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Is foetal hyperexposure to androgens a cause of PCOS?

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TABLE OF CONTENTS

BACKGROUND: Polycystic ovary syndrome (PCOS) is the most common endocrinopathy affecting reproductive-aged women. The pathophysiology of this syndrome is still not completely understood but recent evidence suggests that the intra-uterine environment may be a key factor in the pathogenesis of PCOS, in particular, hyperexposure of the foetus to androgens. High concentrations of maternal serum testosterone during pregnancy have been shown to influence behaviour during childhood, the prevalence of autism disorders and anti-Mullerian hormone (AMH) concentrations in adolescence. They are also thought to re-programme the female reproductive axis to induce the features of PCOS in later life: oligo/anovulation, polycystic ovaries, hyperandrogenism and insulin resistance (IR). Support for this developmental theory for the aetiology of PCOS is gathering momentum, following results from first animal studies and now human data, which lend credence to many aspects of this hypothesis.

OBJECTIVE AND RATIONALE: In this review the recent available evidence is presented to support the hypothesis that hyperandrogenic changes in the intra-uterine environment could play a major part in the aetiological basis of PCOS.

SEARCH METHODS: An extensive PubMED and MEDline database search was conducted. Relevant studies were identified using a combination of search terms: 'polycystic ovary syndrome', 'PCOS', 'aetiology', 'anti-Mullerian hormone', 'AMH', 'pathogenesis', 'kisspeptin', 'hyperandrogenism', 'insulin resistance', 'metabolic factors', 'placenta', 'developmental hypothesis', 'genetic and epigenetic origins'.

OUTCOMES: A total of 82 studies were finally included in this review. There is robust evidence that a hyperandrogenic intra-uterine environment 'programmes' the genes concerned with ovarian steroidogenesis, insulin metabolism, gonadotrophin secretion and ovarian follicle development resulting in the development of PCOS in adult life.

WIDER IMPLICATIONS: Once the evidence supporting this hypothesis has been expanded by additional studies, the door would be open to find innovative treatments and preventative measures for this very prevalent condition. Such measures could considerably ease the human and economic burden that PCOS creates.

Key words: PCOS / developmental theory / aetiology / androgens / anti-Mullerian hormone / kisspeptin / placenta / intra-uterine environment / insulin resistance / epigenetics

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy among reproductive-aged women, affecting 8–18% (Norman *et al.*, 2007; March *et al.*, 2010). According to the Rotterdam consensus criteria, the diagnosis of PCOS requires two of the following three features: oligo-ovulation/anovulation; clinical and/or biochemical hyperadrogenism; polycystic ovaries on ultrasound, in the absence of other endocrinological or gynaecological disorders. Menstrual disturbances and clinical manifestations of hyperandrogenism such as hirsutism (The Amsterdam Consensus, 2012), acne and alopecia (Wijeyaratne *et al.*, 2002; Azziz *et al.*, 2004) are the main clinical features of PCOS. Insulin resistance (IR) is a prevalent finding in PCOS women (although not included in any of the proposed definitions of the syndrome) and is encountered according to various series in 60–80% of PCOS women (Diamanti-Kandarakis *et al.*, 1999; Carmina and Lobo, 2004; DeUgarte *et al.*, 2005). IR is present even in non-obese PCOS women (Dunaif *et al.*, 1989) and contributes to development of the pathophysiology of the syndrome (Diamanti-Kandarakis, 2006). The resulting hyperinsulinemia contributes directly and/or indirectly to the reproductive dysfunction in PCOS as well as to its cardio-metabolic complications (Teede *et al.*, 2006, 2007).

The prevalence of metabolic syndrome is up to 2-fold higher in PCOS than in weight-matched women (Wild *et al.*, 2010). PCOS women have 2.5-fold higher risk for impaired glucose tolerance and 4.5-fold higher risk for Type 2 diabetes (Moran *et al.*, 2010). Blood pressure is increased (Vrbikova *et al.*, 2003; Wild *et al.*, 2011) in PCOS and dyslipidaemia is a common feature of these women, presenting in up to 70% of cases (Wild *et al.*, 2010; Rizzo *et al.*, 2011). The American Association of Clinical Endocrinologists/American College of Endocrinology recommends screening of all women with PCOS (obese and non-obese) for metabolic syndrome and cardiovascular disease (CVD) by the age of 30 years. Additionally ESHRE recommends CVD risk assessment at any age with periodic reassessment, because CVD increases with age and accompanying additive environmental insults (The Amsterdam Consensus, 2012).

Women with PCOS are also at increased risk of mental health disorders such as depression, bipolar disorder, anxiety and eating disorders (Merikangas *et al.*, 1989; Klipstein and Goldberg, 2006; Hollinrake *et al.*, 2007; Rassi *et al.*, 2010; Dokras *et al.*, 2012).

The extent of the human suffering and economic burden of PCOS (Azziz *et al.*, 2005) must encourage research into its origins. Elucidation of these would enable a search for innovative treatments and preventative measures for this very prevalent condition. In this article we examine the evidence suggesting the contribution of hyperexposure of the foetus to androgens as the most probable factor involved in the aetiology of PCOS.

Methods

An extensive PubMED and MEDline database search was conducted until October 2016. Combinations of the following terms were used in our search: 'polycystic ovary syndrome', 'PCOS', 'aetiology', 'anti-Mullerian hormone', 'AMH', 'pathogenesis', 'kisspeptin', 'hyperandrogenism', 'insulin resistance', 'metabolic factors', 'placenta', 'developmental hypothesis', 'genetic and epigenetic origins'. The search was conducted for all the above terms in association with PCOS and aetiology. A total of 7859 papers was the result of our search. The majority of these studies was concerned with pathogenesis, hyperandrogenism and IR but not all of them were relevant to our subject. After excluding the studies with an absence of linkage with the outcome of interest and duplications, 269 studies were reviewed (Fig. 1). Only original articles in English were included and 82 of these studies were used to construct the evidence for this review.

