for AR-mediated androgen actions in the pathogenesis of PCOS metabolic symptoms (Aflatounian et al. 2020). Taken together, these data indicate that the manifestation of different clinical PCOS sub-phenotypes may arise through various steroid signalling pathways, which warrant further investigation.

Location of androgenic target sites in PCOS pathogenesis

Identifying the location of androgenic actions and the site-specific mechanisms involved in PCOS pathogenesis is crucial for the future development of target therapies. It has been proposed that excess levels of androgens acting via the AR at various locations throughout the body including the hypothalamus, ovary, skeletal muscle, adipose tissue or liver are involved in the origins of PCOS. Significant advances in our understanding of the likely mechanisms involved in PCOS pathogenesis have been made by studies combining PCOS mouse models and global and cell-specific ARKO mouse models. To unravel the prominent sites of these AR actions, several studies have induced PCOS in mice with a specific loss of AR function only in the brain, pituitary, granulosa cells, theca cells, adipocytes and liver (Fig. 1). Studies suggest that extra- and not intra-ovarian mechanisms are the major mediators in the development of PCOS. Androgen excess-induced PCOS symptoms in control mice and ovariectomised control mice with transplanted ARKO ovaries, where only the ovaries have non-functional AR signalling, lead to the development of PCOS traits of disrupted cycles. On the other hand, normal cyclicity was retained after induction of PCOS by androgen excess in ovariectomised global ARKO mice with transplanted control ovaries where only the ovaries have functional AR signalling (Caldwell et al. 2017). In particular, the brain has been pinpointed as a key site at the core of PCOS pathogenesis, as a loss of AR signalling only in the brain protects female mice from developing the majority of reproductive and metabolic PCOS traits, including ovulatory dysfunction, increased adiposity, dyslipidaemia, pronounced adipocyte hypertrophy and hepatic steatosis (Abbott 2017, Caldwell et al. 2017). Additionally, a pituitary-specific loss of AR signalling...
protects against the development of cycle irregularity and ovulatory dysfunction (Wang et al. 2019b).

In comparison to the brain-specific ARKO mouse that was protected from developing the majority of PCOS features, DHT-induced PCOS female mice with a loss of AR granulosa cell function still displayed the majority of PCOS characteristics, with the loss of AR granulosa cell actions only protecting against an increase in granulosa cell degeneration (Caldwell et al. 2017). Inactivation of AR signalling only in the theca cells was more beneficial, causing partial prevention against the development of acyclicity, dysfunctional ovulation and infertility in a hyperandrogenic PCOS mouse model (Ma et al. 2017). Interestingly, a mouse model with theca cell targeted deletion of Pten was observed to exhibit the PCOS-like reproductive features of irregular cycles, ovulatory dysfunction and increased testosterone levels (Lan et al. 2017). The lack of Pten caused defective PI3K/Akt signalling in theca cells, indicating ovary-specific Pten signalling may be involved in mediating reproductive features of PCOS (Lan et al. 2017). Additionally, investigations into the potential role of the ovarian stroma are warranted as women with PCOS have an increased volume of ovarian stroma, and more structural collagen and AR are expressed in the fetal stroma (Fowler et al. 2011). Recently, in a novel transgenic mouse model overexpressing CYP17 only in theca cells, the development of hypertrophic stromal cells was attributed to the enrichment of pathways for collagenisation and extracellular matrix organisation (Secchi et al. 2021). Interestingly, this model also displayed the PCOS-like traits of hyperandrogenism and oligoovulation. Thus, the increase in the stroma in this model could potentially be due to an increase in AR-mediated actions due to the increased androgen production (Secchi et al. 2021).

It was recently suggested that expression of PCOS candidate genes during development of the PCOS ovary could be perturbed leading to the adult PCOS phenotype (Hartanti et al. 2020b, Liu et al. 2020). However, how does this sit with the conclusions from cell-specific AR knockout mouse models suggesting that the AR in the ovary is not the major mediator in PCOS? To date, silencing of AR, in PCOS mouse models, has only been performed in follicular cells and not ovarian stroma cells, but in the human fetal ovary, AR is expressed exclusively in the stroma cells (Fowler et al. 2011). Additionally, the AR is expressed relatively late compared to many other PCOS candidate genes (Hartanti et al. 2020b, Liu et al. 2020), which is presumably why prenatal androgen treatment is effective in inducing a PCOS phenotype if administered in the second half of gestation in animal models of PCOS (Stener-Victorin et al. 2020). Hence, although evidence indicates that follicular AR is not a key site involved in the aetiology of PCOS, this does not preclude a potential role for ovarian stroma AR actions and other causes of PCOS that might act in the fetal ovary, especially those that act earlier than AR-mediated actions.

A recent study suggests that hyperandrogenic actions in multiple tissues are involved in the pathogenesis of PCOS. Using a dual knockout mouse model with a loss of AR signalling in the brain and white and brown adipose tissues, Cox et al. observed additional protection from the development of DHT-induced PCOS symptoms, compared to a loss of AR actions in the brain only (Cox et al. 2020). Moreover, the liver has also been pinpointed as a site of AR action involved in the development of PCOS, with liver-specific ARKO female mice observed to be protected from developing DHT-induced glucose and insulin intolerance (Andrisse et al. 2021). Interestingly, it appears that similar shared molecular pathways may be altered by AR signalling across multiple tissues. A study of ovaries in a PCOS rat model recently found that prenatal androgenisation leads to alterations in ovarian lipid metabolism and the PPARγ system, with observed increases in ovarian cholesterol esters, decreased PPARγ mRNA and protein expression and also decreased levels of the master regulator of lipogenesis Srebp1 (sterol regulatory element-binding protein 1) mRNA (AbruZZeSe et al. 2020). Similarly, another study dissecting the effects of hyperandrogenism in the liver also observed a decrease in Srebp1 in the liver of DHT-induced PCOS female mice which resulted in the increased lipid content in the liver (Seidu et al. 2021). Hence, further studies identifying if AR actions within other sites, such as adipocytes and/or skeletal muscle, play a role in the development of PCOS are of interest. Understanding of the downstream mechanisms influenced by the multiple site-specific AR actions involved in PCOS pathogenesis is also important.

