REVIEW

Ovarian and extra-ovarian mediators in the development of polycystic ovary syndrome

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Abstract

Polycystic ovary syndrome (PCOS) is a heterogeneous endocrine disorder affecting women of reproductive age. The origin of PCOS is still not clear and appears to be a function of gene × environment interactions. This review addresses the current knowledge of the genetic and developmental contributions to the etiology of PCOS, the ovarian and extra-ovarian mediators of PCOS and the gaps and key challenges that need to be addressed in the diagnosis, treatment and prevention of PCOS.

Key Words

- ▶ polycystic ovary syndrome
- ▶ ovary
- ▶ follicle
- ▶ granulosa cells
- ▶ developmental programming
- ▶ androgen
- ▶ insulin
- ▶ sheep

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Introduction

Polycystic ovarian syndrome (PCOS) is the most common infertility disorder affecting 5–20% of women in their reproductive age (Azziz *et al.* 2016). Stein and Leventhal described this condition for the first time in 1935 when they identified in seven patients enlarged ovaries in association with menstrual disturbances (most notably amenorrhea), sterility, pain or hyperandrogenism (Stein & Leventhal 1935). Since then PCOS has been recognized as a heterogeneous condition with various consensus meetings establishing diagnostic criteria for the identification and treatment of this condition. The 1990 United States National Institutes of Health (NIH) diagnostic criteria include clinical and/or biochemical signs of hyperandrogenism and menstrual dysfunctions (Zawadzki & Dunaif 1992), while the 2003 Rotterdam consensus requires meeting two of the following features:

clinical and/or biochemical hyperandrogenism, oligo-/an-ovulation or polycystic ovaries (The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2004). The androgen excess society in 2006 also proposed that all features such as clinical and/or biochemical hyperandrogenism and ovarian dysfunction including oligo/anovulation and/or polycystic ovaries should be considered as diagnostic features of PCOS (Azziz *et al.* 2009). All these criteria, regardless of which source, required exclusion of disorders that mimic similar symptoms such as thyroid dysfunction, hyperprolactinemia, adrenal hyperplasia, androgen-secreting tumors, among others. In spite of these, confusion regarding the use of different criteria in diagnosis and research continued, which prompted NIH through its Consensus Development Programs to organize an Evidence-Based Methodology

Table 1 Different PCOS sub-phenotypes for diagnostic criteria recommended by the expert panel at the NIH's Evidence-Based Methodology PCOS Workshop.

Symptoms	Sub-phenotype			
	A	B	C	D
Clinical and biochemical signs of hyperandrogenism	+	+	+	–
Ovulatory dysfunctions	+	+	–	+
Polycystic ovary morphology (PCOM)	+	–	+	+

PCOS Workshop. This workshop panel recommended adoption of the broader 2003 Rotterdam criteria with sub-classification of the PCOS sub-phenotypes as depicted in Table 1 (National Institutes of Health 2012). All in all, PCOS is a complex condition manifesting a wide variety of dysfunctions in addition to those considered as diagnostic criteria. These include (i) cardiometabolic dysfunctions such as basal and glucose-stimulated hyperinsulinemia independent of their BMI (Dunaif *et al.* 1989), insulin resistance (Dunaif *et al.* 2001, DeUgarte *et al.* 2005) and increased risk for cardiovascular diseases (Legro *et al.* 2001, Cobin 2013), (ii) depression (Cooney & Dokras 2017), (iii) gestational diabetes and pre-eclampsia and (iv) poor birth outcomes such as small or large for gestational age newborns, congenital abnormalities and perinatal mortality (Boomsma *et al.* 2006, Qin *et al.* 2013, Doherty *et al.* 2015). This review aims to summarize the potential causes with focus on the ovarian and extra-ovarian factors implicated in the pathogenesis of PCOS.

Origin of PCOS

Because of the heterogeneous nature of this condition, the etiopathology of PCOS is still not clearly identified. Factors ranging from genetic to environmental and/or the interaction between them have been proposed to have a role in the origin of PCOS.

Genetic basis in the origin of PCOS

The observations that there is a high amount of familial aggregation among first-degree female relatives of women with PCOS (Legro *et al.* 1998a) coupled with heritability score of 0.79 for PCOS phenotype in the Dutch twin study (Vink *et al.* 2006) provide credence for the notion that PCOS is heritable (Franks *et al.* 1997). Among the diagnostic characteristics of PCOS, evidence for heritability is strongest for hyperandrogenism (Legro *et al.* 1998a,b). Nonetheless, considering the heterogeneity in

PCOS phenotypes and the varying attributes, it is now not believed to be a monogenic disease (Fenichel *et al.* 2017). In support of this, mutations or polymorphism in several genes have been identified (Dunaif 2016). Both individual gene analysis and genome-wide association studies (GWAS) have identified mutations or polymorphisms in follicle-stimulating hormone receptor (*FSHR*), luteinizing hormone receptor (*LHCGR*), DENN/MADD domain containing 1A (*DENND1A*), RAB5B, member RAS oncogene family (*RAB5B*) and thyroid adenoma associated (*THADA*) gene loci in Hui Chinese and American or European Caucasian individuals with PCOS (Goodarzi *et al.* 2012, Eriksen *et al.* 2013, Louwers *et al.* 2013, Ha *et al.* 2015, Hayes *et al.* 2015). While mutations, polymorphisms and splice variants in genes such as follistatin, fibrillin 3, cytochrome p450 side-chain cleavage (*CYP11A*), insulin receptor, hydroxysteroid dehydrogenase (*HSD*) *17B5*, *HSD17B6* and androgen receptor have also been linked to PCOS, such observations have not been confirmed in large populations or in multiple ethnicities (Azziz 2016, Dunaif 2016). The degree to which each of these genes contributes to the final reproductive and metabolic phenotype of PCOS women remains unclear. For instance, while overexpression and silencing of the polymorphic variant of the *DENND1A* in the human theca cells, the main source of testosterone for the hyperandrogenic phenotype, has been shown to increase and decrease testosterone synthesis, respectively (McAllister *et al.* 2014), to what extent alterations in this is linked to other PCOS symptoms is unclear.

Genetic mouse models involving many of the gene variants linked to PCOS are limited. A transgenic mouse overexpressing the *DENND1A* variant identified in PCOS patients resulted in a hyperandrogenic state with no impact on fertility (Modi 2016); detailed phenotypic assessment of PCOS characteristics is not available for this model. Transgenic mouse models overexpressing LH beta subunit while manifesting chronically elevated LH/testosterone levels and infrequent ovulation produced a cystic, tumorigenic ovarian phenotype (Risma *et al.* 1995). While FSH deficiency and polymorphism in *FSHR* has been linked to PCOS (Dolfin *et al.* 2011), mouse models of FSH deficiency although infertile fail to manifest a hyperandrogenic or multifollicular ovarian phenotype (Burns & Matzuk 2002, Barnett *et al.* 2006). Prevention of the development of PCOS phenotype that normally follow prenatal androgen treatment in global, brain or theca cell-specific androgen receptor (AR) knock-out mice emphasizes a role for AR (Walters & Handelsman 2017). This observation is consistent with polymorphisms in

AR gene being linked to PCOS (Wang *et al.* 2015). These findings from transgenic models combined with the <10% heritability estimate of PCOS-linked loci identified through GWAS (Azziz 2016) suggest involvement of additional loci and factors. Several transgenic models are available that link other loci (nerve growth factor, plasminogen activator inhibitor 1, estrogen receptor alpha) to PCOS characteristics (Barnett *et al.* 2006, Walters *et al.* 2012). However, these loci have not been substantiated in large PCOS cohort studies.

