Pathogenesis of polycystic ovary syndrome: multifactorial assessment from the foetal stage to menopause

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Abstract

Polycystic ovary syndrome (PCOS) is a multifactorial disorder that arises from interactions between genetic, environmental and intra-uterine factors. Small-for-gestational-age (SGA) babies and the daughters of mothers with PCOS represent possible postnatal clinical targets for developmental programming by steroid excess. The presence of excess glucocorticoids and/or androgens during foetal organogenesis and growth might promote changes in gene expression, and these changes might be related to an increase in the risk of PCOS-like reproductive and metabolic disorders in postnatal life, such as rapid growth and weight gain during the first 2 years of life (only in SGA babies), hyperinsulinaemia, adipocyte dysfunction and childhood visceral obesity, premature pubarche and adrenarche (only in SGA babies) and PCOS. In the fourth decade of life, women who have PCOS may be at higher risk for type 2 diabetes mellitus, dyslipidaemia and systemic arterial hypertension, which suggests that these women are also at higher risk for cardiovascular disease during menopause. However, PCOS can also occur in women who were born at appropriate weight for GA or in newborns of women without PCOS, which suggests that genetic variation and environmental factors play important roles in the development and maintenance of PCOS in a population. Genome-wide association studies based on adequate population samples have shown a higher frequency of genetic polymorphisms of the *LHCGR*, *THADA* and *DENND1A* genes in women with PCOS. Genetic studies of PCOS have also included analyses of structural changes in the chromosome based on an assessment of telomere length in single, cross-sectional evaluations, and these studies have produced controversial results. The present narrative review assesses the multifactorial origins of PCOS (including environmental, genetic and intra-uterine factors) and the development of conditions associated with this disorder. It is concluded that although PCOS might originate in the intra-uterine environment through developmental programming by steroid excess, the interaction between genetic and environmental factors is crucial for its appearance. Follow-up studies should be conducted to assess the same populations over their entire lifespans while taking into account different aspects of the pathogenesis of PCOS.

*Reproduction* (2015) **150** R11–R24

Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous and complex endocrine disorder with an estimated prevalence of 5–13.9% in women of reproductive age (Norman *et al.* 2007, Melo *et al.* 2010). In addition to causing reproductive disorders (anovulation and infertility), PCOS may or may not have a clinical and metabolic impact that varies according to ethnicity and geographic region (Tian *et al.* 2006, Norman *et al.* 2007).

Although the clinical manifestations of PCOS appear in adolescence, it has been suggested that the disease has its origins in the intra-uterine environment (de Zegher & Ibáñez 2006). Experimental studies in animals (Abbott *et al.* 2002, 2005) and clinical observations in human subjects (Melo *et al.* 2010) lend support to the hypothesis that developmental programming by steroid excess plays a role in the development of PCOS and its associated disorders at various stages of life (Jaquet *et al.* 2005) (Fig. 1). However, because the interaction between postnatal environmental factors and genetic predisposition are crucial for its occurrence, PCOS clearly has a multifactorial aetiology.

The developmental programming of PCOS represents changes in gene expression that occur following exposure to steroids (mainly glucocorticoids and/or androgens) during critical periods of foetal development. Some evidence suggests that this phenomenon is associated with variable PCOS-related metabolic and reproductive phenotypes in extra-uterine life and that these phenotypes are associated with the stage of
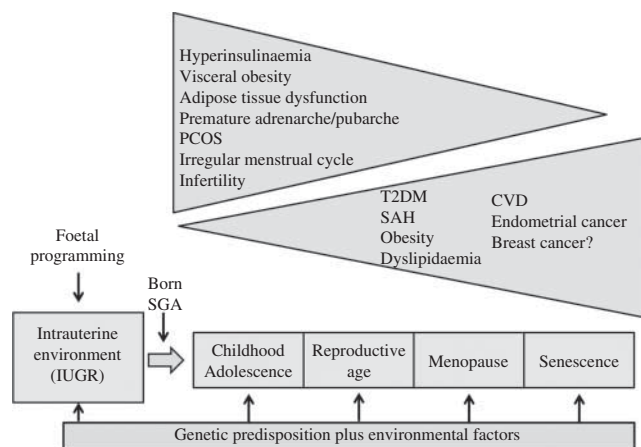
Figure 1 Natural history of PCOS. PCOS has a multifactorial aetiology that includes intra-uterine, genetic and environmental factors which might or might not be interrelated. Although the clinical manifestations of PCOS appear in adolescence, it has been suggested that the natural history of the disease has its origins in the intra-uterine environment through developmental programming. This process might be associated with several of the PCOS-related clinical and metabolic disorders that occur at different stages of life as a function of the type and duration of an individual's exposure to postnatal environmental factors. In addition, other mechanisms (such as genetic predisposition) might play a role in the origin of PCOS, seeing as individuals who allegedly do not undergo developmental programming (appropriate-for-gestational-age subjects or daughters of women without PCOS) can also develop PCOS and its associated comorbidities. Adapted from Galluzzo et al. (2008). IUGR, intra-uterine growth restriction; SGA, small for gestational age; T2DM, type 2 diabetes mellitus; SAH, systemic arterial hypertension; CVD, cardiovascular disease.

pregnancy during which the foetus was exposed to excess steroids (Padmanabhan & Veiga-Lopez 2011, Reynolds et al. 2012).

Developmental programming by glucocorticoid excess might account for the higher risk of PCOS and the associated clinical-metabolic disorders exhibited by small-for-gestational-age (SGA) infants who present compensatory growth. Although women who were born SGA may have an increased risk for developing PCOS, and both PCOS and SGA may lead to a higher risk of cardiovascular disease during old age (Jaquet et al. 2005, Martinez-Aguayo et al. 2007, Bonamy et al. 2008, Anderson et al. 2014), these propositions have not been evaluated in follow-up studies that simultaneously considered subjects who were born SGA and those who had PCOS. Thus, it is possible that individuals born SGA who exhibit compensatory growth, PCOS and cardiovascular and metabolic alterations represent part of a spectrum of abnormalities in developmental programming due to steroid excess.