Several lines of evidence strongly support a key role for aberrant neuroendocrine actions in the origins of PCOS (Walters et al. 2018c, Coutinho & Kauffman 2019, Ruddenklau & Campbell 2019). In particular, the importance of neuroendocrine androgen actions has been reported (Caldwell et al. 2017), with brain circuitry impairments identified across several species of PCOS animal models (Walters et al. 2018c) and abnormal GABAergic wiring to GnRH neurons proposed as a key mechanism involved in the origins of the neuroendocrine dysfunction in PCOS (Sullivan & Moenter 2004, Moore et al. 2015, Silva et al. 2018). The importance of AR-driven actions is further highlighted by the finding that blockade of AR signalling
in PCOS mouse (Silva et al. 2018) and sheep (Sheppard et al. 2011) models with the AR antagonist flutamide reverses the observed abnormal changes in female brain wiring linked to the development of PCOS features.

Collectively, these results support a pivotal role for hyperandrogenism mediating its actions via the AR in the origins of PCOS. Research findings provide strong evidence that while ovarian AR actions may contribute to the generation of reproductive features in PCOS, extra-ovarian AR sites of action appear to be the key drivers in PCOS pathogenesis. In particular, the brain has been highlighted as a prominent site, with neuroendocrine androgen-driven molecular mechanisms likely playing a crucial role in the developmental origins of PCOS symptoms.

Pharmacological treatment of excess androgens in PCOS

Several drugs with anti-androgenic properties and the steroidal contraceptive pill containing progesterin can be used to treat clinical hyperandrogenism and its associated symptoms in PCOS (Teeed et al. 2018). The oral steroidal contraceptive pill via its negative feedback control of pituitary hormonal secretions can reduce the ovarian secretion of androgens and alleviate the severity of the symptoms of hyperandrogenism while also restoring regular menstrual cycles (Dokras 2016). Steroidal AR blockers compete with bioactive androgens for the AR ligand-binding domain. Examples of these include spironolactone and cyproterone acetate and both are reported to be an effective way to significantly decrease hirsutism and acne in women suffering from PCOS (Escobar-Morreale 2018). Spironolactone therapy in some women with PCOS has also been associated with a significant improvement in metabolic phenotype (Zulian et al. 2005). Flutamide is a synthetic nonsteroidal anti-androgen and a competitive antagonist of the AR. Beneficial effects of treatment with flutamide in PCOS patients include reports of a reduction in the degree of hirsutism and acne observed in women with PCOS (Calaf et al. 2007), restoration of menstrual regularity and ovulation (Paradisi et al. 2013) and amelioration of the impaired sensitivity of the GnRH pulse generator to feedback inhibition by oestradiol and progesterone (Eagleson et al. 2000). In addition, independent of changes in weight, flutamide has been demonstrated to improve lipid profile in women with PCOS (Diamanti-Kandarakis et al. 1998). Collectively, findings from the treatment of PCOS patients with anti-androgenic drugs have revealed that overall targeted suppression of androgen excess improves the symptoms of PCOS and therefore supports a key role for androgen excess via AR-mediated actions in the development of a wide range of PCOS symptoms. However, it is worthy to note that these treatments are not curative and there is evidence to suggest that anti-androgens have unacceptable hepatotoxicity (Conway et al. 2014); hence, their benefits are outweighed for use in non-lethal disorders, such as PCOS. Therefore, while treating PCOS with systemic androgen blockade is a logical approach, an improved in-depth knowledge of the physiological mechanisms underpinning the evolution of PCOS is needed to develop a more targeted pharmacological approach in the future.

Insulin

Insulin resistance and aberrant insulin signalling pathways in PCOS

PCOS has a strong metabolic component. Whole-body IR and compensatory hyperinsulinaemia are present in the majority of women with PCOS and are considered to be major drivers of PCOS symptoms. Approximately 38–95% of women with PCOS, depending on the severity of the phenotype, are insulin resistant with a ~25% reduction in insulin sensitivity (Cassar et al. 2016) based upon euglycaemic-hyperinsulinaemic clamp studies (Moghetti et al. 2013, Stepto et al. 2013). Interestingly, the reduction of insulin sensitivity occurs independently of, but is exacerbated by, increased BMI (Stepto et al. 2013). Since even lean women with PCOS can display insulin resistance and other associated metabolic defects (Dunaif et al. 1989, Ciaraldi et al. 2009, Stepto et al. 2013), it is likely that PCOS is associated with unique molecular abnormalities. PCOS-IR has been attributed to post-binding insulin signalling defects in skeletal muscle, adipocytes and skin fibroblasts, which occur independently of obesity or type 2 diabetes (Ciaraldi et al. 1992, Dunaif et al. 1995, 2001).

The studies of the insulin signalling pathways have not produced consistent outcomes. Increased phosphorylation of insulin receptor substrate 1 (IRS-1) on serine 312 and the resultant decrease in the insulin-mediated IRS-1 associated phosphatidylinositol 3-kinase (PI3K) activity has been identified in human PCOS skeletal muscle (Corbould et al. 2005). However, other studies have reported decreased phosphorylation of Akt and AS160 (Akt substrate of 160 kD) (Hojlund et al. 2008) or a decrease in insulin-stimulated phosphorylation of mTOR (Stepto et al. 2020) in overweight/obese women with PCOS. Some other studies reported no differences in the levels of Akt,
Akt phosphorylated at serine 473 or of threonine 308 (Ciaraldi et al. 2009, Hansen et al. 2019), or in the levels of AS160 and insulin receptor or glucose transporter 4 (GLUT4) in skeletal muscle between women with and without PCOS. Instead, a reduction in AMP-activated protein kinase (AMPKα2) protein expression and AMPK threonine 172 phosphorylation has been detected (Hansen et al. 2019). Taken together, despite some discrepancies across studies that need further validation, these findings demonstrate the presence of impaired peripheral insulin signalling at various levels in women with PCOS.

IR in PCOS is suggested to be selective, affecting only metabolic pathways but not other insulin actions in various cell types, including skin fibroblasts (Book & Dunaif 1999), adipocytes (Corbould & Dunaif 2007) and ovarian granulosa cells (Rice et al. 2005). However, in skeletal muscle of women with PCOS, insulin's mitogenic signalling has been reported to be compromised by constitutive activation of MAPK-ERK1/2 signalling (Cusi et al. 2000, Corbould et al. 2006, Rajkhowa et al. 2009). Interestingly, the activation of this pathway is suggested to be involved in increased serine phosphorylation and consequently a decrease in IRS-1-associated PI3-K activity (Corbould et al. 2006).