Proposed theories for the pathogenesis of PCOS

Over the years, many theories have been put forward for the origin(s) of PCOS. These are briefly summarized in Fig. 2 and described in detail below.

Heritability

Increased ovarian androgen production is a central feature of the disturbed endocrine environment in PCOS. Numerous genetic and environmental factors interact and play a role in this disturbance and are at the heart of the underlying pathophysiology of this syndrome (Vink *et al.*, 2006). PCOS is familial in a majority of cases and molecular genetic pathways have been implicated in the metabolic and biochemical alterations associated with PCOS (Escobar-Morreale *et al.*, 2005; Urbanek, 2007). However, it has proven extremely difficult to pin down the genes responsible and many have been proposed and later eliminated. The advent of genome-wide association studies (GWAS) offers some hope, showing a higher frequency of genetic polymorphisms. To date, 15 loci have been identified with genetic susceptibilities for PCOS, including LHCGR (LH/CG receptor), THADA (thyroid adenoma associated) and DENND1A (DENN domain-containing protein 1A) genes (Chen *et al.*, 2011; Shi *et al.*, 2012; Louwers *et al.*, 2013; Hayes *et al.*, 2015), but these and others

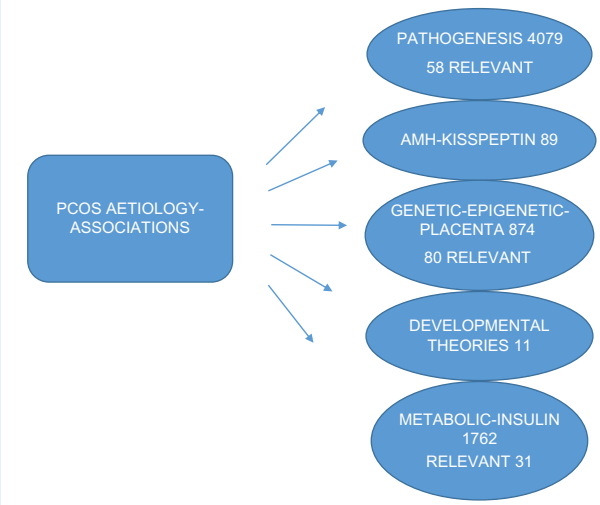

Figure 1 Selection of studies relevant for a review of foetal hyperexposure to androgens and PCOS. PCOS, polycystic ovary syndrome; AMH, anti-Mullerian hormone.

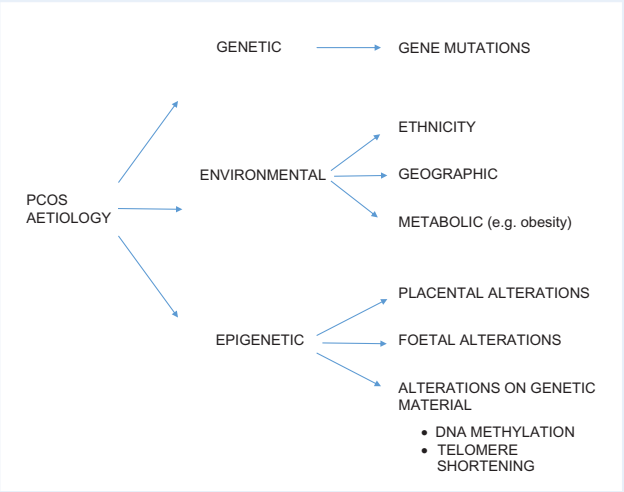

Figure 2 An overview of possible factors involved in the aetiology of PCOS.

have yet to be confirmed. There are discrepancies in the published work on the genetic analysis of PCOS mainly caused by different definitions of PCOS that are used in the studies. Some use the Rotterdam Criteria (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004), for diagnosis of PCOS (Chen *et al.*, 2011; Hwang *et al.*, 2012; Shi *et al.*, 2012; Louwers *et al.*, 2013), whereas some authors use a PCOS diagnosis based on criteria listed by the US National Institutes of Health (NIH) (Goodarzi *et al.*, 2011; Hwang *et al.*, 2012; Jones *et al.*, 2012; Welt *et al.*, 2012; Mutharasan *et al.*, 2013). There are also authors who have not even attempted to standardize the diagnosis of PCOS (Davies *et al.*, 2012; Hizli *et al.*, 2012), and they include in their studies women with polycystic ovaries on ultrasonography only.

Ethnic and geographic heterogeneity of this syndrome is well documented (The Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group, 2012), which in the GWAS further confuses the picture.

The 'missing heritability' as it is becoming known for PCOS, has opened the door for other possible considerations, the most popular of which is an epigenetic phenomenon involving hyperexposure of the foetus to androgens.

It has also been suggested that telomere shortening is another mechanism associated with the pathogenesis of PCOS and its comorbidities (Li *et al.*, 2014). Others suggested DNA methylation as the epigenetic alteration and explanation of developmental programming of PCOS (Li and Huang, 2008; Xu *et al.*, 2011; Wang *et al.*, 2014) (Fig. 2): the latter theory seems to use the most probable contributing factor.