Developmental basis in the origin of PCOS

The observation that individuals born with low birth weight are at high risk for manifestation of cardiometabolic disorders during adulthood led to the developmental origin of health and disease hypothesis by Barker (2004). According to this hypothesis, early fetal exposure to stressors can induce physiological adaptations that fail and manifest as disease during adulthood. The findings that girls born either small or large for their gestational age are at increased risk for developing PCOS during reproductive life (Melo *et al.* 2010, Mumm *et al.* 2013), suggests PCOS could also have developmental basis. Additional support for this premise comes from reports of (1) PCOS phenotype in offspring exposed to excess androgen *in utero*, which occur in conditions such as congenital adrenal hyperplasia (Hague *et al.* 1990), congenital virilizing tumors (Barnes *et al.* 1994) and loss-of-function mutations in aromatase (Morishima *et al.* 1995) or sex hormone-binding globulin gene (SHBG) (Hogeveen *et al.* 2002), (2) elevated second trimester amniotic fluid testosterone levels in PCOS women (Palomba *et al.* 2012) and (3) increased anogenital distance (Wu *et al.* 2017, Barrett *et al.* 2018) and 2nd to 4th finger (2D:4D) ratio (Palomba *et al.* 2012), biomarkers of prenatal androgen exposure in offspring of PCOS women. The developmental origins of PCOS theory is also supported by studies in murine, rodent, sheep and monkey models, which show that administration of steroids such as testosterone, dihydrotestosterone (DHT) or estradiol valerate or steroid synthesis inhibitors during the perinatal period induce the development of PCOS-like phenotype (Abbott *et al.* 2005, Padmanabhan & Veiga-Lopez 2013, Maliqueo *et al.* 2014). For instance, the prenatal testosterone-treated female sheep, the animal model our group works with, manifests fetal growth restriction and is born with low birth weight (Manikkam *et al.* 2004, Steckler *et al.* 2005). As these animals age, they manifest disruptions in neuroendocrine steroid feedback, increased

pituitary sensitivity to gonadotropin-releasing hormone, LH hypersecretion, functional hyperandrogenism, multifollicular ovarian morphology, oligo-/anovulation and insulin resistance. In addition to meeting the diagnostic criteria proposed for PCOS by all agencies, these animals manifest cardiometabolic disruptions such as that seen in women with PCOS (Padmanabhan & Veiga-Lopez 2013, 2014s, Cardoso *et al.* 2015). Similarly, developmental exposure of sheep to bisphenol A (BPA, an environmental endocrine-disrupting compound (EDC) results in low birth weight (Savabieasfahani *et al.* 2006) offspring, which during adulthood manifest hypothalamic, pituitary and ovarian changes that mimic the PCOS phenotype (Veiga-Lopez *et al.* 2014a). Human studies also point to an association between BPA and hyperandrogenism (Rutkowska & Rachon 2014).

While the findings from animal and human disease models suggest that inappropriate developmental exposure to native and environmental steroidal mimics during critical windows of differentiation can result in a PCOS phenotype, this does not necessarily imply that this is the basis for the etiology of human PCOS. For instance, although cordocentesis studies have found 40% of human female fetuses during the second trimester have male circulating levels of testosterone (Beck-Peccoz *et al.* 1991), the prevalence rate for PCOS is far less, only 5–20%. Similarly, the prevalence of PCOS in females who are co-twin with male is not different compared to females born as part of same-sex twin pairs or singletons (Kuijper *et al.* 2009). These observations are at odds with the premise that developmental exposure to excess steroids, by itself, can explain the etiology of PCOS.

Gene-environment interaction

The evidence accumulated so far suggests that the pathogenesis of PCOS is likely complex and quite possibly involve gene $\times$ environment interaction. Such an interaction would not only explain the prevalence estimate of PCOS (5–20%) relative to number of fetuses getting exposed to excess testosterone (40%), but also the differing phenotypic manifestation of PCOS phenotypes. Phenotypic differences in neuroendocrine, ovarian and metabolic defects in animal models following prenatal androgen excess can originate from differences in the timing, duration and degree of exposure. However, the fact that phenotypic differences are also evident among animals subjected to identical exposure paradigms highlights contribution from individual genetic susceptibility to such developmental insults. Such gene $\times$ environment

interactions are likely mediated by epigenetic mechanisms (Barros & Offenbacher 2009), involving changes in DNA methylation, histone acetylation and non-coding RNA expression (Amaral & Mattick 2008, Kim *et al.* 2009). As such the epigenome provides a means to translate the information captured from the environment to heritable changes by turning on or off gene expression patterns.

From a PCOS perspective, epigenetic changes in the AR gene (Hickey *et al.* 2006) and increased presence of markers of epigenomic alterations in the whole blood, ovarian and adipose tissues from PCOS women have been reported (Jones *et al.* 2015, Li *et al.* 2016). Epigenetic modifications have also been observed in prenatal androgenized animal models of PCOS. These include changes in 163 and 325 methylated loci in infant and adult visceral adipose tissues of prenatally androgenized rhesus macaques (Xu *et al.* 2011), changes in expression of miR-497 and miR-15b in fetal ovaries from prenatal testosterone-treated sheep (Luense *et al.* 2011) and hypomethylation of five CpG sites of AR and one single CpG site in Cyp11a1 (CpG +953) in ovaries of prenatal testosterone-treated rats (Xia *et al.* 2015). In this context, it is important to recognize that sex hormones (estrogens and androgens), which are perturbed in PCOS women, are known activators of epigenetic mechanisms (Crews *et al.* 2014, Hunter *et al.* 2015).

Severity of PCOS phenotype

While genetic and developmental insults induce disruptions early in life, various postnatal events such as diet, lifestyle, disease states, stress and environmental exposures can have continuing influence on the disease state. Recently, the idea that these postnatal factors may act as 'second hit' to maintain, unmask or amplify the severity of disease phenotype programmed by genetic or developmental is gaining prominence. Such a phenomenon has been described in the pathogenesis of schizophrenia and cancer (Bayer *et al.* 1999, Tang & Ho 2007). Because PCOS symptoms do not become apparent till puberty, postnatal factors can serve as a second hit in terms of the manifestation or amplification of the severity of the PCOS phenotype. For instance, LH excess and metabolic disruptions that originate from the disruption of the fetal hypothalamic-pituitary-ovarian axis by the maternal hyperandrogenic condition (first hit) themselves can serve as a second hit contributing to the severity of offspring's reproductive and metabolic phenotype (Bremer 2010). Additionally, 38–88% of PCOS patients have obesity with associated metabolic

disorders such as insulin resistance (Diamanti-Kandarakis & Dunaif 2012) and hyperinsulinemia due to insulin resistance can act as a second hit. Hyperinsulinemia increases theca cell androgen production (Nahum *et al.* 1995), which when associated with reduced levels of SHBG – a buffer to sequester free testosterone, can induce hyperandrogenemia (Nestler *et al.* 1991). Studies in animal models provide support for the two hit hypothesis in PCOS pathogenesis. In the prenatal testosterone-treated sheep model of PCOS phenotype, postnatal obesity has been found to act as a second hit to increase the severity of PCOS-like symptoms (Steckler *et al.* 2009). Although not tested, PCOS phenotype has also been postulated to involve both the prenatal testosterone exposure and postnatal adiposity in rhesus macaques (Abbott *et al.* 1998).

Mediators of PCOS

PCOS patients manifest disruptions at both ovarian and extra-ovarian levels. Ovarian changes that contribute to the diagnostic criteria of PCOS include multifollicular appearance, hyperandrogenism, oligo/anovulation and luteal defects. The extra-ovarian changes, although not part of diagnostic criteria, include LH hypersecretion with increased LH/FSH ratio at the neuroendocrine level and hyperinsulinemia, hyperglycemia, dyslipidemia and altered adipokine secretion at the metabolic level. The ovarian and extra-ovarian factors that contribute toward the phenotypic manifestation of PCOS are discussed in detail below.