Although it is accepted that PCOS has a unique IR with post-binding defects in insulin signalling, it is still under debate whether these abnormalities are intrinsic and/or acquired. Some studies have reported that despite the presence of signalling defects, cultured primary myotubes from women with PCOS do not retain the impaired insulin-stimulated glucose uptake (Corbould et al. 2005, Eriksen et al. 2010, McIlvenna et al. 2021, Moreno-Asso et al. 2021), suggesting an interaction between intrinsic and in vivo environmental factors occurs in the development of skeletal muscle IR. Others, however, propose an exclusive role for intrinsic defects in skeletal muscle (Ciaraldi et al. 2009), while adipocytes exhibit normal insulin responsiveness in culture (Corbould & Dunaif 2007, Ciaraldi et al. 2009). These findings infer that the mechanisms leading to IR in PCOS are tissue-specific. Hence, considering the distinct metabolic heterogeneity of PCOS, further mechanistic studies are needed to fully understand the environmental and/or intrinsic factors that act in a tissue-specific manner.

Effects of IR and hyperinsulinaemia on hyperandrogenism

Neither hyperandrogenism nor IR are clinically universal in women with PCOS, implying that both features are key drivers of PCOS acting either alone or in synergy. IR of the peripheral tissues leads to compensatory hyperinsulinaemia (Bergman et al. 2002) and increases hyperandrogenism, which aggravates its inherent metabolic and reproductive dysfunctions, including type 2 diabetes, cardiovascular risk factors and infertility (Diamanti-Kandarakis & Dunaif 2012).

Both insulin and insulin-like growth factor I (IGF-I) participate in the regulation of ovarian steroidogenesis (Ipsa et al. 2019). The IGF-1 effect on ovarian steroidogenesis is amplified by insulin, which induces the up-regulation of type I IGF receptors and inhibits ovarian IGF-binding protein 1 (IGFBP-1) production. This results in increased bioavailability of IGF-I and higher sensitivity of ovarian cells to IGF signalling (Poretsky et al. 1996a,b). Interestingly, insulin’s mitogenic and steroidogenic action is selectively increased in PCOS ovaries, despite its impaired metabolic signalling (Wu et al. 2003, Rice et al. 2005). In vitro studies in human granulosa cells suggest that insulin-induced steroidogenesis is independent of PI3K and MAPK pathway, while similar effects by IGF-1 are largely MAPK dependent (Poretsky et al. 2001, Seto-Young et al. 2003). At the same time, both insulin and IGF-1 can also stimulate testosterone production by human thecal cells in women with PCOS (Nestler et al. 1998).

While in vitro experiments provide an approach to assess the relationship between hyperinsulinaemia and hyperandrogenism, studying this relationship in clinical studies is more challenging. It is known that serum levels of insulin and androgens are positively correlated in women with PCOS (Burghen et al. 1980). However, it is not possible to administer high levels of insulin to healthy individuals for an extended period of time; thus, clinical studies have utilised only short-term insulin infusions to investigate the role of hyperinsulinaemia on the production of androgens. Findings infer that sustained high levels of insulin significantly increase the levels of androstenedione and testosterone (Micic et al. 1988, Fox et al. 1993). Short- and long-term administration of insulin sensitisers in women with PCOS, such as metformin or thiazolidinedione drugs, show a concomitant increase in insulin sensitivity and decreased androgen production, improving menstrual cycle regularity and alleviating androgen-excess symptoms (Velazquez et al. 1994, Dunaif et al. 1996, la Marca et al. 2000, Moghetti et al. 2000, Azziz et al. 2001). These studies provide strong evidence that hyperinsulinaemia can directly influence hyperandrogenaemia in PCOS.

Hyperinsulinaemia is also associated with increased serum free testosterone levels brought about by lower levels of circulating sex hormone-binding globulin (SHBG). Studies in mice suggest that insulin reduces
hepatocyte nuclear factor-4α (HNF4α) expression in the liver by upregulating its negative transcriptional regulator, the sterol regulatory element-binding protein (SREBP) (Xie et al. 2009). This decrease in HNF4α consequently reduces SHBG synthesis and hence the levels of circulating SHBG, resulting in an increase in free testosterone levels (Selva et al. 2007) (Fig. 2). However, this concept that insulin directly regulates SHBG synthesis by the liver has been recently challenged, proposing that low-grade inflammation is the main risk factor for low plasma levels of SHBG in metabolic diseases, including PCOS (Simó et al. 2015).

**Effect of hyperandrogenism on IR and hyperinsulinaemia**

Hyperandrogenaemia is associated with metabolic dysfunction in PCOS (O’Reilly et al. 2014), but the mechanism(s) by which hyperandrogenism aggravates IR or hyperinsulinaemia remain(s) unclear. A recent study has suggested that serum androgen levels may be associated with impaired insulin clearance in PCOS rather than an increase in insulin secretion in contributing to hyperinsulinaemia (Tosi et al. 2020). Other studies support a direct role for androgen excess in causing beta-cell dysfunction and increasing insulin secretion in both obese and lean women with PCOS (Dunaif & Finegood 1996, Vrbikova et al. 2002) (Fig. 2). Androgens can also directly modulate insulin action in classic target tissues (skeletal muscle and adipocytes), with some pharmacological studies reporting improved insulin sensitivity in women with PCOS when reducing androgen levels or actions (Diamanti-Kandarakis & Dunail 2012).

Testosterone can induce IR in cultured mature adipocytes from healthy women (Corbould 2007). In normal-weight PCOS women, circulating levels of testosterone are positively correlated with increased intra-abdominal fat deposition and greater proportion of small s.c. abdominal adipocytes (Dumesic et al. 2016). This may promote ectopic fat accumulation, leading to lipotoxicity and consequent metabolic dysfunction (Fig. 2). Consistent with this theory, chronic administration of the androgen blocker, flutamide, to women with PCOS has been associated with a reduction in abdominal fat depots (Gambineri et al. 2006). In animal models, testosterone administration for 12 weeks to adult female rats caused hyperinsulinaemia, reduced whole-body insulin sensitivity and caused an inhibition of glycogen synthesis in skeletal muscle (Holmang et al. 1990). Characterisation of a DHEA-induced mouse model of PCOS revealed whole-body IR and impaired skeletal muscle insulin signalling with reduced insulin-induced phosphorylation of Akt and GLUT4 translocation, increased insulin-induced activation of mTOR/S6K1

![Figure 2](https://joe.bioscientifica.com)

**Figure 2**
Schematic representation of proposed IR–hyperandrogenism interaction in PCOS. HPO, hypothalamo-ovarian axis.
and impaired mitochondrial function (Song et al. 2018) (Fig. 2). A recent metabolomic analysis of skeletal muscle in this same DHEA-induced PCOS mouse model identified alterations in several metabolic pathways, highlighting the role of mitochondrial impairment as an androgen-induced mechanism of IR in PCOS (Shen et al. 2019). However, in vitro studies investigating the direct effect of testosterone on primary cultured myotubes and C2C12 cells are contradictory. Studies in primary myotubes from women with PCOS found no changes in insulin-stimulated glucose uptake (Eriksen et al. 2014), while studies in primary rat myotubes and C2C12 suggest that testosterone impairs insulin signalling through phosphorylation of IRS-1 Ser636/639, activates mTORC1 and reduces mitochondria function and plasma membrane GLUT4 expression in skeletal muscle (Allemand et al. 2009, Song et al. 2018).