Prenatal exposure to androgens

Although PCOS is clearly a hereditable syndrome in the majority of cases, the search for associated genes has not yet borne fruit. The present thinking leans more towards an epigenetic phenomenon involving a hyperexposure of the female foetus *in utero* to testosterone influencing the expression of genes, mainly those concerned with ovarian steroid production, insulin action and GnRH pulsatility. This developmental theory of the origin of PCOS has been mainly conceived by the seminal work of Abbott *et al.* (2005) who injected pregnant monkeys with testosterone at two stages during their pregnancy. When followed up beyond puberty and compared with controls, many developed polycystic ovaries, high serum LH concentrations and IR. Further credence for the ability of high levels of maternal testosterone during pregnancy to influence adult life are evidenced by the facts, mentioned above, that girls so exposed during intra-uterine life exhibit tom-boy behaviour (Hines *et al.*, 2002), an increased prevalence of autism spectrum disorders (ASDs) (Kosidou *et al.*, 2016) and high anti-Mullerian hormone (AMH) serum levels in adolescence (Hart *et al.*, 2010).

In studies in the rhesus monkey, prenatal exposure of experimental animals to androgens produces endocrine and metabolic alterations in offspring resembling those in PCOS (Abbott *et al.*, 2008; Wu *et al.*, 2010; Padmanabhan *et al.*, 2013). There is evidence of disordered ovarian function, impaired fertility, hypersecretion of LH and IR. These observations suggest that exposure of each part of the foetal hypothalamic-pituitary-ovarian axis to excess androgens can set up a series of events which result in both the reproductive and metabolic consequences of PCOS. Furthermore, exogenous androgens have been shown to affect ovarian follicular development both in the rhesus monkey (Vendola *et al.*, 1999) and in the sheep. Studies of the ovaries of the prenatally androgenized ewe (Birch *et al.*, 2001) indicate that exposure to androgens *in utero* influences the dynamics of early follicular development, and might explain the disordered pattern of folliculogenesis, which is characteristic of the polycystic ovary.

The hypothesis of the interaction of genetic factors with the environment as a developmental theory for PCOS was nicely described by Franks *et al.* (2006). Polycystic ovaries have been noted in girls before the onset of puberty suggesting that the origin of the syndrome depends on 'programming' of ovarian morphology and function at a much earlier stage of development—perhaps even *in utero*, during ovarian development and oogenesis (Webber *et al.*, 2003). Indeed maternal circulating total testosterone levels at 18 weeks of gestation were statistically significantly correlated with early follicular-phase circulating AMH levels in female adolescent offspring (Hart *et al.*, 2010). The Hart *et al.* (2010) study provides evidence that the uterine environment could 'programme' ovaries in the human at a very early stage. In fact there could be a critical window of susceptibility during differentiation of organs and systems that could be related to pathogenesis of PCOS, as seen in animal studies (Birch *et al.*, 2003). For example, earlier exposure to androgens, prenatally, creates phenotypically virilised females in sheep (Steckler *et al.*, 2007) while later exposure creates PCOS phenotypes with irregular ovulation and lower fertility rates compared to controls (Wood and Foster, 1998). Although animal studies laid the foundations of the developmental hypothesis for PCOS aetiology, in humans this theory was doubted for many years as the human foetus is protected from the effects of excessive maternal androgens by a combination of high concentrations of plasma binding proteins and a high level of placental aromatase activity. In human pregnancy, androgens are metabolized to oestrogens by placental P450 aromatase (Thompson and Siiteri, 1974). Thus, even in women with androgen-secreting tumours in pregnancy, it is very unusual to see virilization of a female foetus (McClamrock and Adashi, 1992). On the other hand, placental

expression of oestrogen and androgen receptors is increased in prenatally androgenized rats (Sun *et al.*, 2012), suggesting higher placental sensitivity to sex steroids. Moreover, female subjects with classic congenital adrenal hyperplasia caused by 21-hydroxylase deficiency who are exposed to excessive prenatal androgen concentrations, which likely mis-programme the hypothalamus, display features of LH hypersecretion and reproductive dysfunction that are similar to those of PCOS patients even after eliminating the hyperandrogenemia with postnatal therapies (Hague *et al.*, 1990; Barnes *et al.*, 1994; Morishima *et al.*, 1995).

The hormonal alterations during pregnancy in PCOS women show that the uterine environment in PCOS is most probably hyperandrogenic. The results of initial autopsies of placentae from women with PCOS, showing macroscopic and microscopic alterations compared to healthy controls (Palomba *et al.*, 2013), were also confirmed by altered histopathological findings in PCOS placentae in recent studies (Maliqueo *et al.*, 2013).

When placental tissues from women with PCOS and healthy women were compared, changes in the activities of important enzymes for steroid synthesis were noticed. Specifically, higher 3β-HSD-1 activity and lower P450 aromatase activity were seen in placenta tissue of women with PCOS. According to the authors, these alterations could increase androgen production during pregnancy, as these enzymes are principally linked to placental steroidogenesis (Maliqueo *et al.*, 2013). Earlier it had been reported that female foetuses of women with defects in the P450 aromatase gene and sex hormone-binding globulin gene, which are rare conditions that cause androgenization, also develop PCOS later in life (Morishima *et al.*, 1995). It seems that altered placental steroidogenesis could play a key role in PCOS pathogenesis. In another study placental signal transducer and activator of transcription 3 (P-STAT3) (Tyr-705) was increased in women with PCOS ($P = 0.05$) versus controls. STAT3 and mechanistic target of rapamycin (mTOR) are important pathways in the regulation of placental nutrient transport and foetal growth (Roos *et al.*, 2007; Maymo *et al.*, 2011). STAT3 is activated by multiple factors, including leptin and cytokines (Aggarwal *et al.*, 2009). This phenomenon seems not to be directly related to alter circulating sex steroid concentrations. However, the authors could not rule out a higher sensitivity of placental tissue to the steroid actions, as was also suggested in animal studies, where placental expression of oestrogen and androgen receptors was increased in prenatally androgenized rats (Sun *et al.*, 2012). Moreover, in rodents, prenatal testosterone exposure reduces foetal and placental growth (Sathishkumar *et al.*, 2011; Sun *et al.*, 2012), which in turn have been linked to decreased amino acid transfer (Sathishkumar *et al.*, 2011). Data from other studies (Abbott *et al.*, 2010) suggest that foetal exposure to testosterone induces increased foetal growth in certain respects and diminishes circulating foetal free fatty acids, also implicating placental dysfunction but without foetal growth restriction. Experimentally induced foetal androgen excess may result in transient hyperglycemic episodes in the intrauterine environment that are sufficient to induce relative increases in pancreatic function in prenatally androgenized infants, suggesting in this nonhuman primate model that differential programming of insulin action and secretion may precede adult metabolic dysfunction.