Ovarian mediators of PCOS

Women with PCOS are characterized by multifollicular ovarian morphology and ovarian enlargement (an increase in ovarian area and volume). Polycystic (multifollicular) ovarian morphology (PCOM) is a criterion for the diagnosis of PCOS as defined by the 2003 Rotterdam consensus and the 2006 Androgen Excess and PCOS Society criteria and recent NIH consensus meeting (Dewailly *et al.* 2014, Azziz *et al.* 2016). Earlier ovarian studies in humans were confined to wedge resection or postmortem tissues and these studies have shown that the polycystic ovary (PCO, a misnomer terminology) appearance results from presence of 10–12 growing follicles that measure <10mm in size along with increase in stromal hypertrophy (Hughesdon 1982). With advance in non-invasive imaging tools such as transvaginal ultrasound, follicle count thresholds for distinguishing PCOM ovaries from normal ovaries have changed over time. The most recent recommendation by

the task force of the Androgen Excess and the Polycystic Ovary Syndrome Society (AE-PCOS) is a threshold setting of >25 follicles, when scanned with transducer frequency ≥ 8 MHz (Dewailly *et al.* 2014).

Most studies addressing follicular number and distribution have involved single time point scanning or histological observations with postmortem ovaries. These studies have concluded ovaries of PCOS women are characterized not only by increased number of antral follicles (Dewailly *et al.* 2014), but also a reduction in number of primordial follicles (Webber *et al.* 2003) and follicular arrest (Franks *et al.* 2008). The PCOM phenotype in PCOS women can therefore arise from (1) enhanced recruitment and (2) follicular persistence due to arrest in follicular development, premature luteinization and reduced state of atresia (Franks *et al.* 2008). Additionally, women with PCOS undergoing *in vitro* fertilization have been found to produce more number of oocytes, which are often of poor quality thus contributing to the reduced fertilization, cleavage and implantation rates (Qiao & Feng 2011). The potential ovarian mediators of each of these aspects of ovarian disruptions are discussed below and summarized in Table 2.

Follicular activation/recruitment

Ovaries have finite number of primordial follicles at birth that form the ovarian follicular pool or reserve where they remain in a quiescent state. The transition of quiescent primordial follicles in the ovary to primary follicles that initiates the growing phase is referred to as follicular activation or recruitment (Fig. 1). During each reproductive cycle, a few follicles from the primordial pool undergo activation/recruitment, of which only a few undergo further follicular development into preantral follicles with others undergoing atresia through programmed cell death (Pru & Tilly 2001). The follicular

activation process is tightly controlled by paracrine and autocrine factors such as transforming growth factor (TGF) family members $\text{TGF}\alpha$, $\text{TGF}\beta$, bone morphogenetic protein 4 (BMP4) and anti-Müllerian hormone (AMH), growth factors or cytokines such as kit ligand (KITL), fibroblast growth factor (FGF) and leukemia inhibitory factor (LIF) and steroid hormones (Skinner 2005). These growth factors either activate (KITL, FGF, $\text{TGF}\alpha$, LIF and BMP4) or inhibit (AMH and $\text{TGF}\beta$) the activation process so the balance between them likely determine the direction of the activation process (Gougeon 2010). Additionally, presence of factors that regulate the bioavailability of these growth factors, for example, fibrillins that sequester TGF family members (Bastian *et al.* 2016), can also govern the follicular activation process. Genetic studies showing increased follicular activation in protein kinase B (AKT) inhibitor, phosphatase and tensin homolog (*Pten*) (Reddy *et al.* 2008) and AKT-dependent transcription factor forkhead box O3a (*Foxo3a*) (Castrillon *et al.* 2003) knockout mouse have shed light on these cell signaling pathways. Androgens can also induce follicular activation via stimulation of AKT signaling and inhibition of FOXO3A protein (Lebbe & Woodruff 2013). In addition, anti-apoptotic proteins such as B-cell lymphoma-2 (BCL2) promote follicular survival and increase the rate of activation, while pro-apoptotic proteins such as BCL2-associated X protein (BAX) decrease the rate of activation by increasing follicular atresia (Hussein 2005).

The suggestion of increased follicular recruitment in PCOS came from initial histological studies by Hughesdon (Hughesdon 1982) and later confirmed by others (Webber *et al.* 2003, Maciel *et al.* 2004), which indicated that ovaries from PCOS women have increased number of primary follicles. Although the reason for such increase is still not clear, the identification that one of the PCOS susceptibility locus D19S884 allele 8 that maps to

Figure 1

Schematic showing the stages of follicular development in normal ovary.

Table 2 Ovarian factors that contribute to PCOS phenotype.

Factor	Changes observed in PCOS patients (references)	Ovary-specific mechanisms	Role
Follicular activation/recruitment			
AMH	Low AMH expression in primordial and transitional follicles (Stubbs et al. 2005)	Inhibits follicular activation	Inhibits activation
FBN3	Polymorphisms in D19S884 allele 8 that maps to fibrillin 3 (FBN3) gene (Urbanek et al. 1999)	Regulates bioavailability of TGF family members	May contribute to activation of follicles
Androgens	General and ovarian hyperandrogenism (reviewed in Franks et al. 2008 , Dewailly et al. 2016)	Stimulates AKT pathway that leads to inactivation of FOXO3	Increases activation
Follicular persistence/arrest			
Androgens	General and ovarian hyperandrogenism (reviewed in Dewailly et al. 2016)	Contributes to increase FSHR and LHR expression that leads to induction of premature luteinization	Causes arrest
Estrogens	Increased or low follicular fluid levels (reviewed in Franks et al. 2008)	Increased levels reduce pituitary FSH secretion and low levels can inhibit follicular growth	Contributes to arrest
Activin	Decreased follicular fluid and serum levels (Norman et al. 2001)	Promotes pituitary FSH secretion and ovarian FSH action	Prevents arrest
Follistatin	Increased follicular fluid and serum levels (Erickson et al. 1995)	Inhibits activin	Contributes to arrest
Inhibin	Decreased inhibin A and B forms in follicular fluid levels; inhibits activins (Magoffin & Jakimiuk 1998 , Welt et al. 2005)	Decreases pituitary FSH secretion	Causes arrest
AMH	Increased antral follicular fluid levels (Fallat et al. 1997 , Desforges-Bullet et al. 2010)	Reduces sensitivity to FSH	Causes arrest
IGF	Increased follicular fluid levels (Eden et al. 1990)	In excess levels may inhibit follicular growth	Contributes to arrest
IGFBP	Decreased IGFBP1 and 4 follicular fluid levels (Holly et al. 1990 , Cataldo & Giudice 1992)	Regulates IGF bioavailability	Contributes to arrest
EGF	Increased follicular fluid levels (Volpe et al. 1991)	Induces premature luteinization	Causes arrest
NGF	High levels in follicular fluid (Dissen et al. 2009)	Unknown	Causes arrest
VEGF	High levels in follicular fluid	Induces premature luteinization	Causes arrest
	Increased granulosa cell and reduced theca cell protein content (Savchev et al. 2010 , Ferrara et al. 2003)		
EB-VEGF	Increased expression in stromal theca-interna cells (Ferrara et al. 2003)	Promotes VEGF expression	Contributes to arrest
FGF	Low levels in follicular fluid levels (Hammadeh et al. 2003)	Induces premature luteinization	Causes arrest
	Elevated levels in follicular fluid (Artini et al. 2006)		
Adiponectin	Low levels of high molecular weight form in follicular fluid; decreased adiponectin and its receptor expression in granulosa cells. (Artimani et al. 2016)	Promotes theca cell androgen synthesis	Causes arrest
TNF	High in follicular fluid (Amato et al. 2003)	Participates in granulosa cell differentiation, proliferation and apoptosis	Contributes to arrest
IL	High follicular fluid IL6 and 13 and low follicular fluid IL12 (Amato et al. 2003)	Reduces estradiol synthesis	Contributes to arrest
MMP	No change in MMP2 and 9 activities in follicular fluid (Lahav-Baratz et al. 2003); higher follicular fluid content of MMP2 and 9 and increased expression of MMP9 in granulosa cells (Shalev et al. 2001)	Induces premature luteinization	Contributes to arrest
TIMP	Low TIMP1 levels in follicular fluid (Lahav-Baratz et al. 2003); no change in expression in granulosa cells (Shalev et al. 2001)	Regulates MMP function	Contributes to arrest

(Continued)

Table 2 Continued.