Investigation of the effects of androgen administration on insulin sensitivity in women is limited. Daily i.m. administration of testosterone esters for 2 weeks in non-PCOS women resulted in IR (Polderman et al. 1994). Similarly, short-term methyltestosterone administration in healthy non-obese women reduced whole-body insulin sensitivity but did not alter hepatic insulin sensitivity (Diamond et al. 1998). Intervention studies using euglycaemic–hyperinsulinaemic clamps to investigate the impact of androgen blockers or GnRH analogue administration in PCOS women have reported conflicting results on insulin sensitivity (Diamanti-Kandarakis et al. 1995, Lasco et al. 1995, Moghetti et al. 1996, Dahlgren et al. 1998, Gambineri et al. 2004). While leuprolide, flutamide, goserelin and buserelin treatment studies reported significant improvements in insulin sensitivity (Lasco et al. 1995, Moghetti et al. 1996, Dahlgren et al. 1998), other intervention studies with flutamide did not appear to impact insulin sensitivity in women with PCOS (Diamanti-Kandarakis et al. 1995, Gambineri et al. 2004). Most of these studies, however, were only short-term, and the duration of treatment may be critical, as observed in an intervention study in PCOS women, where insulin sensitivity was significantly improved after 12 months of flutamide treatment but not after 6 months (Gambineri et al. 2006).

Regardless of the pathogenesis of increased insulin and androgens, a vicious cycle is potentially established whereby IR–hyperinsulinaemia–hyperandrogenism appears to mutually reinforce each other to accentuate the symptoms of PCOS. However, the specific underlying mechanisms linking the severity of hyperandrogenism with metabolic dysfunction and IR in women with PCOS remain to be better understood. Therefore, further long-term interventions investigating the role of androgens on metabolic IR in women with PCOS are needed.

**Insulin sensitisers and lifestyle intervention in the management of IR in PCOS**

Insulin sensitisers and lifestyle interventions, such as exercise, are established to be the first-line therapy to improve metabolic health in PCOS. However, these approaches still remain suboptimal at restoring the key metabolic features of PCOS, as at present they have only been demonstrated to improve rather than normalise insulin sensitivity (Hutchison et al. 2011, Harrison et al. 2012, Stepto et al. 2019, Xing et al. 2020). Metformin is the most common insulin sensitisier and has been prescribed to women with PCOS for the last two decades. Besides its insulin-sensitising actions, it can lower androgens and restore ovulation. There is growing evidence that metformin has a direct effect on ovarian steroidogenesis and thus on hyperandrogenic symptoms independently of its insulin-dependent effects, but its mechanisms of action remain unclear (Arlt et al. 2001, Mansfield et al. 2003, Hirsch et al. 2012, Kurzthaler et al. 2014). Metformin’s main metabolic action is the inhibition of the mitochondrial complex I, compromising ATP production and thus increasing the intracellular AMP:ATP ratio. These changes suppress hepatic glucose production through AMPK-dependent and independent pathways (Rena et al. 2017). Metformin also enhances insulin action at the post-receptor level in peripheral tissues, mainly through AMPK activation (Musì et al. 2002). Although controversies remain about the exact regulated pathways, it is proposed that activated pathways result in the inhibition of mTOR-S6K1, causing decreased serine phosphorylation of IRS-1 and consequent increases in IRS/P13K-dependent signalling (Musì et al. 2002, Kalender et al. 2010). Thereby, metformin enhances insulin sensitivity and insulin-stimulated glucose uptake in peripheral tissues, resulting in reduced circulating glucose and insulin levels (Bailey 1992, Bailey & Turner 1996). Also, metformin has been reported to promote fatty acid oxidation in peripheral tissues by reducing lipid synthesis through AMPK-induced inactivation of acetyl-CoA-carboxylase (Fullerton et al. 2013, O’Neill et al. 2014). Thus, in women with PCOS, metformin can reduce the risk of obesity, improve insulin sensitivity and reduce hyperinsulinaemia.

Although metformin has been shown to improve metabolic health in PCOS, for an effective long-term health improvement, especially for those who are overweight or obese, lifestyle intervention is regarded as essential. Indeed,
a long-term follow-up study in a high-risk population for type 2 diabetes reported a 58% reduced incidence following lifestyle changes compared to a 31% reduction by metformin, indicating that lifestyle intervention can be significantly more effective than metformin in preventing this metabolic disorder (Knowler et al. 2002). Particularly, exercise is a great therapeutic approach to improve peripheral insulin sensitivity as skeletal muscle accounts for ~85% of whole body insulin-induced glucose uptake (DeFronzo et al. 1981). In PCOS, long-term aerobic exercise has been demonstrated to improve insulin sensitivity and reduce cardiometabolic risk factors such as visceral fat and triglycerides, despite no significant differences in weight loss (Hutchison et al. 2011, Almenning et al. 2015, Patten et al. 2020, 2022). This highlights the extensive benefit of exercise training as a key lifestyle intervention for improving cardiometabolic health in women with PCOS, regardless of BMI changes. Muscle contraction induces complex molecular signalling through the activation of several kinases including AMPK, CaMKII, MAPKs and other molecules that control a network of transcription factors and regulators of mitochondrial biogenesis, fatty acid metabolism, glucose uptake and insulin sensitivity (Egan et al. 2016). In particular, exercise intervention in women with PCOS results in improved skeletal muscle insulin signalling with increased phosphorylation of AMPK threonine 172 and Akt serine 473/308, reduced phosphorylation of the IRS-1 serine 312 and increased GLUT4 translocation (Dantas et al. 2015). Similarly, exercise also has a positive impact on the inflammatory state which is implicated in cardiometabolic dysfunction in PCOS by increasing the anti-inflammatory factors IL-6, IL-10 and IL-4 and reducing pro-inflammatory factors, TNFα, IKKα/β and JNK, in skeletal muscle (Dantas et al. 2019).