Besides placenta, hyperandrogenemia may actually affect the foetal ovary specifically during the second trimester when changes in the ovaries and number of follicles occur.

Human ovarian folliculogenesis begins in early foetal development, when germ cells migrate to the gonadal ridge and multiply by mitosis until about 20 weeks of gestation, reaching a maximum number of about 7 million (Adashi, 1996). Oogonial mitosis becomes superimposed with meiosis between 14 and 26 weeks of gestation, as ovigerous cords, packed with oogonia and oocytes, develop into abundant primordial and primary follicles, along with occasional secondary follicles (Cole *et al.*, 2006). By mid-gestation, the human foetal ovary has the capacity to produce and detect sex steroids, including androgen and oestrogen (Payne and Jaffe, 1974; George and Wilson, 1978; Wilson and Jawad, 1979; Voutilainen and Miller, 1986; Shifren *et al.*, 1993; Cole *et al.*, 2006; Fowler *et al.*, 2011), with oestrogen believed to regulate folliculogenesis and oocyte development *in utero* (Reyes *et al.*, 1973; Pepe *et al.*, 2006; Albrecht and Pepe, 2010). Together, endocrine (i.e. gonadotrophins) and paracrine facilitators of follicular growth [i.e. androgens, growth factors such as activin and insulin-like growth factors (IGF)] interact with survival and atresia factors to establish the maximal germ cell endowment of the foetal ovary, which then diminishes to about 1–2 million at birth and 300 000 by menarche (Adashi, 1996; Mesiano, 2009).

At mid-gestation, human foetal ovaries also have several steroidogenic enzymes, genes encoding multiple steroid signalling pathways and receptors to steroids, insulin, IGF-I and IGF-II (Voutilainen and Miller, 1986; Shifren *et al.*, 1993; Cole *et al.*, 2006; Fowler *et al.*, 2011). Cultured human foetal ovaries at this gestational age can metabolize pregnenolone sulphate to dehydroepiandrosterone (DHEA) and androstenedione (Payne and Jaffe, 1974) and also can secrete DHEA, progesterone and estrone, with lesser amounts of androstenedione, estradiol and testosterone (Wilson and Jawad, 1979).

Therefore, mid-gestational human foetal ovaries may produce androgens *in vivo*, particularly in response to insulin, which may contribute to wide variation in foetal androgen production, as evidenced by 40% of mid-gestational female foetuses having elevated serum androgen levels, into the normal male range (Reyes *et al.*, 1973; Beck Peccoz *et al.*, 1991). This hypothesis agrees with previous reports in diabetic women of elevated amniotic fluid testosterone levels (Barbieri *et al.*, 1986), along with findings of hirsutism, ovarian theca-lutein cysts and thecal cell hyperplasia in their female stillbirth offspring (Driscoll *et al.*, 1960; Hultquist and Olding, 1981; Dumesic *et al.*, 2014).

Additionally, according to human and animal studies (Rajpert-De Meyts *et al.*, 1999; Pellatt *et al.*, 2007; Veiga-Lopez *et al.*, 2012), prenatal testosterone exposure is shown to create changes in AMH expression in preantral and antral follicles in adult ovaries. Especially as seen in sheep (Veiga-Lopez *et al.*, 2012), the epigenetic action of androgens creates a reduction in AMH expression in large preantral follicles in the prenatal testosterone-treated group, a stage when AMH levels are high in control ovaries (Weenen *et al.*, 2004), and may therefore help overcome AMH inhibition of FSH sensitivity. This in turn would increase the number of follicles being recruited thus contributing to the multifollicular morphology of prenatal testosterone-treated females (West *et al.*, 2001; Smith *et al.*, 2009). AMH methylation or altered steroid receptor balance in the foetal ovary (granulosa cells) due to increased androgens could be a possible mechanism in PCOS pathogenesis.

Genetic programming *in utero*

Besides a genetic predisposition, environmental exposure and specifically the intra-uterine environment is also thought to play a major role in PCOS development. Although PCOS is clearly familial in a large majority of cases, many years of searching for the culprit polymorphisms or combination of causative genes have not produced cogent results. This has led to the developmental theory of PCOS, based on the Barker hypothesis (Barker, 1990), that the offending genes are programmed by hyperexposure to androgens *in utero* (Abbott *et al.*, 2002).