Factor	Changes observed in PCOS patients (references)	Ovary-specific mechanisms	Role
Fas	Decreased serum and follicular fluid levels of soluble Fas	Reduced levels decrease atresia	Contributes to arrest
MicroRNAs	Promotes apoptosis (Onalan <i>et al.</i> 2005) miR-320a down-regulated in cumulus cells – targets RUNX2 which regulates steroidogenesis and granulosa cell differentiation (Zhang <i>et al.</i> 2017) miR-483 down-regulated in ovarian cortex – targets IGF1 (Xiang <i>et al.</i> 2016) miR-145 down-regulated in granulosa cells – targets insulin receptor substrate 1 (IRS1) that regulates granulosa cell proliferation (Cai <i>et al.</i> 2017) Decreased follicular fluid levels of miR-93 and miR-21 – targets TGF beta family members (Naji <i>et al.</i> 2017) miRNAs 200a-3p, 10b-3p, 200b-3p, 29c-3p, 99a-3p, and 125a-5p are elevated and miR-105-3p is decreased in follicular fluid – many of these are associated with androgen synthesis (Xue <i>et al.</i> 2017) Decreased miR-145 and miR-182 in granulosa cells and increased miR-182 in follicular fluid (Naji <i>et al.</i> 2018)	Variable mechanism depending on their gene targets	Variable outcomes depending on their gene targets
WNT/frizzled/ β -catenin	Frizzled 3 expression increased in cumulus cells (Qaio <i>et al.</i> 2017)	Decreases estradiol synthesis	Causes arrest
FOXO3	Increased FOXO3 mRNA and protein and decreased phospho FOXO3 in granulosa cells from antral follicles (Mikaeili <i>et al.</i> 2016)	Promotes apoptosis of granulosa cells, participates in follicular atresia	Causes follicular persistence
Oocyte quality			
GDF9	Reduced cumulus cells and normal oocyte expression of mRNA (Zhao <i>et al.</i> 2010)	Participates in oocyte maturation	Promotes oocyte quality
BMP15	No change in oocyte or cumulus expression of mRNA (Zhao <i>et al.</i> 2010)	Participates in oocyte maturation	Promotes oocyte quality
BDNF	Increased follicular fluid levels (Johnstone <i>et al.</i> 2008)	Unknown	Promotes oocyte quality
FGF	Low levels in follicular fluid levels (Hammadeh <i>et al.</i> 2003)	Unknown	Reduces oocyte quality
EGF	Elevated levels in follicular fluid (Artini <i>et al.</i> 2006) Increased follicular fluid levels (Volpe <i>et al.</i> 1991)	Involved in oocyte maturation	May promote oocyte quality
IGF	Increased follicular fluid levels (Eden <i>et al.</i> 1990)	May be involved in oocyte maturation	May promote oocyte quality
VEGF	High levels in follicular fluid (Savchev <i>et al.</i> 2010)	Stimulates maturation of oocytes	Promotes oocyte quality
AMH	Increased antral follicular fluid levels (Fallat <i>et al.</i> 1997, Desforges-Bullet <i>et al.</i> 2010)	May participate in oocyte maturation	Reduces oocyte quality
IL	High follicular fluid IL6 and 13 and low follicular fluid IL12 (Amato <i>et al.</i> 2003)	May participate in oocyte maturation	Reduces oocyte quality
LIF	Low follicular fluid levels (Ledee-Bataille <i>et al.</i> 2001)	Promotes oocyte maturation	Promotes oocyte quality
TNF	High follicular fluid (Amato <i>et al.</i> 2003)	Decreases oocyte maturation and induces abnormal chromosomal alignment and cytoskeleton structure in oocyte	Reduces oocyte quality
CRF	Low follicular fluid levels (Mastorakos <i>et al.</i> 1994)	Unknown	Promotes oocyte quality

AMH, anti-Müllerian hormone; FBN3, fibrillin; IGF, insulin-like growth factor; IGFBP, IGF binding proteins; EGF, epidermal growth factor; NGF, nerve growth factor; VEGF, vascular endothelial growth factor; EB-VEGF, endocrine-gland derived VEGF; FGF, fibroblast growth factor; TNF, tumor necrosis factor alpha; IL, interleukins; MMP, matrix metalloproteinase; TIMP, tissue inhibitors of MMP; FOXO3, forkhead box O3 transcription factor; GDF9, growth differentiation factor 9; BMP15, bone morphogenetic protein 15; BDNF, Brain-derived neurotrophic factor; LIF, Leukemia inhibiting factor; CRF, Corticotrophin-releasing hormones.

fibrillin 3 (*FBN3*) gene (Urbanek *et al.* 1999) sheds some light on ovarian factors involved during early follicular development. Considering that (1) the expression of *FBN3* is restricted to perifollicular stromal area of the follicles transitioning from primordial to primary stage (Jordan *et al.* 2010), (2) its expression is highest in the first trimester fetal ovaries (Hatzirodos *et al.* 2011) and (3) fibrillins can sequester TGFs (Bastian *et al.* 2016), *FBN3* has the potential to alter the follicular developmental trajectory by altering bioavailability of TGF members. In addition, lower expression of *AMH* in primordial and primordial to primary transitional follicles in ovaries from PCOS women compared with normal women (Stubbs *et al.* 2005) coupled with findings of increased follicular activation evidenced in *Amh* null mouse (Durlinger *et al.* 1999), and the ability of *AMH* to inhibit the number of early growing follicles from mouse ovaries cultured *in vitro* (Durlinger *et al.* 1999) indicate lower *AMH* expression to be conducive to follicular activation in the PCOS ovary. As such, the lower number of atretic early growing follicles in PCOS ovarian tissue (Webber *et al.* 2007) in the face of low *AMH* levels could promote follicular activation/recruitment and survival thus increasing the cohort of early growing follicles available for further differentiation (Fig. 2) (Franks *et al.* 2008).

Support for enhanced recruitment also comes from prenatal testosterone-treated Suffolk (Steckler *et al.* 2005, Smith *et al.* 2009) and Poll Dorset (Forsdike *et al.* 2007) sheep models of PCOS, which manifest reduced primordial follicles with corresponding increase in growing follicles. In addition to this morphological evidence, (1) reduced number of early growing follicles staining positively for the follicular activation inhibitor, *AMH*, in ovaries from both of these prenatal testosterone-treated sheep breeds (Bull *et al.* 2004, Veiga-Lopez *et al.* 2012), (2) increased presence of *AR* protein in the fetal granulosa and stromal cells of the prenatal testosterone-treated Suffolk sheep (Ortega *et al.* 2009) indicative of increased androgen signaling and (3) reduced expression of pro-apoptotic protein *BAX* in granulosa cells of fetal primordial and primary follicles (Salveti *et al.* 2012) are all supportive of increased follicular activation (Fig. 2). All in all, studies with sheep models of PCOS phenotype, as is the case with human PCOS provide support for involvement of paracrine factors and apoptotic machinery in increasing follicular recruitment.