Developmental programming by androgen excess during pregnancy could occur in women with obesity, type 2 diabetes mellitus (DM), insulin resistance (IR) (Escobar-Morreale et al. 2014), excessive weight gain during pregnancy, PCOS and/or any other situation associated with hyperandrogenism (Sir-Petermann et al.

2009) and could result in an increased risk of PCOS and/or associated clinical and metabolic comorbidities in their offspring (Sir-Petermann et al. 2009, Padmanabhan & Veiga-Lopez 2011, Escobar-Morreale et al. 2014). Experimental studies show that androgen excess during intra-uterine development may also be associated with intra-uterine growth restriction (IUGR) (Beckett et al. 2014), hyperinsulinaemia, visceral obesity in childhood and PCOS-like reproductive manifestations in women of reproductive age (Abbott et al. 2005, Padmanabhan & Veiga-Lopez 2011). Notwithstanding this association, individuals who apparently did not undergo developmental programming (subjects with birth weights appropriate for their gestational age (AGA) and daughters of women without hyperandrogenism during pregnancy) can also develop PCOS (Melo et al. 2010). This observation suggests that genetic variation and environmental factors play important roles in the development of PCOS. Genome-wide association studies (GWAS) have shown a higher frequency of genetic polymorphisms of the LHCGR, THADA and DENND1A genes in women with PCOS (Chen et al. 2011, Shi et al. 2012, Louwers et al. 2013). Genetic factors associated with a higher frequency of PCOS also include structural changes in chromosomes that have been analysed in cross-sectional studies by assessing telomere length at a single time point. These analyses have presented controversial results, and additional studies involving the longitudinal assessment of telomere length dynamics in women with PCOS at different stages of life are needed.

Existing studies of the pathogenesis of PCOS have limitations. Because there are three supported definitions of PCOS (Zawadski & Dunaif 1992, Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2004, Azziz et al. 2006), comparing the results reported in different publications is somewhat difficult. Whereas some authors use a PCOS diagnosis based on criteria listed by the National Institutes of Health (NIH) (Goodarzi et al. 2012, Jones et al. 2012, Welt et al. 2012, Hwang et al. 2012, Mutharasan et al. 2013), others use the criteria proposed by the American Society of Human Reproduction/European Society of Human Reproduction and Embryology (Chen et al. 2011, Shi et al. 2012, Hwang et al. 2012, Louwers et al. 2013), which thereby intensifies discrepancies in the published work on the genetic analysis of PCOS. Some authors have not even attempted to standardise 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, a finding that alone does not increase the risk for PCOS-associated conditions (Cresswell et al. 1997), or they collect retrospective data from medical records that antedate the publication of the first consensus on PCOS by the NIH (Mumm et al. 2013).

The Rotterdam consensus criteria for the diagnosis of PCOS (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2004) defines some non-hyperandrogenic phenotypes (menstrual irregularity plus polycystic ovaries) as non-existent diagnoses according to the criteria formulated by the NIH (Zawadski & Dunaif 1992) and the Androgen Excess Society (Azziz *et al.* 2006). Because the cardio-metabolic profile of PCOS phenotypes characterised by hyperandrogenism and chronic anovulation is poorer, the inclusion of non-hyperandrogenic phenotypes might favour the occurrence of bias in the assessment of the aetiopathogenesis of PCOS and its relationship to cardiovascular comorbidities during late stages of life (Melo *et al.* 2011, Daan *et al.* 2014). Moreover, most of the existing studies are multicentre cross-sectional or case–control studies in which women at different stages of life and who have a varied prevalence of comorbidities associated with PCOS were analysed (Michelmore *et al.* 2001, Ibáñez *et al.* 2008, Legro *et al.* 2010, Chen *et al.* 2011, Hizli *et al.* 2012, Hwang *et al.* 2012, Jones *et al.* 2012, Shi *et al.* 2012, Louwers *et al.* 2013, Shayeb *et al.* 2014).

The ethnic and geographic heterogeneity of PCOS demonstrates that this disorder is associated with environmental factors (Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group 2012). The identification of geographical variations in the morbidity profiles of women with PCOS is therefore essential for establishing preventive measures to improve their health. In this context, birth cohorts seem to minimise these limitations because they include women of the same age range who are exposed to similar social, cultural and geographic factors. Moreover, birth cohort studies favour the simultaneous and prospective assessment of a variety of environmental, genetic and intra-uterine factors that may be associated with the development of PCOS and its clinical/metabolic changes at all stages of a woman's life.

The aim of the present narrative review is to assess the multifactorial origins of PCOS and to explore how its origin involves the interaction of environmental, genetic and intra-uterine factors over a lifespan. With respect to genetic studies of PCOS, only GWAS published before November 2014 were included in the present review, because the external validity of the population case series of such studies is adequate. No study has yet assessed the environmental factors directly associated with PCOS, so articles on lifestyle modifications and theories based on experimental studies were considered. Articles were searched in the PubMed database relative to observational studies published in English from January 1990 to November 2014. The search keywords were (polycystic ovary syndrome AND (foetal programming OR genetic susceptibility)), (polycystic ovary syndrome AND foetal programming AND (androgen OR glucocorticoids)), (polycystic ovary syndrome AND (low birth weight

OR birth weight OR small for gestational age)), (polycystic ovary syndrome AND (association studies, genome-wide OR association study, genome-wide OR genome-wide association studies)) and (polycystic ovary syndrome AND (gene environment interaction OR environment OR lifestyle)). A total of 1114 articles were located. Of them, 16 observational studies were included in the present review; four of these were relevant to the genetic component of PCOS, and 12 were relevant to intra-uterine factors associated with PCOS.

Environmental factors associated with PCOS

Environmental factors associated with PCOS can be classified as prenatal (foetal developmental programming) or postnatal (diet, obesity, sedentary lifestyle, environmental toxins and prescription drugs) (Diamanti-Kandarakis *et al.* 2006). Evidence suggests that environmental stimuli can both mimic hormonal actions and activate pre-existing, predisposing factors that trigger the endocrine activity characteristic of PCOS (Escobar-Morreale *et al.* 2005, Norman *et al.* 2007). Dietary habits, exercise and cultural, social and economic factors might modify environmental exposure. For that reason, among others, the prevalence of the metabolic conditions associated with PCOS (obesity, metabolic syndrome and disorders of glucose metabolism) might vary as a function of the type of environmental exposure, especially in racially mixed populations that do not have a predominant genetic background. Thus, although environmental factors cannot be homogeneous in studies of the pathogenesis of PCOS in human subjects, the internal validity of such studies can be increased by including women of the same ethnicity who are from the same geographic area.