There is increasing evidence that women with PCOS expose their foetuses to a hyperandrogenic environment *in utero* (Sir-Petermann *et al.*, 2002; Falbo *et al.*, 2010). Umbilical vein testosterone in female infants born to mothers with PCOS is elevated (Barry *et al.*, 2010; Mehrabian and Kelishadi, 2012), but there is some heterogeneity among studies (Anderson *et al.*, 2010). Second trimester amniotic fluid testosterone levels are elevated in female foetuses of PCOS compared to normal mothers (Palomba *et al.*, 2012) suggesting that androgen overproduction can occur during human female foetal development under certain pregnancy conditions (Goodarzi *et al.*, 2011; Dumesic *et al.*, 2014). Evidence suggests that this hyperexposure to testosterone *in utero* can have an influence in later life. For example, when serum and amniotic fluid androgen concentrations were high during pregnancies with a female foetus, pre-school age girls behaved like boys, with distinct masculine type behavioural traits compared with female controls of the same age (Hines *et al.*, 2002).

Some recent studies have also shown increased risk of autism in children born to mothers with PCOS. The underlying reason is suggested to be increased androgens *in utero*. In a case-control study nested within the total population of Sweden (children aged 4–17 years old who were born in Sweden from 1984 to 2007) consisting of 23 748 ASD cases and 208 796 controls, maternal PCOS increased the odds of ASD in the offspring by 59%, after adjustment for confounders (odds ratio (OR) 1.59, 95% CI 1.34–1.88). The odds of offspring ASD were further increased among mothers with both PCOS and obesity, a condition common to PCOS that is related to more severe hyperandrogenemia (OR 2.13, 95% CI 1.46–3.10) (Kosidou *et al.*, 2016).

Additionally, a study examining a large bio-bank of amniocentesis samples from the Danish Historic Birth Cohort, in Copenhagen, Denmark, of children diagnosed later in life with autism and compared to controls concluded that foetal steroidogenic activity is elevated in autism. The researchers found that amniotic fluid steroid hormones are elevated in those who later received diagnoses on the autism spectrum. Rather than the abnormality being restricted to a specific steroid hormone, a latent steroidogenic factor is elevated, which includes all hormones in the Δ4 pathway, as well as cortisol. The authors suggested that the mechanism involved was dysregulation of pathways mediated by cytochrome P450-containing enzymes that catalyse the conversion of hormones along the Δ4 and glucocorticoid pathways (Baron-Cohen *et al.*, 2015).

Prior evidence pointing toward the importance of such enzymes was previously found via genetic associations between autism and single-nucleotide polymorphisms in CYP17A1, CYP19A1 and CYP11B1 genes (Chakrabarti *et al.*, 2009). These enzymes, though present, showed an altered expression in PCOS patients as well (Jakimiuk *et al.*, 2001; Medeiros *et al.*, 2013; Garg and Merhi, 2016).

Androgens in humans are derived from the Δ5 pathway and it has been suggested that more research should be carried out on this pathway to obtain a clearer view of the production of androgens *in utero* (Conley and Bird, 1997). While there are robust sex differences in amniotic fluid testosterone in this cohort suggesting that the foetus is a primary source, some research has documented correlations between amniotic fluid levels and maternal plasma that would suggest that maternal–placental–foetal transfer is another possible source (Sarkar *et al.*, 2007a,b; Glover *et al.*, 2009).

Altered P450 aromatase activity is seen in placental tissue of women with PCOS (Maliqueo *et al.*, 2013), which would contribute to a hyperandrogenic uterine environment in PCOS.

Prenatal exposure of experimental animals to androgens produces endocrine and metabolic alterations in offspring resembling those in PCOS (Abbott *et al.*, 2008; Wu *et al.*, 2010; Maliqueo *et al.*, 2013; Padmanabhan *et al.*, 2013). These latest studies clearly suggest that foetal over-exposure to androgens could be the main factor in PCOS pathogenesis.

Metabolic factors

IR is a prevalent metabolic feature in PCOS and is suggested to play some role in the pathogenesis of PCOS. Insulin has been shown to inhibit aromatase activity in human cytotrophoblasts (Nestler, 1987) and hyperandogenemia increases IR. For example, gestational testosterone excess leads to maternal hyperinsulinemia in sheep (Abi-Salloum *et al.*, 2015). Additionally, prenatally androgenized female monkeys exhibit enhanced insulin secretion from pancreatic β-cells. Therefore, an excess of maternal-foetal androgen may induce relative insulin hypersecretion in exposed female foetuses and infants. A recent meta-analysis (Yu *et al.*, 2016) showed that PCOS in pregnancy had little or no effect on outcome for large-for-gestational age, small-for-gestational age and foetal growth restriction. However, PCOS in pregnancy was associated with greater risk of gestational diabetes mellitus (GDM), pre-eclampsia, pregnancy induced hypertension, preterm delivery, Caesarean delivery, miscarriage, hypoglycemia and perinatal death.

However, an important question is whether IR or hyperinsulinaemia on its own could be a factor in the aetiology of PCOS. Studies shows that foetuses of diabetic mothers using insulin have increased levels of macrosomia and foetal pancreatic β-cell hyperplasia, as well as hirsutism, ovarian theca-lutein cysts, ovarian theca cell hyperplasia, and high tesosterone and hCG levels in the amniotic fluid (Dumesic *et al.*, 2007). However, it seems that hyperinsulinemia itself could not lead to PCOS as diabetic mothers-without PCOS-do not seem to have any preponderance to deliver PCOS daughters. Thus hyperinsulinemia could have a synergistic role in pathogenesis of PCOS but it does not seem to be the main factor for the syndrome.