Follicular persistence

The activated primary follicles differentiate and grow to become preantral follicles. *In vitro* studies in non-human primates have found that *AMH* supports preantral

Figure 2

Schematic showing directionality of changes in factors contributing to disruptions in the follicular activation/recruitment in PCOS women and animal models of PCOS.

follicular growth (Xu *et al.* 2018). As such, *AMH* appears to have opposing effects on primordial and preantral follicles, namely one of inhibition on primordial to primary transition but stimulation on survival and growth of preantral follicles. Preantral follicles continue to develop under the stimulation of *FSH* into antral follicles that transition through small to large antral follicle stages. The fate of antral follicles is to either undergo atresia or mature to become preovulatory follicles that ovulate in response to *LH* surge and luteinize (Fig. 1). As such, the persistence of small antral follicles leading to the PCOM can arise from arrest in antral follicular growth, premature luteinization and reduced rate of atresia.

(i) *Arrest in antral follicular development*: Because *FSH* is a major regulator of antral follicular development, reduction in factors that promote sensitivity of antral follicles to *FSH* such as activin and insulin-like growth factor (*IGF*)

or increase in factors that inhibits sensitivity to FSH such as inhibins, follistatin and AMH (Mazerbourg *et al.* 2003, Knight *et al.* 2012, Visser & Themmen 2014) or IGF-binding proteins (IGFBP) that regulate bioavailability of IGFs (Kwintkiewicz & Giudice 2009) can arrest follicular growth. Other factors that can contribute to follicular growth arrest include epidermal growth factor (EGF), nerve growth factor (NGF) and tumor necrosis factor alpha (TNF) (Jonard & Dewailly 2004).

Lower levels of activin (Norman *et al.* 2001) coupled with higher follistatin (Erickson *et al.* 1995) and AMH levels in follicular fluid of women with PCOS (Fallat *et al.* 1997, Desforbes-Bullet *et al.* 2010) are consistent with reduced FSH sensitivity and growth arrest (Fig. 3). In addition, higher antral follicular fluid levels of EGFs (Volpe *et al.* 1991), NGF (Disseen *et al.* 2009) and TNF (Amato *et al.* 2003) also contribute to antral follicular growth arrest/follicular persistence. While the high levels of IGF1 found in PCOS follicles (Eden *et al.* 1990) are inconsistent with growth arrest, their action may have been offset by parallel increases in IGF-binding proteins (IGFBP) (Holly *et al.* 1990, Cataldo & Giudice 1992).

The prenatal T-treated sheep model of PCOS, only model where serial ultrasonographic scans have been performed for over 25 days, has provided evidence in support of antral follicles surviving for longer periods (Manikkam *et al.* 2006, Veiga-Lopez *et al.* 2014b) without further growth, supportive of follicular arrest and persistence. Intra-follicular changes observed in prenatal testosterone-treated sheep namely increased follistatin and reduced activin β B mRNA levels (West *et al.* 2001) and increased AMH protein content in granulosa cells (Veiga-Lopez *et al.* 2012) and TNF in the theca cells (Puttabyatappa *et al.* 2017b) of antral follicles, parallel what has been observed in PCOS women. Altered steroid receptor balance with an increase in estrogen receptor alpha (ESR1) and AR and decrease in estrogen receptor beta (ESR2) protein content in granulosa cells of antral follicles (Ortega *et al.* 2009) leading to reduced estrogen action are consistent with impaired antral follicle growth (Volpe *et al.* 1991). These findings are consistent with increase in negative mediators of follicular FSH sensitivity and reduced antral follicle growth contributing to the follicular arrest in prenatal testosterone-treated sheep (Fig. 3).

(ii) *Premature luteinization*: The growth of the antral follicles that develop from small to large antral and preovulatory stage terminates with the follicle differentiating under the influence of ovulatory gonadotropin surge into corpus luteum through a process known as luteinization. The intra-ovarian factors that

Figure 3

Schematic showing directionality of changes in factors contributing to follicular persistence in PCOS women and animal models of PCOS.

promote luteinization are androgens (Nielsen *et al.* 2011), adiponectin (Palin *et al.* 2012), proteases such as matrix metalloproteases (MMP), growth factors such as epidermal growth factors (EGF), vascular endothelial growth factors (VEGF), FGFs and cytokines such as interleukins and LIF (Giovanni Artini *et al.* 2007). The increases in these factors during the small antral stage can induce premature luteinization (Franks *et al.* 2008, Dewailly *et al.* 2016) thus contributing to the follicular persistence.

Thecal androgen excess (Gilling-Smith *et al.* 1994), premature expression of LH receptors possibly the consequence of the hyperandrogenic status (Willis *et al.* 1998, Dewailly *et al.* 2016), increased follicular fluid EGF (Volpe *et al.* 1991) and VEGF (Ferrara *et al.* 2003) and increased granulosa cell expression of VEGF and endocrine gland-derived VEGF (EB-VEGF) (Ferrara *et al.* 2003), increased MMP2 and 9 and reduced tissue inhibitor of MMPs (TIMP1) content in the follicular fluid and increased MMP9 but not TIMP1 expression in granulosa cells (Shalev *et al.* 2001, Lahav-Baratz *et al.* 2003) and increase in cytokines interleukin 6 and TNF (Amato *et al.* 2003) evidenced in women with PCOS are all consistent with premature luteinization and follicular persistence.

Similarly, the increases in AR (Ortega *et al.* 2009), VEGF and its receptor VEGFR3 (Ortega *et al.* 2015) and increase in MMP9 with reduction in TIMP1 and matrix proteins laminin B and collagen protein observed in antral follicles of prenatal testosterone-treated sheep (Puttabyatappa

et al. 2017b) are consistent with premature luteinization in this animal model of PCOS.

(iii) *Follicular atresia*: Follicular development involves cyclical recruitment of a cohort of small antral follicles out of which one of them (in monoovulatory and several in litter-bearing species) emerge to be dominant that goes on to ovulate while the rest of them undergo atresia through apoptosis (Fig. 1) (Pru & Tilly 2001). Increased expression of growth factors such as EGF and IGF that promote cell survival (Homburg & Amsterdam 1998) and loss of balance between pro-apoptotic proteins such as Fas and BCL2-associated X, apoptosis regulator (BAX) and anti-apoptotic proteins such as BCL2 (Escobar *et al.* 2011) can disrupt the atretic process contributing to follicular persistence and accumulation leading to the PCOM phenotype.

Consistent with the decreased atresia providing a basis for accumulation of follicles leading to PCOM phenotype DNA fragmentation, soluble Fas (sFas) and soluble Fas ligand (sFasL), markers of apoptosis were lower in granulosa cells from PCOS patients (Onalan *et al.* 2005). As such, low follicular fluid levels of soluble Fas (Onalan *et al.* 2005) coupled with high levels of growth factors EGF (Volpe *et al.* 1991) and IGF1 (Eden *et al.* 1990) in PCOS patients are consistent with reduced atresia and increased follicular survival leading to persistence.

Similarly, imbalance in opposing proliferative and apoptotic signals represented as an increase in expression of the proliferative marker PCNA and decrease in BCL2 and activated caspase-3 in granulosa cells (Salveti *et al.* 2012) coupled with increased TNF in theca cells of antral follicles (Puttabyatappa *et al.* 2017b) in the sheep model of PCOS phenotype are also consistent with reduced atresia contributing to accumulation of antral follicles.