It has been suggested that PCOS presents a non-genetic inheritance pattern in populations with a poor lifestyle (high-saturated-fat diet, sedentary lifestyle, alcoholism and smoking). In such populations, changes in the foetal–placental unit, the onset of IUGR and the frequency of SGA newborns may occur. Hyperinsulinaemia and visceral obesity are more likely to develop during childhood and to culminate in a higher prevalence of IR, systemic arterial hypertension (SAH) and hyperandrogenism (Ibáñez *et al.* 2001) in reproductive-age women. During pregnancy, women who were born SGA also present a higher risk for placental disorders and the delivery of SGA newborns, which suggests a non-genetic inheritance pattern of PCOS. If they maintained a proper lifestyle throughout their childhood and their reproductive period, these women would not experience placental changes; thus, the process described earlier would be interrupted, and their children would be born AGA (Fig. 2) (Escobar-Morreale *et al.* 2005). Studies of familial aggregation in
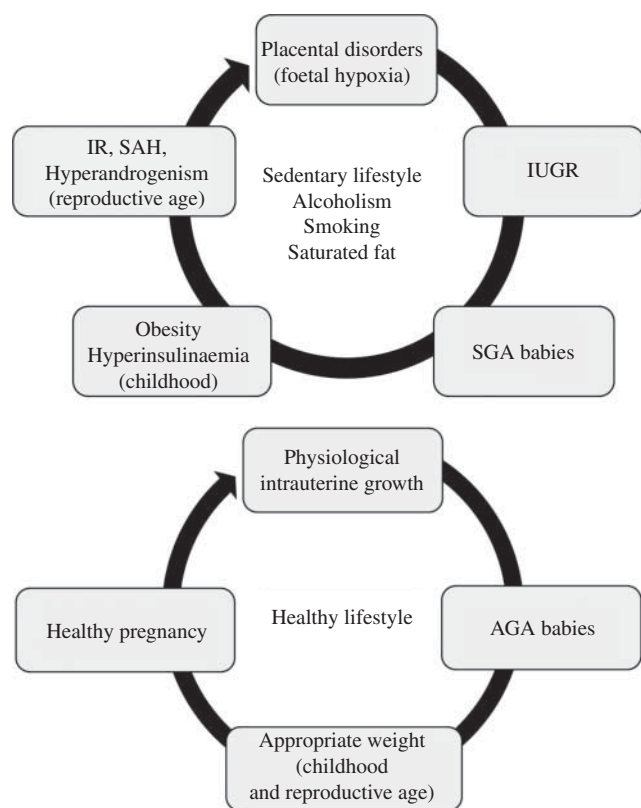
Figure 2 Environmental factors in the pathogenesis of polycystic ovary syndrome. Adapted from Escobar-Morreale *et al.* (2005). IUGR, intrauterine growth restriction; IR, insulin resistance; SAH, systemic arterial hypertension; SGA, small for gestational age; AGA, appropriate for gestational age.

which different members of the same family are considered could establish the potential influence of the genetic component in the cascade of events described earlier.

The relevance of environmental factors to the development of PCOS is evidenced by the effect of lifestyle changes on obesity, which is a highly prevalent comorbidity among women with PCOS. Such measures might alter the phenotypic expression of PCOS. Reduction in body weight is the first line of treatment in obese women with PCOS. A loss of 2–5% of body weight reduces hyperinsulinaemia, increases sex hormone-binding globulin (SHBG) levels, reduces free androgen levels, restores the ovulatory cycle and improves clinical hyperandrogenism and metabolic disorders, such as dyslipidaemia (Thessaloniki ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2008, Moran *et al.* 2011). Contrarily, diets that are high in saturated fat and sedentary lifestyles are associated with the development of PCOS and its metabolic consequences, mainly obesity (Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group 2012).

Genetic factors associated with PCOS

Familial aggregation studies of PCOS suggest that it is an inherited disorder. Genetic inheritance of the condition has been demonstrated in studies of twins in which women with monozygotic twin sisters affected by PCOS were shown to have twice the risk of developing the disorder by the time they reached reproductive age (Vink *et al.* 2006). Studies of first-degree relatives of women with PCOS demonstrated an increased incidence of abnormal glucose metabolism and metabolic syndrome in men, which suggests a genetic background effect (Yildiz *et al.* 2003). Despite these considerations, the absence of a male PCOS phenotype, differences in the methodologies used in the studies, which were case–controlled and did not consider the multifactorial aspects of the aetiology of PCOS, fertility problems in women with PCOS and the irreproducibility of the results obtained in non-familial studies limit the internal and external validity of current assessments of the genetic components of PCOS.

Although gene variants of hundreds of coding genes associated with the clinical and laboratory features of PCOS (genes related to hyperandrogenism, IR, SHBG, gonadotrophins, metabolic and inflammatory markers and obesity, among others) have been demonstrated in specific populations (Escobar-Morreale *et al.* 2005), these results present low external validity. A group of researchers from China conducted the first large GWAS on the human genome in Chinese women with PCOS and demonstrated a higher frequency of genetic polymorphism at 2p16.3 (rs13405728), 2p21 (rs13429458) and 9q33.3 (rs2479106) in women with PCOS. These single-nucleotide polymorphisms (SNPs) were related to the following genes: *LHCGR* (the LH/hCG receptor gene, which is associated with increased luteinising hormone (LH), enlarged ovaries, oligomenorrhea, resistance to LH or human chorionic gonadotrophin (HCG) and infertility), *DENND1A* (a gene that codes for a modifier of guanine that is associated with multiple organ dysfunction, including dysfunction of the ovary, hypothalamus, pituitary and adrenal glands and tissue-specific responses to insulin, type 2 DM and obesity) and *THADA* (a thyroid adenoma gene associated with disorders of glucose metabolism, polycystic ovarian morphology, hypersecretion of LH, hyperandrogenism and dyslipidaemia) in the PCOS group (Chen *et al.* 2011). However, the cases included in this initial evaluation were insufficient to establish genetic susceptibility to PCOS, which requires the evaluation of other polymorphisms in a larger sample. Subsequently, in 2254 cases of PCOS vs 3001 controls (women without PCOS), eight new loci were identified by the Chinese group. These included *C9orf3*, which is associated with hyperandrogenism and is located at 9q22.32 (rs3802457), *YAP1*, a gene associated with cell proliferation and apoptosis at 11q22.1 (rs1894116), *RAB5B* and