Increased maternal testosterone concentrations appear to alter placenta function by affecting amino acid nutrient delivery to the foetus by down regulating specific amino acid transporter activity (Sathishkumar *et al.*, 2011), diminishing circulating foetal free fatty acids (Abbott *et al.*, 2010) and increasing also the risk of pre-eclampsia. There have been a number of reports that women with pre-eclampsia have higher plasma testosterone levels compared with

those of healthy pregnant women (Laivuori *et al.*, 1998; Acromite *et al.*, 1999; Serin *et al.*, 2001; Steier *et al.*, 2002; Troisi *et al.*, 2003; Atamer *et al.*, 2004; Baksu *et al.*, 2004; Carlsen *et al.*, 2005; Gerulewicz-Vannini *et al.*, 2006; Salamalekis *et al.*, 2006; Ghorashi and Sheikhvatan, 2008; Hsu *et al.*, 2009; Sathishkumar *et al.*, 2012). It seems that increased androgens induce an altered placental function or metabolic profile. Hyperinsulinemia, pre-eclampsia and diminished nutrient transfer ay 'programme' a pathological metabolic profile in later life.

The thrifty hypothesis supports an impaired intrauterine nutritional environment that could trigger metabolic disorders in adult life. For example, growth restricted foetuses later in life will develop IR and have an increased prevalence of metabolic syndrome when exposed to normal nutritional conditions (Dumesic *et al.*, 2007) and these foetuses could also develop PCOS in the future. Again owing to hyperinsulinaemia, steroidogenesis is increased in the ovaries, leading possibly to hyperandrogenemia in later life for female foetuses with intrauterine growth restriction (IUGR), concluding in clinical manifestations of PCOS. Evidence is lacking on sex hormonal environment and on placental steroid function in IUGR and this theory cannot be supported robustly. On the other hand it is clearer that maternal androgen excess is related to PCOS programming because of placental alterations, while metabolic factors though present sometimes may not play a part.

Role of AMH

AMH is an important regulator of folliculogenesis in the ovaries (Visser *et al.*, 2006). It is secreted by granulosa cells of the ovarian follicles and AMH acts as an important inhibitory factor for follicular growth.

It is well known that PCOS ovaries comprise a higher number of preantral and small antral follicles (Hughesdon, 1982; Franks *et al.*, 2000, 2006; Webber *et al.*, 2003), and serum AMH concentration is consequently elevated in PCOS women compared to women with normal ovaries (Fallat *et al.*, 1997; Laven *et al.*, 2004; Mulders *et al.*, 2004; Pellatt *et al.*, 2007). However, it is not merely the increased number of follicles and increased granulosa cell mass that contribute to raised AMH concentrations in PCOS but also greater production by individual granulosa cells (Pellatt *et al.*, 2007; Catteau-Jonard *et al.*, 2008; Bhide *et al.*, 2015).

AMH concentrations are correlated with the degree of ovulatory dysfunction. The concentration of AMH in follicular fluid from women with anovulatory PCOS was found to be 5-fold greater compared with ovulatory women (Das *et al.*, 2008). Laven *et al.* (2004) showed that normogonadotrophic anovulatory women with or without PCOS have higher AMH concentrations than their normo-ovulatory counterparts and that serum AMH is correlated with menstrual cycle duration. Moreover, Pellatt *et al.* (2010) have suggested that PCOS can be divided into anovulatory and ovulatory based on the serum AMH concentrations, as women with anovulatory PCOS were found to have 18 times higher AMH concentrations than the women with ovulatory PCOS, with no overlap. Further, Tal *et al.* (2014) reported that serum AMH concentration had a strong predictive ability for amenorrhoea in their study population of women with elevated AMH, having 91.7% specificity and 79.4% sensitivity in predicting amenorrhoea when the threshold AMH concentration was 11.4 ng/ml. These observations suggest that anovulatory PCOS women have an increased number of AMH-producing small antral follicles (2–5 mm), presumably creating an extreme, AMH-dominated micro-environment, which impairs the action of FSH on the selectable follicles leading to anovulation. AMH counteracts the actions of FSH on aromatase activity and in the development of an ovulatory follicle (Pellatt *et al.*, 2010). However, these observations provide only indirect evidence for an AMH role in PCOS-related anovulation. While it is likely that elevated AMH contributes to the pathogenesis of anovulation in PCOS, the cause(s) of its increased production remain unknown (Garg and Tal, 2016). A recent paper (Cimino *et al.*, 2016) demonstrated that a significant subset of GnRH neurons both in mice and humans express the AMH receptor, and that AMH potently activates GnRH neuron firing in mice. By conducting *in vivo* and *in vitro* experiments (in mice), the authors showed that AMH increases GnRH-dependent LH pulsatility and secretion, supporting a central action of AMH on GnRH neurons. These findings raise the intriguing hypothesis that AMH-dependent regulation of GnRH release could be involved in the pathophysiology of PCOS.

However, some additional factors which are also closely related to PCOS pathophysiology, such as increased LH, androgen levels and IR, may be implicated in affecting AMH (Homburg and Crawford, 2014; Garg and Tal, 2016).

Androgens play a role in stimulating the early (FSH independent) stages of follicular growth (Vendola *et al.*, 1998; Weil *et al.*, 1999) and may thus contribute to increased AMH production. Several studies have shown that serum AMH is correlated with LH and androgen levels (Pigny *et al.*, 2003; Eldar-Geva *et al.*, 2005; Piouka *et al.*, 2009; Lin *et al.*, 2011; Homburg *et al.*, 2013; Tal *et al.*, 2014).