International evidence-based guidelines acknowledge that the combined intervention of metformin and lifestyle should be considered for the management of weight and metabolic outcomes in PCOS (Teede et al. 2018). However, metformin may interfere with some of the exercise training-induced adaptations and attenuate its beneficial effect on decreasing cardiovascular disease risk factors (Malin et al. 2013). This interference is suggested to occur through mitochondrial complex I inhibition, which despite enhancing glucose uptake, causes a negative impact on mitochondrial respiratory function and cardiorespiratory fitness (Konopka et al. 2019). In PCOS, clinical studies informing on the combined effect of metformin and lifestyle are limited, with a small sample size and short duration. Except for weight loss, the majority of studies show no additional beneficial impact of metformin on metabolic, reproductive and psychological outcomes compared to lifestyle alone (Ladson et al. 2011, Naderpoor et al. 2015). Future studies should clarify the long-term impact of these interventions in the management of PCOS.

Anti-Müllerian hormone

Anti-Müllerian hormone expression and biosynthesis

Anti-Müllerian hormone (AMH) is a TGFβ superfamily member that signals by binding to the AMH type 2 receptor (AMHR2) in tandem with a type 1 receptor (BMPR1A, ACVR1 and possibly BMPR1B) (Orvis et al. 2008). The intracellular domain of the activated receptor complex phosphorylates serine/threonine residues on smad1, smad5 or smad9 (Shi & Massague 2003). These transcription factors then bind to smad 4, facilitating the translocation of these complexes to the nucleus where they regulate gene transcription (Shi & Massague 2003). In females, AMH is primarily expressed in the granulosa cells of developing ovarian follicles from the primary follicle stage onwards (Weenen et al. 2004). Peak AMH expression occurs in the granulosa cells of large preantral and small antral follicles, with a subsequent, gradual decline in the large antral and preovulatory stages (Weenen et al. 2004, Jeppesen et al. 2013). AMHR2 expression follows an almost identical expression pattern (Baarends et al. 1995), with two exceptions; low levels of AMHR2 are expressed in the pregranulosa cells of primordial follicles (Kano et al. 2017) and also in the theca cells of developing follicles (Cheon et al. 2018). AMH is secreted from the ovary into the blood, and serum concentrations are used as a clinical biomarker to predict the number of developing follicles in the ovaries (Dewailly et al. 2014).

Roles of AMH in females

AMH has multiple roles in the ovary. Primordial follicle activation is inhibited in the presence of AMH (Hayes et al. 2016, Kano et al. 2017, Pankhurst et al. 2018) and accelerated in its absence (Durlinger et al. 1999, Guo & Pankhurst 2020). At the primary follicle stage, elevated AMH reduces the number of follicles that progress to the preantral stage (Park et al. 2011, Pankhurst et al. 2018). At the preantral/ small antral stages of follicle growth, the role of AMH becomes less clear. A leading theory is that AMH suppresses the response to FSH (Visser et al. 2006). This is based on early observations that AMH inhibits FSH-mediated follicular growth in vitro (Durlinger et al. 2001). FSH-mediated cAMP
production is also inhibited by AMH via up-regulation of miRNA-181a and miRNA-181b expression in granulosa cells from preantral follicles (Hayes et al. 2016). However, numerous studies provide evidence that AMH promotes preantral follicle growth (McGee et al. 2001, Xu et al. 2016, 2018a,b, Baba et al. 2017) which is not consistent with FSH suppression. In the larger antral stages of follicle growth, AMH is not normally at high levels, and in this situation, AMH signalling appears to be detrimental to follicle development (Park et al. 2011, Xu et al. 2016).

AMH induces a robust and reproducible decrease in cytochrome P450 aromatase or oestradiol production in granulosa cells derived from preantral and small antral follicles (Vigier et al. 1989, Campbell et al. 2012, Rodrigues et al. 2015, Xu et al. 2016). These stages correspond to the period of maximal AMH expression and may represent a mechanism to prevent excessive oestradiol production, as this is typically only a characteristic of large developing follicles (Jeppesen et al. 2013). Interestingly, exogenous AMH can still inhibit aromatase expression or oestradiol synthesis in granulosa lutein cells from preovulatory follicles (Grossman et al. 2008, Pellatt et al. 2011, Chang et al. 2013, Prapa et al. 2015, Sacchi et al. 2016), even though these follicles do not typically produce large quantities of AMH. The suppression of aromatase by AMH has only been observed in granulosa cell cultures when co-treated with FSH. However, granulosa cell aromatase is not expressed in the absence of FSH or LH signalling (Grossman et al. 2008, Chang et al. 2013, Prapa et al. 2015, Sacchi et al. 2016), and it has not been determined whether AMH inhibits aromatase expression directly or via upstream suppression of FSH signalling.

AMH and AMHR2 expression has been discovered in CNS neurons (Wang et al. 2005), including the GnRH-secreting neurons in the hypothalamus (Cimino et al. 2016). GnRH neurons process signals that terminate outside the blood–brain barrier (Herde et al. 2011), suggesting that GnRH neurons can also respond to gonad-derived AMH in circulation. Exogenous application of AMH increases the rate of pulsatile GnRH secretion in cultures of brain slices and increases LH secretion in vivo (Cimino et al. 2016). However, elevated circulating AMH levels in female mice do not induce a PCOS-like phenotype when introduced in adulthood (Kano et al. 2017, Pankhurst et al. 2018).

**AMH in women with PCOS**

Increased serum AMH has been proposed as a potential alternative to ultrasound for assessing polycystic ovary morphology in adults, pending further evaluation (Teede et al. 2018). Elevated serum AMH is observed in women with PCOS, particularly in patients with PCOM (Sahmay et al. 2013). However, elevated serum AMH also occurs in women who have large numbers of developing follicles but no other PCOS symptoms, apparently representing healthy women with large ovarian reserves (Dewailly et al. 2014).

AMH expression differs in the follicles of the women with PCOS (with PCOM), as each granulosa cell expresses AMH mRNA at a higher level (Pierre et al. 2017). This leads to higher follicular-fluid AMH concentrations in early antral and preovulatory follicles (Fallat et al. 1997, Catteau-Jonard et al. 2008, Das et al. 2008) and higher serum AMH/antral follicle count ratios (Alebic et al. 2015, Bhide et al. 2015). AMH does not undergo the normal down-regulation in the preovulatory follicles of women with PCOS (Fallat et al. 1997, Pellatt et al. 2007, Catteau-Jonard et al. 2008), which may involve an abnormal granulosa cell response to LH (Pierre et al. 2013).