SUOX, two genes associated with type 1 DM at 12q13.2 (rs705702), *HMGA2*, a gene associated with infertility at 12q14.3 (rs22720), *TOX3*, a gene associated with DNA modification at 16q12.1 (rs4784165), *INSR*, a gene associated with IR at 19p13.3 (rs205980), *SUMO1P1* and *ZNF217*, two genes associated with telomere dysfunction at 20q13.2 (rs6022786) and *LHCGR* at 2p16.3 (rs2268361). These new findings have demonstrated other genetic variants related to glucose metabolism disorders, sex hormone functions, insulin/calcium signalling and endocytosis. The discovery of these new loci confirms the genetic predisposition to PCOS in Chinese women (Shi *et al.* 2012).

Although the findings obtained in Chinese populations have been confirmed in women with PCOS in the USA with respect to variants in the *DENND1A* (Goodarzi *et al.* 2012, Welt *et al.* 2012), *THADA* (Goodarzi *et al.* 2012), *LHCGR* and *FSHR* (Mutharasan *et al.* 2013) genes as well as in European populations (12 genetic variants in the *LHCGR*, *THADA*, *DENND1A*, *FSHR*, *c9orf3*, *YAP1*, *RAB5B/SUOX* and

SUMO1P1 loci) (Louwers *et al.* 2013). A GWAS study conducted by a Korean group failed to confirm these results and found that only the glycogen synthase 2 (*GYS2*) gene could be linked to PCOS and its metabolic complications (Hwang *et al.* 2012). Another GWAS study conducted in the USA that analysed genes that code for proteins associated with metabolic and cardiovascular abnormalities also did not demonstrate a hereditary component of PCOS (Jones *et al.* 2012). These discrepancies may occur because genetic background varies according to ethnicity. Furthermore, some multicentre case–control studies included women at stages of life that ranged from adolescence to menopause who had heterogeneous social/cultural lifestyles and different clinical and metabolic manifestations of PCOS (certain patients were too young to present comorbidities). In addition, these studies did not consider other aspects of the pathogenesis of PCOS, such as birth weight and environmental factors. Table 1 presents data from GWAS studies of women with PCOS.

Table 1 Genome-wide association study in women with polycystic ovary syndrome[a].

Author	Population	Genes	Loci
Chen *et al.* (2011) (China) (GWAS1)	PCOS: 744 vs control: 895 Replication 1 (North China) PCOS: 2840 vs control: 5012 Replication 2 (South China) PCOS: 498 vs control: 780	*DENND1A* (9q33.3) *LHCGR* (2p16.3) *THADA* (2p21)	rs2479106 rs13405728 rs13429458
Shi *et al.* (2012) (China) (GWAS2) and (Meta-analysis GWAS1 and 2)	GWAS2 PCOS: 1510 vs control: 2106 Meta-analysis (GWAS1 and 2) PCOS: 2254 vs control: 3001	*C9orf3* (9q22.32) *HMGA2* (12q14.3) *INSR* (19p13.3) *LHCGR* (2p16.3) *RAB5B* and *SUOX* (12q13.2) *SUMO1P1* and *ZNF217* (20q13.2) *TOX3* (16q12.1) *YAP1* (11q22.1)	rs3802457 rs2272046 rs205980 rs2268361 rs705702 rs6022786 rs4784165 rs1894116
Jones *et al.* (2012) (USA)	PCOS: 443 vs control: 193	None	None
Hwang *et al.* (2012) (Korea)	PCOS: 774 vs control: 967	*GYS2*	rs6487237, rs7485509, rs10841843
Louwers *et al.* (2013) (The Netherlands/Dutch study) and (Meta-analysis of the population of USA, China and The Netherlands)	Dutch study PCOS: 703 vs control: 2164 Meta-analysis (USA, China and The Netherlands) PCOS: 2254 vs control: 3001	*C9orf3* *DENND1A* *THADA* *YAP1* Meta-analysis *C9orf3* *DENND1A* *FSHR* *LHCGR* *RAB5B* and *SUOX* *SUMO1P1* *THADA* *YAP1*	rs4385527 rs10986105 rs12468395, rs12478601 rs1894116 rs2349415, rs4385527 rs10818854, rs10986105 rs2268361 rs13405728 rs1894116 rs705702, rs6022786 rs12468394, rs12478601 rs3802457

GWAS, genome-wide association study; *LHCGR*, luteinising hormone/human chorionic gonadotrophin receptor gene; *THADA*, thyroid adenoma-associated gene; *DENND1A*, gene modifier of guanine; *C9orf3*, chromosome 9 open reading frame 3; *YAP1*, yes-associated protein1; *RAB5B*, oncogene family; *SUOX*, sulphite oxidase gene; *HMGA2*, high-mobility group AT-hook2; *TOX3*, TOX high-mobility group box family member3; *INSR*, insulin receptor gene; *ZNF217*, zinc finger protein 217; *GYS2*, glycogen synthase 2 (liver); *FSHR*, follicle-stimulating hormone receptor gene.
[a]All of the studies mentioned were case–control studies. This table includes only the genome-wide association studies on women with PCOS.