Another candidate that may contribute to elevated AMH in PCOS is insulin. Examining markers of IR using homeostatic model assessment (HOMA-IR) and fasting insulin levels in women with PCOS showed they both were positively correlated to AMH (La Marca *et al.*, 2004a,b; Nardo *et al.*, 2009). This could be explained by an increased androgen production in the presence of hyperinsulinaemia in PCOS that acts on increasing AMH production from granulosa cells (Park *et al.*, 2010). It is interesting that weight reduction in PCOS women reduces serum AMH levels (Bhandari *et al.*, 2016) providing further evidence that improving IR and a consequent reduction of androgens as a result of weight loss can actually reduce AMH levels.

Clearly, AMH concentrations are positively correlated with the severity of the manifestations of PCOS, including oligo/amenorrhoea, hyperandrogenism and polycystic ovary morphology (Pigny *et al.*, 2003; Eldar-Geva *et al.*, 2005; Homburg *et al.*, 2013; Tal *et al.*, 2014) lending support to the notion that AMH is not only a biomarker of the syndrome but also actually contributes to the pathogenesis of PCOS (Homburg and Crawford, 2014; Garg and Tal, 2016).

The positive correlation between testosterone and AMH concentrations and the fact that maternal testosterone serum concentrations at 18 weeks gestation correlate with serum AMH concentrations in female adolescents (Hart *et al.*, 2010) could theoretically signal an epigenetic phenomenon involving the hypo-methylation of the AMH gene by testosterone resulting in raised AMH production (Li and Huang, 2008; Xu *et al.*, 2011; Wang *et al.*, 2014).

The role of kisspeptin

Kisspeptin, a hypothalamic peptide coded by the KISS1 gene, is a novel neuromodulator that acts upstream of GnRH, and is sensitive to sex steroid feedback and metabolic cues. Kisspeptin is now recognized as a crucial regulator of the onset of puberty, the regulation of sex hormone-mediated secretion of gonadotrophins and the control of fertility (Pinilla *et al.*, 2012). Kisspeptin acts upstream of GnRH and, following paracrine stimulatory and inhibitory inputs from neurokinin B and dynorphin (KNDy neuropeptides), signal directly to GnRH neurones to control pulsatile GnRH release. When administered to humans in different isoforms, routes and doses, kisspeptin robustly stimulates LH secretion and LH pulse frequency (Clarke and Cummins, 1985; George and Seminara, 2012; Skorupskaite *et al.*, 2014).

Animal studies have shown that inappropriate exposures to sex steroids, mainly androgen excess, during early stages of development can be linked to the pathogenesis of PCOS. It is tempting to speculate that one of the mechanisms contributing to the neuroendocrine alterations observed in PCOS is the perturbed organization of the hypothalamic Kiss1 system, due to an altered sex steroid milieu during critical developmental windows.

The first experimental evidence for the organizing effects of sex steroids on the Kiss1 system was provided by studies using rats as a model. For example, neonatal exposure to high doses of androgen, such as testosterone propionate, of female rodents has been shown to prevent the capacity of oestrogen to induce positive feedback and therefore to evoke the preovulatory surges of gonadotrophins later in life, thus confirming that androgen excess during early development in the female can alter the normal process of ovulation (Garcia-Galiano *et al.*, 2012). Indeed, the neonatally androgenized female rat displays metabolic and reproductive features that resemble those of patients with PCOS. These findings were confirmed in other studies (Gonzalez-Martinez *et al.*, 2008; Homma *et al.*, 2009, Gill *et al.*, 2010) that have all documented that proper maturation of Kiss1 neuronal populations is key for brain sex differentiation, and requires a finely regulated sex steroid input in both sexes. However, in primates including monkeys and humans, foetal testosterone exposure does not alter the female ability to demonstrate preovulatory LH surges and this mechanism functions normally. In anovulatory PCOS women both aromatase inhibitors and clomiphene citrate are capable of producing ovulation by inducing an FSH release with consequent follicular growth evoking a preovulatory LH surge by the proactive feedback of oestrogens. It is the impairment of follicular growth and its cause that is the real issue causing the anovulation of PCOS (see section on AMH).

In more recent studies, morphological changes were seen in Kisspeptin/neurokinin B/dynorphin (KNDy) neurons of the arcuate nucleus of the hypothalamus with changes in synaptic inputs onto KNDy neurons and preoptic area kisspeptin neurons in sheep after prenatal testosterone treatment (Cernea *et al.*, 2015). Additionally a rat model of PCOS showed an increase of kisspeptin-positive cells in the hypothalamus (Kondo *et al.*, 2016). Taken together these studies provide more evidence that prenatal exposure to androgens causes changes in the areas of the brain that regulate the menstrual cycle and 'programmes' the female reproductive axis in general.

Conclusion

PCOS is a condition that affects a large number of reproductive age women and has several implications on health, reproduction and quality of life but we are still unclear of its origin. Once known, treatments targeting the exact origin of the syndrome could play a key role in improving the quality of life of women with PCOS.