Oocyte quality

Ovarian folliculogenesis and oogenesis occurs in parallel with follicular somatic cells and oocytes influencing each other during the developmental process. Although oocyte meiotic arrest occurs during early stages of follicle formation, oocyte-secreted factors influence follicle growth (Eppig 2001). While the granulosa cell secretions provide nutrient support and maintain meiotic arrest till induction of ovulation (Albertini 2015), factors produced by follicular somatic cells such as EGFs (Hsieh *et al.* 2009), IGFs (Wang & Sun 2007), FGFs (Artini *et al.* 2006), brain-derived neurotrophic factor (BDNF) (Kawamura *et al.* 2005), VEGF (Luo *et al.* 2002) and LIF (Ledee-Bataille *et al.* 2001) and oocyte-derived factors GDF9 and BMP15 (Hussein *et al.* 2006) are associated with good oocyte

quality. Factors such as androgens (Teissier *et al.* 2000), AMH (Fallat *et al.* 1997), TNF (Ma *et al.* 2010) and cytokine IL6, IL12 and IL13 (Gazvani *et al.* 2000, Amato *et al.* 2003) and corticotrophin-releasing hormones (CRF) (Mastorakos *et al.* 1994) are associated with poor oocyte quality.

Several studies have elaborated on the poor oocyte quality of women with PCOS (Franks *et al.* 2003, Qiao & Feng 2011). For instance, ovulation induction through stimulation cycles in anovulatory PCOS has been shown to produce increased number of oocytes (Qiao & Feng 2011) but of smaller oocyte size (Marquard *et al.* 2011). Excess ovarian androgen (Gilling-Smith *et al.* 1994), and follicular fluid FGF (Artini *et al.* 2006), TNF, IL6 and IL13 (Amato *et al.* 2003) and low follicular fluid levels of LIF (Ledee-Bataille *et al.* 2001) and CRF (Mastorakos *et al.* 1994) and reduced expression of GDF9 in cumulus cells (Zhao *et al.* 2010) of PCOS women (Table 2) are consistent with poor oocyte quality (Qiao & Feng 2011). On the contrary, increased follicular fluid content of EGF (Volpe *et al.* 1991), VEGF (Ferrara *et al.* 2003), IGF1 (Eden *et al.* 1990) and BDNF (Johnstone *et al.* 2008) are inconsistent with poor oocyte quality in PCOS patients. These data indicate that the balance between factors that promote oocyte quality and those that reduce quality may be shifted to the negative side in women with PCOS thus contributing to reduced oocyte competence and fertility outcomes in PCOS patients (Franks *et al.* 1998, Franks *et al.* 2003). Differential expression of genes in oocytes from PCOS women indicative of defects in meiosis or early embryonic development (Wood *et al.* 2007) provide further support for this premise. Additionally, fertilization rates in PCOS women in IVF settings have yielded variable results ranging from no effect to higher rates of fertilization (Sermondade *et al.* 2013). While the pregnancy rates among PCOS women undergoing IVF have been found to be similar to the non-PCOS women undergoing IVF (Heijnen *et al.* 2006), various meta-analysis have shown that infants born to women with PCOS are at higher risk for preterm birth, low birth weight, perinatal mortality, congenital abnormalities and likelihood of birth by Cesarean section (Boomsma *et al.* 2006, Qin *et al.* 2013). These findings raise concerns regarding the quality of oocytes used in IVF settings.

While the factors that regulate oocyte maturation and competency have not been well characterized in most animal models of PCOS, reduced oocyte competency have been reported in prenatally androgenized rhesus macaques (Dumesic *et al.* 2002). In the sheep model of PCOS, oocyte competence has not been directly examined

but mating studies showing reduced pregnancy rate (Steckler *et al.* 2007) suggest poor oocyte quality may also be a contributing factor in this model. An increase in VEGF (Ortega *et al.* 2015), which parallel what is seen in women with PCOS (Ferrara *et al.* 2003), is not consistent with poor oocyte quality; however, its beneficial effect appears to be offset by the increase in granulosa cell AMH (Veiga-Lopez *et al.* 2012) and thecal cell TNF (Puttabyatappa *et al.* 2017b).

Extra-ovarian mediators of PCOS

In addition to the ovarian mediators discussed earlier, disruptions in extra-ovarian mediators (Fig. 4) such as neuroendocrine secretions from the hypothalamo-pituitary axis that act on the ovary to regulate ovarian steroidogenesis, follicular development, ovulation and corpus luteum formation can play an important role in the pathogenesis of PCOS. The main neuroendocrine changes observed in PCOS women are high LH and subnormal FSH levels leading to higher LH/FSH ratio, the consequence of disruptions in steroid-negative feedback mechanisms and reduced pituitary sensitivity to GnRH (Balén *et al.* 1993, Taylor *et al.* 1997, McCartney *et al.* 2002, Gill & Hall 2014). In addition, metabolic factors such as insulin and adiponectin can also influence ovarian function with the former serving as a co-gonadotropin and the latter influencing sensitivity to insulin. Consistent with this,

hyperinsulinemia is the main metabolic abnormality observed in majority of the patients with PCOS (Diamanti-Kandarakis & Dunaif 2012) along with hyperglycemia, dyslipidemia and hypoadiponectinemia (Palin *et al.* 2012, Churchill *et al.* 2015). While ovary is the major contributor for the hyperandrogenic state, increased circulating adrenal androgen DHEAS levels present in 15–45% in women with PCOS (Luque-Ramírez & Escobar-Morreale 2016) indicates adrenal factors also play an important role in the development of PCOS. Further, growing evidence also point to role of other factors, such as environmental chemicals and lifestyle, in the manifestation of PCOS.

Neuroendocrine mediators

The ovarian function is tightly regulated by the integration of the HPO axis through feed-forward and negative and positive feedback loops. The hypothalamic secretion of GnRH on the pituitary and anterior pituitary secretion of FSH and LH on the ovary form the feed-forward response. The ovarian steroids androgens, estradiol and progesterone and peptide hormones such as inhibin and follistatin produced by the ovary and the pituitary provide the negative feedback loop to the hypothalamo-pituitary axis. The mid-cycle rise in estradiol drives the positive feedback at the level of the hypothalamus and pituitary to cause surge release of LH that triggers ovulation. Disruptions in the feed-forward and feedback mechanism will influence LH and FSH, the

Figure 4

Schematic showing extra-ovarian factors contributing to ovarian disruptions in PCOS women and animal models of PCOS. Images used in this figure were sourced from www.pixabay.com, www.publicdomainvectors.org, commons.wikimedia.org and www.openclipart.org.

main hypothalamo–pituitary regulators of ovary, which in turn will have a negative impact on ovarian function.

Neuroendocrine studies carried out in PCOS women provide evidence in support of abnormalities in the hypothalamic–pituitary axis contributing to the pathogenesis of PCOS. The increase in frequency of GnRH release assessed using LH as a bioassay (Pagan *et al.* 2006) and exaggerated LH response to exogenous GnRH (Gill & Hall 2014) together appear to underlie the LH hypersecretion in women with PCOS. The increases in GnRH/LH in turn appear to be a function of reduced sensitivity to steroid negative feedback (Marshall & Eagleson 1999, Burt Solorzano *et al.* 2012). Findings that high concentrations of estradiol and progesterone are required to reduce pulsatile LH release (Pastor *et al.* 1998) and steroid sensitivity can be restored with androgen antagonist flutamide treatment (Eagleson *et al.* 2000) do provide evidence in support of compromised steroid negative feedback sensitivity in PCOS patients. While pituitary levels of activin and follistatin are not known, the observations that PCOS patients have low circulating levels of activin (Norman *et al.* 2001) and increased follicular fluid follistatin (Erickson *et al.* 1995) are consistent with the low FSH levels.