Genetic studies of PCOS also include the analysis of structural/anatomical changes in chromosomes (changes in non-coding sequences) based on the assessment of telomere length. The telomere is a structure located at the end of the chromosome that is involved in chromatin organisation, the control of cell proliferation, the preservation of genome integrity and the stability and prevention of chromosome fusion (Allsopp *et al.* 1992). Physiologically, telomere length decreases with ageing. However, this process can be accelerated by cardiovascular disease, SAH, hypercholesterolaemia and type 2 DM, conditions that are associated with an inflammatory and oxidative environment (Fuster & Andres 2006). Because women with PCOS are at greater risk for these clinical and metabolic conditions, and because PCOS is associated with inflammation and oxidative stress (Cussons *et al.* 2006), telomere shortening may be another mechanism associated with the pathogenesis of PCOS and its comorbidities. In this context, a Chinese group showed that women with PCOS have shorter telomeres than do subjects without PCOS in a single-assessment, cross-sectional study of women of various ages (PCOS: $n = 698$ vs control: $n = 611$) (Li *et al.* 2014). These data were not confirmed by other investigators who assessed Brazilian women with PCOS (PCOS: $n = 150$ vs control: $n = 124$) (Pedroso *et al.* 2014). The discrepancy might occur because the two studies compared women at different stages of life (reproductive age and menopause) and the assessments of women at reproductive age might have occurred too early to detect differences in telomere length between women with PCOS and those without PCOS. Birth cohort studies may be more suitable for elucidating the dynamic of telomere shortening because this methodology can assess the same population at different stages of life (lifespan assessment).

Role of developmental programming in the pathogenesis of PCOS

Developmental programming refers to changes in gene expression that result from the presence of increased levels of steroid hormones in the foetal circulation at critical stages of foetal development and that result in permanent structural and functional modifications of the body organs. This phenomenon might be caused either by the presence of excess glucocorticoids (resulting from foetal hypoxia and IUGR) (Wells 2011, Longo *et al.* 2013) or by the elevation of maternal androgen levels during pregnancy (Padmanabhan & Veiga-Lopez 2011, Escobar-Morreale *et al.* 2014). In addition to anatomic and functional changes in organs and organ systems, developmental programming might also be associated with the programming of endocrine pathways and thus also with clinical, metabolic and reproductive changes during postnatal life.

Changes in gene expression are necessary for the physiological development of the foetus. However, under unfavourable intra-uterine conditions, excessive steroid levels might induce alterations in gene expression that can result in epigenetic modifications (hereditary changes in the genome that do not involve any alteration in the DNA nucleotide sequence and are transmitted during cell division). DNA methylation, in which the chemical structure of cytosine is modified through the addition of a methyl group (CH_3), is one such epigenetic alteration (Li & Huang 2008). Xu *et al.* (2011) have shown that exposure of foetal female monkeys to testosterone excess changes the epigenome of their visceral fat cells. Therefore, epigenetic alterations might represent the molecular basis of developmental programming related to the reproductive and metabolic phenotypes exhibited by women with PCOS over the course of their lives (Wang *et al.* 2014).

Developmental programming by glucocorticoid excess

The proper growth of the foetus results from a balance between anabolic and catabolic processes that occur during the functional maturation and differentiation of foetal organs and tissues. If foetal hypoxia develops during this period because of dietary restrictions and/or maternal or placental disease, the catabolic process will predominate, which results in IUGR and ultimately an SGA newborn. To assure survival and reduce energy expenditure ('thrifty phenotype') (Barker 1995), foetal blood flow is redirected to essential organs (heart, brain and adrenal glands) in a phenomenon called centralisation. Consequently, there is an increased production of glucocorticoids because of the hyperactivity of the hypothalamic–pituitary–adrenal (HPA) axis, which results in epigenetic modifications (Wells 2011, Longo *et al.* 2013).

Clinical assessments of the effects of developmental programming that result from glucocorticoid excess on the development of disease at later stages of life are hindered by the multifactorial aetiology of most reproductive, metabolic and cardiovascular diseases. However, studies with experimental animals have found that foetuses with IUGR caused by placental insufficiency or maternal malnutrition were born SGA and that these animals showed a predisposition to developing pathologies in postnatal life after compensatory growth during the first 2 years of life (Bloomfield *et al.* 2003, Reynolds 2012). Clinical data show that compensatory growth might also be associated with the development of comorbidities in humans, such as precocious puberty (Ibáñez *et al.* 1998), SAH (Elting *et al.* 2001), cardiovascular disease (Bonamy *et al.* 2008), type 2 DM, glucose intolerance (Willemsen *et al.* 2008), dyslipidaemia, obesity (Martinez-Aguayo *et al.* 2007),

metabolic syndrome (Jaquet *et al.* 2005) and PCOS (Pandolfi *et al.* 2008, Melo *et al.* 2010, Hizli *et al.* 2012). Thus, children born SGA would exhibit a clinical marker of developmental programming by glucocorticoids associated with the development of PCOS and its associated comorbidities, although SGA may also be constitutional (variation of normal).

Developmental programming by androgen excess

In addition to elevated glucocorticoid levels, hyperactivity of the HPA axis secondary to IUGR might also favour the occurrence of hyperandrogenism resulting from overactivity of the adrenal gland. Excessive levels of adrenal androgens may alter gene expression in such a way as to favour the development of the reproductive and metabolic phenotypes of PCOS in animals (Reynolds 2012). Among other factors, this mechanism might account for the higher risk of PCOS and associated clinical/metabolic conditions exhibited by the offspring of mothers with PCOS (Padmanabhan & Veiga-Lopez 2011). Adrenal androgen excess in the maternal circulation occurs in the absence of IUGR (a characteristic that has been shown in animal models) (Zhou *et al.* 2005), and hyperandrogenism might be consequence of obesity, DM, IR (Escobar-Morreale *et al.* 2014), PCOS and/or any other condition associated with androgen excess (Sir-Petermann *et al.* 2009).

Obesity (Chandrasekaran *et al.* 2014), DM, IR and excessive weight gain during pregnancy (Xiang *et al.* 2015) are predictors of large-for-gestational-age (LGA) offspring. Because these conditions may also be associated with hyperandrogenism (Macut *et al.* 2014), LGA babies might exhibit a higher risk for PCOS via developmental programming by androgen excess, but this hypothesis remains to be confirmed through clinical studies. Mumm *et al.* (2013) found that the risk of PCOS was not higher among LGA female babies of mothers with type 2 DM. However, those authors used data they had located through a search of medical records using International Classification of Diseases (ICD) codes that was performed before the publication of any consensus on the diagnostic criteria for PCOS.