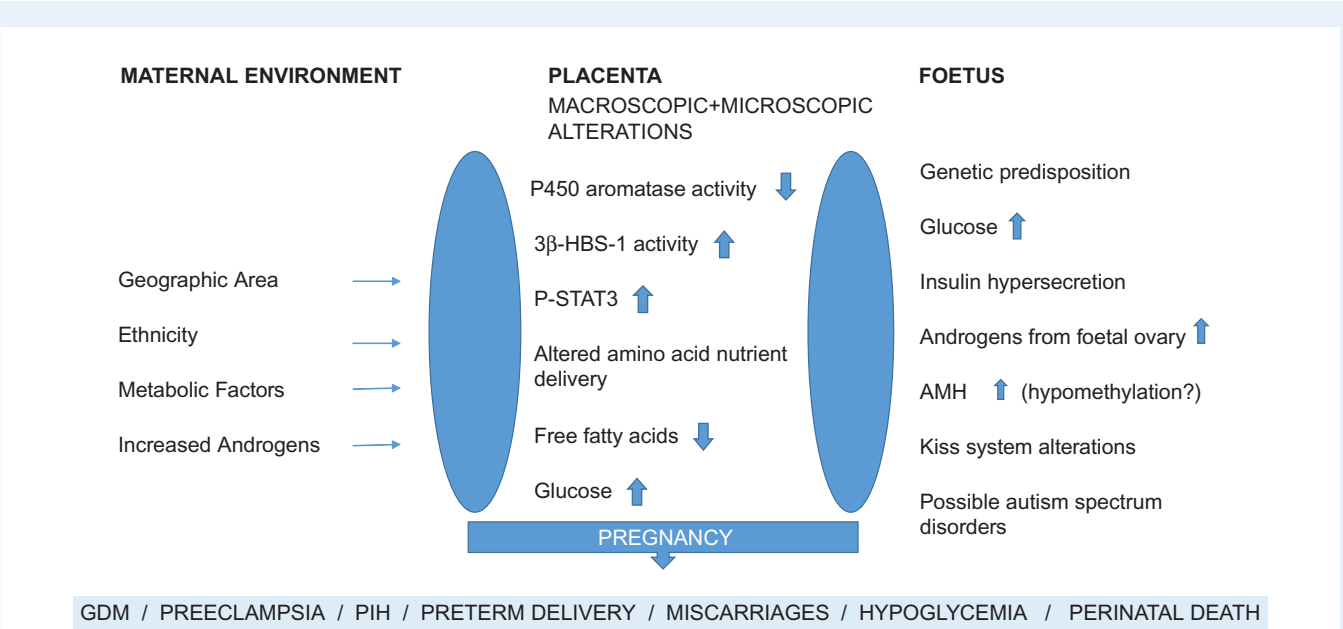

Figure 3 A current proposal for the influence of the maternal environment of a woman with PCOS on the placenta, foetus and pregnancy complications. GDM, gestational diabetes; PIH, pregnancy induced hypertension; STAT3, signal transducer and activator of transcription 3.

According to the recent available data, the aetiology of PCOS could be explained by the developmental theory based on the principles of the Barker hypothesis (Barker, 1990). Exposure of the female foetus to high levels of androgens during intrauterine life may well predispose to the development of PCOS following puberty. The studies on pregnant Rhesus monkeys, injected with testosterone during pregnancy with a follow-up of the offspring who developed polycystic ovaries, oligomenorrhea, high LH levels and IR constitute the strongest evidence for this theory to date (Abbott *et al.*, 2008).

Evidence suggests that the intrauterine environment in pregnant PCOS women is hyperandrogenic. (Palomba *et al.*, 2012). This hyperandrogenism could be caused by raised maternal androgen concentrations, from the foetal adrenal/ovary or, most likely, from a dysfunctional placenta. Under normal conditions, maternal androgens or foetal adrenal androgens are rapidly converted to oestrogens by the activity of the placental enzyme aromatase. Specifically, a higher 3β-HSD-1 activity and lower P450 aromatase activity were seen in placental tissue of women with PCOS, which would increase androgen production during pregnancy (Maliqueo *et al.*, 2013).

It is known that PCOS is largely familial but no clear genetic mutations have been associated with the syndrome. Hypothetically, epigenetic factors such as methylation of relevant genes for PCOS could play an important role in its origin. It is known that methylation plays a critical role in the regulation of gene expression (Li and Huang, 2008; Xu *et al.*, 2011; Wang *et al.*, 2014). AMH has an enigmatic role in PCOS pathogenesis and increased AMH is a common feature of PCOS. Hypo-methylation of the AMH gene possibly causes an intrinsic over-expression of the AMH gene and increased production of AMH in PCOS. Increased AMH seems involved in anovulation by inhibiting FSH action in promoting follicle growth, and AMH is positively correlated with testosterone and LH serum concentrations (Pigny *et al.*, 2003; Eldar-Geva *et al.*, 2005; Piouka *et al.*, 2009; Lin *et al.*, 2011; Homburg *et al.*, 2013; Tal *et al.*, 2014). It could be hypothesized that AMH methylation could represent another epigenetic alteration, related to a disturbed hormonal environment *in utero* in PCOS.

Kisspeptin may be an additional part of the causal chain as it was shown to play a crucial role in maturation of the female reproductive axis when animals were exposed to a hyperandrogenic intra-uterine environment resulting in increased GnRH pulsatility and consequently LH secretion (Clarke and Cummins, 1985; Garcia-Galiano *et al.*, 2012; George and Seminara, 2012; Skorupskaite *et al.*, 2014). This disturbed environment could directly have an influence on the ovary during the prenatal period and create the characteristic morphology of PCOS. There is a possibility that selection of the number of oocytes/follicles that will survive to puberty is arranged during the prenatal period and a higher number of oocytes/follicles with more surrounding granulosa cells and finally higher levels of AMH will be seen in the girls born from a hyperandrogenic uterine environment.

Finally, data from animal experiments suggest that a hyperandrogenic intrauterine environment also predisposes to IR in the offspring of PCOS mothers, eventually creating the metabolic features of PCOS. Figure 3 summarizes the influence of the maternal environment of a woman with PCOS on the placenta, foetus and pregnancy.

The evidence described here, taken together, presents a convincing case that foetal hyperexposure to androgens *in utero* plays a central role in the aetiology of PCOS.

Authors' roles

R.H. designed and supervised the review, the data collection and critically revised the manuscript. P.F. performed the search, wrote the original draft and collected the references.

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Conflict of interest

None declared.

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