Analogous scenario also exists in prenatal testosterone-treated animal models (Cardoso *et al.* 2015, Abbott *et al.* 2016). For instance, consistent with disruptions in the feedback mechanisms at pituitary and hypothalamic levels, prenatal testosterone-treated sheep manifest increased pituitary sensitivity to GnRH (Manikkam *et al.* 2008), reduced sensitivity to estradiol negative (Wood & Foster 1998, Sarma *et al.* 2005), estradiol positive (Wood & Foster 1998, Sharma *et al.* 2002, Unsworth *et al.* 2005) and progesterone negative feedback (Robinson *et al.* 1999, Veiga-Lopez *et al.* 2009). Neuroanatomical studies support neuropeptide imbalance in the KNDy (kisspeptin, neurokinin B and dynorphin) neurons reflected as reduced inhibitory (dynorphin) and no change in stimulatory (kisspeptin) (Cheng *et al.* 2010) neuropeptides as a potential mediator of the decreased ability of progesterone to exert negative feedback effect on GnRH/LH secretion. Similar disruptions in estradiol and progesterone feedbacks have also been observed in prenatal testosterone-treated macaque (Abbott *et al.* 2016) and rodent (Moore *et al.* 2015) models. Furthermore, the protection from development of PCOS-like phenotype induced by prenatal DHT treatment in brain-specific AR-knockout mouse (Caldwell *et al.* 2017) indicates a neuroendocrine role for androgens in disrupting the HPO axis.

Metabolic mediators

Insulin is the major metabolic hormone that regulates glucose homeostasis and lipid metabolism in the body. When cell or tissue requires excess insulin to respond normally, it develops insulin resistance and as pancreatic beta cells respond with production of more insulin compensatory hyperinsulinemia develops. Hyperinsulinemia can induce hyperandrogenism by either directly stimulating ovarian androgen production (Hernandez *et al.* 1988) or indirectly through (1) enhancement of gonadotropin secretion from pituitary (Adashi *et al.* 1981), (2) intensifying gonadotropin action at the ovary (Cara & Rosenfield 1988) or (3) increasing bioavailability of androgens through inhibition of liver sex hormone-binding globulin (SHBG) production (Nestler *et al.* 1991). In addition, hyperinsulinemic state together with hyperandrogenemia can increase FSH induction of LH receptor in granulosa cells of antral follicles and also LH action to induce premature luteinization (Willis *et al.* 1996). Hyperinsulinemia and insulin resistance evidenced in metabolic diseases are commonly associated with inflammatory state, hypoadiponectinemia and dyslipidemia, which can indirectly influence ovarian function by modulating insulin and gonadotropin action (Palin *et al.* 2012, Macut *et al.* 2013, Vassilatou 2014, Moran *et al.* 2015, De Leo *et al.* 2016).

Although 60–70% of women with PCOS are obese or overweight (Moran *et al.* 2015, Naderpoor *et al.* 2015) and obesity is associated with insulin resistance (Esser *et al.* 2014) the observation that majority of lean women with PCOS also manifest insulin resistance (Yildizhan *et al.* 2016) support a role for hyperinsulinemia in the manifestation of PCOS. The increased prevalence of chronic low-grade inflammation (Boots & Jungheim 2015), dyslipidemia (Couto Alves *et al.* 2017), hypoadiponectinemia (Manneras-Holm *et al.* 2011) and NAFLD (Makri & Tziomalos 2017) in PCOS patients, features that can negatively affect HPO axis, are also consistent with the role of metabolic factors contributing to the development of PCOS.

Similar to women with PCOS, prenatal testosterone-treated sheep also manifest reduced peripheral insulin sensitivity and hyperinsulinemia (DeHaan *et al.* 1990, Hansen *et al.* 1995, Recabarren *et al.* 2005, Padmanabhan *et al.* 2010), dyslipidemia (Veiga-Lopez *et al.* 2013, Puttabyatappa *et al.* 2017a) and hepatic lipid accumulation (Puttabyatappa *et al.* 2017a). Hyperinsulinemia with increased adiposity is also a feature of prenatal testosterone-treated macaque (Abbott *et al.* 1998) and rodent models (Roland *et al.* 2010). Hypoadiponectinemia, a feature seen in women with PCOS,

was observed in DHT-treated mouse model (Benrick *et al.* 2017) but not in prenatal testosterone-treated sheep (Puttabyatappa *et al.* 2017a).

Adrenal mediators

Although ovarian theca cell androgen production is a major source of hyperandrogenemia, adrenal production of DHEAS accounts for hyperandrogenism in about 15–45% of the women with PCOS (Luque-Ramírez & Escobar-Morreale 2016). The presence of high DHEAS among sisters and daughters of patients with PCOS suggests that adrenal hyperandrogenism (AH) may be an inherited trait (Maliqueo *et al.* 2009, Yildizhan *et al.* 2016). The cause for AH among PCOS patients is either adrenal hyper-responsiveness to adrenocorticotrophic hormone (ACTH) (Moran *et al.* 2005) or increased ACTH drive to adrenal due to reduced negative feedback stemming from decreased hyperinsulinemia-induced hepatic cortisol regeneration (Rodin *et al.* 1994).

Most of the data on the developmental programming of AH has come from prenatal androgen treated monkeys. Prenatally androgenized female rhesus macaques show enhanced basal and adrenocorticotrophic hormone (ACTH)-stimulated adrenal DHEA production suggesting adrenal defect in this model (Zhou *et al.* 2005). Hyperinsulinemia and increased adiposity observed in this model has been proposed as the potential mediator of AH (Abbott *et al.* 2009) and the observation that insulin sensitizer pioglitazone normalizes DHEAS response to ACTH stimulation confirms such assertions (Zhou *et al.* 2007).

Other mediators

In addition to ovarian and extra-ovarian mediators, other factors that can influence the ovarian function include environmental chemicals and lifestyle. Environmental cues play an important role in the regulation of ovarian function and its influence is integrated through the HPO axis (Vermeulen 1993). Environmental chemicals especially endocrine disruptors with steroid potential such as phthalates and bisphenol A (BPA) can alter the HPO functions by disrupting the steroidal feedbacks at the hypothalamus and pituitary level and steroid action at the level of the ovary (Peretz *et al.* 2014, Gore *et al.* 2015). Both under- and overnutrition, through production of stress steroids (Whirledge & Cidlowski 2010), sex steroids (Whyte *et al.* 2007, Mossa *et al.* 2013) or metabolic factors (Duque-Guimaraes & Ozanne 2013), can have a bearing on ovarian functions (Evans & Anderson 2017). Lifestyle factors such as a socioeconomic status, neighborhood

one lives in, stress and sedentariness can also impact the ovarian function through adverse health behavior that can lead to excessive weight gain, activation of the stress hormone axis or increased exposure to environmental chemicals (Rosmond 2005, Beydoun & Wang 2010, Nelson *et al.* 2012).

The identification of environmental chemicals with steroidogenic potential such as perfluorooctanoate, polychlorinated biphenyls, pesticides, polycyclic aromatic hydrocarbons and BPA (Kandaraki *et al.* 2011, Vagi *et al.* 2014) in PCOS women raise concerns regarding risks posed by these chemicals in development of PCOS. In support of this increased level of BPA has been associated with hyperandrogenic status (Rutkowska & Rachon 2014). Likewise, overnutrition and sedentary lifestyle likely contribute to the observation that 60–70% of women with PCOS are obese. A contributory role for nutrition is emphasized by the fact that dietary changes and weight loss ameliorates PCOS symptoms (Huber-Buchholz *et al.* 1999, Merkin *et al.* 2016).