Based on the results of experiments conducted in animals, it has been established that the stage of pregnancy during which exposure to androgen excess occurs is crucial for determining the reproductive and metabolic phenotypes associated with PCOS. PCOS-like reproductive disorders were found to predominate in animals that had been treated with androgens during both the first and second halves of pregnancy, whereas problems related to glucose metabolism and visceral obesity predominated in animals that had been treated with androgens during the first half of pregnancy (Abbott *et al.* 2005; Table 2).

Table 2 Main reproductive and metabolic disorders associated with developmental programming by androgen excess in monkeys[a].

Reproductive	Metabolic
Anovulation	Insulin resistance
Ovarian hyperandrogenism	Glucose intolerance
Polycystic ovaries	Type 2 diabetes mellitus
LH hypersecretion	Visceral obesity
Infertility	Dyslipidaemia

LH, luteinising hormone.
[a]PCOS-like reproductive disorders occur in the offspring of animals treated with androgens in both the first and second halves of pregnancy, whereas PCOS-related metabolic disorders appear in the offspring of animals treated with androgens at the beginning of pregnancy.

Developmental programming related to PCOS manifestations: aspects relevant to childhood and adolescence

Approximately 90% of SGA children present rapid growth and weight gain during the first 2 years of life (compensatory growth). At the end of this period, the growth curves and development of these children are similar to the patterns observed in children born AGA (Albertsson-Wikland *et al.* 1998). Compensatory growth is associated with hyperinsulinaemia, central obesity and adipose tissue dysfunction in childhood, factors that can alter the distribution of body fat and accelerate the onset of adrenarche and puberty, especially pubarche (Ibáñez *et al.* 1998).

During the peripubertal period, increased levels of leptin caused by dysfunctions in adipose tissue are associated with the hypersecretion of LH and the development of ovarian hyperandrogenism. Consequently, anovulatory cycles are more frequently observed in women who were born SGA (Ibáñez *et al.* 2002), which suggests that these women have a higher risk of developing PCOS. In addition, insulin acts on the ovarian theca through insulin-like growth factor 1 (IGF1), which results in increased levels of ovarian androgens. This process occurs because hyperinsulinaemia decreases the hepatic synthesis of IGF binding protein 1 (IGFBP1), which thus increases the free fraction of IGF1 (Poretsky *et al.* 1999). A reduction in the hepatic synthesis of SHBG, with a resulting increase in the levels of the free fraction of circulating androgens in SGA women, is another process that may be associated with hyperinsulinaemia (Ibáñez *et al.* 1998, 2002). Foetuses of animals exposed to androgen excess at the beginning of pregnancy may also exhibit an increased risk of hyperinsulinaemia and visceral obesity in infancy (Escobar-Morreale *et al.* 2014). Although developmental programming by androgen excess might be associated with IUGR in rodents (Sathishkumar *et al.* 2011) and sheep (Beckett *et al.* 2014) and with low birth weight (LBW), these characteristics are not frequently found in that process in non-human primate models for PCOS (Abbott *et al.* 2010).

Development programming related to PCOS manifestations: aspects relevant to reproductive age

The hyperinsulinaemia and hyperandrogenism observed during and after the immature stage of development of the HPA in SGA women, which is associated with a higher frequency of anovulatory cycles (Ibáñez *et al.* 2002), characterises SGA women as a group that is at risk for developing PCOS at reproductive age (Fig. 3). During the third decade of life, this risk can be twice as high in women born SGA as it is in women born AGA (Melo *et al.* 2010). However, PCOS does not occur in all women born SGA (Laitinen *et al.* 2003, Mumm *et al.* 2013), and
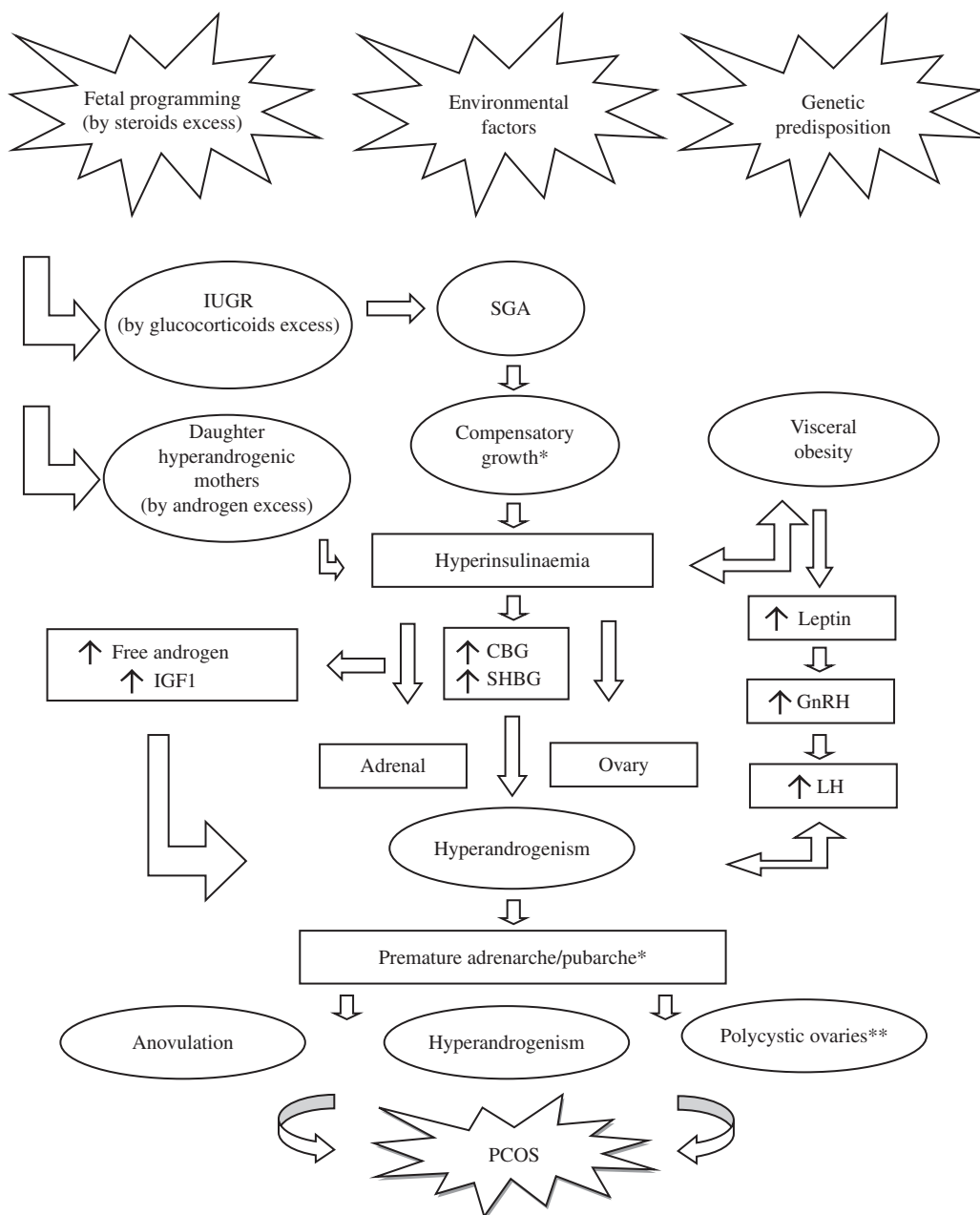
Figure 3 Aetiology of PCOS: multifactorial assessment from the intra-uterine environment to reproductive age. *Events associated only with born SGA, which suggests later phenotypes of developmental programming by glucocorticoid excess. **Characteristic shown only in daughters of hyperandrogenic mothers, which suggests manifestation of developmental programming by androgen excess. The other features may be associated with the intra-uterine effects of both steroids. IUGR, intra-uterine growth restriction; SGA, small for gestational age; CBG, corticosteroid binding globulin; SHBG, sex hormone-binding globulin; GnRH, gonadotrophin-releasing hormone; LH, luteinising hormone; IGF1, insulin-like growth factor 1; PCOS, polycystic ovary syndrome.