Elevated androgen levels are unlikely to be the cause of excessive AMH production in PCOS, for if anything, androgen tends to reduce granulosa cell AMH expression (Crisosto et al. 2009, Laird et al. 2017). Female-to-male transsexual patients receiving testosterone and an aromatase inhibitor letrozole also experience reductions in serum AMH (Caanen et al. 2015). However, altered oestrogen signalling may be involved in this dysregulation of AMH production. ERα-signalling stimulates AMH expression in granulosa cells, while ERβ-signalling results in down-regulation (Grynberg et al. 2012). ERα and ERβ are expressed at similar levels in small antral follicles but in PCOS patients ERβ expression has been reported to be reduced (Jakimiuk et al. 2002). A reduction in the suppressive effects of ERβ in PCOS may explain the elevated AMH expression in developing follicles (Fig. 3). Over-production of AMH has the capacity to contribute to the androgen/oestrogen imbalance in PCOS through aromatase down-regulation. However, the overexpression of AMH is hypothesised to arise from an altered ERα/ERβ ratio and thus the current evidence seems to suggest this is a consequence of PCOS, rather than an initial cause. Unfortunately, more clarity on the interaction between AMH and FSH, particularly at the preantral follicle stage, is needed to determine whether it affects ovarian function in PCOS.

**AMH induces PCOS-like phenotypes in offspring**

Recent studies into the heritability of PCOS have focused on the role of maternal AMH. Maternal injection of
mice with recombinant AMH in late pregnancy induces an androgen-mediated PCOS-like effect in the female offspring which includes elevated serum testosterone, ovarian cysts, arrested follicle development, dysregulated ovarian cycles, reduced ovulation rates and metabolic dysfunction (Tata et al. 2018, Mimouni et al. 2021). AMH was shown to interact with maternal GnRH neurons to induce LH secretion during pregnancy, which in turn, induces placental production of androgens by driving down placental aromatase expression (Tata et al. 2018). Thus, AMH administration in pregnant mice appears to cause a PCOS-like phenotype in offspring due to increased prenatal exposure to androgens.

In women, serum AMH levels decline during pregnancy (Pankhurst et al. 2016) but only a limited number of studies have characterised this in women with PCOS. One study showed that serum AMH levels at 16–19 weeks of gestation are higher in women with PCOS than in controls (Tata et al. 2018). Similarly, another study observed elevated serum AMH in pregnant women with PCOS in the third trimester relative to controls (Piltonen et al. 2019). However, Koninger et al. observed no difference in the second or third trimesters (Koninger et al. 2018). Both studies indicate that pregnancy reduces serum AMH levels in women with PCOS and controls, which appears to reduce the differences that exist prior to pregnancy. Furthermore, LH was not detected in the serum of women with or without PCOS at 9 months of pregnancy (Piltonen et al. 2019); hence, there is currently little evidence that AMH induces maternal LH secretion during the later stages human pregnancy.

While animal models demonstrate that elevated maternal AMH can induce a PCOS-like phenotype in mouse offspring the question remains, does this occur naturally in humans? In human pregnancy, the corpus luteum is rescued by chorionic gonadotrophin produced by the early placenta. Chorionic gonadotrophin and LH both bind to the same receptor (LHCGR) which is expressed in luteal cells. In mice, however, there is no analogous system to chorionic gonadotrophin and instead prolactin and placental lactogens maintain the corpora lutea (Gunnet & Freeman 1983, Ormandy et al. 1997, Bachelot et al. 2009). Therefore, it is unlikely that humans have the same susceptibility to excessive LH secretion from the pituitary because human pregnancy is characterised by high levels of chorionic gonadotrophin, and activation of the LHCGR is a normal feature of human pregnancy.

The AMH-induced model of PCOS is unique in that it induces a PCOS-like phenotype with features from all three diagnostic criteria and metabolic dysfunction. Crucially, the model does not require ongoing administration of drugs (e.g. testosterone implants) to maintain the phenotype, which has enabled its use to demonstrate the transgenerational inheritance of PCOS-like traits in mice (Mimouni et al. 2021). The features of this model provide a promising avenue for further investigation of PCOS pathology. Current evidence suggests that the model utilises AMH to release LH which induces elevated placental androgen production, supporting hyperandrogenism.

Figure 3
AMH interacts with steroidal signalling pathways in granulosa cells. In normal granulosa cells, oestrogen signalling through ERβ leads to suppression of AMH expression and AMH-signalling in turn, suppresses aromatase expression. This suggests that aromatase, ERβ and AMH form a negative feedback loop that helps to maintain homeostasis within the granulosa cell. In the granulosa cells of women with PCOS, reduced ERβ expression leads to reduced suppression of AMH expression. The subsequent increase in AMH signalling leads to inhibition of aromatase expression. E2, oestradiol; A, androgen; ERα, oestrogen receptor-α; ERβ, oestrogen receptor-β; AMH, anti-Müllerian hormone.
as a cause of human PCOS (Maliqueo et al. 2013). More evidence in humans is needed to demonstrate whether AMH has a causative role in modulating steroidogenesis in the placenta of female fetuses that go on to develop PCOS.

### Transforming growth factor β

Some of the cardinal features of PCOS ovaries are their increased production of androgens, the presence of many antral follicles and their very fibrous nature with expanded, rigid and collagen-rich stroma and tunica albuginea (Stein & Leventhal 1935, Hughesdon 1982, Govinden & Bhoola 2003, Verrecchia & Mauvel 2004, Kisseleva & Brenner 2007). The production and deposition of collagen in stroma, particularly in fibrotic tissues (Chapman 2004, Christner & Ayitey 2006), is driven by TGFβ. In adult fibroblasts, TGFβ also stimulates their replication and production of collagen leading to an expanded fibrous stroma. Fibrosis is the replacement of specialised cells in organs with stromal fibroblasts and their extracellular matrix including collagens type I and III. This usually follows inflammation or tissue injury. However, it is not clear if the PCOS ovary is fibrotic or merely fibrous with the stroma having been altered in some way when it first developed in the ovary. It is also not clear what the consequences of a fibrous stroma in ovaries are. However, it has been suggested that it could physically restrict the growth of follicles and stimulate androgen production in the theca interna resulting in the PCOS reproductive phenotype of chronic hyperandrogenism (Raja-Khan et al. 2014).

In the stroma, TGFβ activity is regulated by the extracellular matrix fibrillins 1–3 (Ramirez & Pereira 1999, Kielty et al. 2002). Fibrillins achieve this by binding the latent TGFβ binding proteins (LTBPs 1–4), which are chaperones and are bound to and co-secreted with TGFβs. The expression of TGFβ signalling molecules in fetal ovaries during gestation is dynamic. Some are significantly correlated with gestational age either positively (LTBP1, LTBP2, LTBP3, LTBP4, FBN1, TGFβ2, TGFβ3, TGFβ2, TGFβ3 and TGFβ111) or negatively (FBN3, TGFβ3L, TGFβ1 and TGFβ1) (Hatzirodotos et al. 2011, 2019, Azumah et al. 2022).