Although these observations from prospective and retrospective studies in humans suggest a role for environmental and nutritional factors in the development of PCOS, causative role for these come from studies in animal models. For example, prenatal BPA treatment in sheep induced neuroendocrine and ovarian changes that mimic PCOS patients (Savabieasfahani *et al.* 2006, Veiga-Lopez *et al.* 2014a). Similarly, postnatal overfeeding was found to amplify the reproductive phenotype of the sheep model of PCOS, leading to anovulation (Steckler *et al.* 2009).

Ovulatory and luteal defects

An estimated 40% of PCOS patients manifest infertility that arise due to infrequent or absent ovulation or luteal phase deficiency (Teede *et al.* 2010, Boutzios *et al.* 2013). Majority of the anovulatory PCOS phenotype is associated with accumulation of 2–8mm antral follicles that fail to undergo follicle selection and dominance but persist due to excess production of AMH or premature acquisition of LH receptors in the granulosa cells. Normalization of HPO axis or metabolic functions has been shown to be effective in achieving ovulation (Legro 2016). Because PCOS patients have generally high LH and low FSH, utilization of estrogen receptor antagonists or aromatase inhibitors that reduce negative feedback of the estrogens on pituitary FSH secretion or exogenous FSH supplementation aid in ovulation induction (Jayasena & Franks 2014,

Legro 2016). Improving metabolic functions through administration of metformin, thiazolidinediones or lifestyle improvements such as dietary change, exercise and bariatric surgery have also been shown to improve ovulation induction (Balen *et al.* 2016). Infertility due to luteal phase deficiency is also evident in ovulatory PCOS patients who have low levels of progesterone during early luteal phase (Joseph-Horne *et al.* 2002). This low progesterone levels may arise due to abnormal synthesis of progesterone by granulosa cells (Doldi *et al.* 1998). Because high concentrations of progesterone are required to reduce LH release in PCOS patients (Pastor *et al.* 1998) supplementation of progesterone is a therapeutic approach to help normalize LH secretion and promote implantation (Unfer *et al.* 2005).

In the sheep model of PCOS, similar rescue of ovarian function by exogenous gonadotropin administration (Steckler *et al.* 2008), cyclic progesterone supplementation (Manikkam *et al.* 2006) or gestational rosiglitazone (an insulin sensitizing thiazolidinedione) treatment (Veiga-Lopez *et al.* 2010) or normalization of LH hypersecretion by postnatal rosiglitazone intervention (Cardoso *et al.* 2016) emphasize the role played by steroidal and metabolic factors in the development and maintenance of the pathology. The specific changes in intra- and extra-ovarian factors that contribute to the success of these interventions on follicular selection and dominance needs further investigation.

Conclusions

As discussed above, multiple factors that impact neuroendocrine, ovarian and metabolic functions are involved in the development of PCOS phenotype. The ovarian defects associated with PCOS are impacted by various intra- and extra-ovarian factors that influence follicular developmental process at multiple steps leading to increased recruitment, failure to achieve dominance or undergo atresia resulting in antral follicular developmental arrest. Differences in these multitudes of factors among the subtypes of PCOS (Table 1) may shed light on the mediators of phenotypic differences in these sub classes. However, several challenges still exist in 1) understanding the etiology of various subtypes of PCOS and lifelong consequences of PCOS, 2) developing interventions to prevent transmission of PCOS traits, 3) identifying optimal treatment strategies, and 4) complete phenotyping of the male counterpart of PCOS.

Development of preventive and intervention strategies require complete understanding of the

underlying ovarian and extra-ovarian mechanisms contributing to the development of the PCOS phenotype. While genetic studies explain only a small percentage of PCOS prevalence, disease gene mapping in a larger sample size that includes women from multiple ethnic groups to identify other loci involved in the development of PCOS are required. In terms of using animal models to probe underlying mechanisms and identifying additional ovarian and extra-ovarian factors, there is a great need to expand studies to precocial species that have similar developmental trajectory of organ systems as in humans.

In view of the potential for PCOS traits to be passed on to subsequent generations and the findings of microRNA expression (Table 2) and epigenetic changes in PCOS patients (Jones *et al.* 2015, Li *et al.* 2016) and animal models of PCOS (Luense *et al.* 2011, Xu *et al.* 2011), it is important to determine if transmission across generations involve transgenerational transmission of PCOS traits or they merely reflect repetitive multigenerational transfer of traits due to programmed manifestation of hyperandrogenism and hyperinsulinemia that serve as repetitive programmers from generation to generation. For instance, PCOS women during pregnancy manifest hyperandrogenemia and hyperinsulinemia (Sir-Petermann *et al.* 2009) that could program PCOS phenotype in the genetically-susceptible offspring. Studies in animal models also support that multi- and transgenerational transfer is possible. Studies in rodents have provided evidence of transgenerational transfer of reproductive and metabolic traits following exposure to experimental chemicals (Guerrero-Bosagna 2016). To what extent insulin sensitivity changes of prenatal testosterone-treated F1 female sheep that are evident also in the F2 female sheep offspring (Burns *et al.* 2016) reflect repetitive hyperandrogenic status remains to be determined. Therefore, in addition to long-term follow-up of offspring of PCOS women to determine which of the PCOS traits get passed on, studies across several generations that include careful phenotyping of each generation are required in animal models to determine the mechanisms by which traits are passed on from generation to generation.

Important are also studies targeted toward development of optimal interventions for not just therapeutic but also preventive strategies. Continued efforts to identify factors and mechanisms involved in the origin of PCOS would be of immense benefit in this regard. In humans, safe intervention strategies that improve inflammatory, oxidative stress, and dyslipidemic state and/or reduce exposure to environmental endocrine

disrupting chemicals are needed to prevent the ovarian and extra-ovarian pathologies that contribute to the development of PCOS phenotype and improve women's reproductive health. Animal models are a great resource in this regard for assessing the effectiveness of such interventions before their implementation in patients.

An emerging area of concern is also the long-term health of the PCOS women. As they age, PCOS women have shown improvements in menstrual cyclicity (Elting *et al.* 2000), hyperandrogenemia and insulin resistance (Brown *et al.* 2011). Although perimenopausal women are found to have increased prevalence of hypertension and diabetes mellitus (Dahlgren *et al.* 1992), a study with small sample size found the prevalence of cardiovascular diseases did not differ between general population and women previously diagnosed with PCOS during postmenopausal period (Merz *et al.* 2016). Studies with larger sample size are needed to determine if women with PCOS manifest higher prevalence of chronic cardiometabolic complications such as obesity, diabetes, and cardiovascular diseases during later life and the contributing factors.

Although, PCOS is generally known as a reproductive disorder in women, the metabolic complications characteristic of PCOS women are also found in male relatives and offspring of PCOS women. The observations that male relatives of PCOS women have premature male baldness (Ferriman & Purdie 1979) and endocrine changes such as increased dehydroepiandrosterone sulfate (DHEAS), increased AMH, low SHBG, insulin resistance and abnormal gonadotropin secretion (Cannarella *et al.* 2018) support a male PCOS-like phenotype. Studies in prenatal T-treated male sheep also found reduced sperm cell count (Recabarren *et al.* 2008) and motility as well as testicular defects (Rojas-Garcia *et al.* 2010). Therefore, detailed characterization of the male complement of PCOS phenotype is required.

In conclusion, the different phenotypes of PCOS that contributes to the heterogeneous nature of this syndrome presents challenges in understanding the disease development and treatment. Meeting these challenges through research in humans and animal models will help in developing successful strategies to not only treat but also to prevent the development of PCOS in subsequent generations.

Declaration of interest

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

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