women born AGA or daughters of women without PCOS can also develop this disorder, which suggests a multifactorial aetiology of PCOS (Melo *et al.* 2010).

Because women born SGA and the daughters of hyperandrogenic women show higher frequencies of hyperinsulinaemia, visceral obesity, adipose tissue dysfunction and IR during childhood, these characteristics could be considered early clinical markers of the development of SAH, dyslipidaemia, metabolic syndrome and type 2 DM related to PCOS. Typically, these metabolic comorbidities are more prevalent after the age of 40 in the general population without PCOS. However, PCOS women, especially obese women with family histories of type 2 DM, histories of gestational diabetes, acanthosis nigricans or irregular menstrual cycles and hyperandrogenism, begin to present these comorbidities beginning in the fourth decade of life (Elting *et al.* 2001, Moran *et al.* 2010).

In addition to the multiple aetiological aspects of PCOS, ethnicity and geographical region are relevant factors in the study of birth weight and the development of PCOS. Although SGA women in Brazil (Melo *et al.* 2010) and those with LBW in Italy (Pandolfi *et al.* 2008) and Turkey (Hizli *et al.* 2012) present a higher risk for developing PCOS, these findings were not confirmed in individuals from the USA (Legro *et al.* 2010), UK (Cresswell *et al.* 1997, Michelmore *et al.* 2001, Shayeb *et al.* 2014), The Netherlands (Sadrzadeh *et al.* 2003), Finland (Laitinen *et al.* 2003), Spain (Ibáñez *et al.* 2008) or Denmark (Mumm *et al.* 2013). One study conducted in Australia analysed birth weight as a continuous variable and found that each 100 g increase in birth weight increased the risk of hyperandrogenism. The authors of that study also found that the subjects' thinness was related to PCOS symptoms and to IR (Davies *et al.* 2012; Table 3). These apparent regional differences may result from the limitations of these studies, which include the use of varying definitions of PCOS (Cresswell *et al.* 1997, Laitinen *et al.* 2003, Sadrzadeh *et al.* 2003, Ibáñez *et al.* 2008, Hizli *et al.* 2012, Mumm *et al.* 2013), the inclusion of women using hormonal contraceptives (Ibáñez *et al.* 2007, Davies *et al.* 2012, Mumm *et al.* 2013), the small numbers of participants (Ibáñez *et al.* 2001, Pandolfi *et al.* 2008), the use of self-reported birth data (Laitinen *et al.* 2003, Sadrzadeh *et al.* 2003, Legro *et al.* 2010, Hizli *et al.* 2012), the inclusion of women with immature HPG axes (Ibáñez *et al.* 2001) and the absence of compensatory growth assessments (Cresswell *et al.* 1997, Michelmore *et al.* 2001, Laitinen *et al.* 2003, Sadrzadeh *et al.* 2003, Ibáñez *et al.* 2008, Pandolfi *et al.* 2008, Legro *et al.* 2010, Melo *et al.* 2010, Hizli *et al.* 2012, Mumm *et al.* 2013, Shayeb *et al.* 2014). The use of birth weight classifications as an exposure factor across studies was perhaps the most important limitation, because not all of the studies adjusted the subjects' birth weight for GA. For that reason, some of the studies included preterm

newborn infants. This feature might indicate the presence of selection bias (Cresswell *et al.* 1997, Michelmore *et al.* 2001, Sadrzadeh *et al.* 2003, Ibáñez *et al.* 2008, Legro *et al.* 2010, Hizli *et al.* 2012, Mumm *et al.* 2013, Shayeb *et al.* 2014), especially considering that the functional maturation of organs and tissues of a preterm newborn is completed during postnatal life (Ben 2008).