In a genetic screen of families with PCOS, allele 8 of the dinucleotide repeat D19S884 found in an intron of fibrillin 3 showed genetic linkage with PCOS (Stewart et al. 2006). Many attempts to discover where fibrillin 3 is expressed in adult control and PCOS ovaries were largely unsuccessful (Prodoehl et al. 2009). However, it was subsequently discovered that fibrillin 3 is expressed in bovine and human fetal ovaries and specifically in the developing stroma as it first penetrates from the mesonephros (Hatzirodotos et al. 2011, Hummitzsch et al. 2013). This penetration of the stroma in this early phase is critical for the formation of the ovary and its oovigerous cords, surface epithelium and follicles (Hummitzsch et al. 2013, 2015, Hartanti et al. 2019, 2020a). Fibrillin 3 is also expressed in other fetal tissues but has not been examined in the fetal liver, muscle, endocrine pancreas or adipose tissue (Sabatier et al. 2011) – all tissues likely involved in PCOS metabolic pathology.

Genome-wide association studies (GWAS) have identified a number of loci associated with PCOS (Jones & Goodarzi 2016). There are about 25 genes in or near these loci. We recently identified that PCOS candidate genes in these loci were not differentially expressed in adult human PCOS ovaries (Liu et al. 2020) but were dynamically expressed in developing human and bovine fetal ovaries (Hartanti et al. 2020b, Liu et al. 2020). The expression of many of these genes correlated with each other and either positively or negatively with gestational age (Hartanti et al. 2020b, Liu et al. 2020). The expression of fibrillin 3 in the bovine fetal ovary is correlated with the expression of other PCOS candidate genes including GATA4, HMGA2, TOX3, DENND1A.X1,2,3,4 and ERBB3 and in the human fetal ovary with GATA4, HMGA2, DENND1A.V1-7, DENND1A.V1,3,4, ERBB3.V1, ERBB3.VS and FSHR (Hartanti et al. 2020b, Liu et al. 2020). Importantly, TGFβ treatment has been shown to inhibit the expression of 7 (INSR, C8H9orf3, RADS0 ERBB3, NEIL2, IRF1 and ZBTB16) of the PCOS candidate genes in cultured bovine fetal ovarian stromal cells (Hartanti et al. 2020b, Azumah et al. 2022). TGFβ also inhibited the expression of AR and stimulated the expression of an AR cofactor called TGFβ1 inducible protein 1 (TGFβI11 or hic5) (Hartanti et al. 2020b). It was concluded that altered TGFβ signalling could be involved in the fetal predisposition to PCOS or at least in the development of polycystic ovaries (Azumah et al. 2022).

While the ovaries of PCOS women are more fibrous, recent evidence suggests other tissues are too. Expression of the genes encoding collagens type IA2 and type IIIA1, decorin, l lysyl oxidase and TGFβ receptor 2 are elevated in skeletal muscle of overweight women with PCOS compared to BMI-matched controls (Stepto et al. 2020). These genes are active in the stroma and contribute to its fibrous nature. The type 2 TGFβ receptor drives collagen production, the collagen type I and III are the structural collagens of stroma, decorin is involved in assembly of these collagens into fibres and lysyl oxidase is involved in the cross linking of the collagens to each
other. Interestingly, it has been shown that inhibiting the activity of TGFβ in mice prevents diet-induced obesity and the development of type 2 diabetes (Yadav et al. 2011). Additionally, circulating levels of TGFβ have been shown in humans to be associated with fat mass, fasting insulin levels and HOMA insulin resistance index (Yadav et al. 2011). Circulating levels of TGFβ2 were higher in PCOS women who lacked allele 8 of D19S884 compared with control women who also lacked allele 8 of D19S884 (Raja-Khan et al. 2010), and in control women, TGFβ2 was significantly correlated with testosterone (Raja-Khan et al. 2014). These findings infer that TGFβ may also play a role in the pathogenesis of the metabolic traits of PCOS.

Using a PCOS model of prenatally androgenised monkeys to examine differentially methylated loci in adipose tissues, it was identified that many genes affected are involved in TGFβ signalling (Xu et al. 2011). This suggests two things, firstly that TGFβ signalling is important in the aetiology of PCOS and secondly it further supports the theory of a fetal origin of PCOS. In a study assessing birth characteristics of women who later developed symptoms of PCOS, a 1-unit decrease in the ponderal index (weight kg/height m²) at birth increased the risk of having all three PCOS symptoms (hyperandrogenism, menstrual dysfunction and polycystic ovaries) by 21% (Davies et al. 2012). Low birthweight or thinness at birth in babies who are of relatively normal birthweight (as indicated by a low ponderal index) reflects a lack of muscle growth (Phillips et al. 1994). Thus, it is widely agreed now that the first ‘hit’ of the ‘two hit’ hypothesis (Diamanti-Kandarakis et al. 2008, Franks 2008) on the development of PCOS can arise during fetal development. Note for clarity that our hypothesis on ovarian-derived features of PCOS is that the excess stroma is produced during fetal development. This is possibly different to the commonly known fibrosis, where stroma expands in adult organs in response to inflammation or tissue injury.

Overall, evidence to date supports a role for stroma and TGFβ in the pathogenesis of PCOS and the strongest evidence comes from the ovary where TGFβ can regulate not only the expression of AR and an AR cofactor but also 7 of the 25 PCOS candidate genes, at least in vitro. Additionally, all the components of the TGFβ signalling pathways are expressed in the fetal ovary and dynamically so. These studies are important as they link the fetal origins of PCOS, their genetic origins and the ovarian phenotype of PCOS. These studies should be expanded to other fetal organs, especially those involved in the aetiology of PCOS in particular skeletal muscle and adipose tissues. However, how or what could alter TGFβ signalling to bring about a predisposition to PCOS or at the least polycystic ovaries is key to identifying potential causes of PCOS.

Implications for the future

Hyperandrogenism is a major feature of PCOS and there is now substantial evidence pointing towards an important role for androgenic actions mediated via the AR in the development and progression of PCOS. Systemic treatment with present generations of anti-androgens is not a viable option due to unacceptable liver toxicity that preclude their use for non-lethal chronic disorders. Therefore, more targeted approaches are required. Moreover, targeted treatment of hyperandrogenism with the current inhibitors of cytochrome P450 17α-hydroxylase, 17,20-lyase used for castrate-resistant prostate cancer, such as abiraterone, is also not currently safe to use in women with PCOS. These have significant side effects by inhibiting cortisol production as well as androgen production (Yin & Hu 2014). Abiraterone is an active site inhibitor and hence the alternative of targeting just 17,20-lyase activity should, in theory, be possible by producing a drug that inhibits androgen production without affecting cortisol production.

Recent research is trying to identify specific sites and mechanisms to target in the development of pharmacological strategies able to suppress excess androgenic effects in women with PCOS. The fact that a specific loss of AR signalling in the brain protects hyperandrogenised PCOS mice against the development of key reproductive and metabolic PCOS traits indicates that the development of PCOS in patients may be mediated via AR-regulated central mechanisms (Caldwell et al. 2017). One novel approach would be to target AR-driven neuroendocrine pathways as strong evidence pinpoints the brain as a key site involved in PCOS pathogenesis (Caldwell et al. 2017). One neuronal network that AR-mediated signalling is known to play a role in is the regulation of the kisspeptin-neurokinin B (NKB)-dynorphin ‘KNDy’ system (Walters et al. 2018d). Rodent and sheep hyperandrogenised PCOS animal models display changes in KNDy expression and circuitry (Brown et al. 2012, Cernea et al. 2015, Kauffman et al. 2015, Osuka et al. 2017), implying that the KNDy system may be an attractive therapeutic target to modulate AR-driven neuroendocrine activity in PCOS patients. Indeed, increased kisspeptin has been reported in several populations of PCOS patients (Albalawi et al. 2018, Katulski et al. 2018, Umayal et al. 2019). Furthermore, findings from a recent clinical study...
support modulation of the KNDy system as a potential new target for the development of treatment for PCOS, as it was shown that treatment of PCOS patients with an NKB receptor antagonist reduced LH pulse frequency and LH and testosterone concentrations (George et al. 2016, Skorupskaite et al. 2020). Moreover, in hyperandrogenised PCOS mice, it was found that treatment with the NKB receptor antagonist improved aberrant PCOS metabolic status by decreasing body weight, adiposity and adipocyte hypertrophy, thus implying that the NKB pathway is also involved in mediating metabolic characteristics of PCOS (Sucquart et al. 2021). Furthermore, neuropeptide Y/agouti-related peptide (NPY/AgRP) neurons play an important role in regulating energy balance and androgenised ewes display an increase in NPY/AgRP cell number and fibre projections (Sheppard et al. 2011), which is blocked by treatment with the AR antagonist flutamide. This indicates that central AR actions may also be useful to target in the treatment of PCOS-associated metabolic dysfunction. However, based on the findings that a loss of AR in adipocytes and the brain provided additional protection to the development of PCOS than brain alone, other sites of AR actions should also be considered. Indeed, the adipokine adiponectin is decreased in PCOS women (Manners-Holm et al. 2011) and in animal models of PCOS (Caldwell et al. 2017). Exogenous adiponectin treatment ameliorates several key PCOS traits (Yuan et al. 2016) and overexpression protects mice from the induction of androgen excess metabolic PCOS traits (Benrick et al. 2017). Hence, modulation of adipose function, in particular adipokines such as adiponectin, may provide a promising novel therapeutic strategy for the management of PCOS.

Targeting TGFβ signalling in women with PCOS may also be a realistic option soon. Inhibitors of TGFβ signalling are under development for the treatment of fibroses, particularly for the heart, liver, lung and kidney (Li et al. 2017). Some of these are targeting TGFβ ligand-binding directly and some targeting the SMADs downstream of TGFβ receptor activation (Li et al. 2017). The drugs, while usually tested for organ-specific treatments in clinical trials, are, as expected, proving to be effective across organs, given that the processes are similar across organs (Distler et al. 2019). While some of these are monoclonal antibodies and are likely to be prohibitively expensive to treat PCOS, some are small molecule inhibitors or peptides. It should be tempered though that such an approach may not be viable in treating PCOS especially if the first ‘hit’ (Diamanti-Kandarakis et al. 2008, Franks 2008) occurs in fetal life or proves to be irreversible.

Targeting AMH signalling in some manner, if at all, is not likely in the short term. While the mouse model of PCOS altering AMH signalling might be a useful experimental tool, more needs to be known about AMH specifically in women with PCOS and the effects on offspring. In any event, there might be a need for agonists and antagonists to be developed and their specificity and safety determined especially if used in pregnant women with PCOS.

Another key research area that requires a better understanding is the mechanisms leading to IR in PCOS. Although to date findings are still inconclusive, especially in peripheral tissues such as skeletal muscle, evidence points to tissue-specific IR mechanisms. Similarly, discussions around the existence of intrinsic defects and/or the role of circulating factors/hormones, including androgens and AMH, in the PCOS-IR are still not clear. Therefore, considering the wide heterogeneity of PCOS, further clinical and mechanistic studies using tissue-specific integrative ‘omics’ analyses will provide vital data to fully understand the unascertained regulation of IR in women with PCOS.

Large-scale human clinical studies should clarify the long-term impact of pharmacological and lifestyle interventions (diet and/or exercise) in the management of PCOS to ultimately improve the metabolic and reproductive health of these women. Exploring the molecular changes induced by these interventions will be essential to improve existing lifestyle approaches and develop new therapeutic strategies.

Mendelian randomisation is a genetic epidemiological approach and recent studies have used GWAS data from PCOS cohorts and compared them with GWAS data from cohorts associated with other diseases or metabolites (Sun et al. 2020, Zhu & Goodarzi 2022). These studies aim to identify causes and consequences of PCOS that, whilst they might even be environmental, operate via genetic linkage. They are not without caveats, especially where there is pleiotrophy. A recent review of such studies identified that ‘obesity, testosterone levels, fasting insulin, serum sex hormone-binding globulin concentrations, menopause timing, male-pattern balding and depression may play a causal role in PCOS’ (Zhu & Goodarzi 2021).

Summary and conclusions

There is an unmet need for the development of effective interventions for PCOS largely because the aetiology of PCOS is relatively unknown. In recent times, there have been significant advances in our understanding of
pathways likely involved in PCOS pathogenesis, namely the potential roles of androgens, insulin, AMH and TGFβ. Future collective efforts between basic, clinical and epidemiological researchers are required to improve our understanding of how these pathways may interlink to initiate PCOS, and with this knowledge, we can hope the development of effective strategies specifically approved for women with PCOS will be achieved.

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

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