There are two main birth weight classifications: i) the classification of birth weight based on GA (AGA: birth weight between P10 and P90 for GA; SGA: birth weight below P10 for GA; LGA: birth weight above P90 for GA), which is the most frequently used classification to confirm IUGR using postnatal data (birth weight, GA and newborn sex), can be used to determine the effects of foetal growth deficits regardless of prematurity (Battaglia & Lubchenco 1967) and is crucial for reducing bias (prematurity, for example) in the interpretation of results; and ii) the classification of birth weight independent of GA (macrossomic: birth weight ≥4000 g; appropriate: 3000–3999 g; inadequate: 2500–2999 g; LBW: 1500–2499 g; very LBW <1500 g; extremely LBW: <1000 g) (FIGO 1977). This information is an important health indicator that reflects the living conditions, nutrition and access to healthcare services in a population, which is extremely important when prenatal data are not available (Paneth 1995). However, birth weight data alone cannot determine preterm individuals; thus, this classification is inappropriate for studying the relationship between birth weight and the prevalence of disease at different stages of life. For example, a newborn weighing 2300 g can be either a preterm AGA newborn or a full-term SGA newborn.

Development programming related to PCOS manifestations: aspects relevant to menopause

Subclinical cardiovascular disease is common in reproductive-age women with PCOS (increased carotid intima-media thickness, reduced arterial elasticity and calcification of the coronary artery and aorta), regardless of the presence of obesity, IR or SAH (Talbott *et al.* 2004, Luque-Ramírez *et al.* 2007). The characteristics associated with early exposure to cardiovascular risk factors in inflammatory and oxidative environments during the reproductive years suggest an increased risk of cardiovascular events for patients in menopause and for elderly patients with PCOS (Cussons *et al.* 2006). However, the multifactorial aetiology of cardiovascular disease and the lack of well-designed prospective studies in which these women were assessed at different stages of life make conclusions regarding this possible association difficult. Although currently available evidence suggests that women with PCOS have twice the risk for cardiovascular events than that of the population without PCOS, this finding has some limitations: i) the lack of a

Table 3 Implications of birth weight in the aetiology of polycystic ovary syndrome.

Author	Study design	Population	Diagnosis of PCOS	Birth weight classification in relation gestational age	Association of SGA or LBW and PCOS RR (95% CI)
Cresswell et al. (1997) (UK)	Birth cohort	Polycystic ovaries: 49 Normal ovaries: 186 (born term and preterm)	Not mentioned	No (birth weight records in medical reports)	No (RR not evaluated) High birth weight was related to PCOS
Michelmore et al. (2001) (UK)	Cross-sectional	Polycystic ovaries: 74 Normal ovaries: 150 (born term and preterm)	Hyperandrogenism, menstrual irregularity and polycystic ovaries (prior to the Rotterdam consensus)	No (birth weight self-reported)	No (higher birth was related to polycystic ovaries)
Sadrzadeh et al. (2003) (The Netherlands)	Retrospective cohort (IVF centres)	PCOS: 265 (LBW: 27, ABW: 173, Unknown: 65) Control: 957 Oocyte donation: 98 Low responders: 351 (born term and preterm)	Not mentioned	No (birth weight self-reported)	No (LBW) 0.88 (0.53–1.48)
Laitinen et al. (2003) (Finland)	Birth cohort	SGA: 144 AGA: 1863 LBW: 71 No LBW: 1936 (born term and preterm)	Only self-reported symptoms of hirsutism and/or menstrual disturbances	Yes (two classifications) (birth weight records in medical reports)	No (SGA) 1.0 (0.76–1.33) No (LBW) 0.80 (0.51–1.26)
Ibáñez et al. (2008) (Spain)	Cross-sectional	Polycystic ovaries: 33 No polycystic ovaries: 53	Not mentioned	No (birth weight records in medical reports)	No (higher weight was related to polycystic ovaries)
Pandolfi et al. (2008) (Italy)	Birth cohort	LBW: 35 (SGA: 19 born term or preterm plus AGA: 16 born preterm) Control: 35 born term AGA	NIH	Yes (two classifications) (birth weight records in medical reports)	Yes Prevalence PCOS (SGA and AGA) LBW group: 40% Control group: 5.7% (RR not evaluated)
Legro et al. (2010) (USA)	Case–control	PCOS: 467 Control: 86 LBW: 46 NBW: 390 HBW: 31 (born term and preterm)	NIH	No (birth weight self-reported)	No (LBW) (OR not evaluated)
Melo et al. (2010) (Brazil)	Birth cohort	SGA: 43 AGA: 122 (born term)	Rotterdam and NIH consensus	Yes (birth weight records in medical reports)	Yes (Rotterdam consensus) 2.44 (1.39–4.28) Yes (NIH consensus) 1.66 (0.9–3.05)
Davies et al. (2012) (Australia)	Birth cohort	948 singleton births (users or non-users of hormonal contraceptives)	Only self-reported symptoms (270 women had a blood sample collected and 112 had a transvaginal ultrasound)	No (birth weight records in medical reports) Weight was available as a continuous variable	Although each 100 g increase in birth weight increased the risk of hyperandrogenism, thinness was related to PCOS symptoms and insulin resistance
Mumm et al. (2013) (Denmark)	Birth cohort	Birth weight (g): *<2500: 29 535 *2000–2999: 90 176 *3000–3499: 200 370 *3500–3999: 151 527 *4000–4499: 44 953 *≥4500: 7196 (born term and preterm)	Not mentioned (included cases of hirsutism and PCOS based on ICD)	Yes (birth weight records in medical reports)	No RR: 1.57 (1.21–2.03) (high birth weight ≥4500 g related to PCOS)

Reference	Study type	Birth weight	Diagnosis criteria	Birth weight source	Association
Hizli *et al.* (2012) (Turkey)	Cross-sectional	Birth weight (g): *<2500: 174 *2500–4000: 925 *>4000: 210 (term and preterm)	Only self-reported symptoms of hirsutism, acne and/or menstrual disturbances	No (birth weight self-reported)	Yes (LBW)
Shayeb *et al.* (2014) (UK)	Case–control	Anovulatory: 465 (PCOS and no PCOS) Control 1: 547 (ovulatory infertile) Control 2: 17 698 (fertile) (born term and preterm)	Rotterdam consensus	No (birth weight records in medical reports)	No OR: 1.73 (1.06–2.83) in relation to control 1 OR: 1.15 (0.75–1.75) in relation to control 2

PCOS, polycystic ovary syndrome; SGA, small for gestational age; LBW, low birth weight; RR, relative risk; ABW, appropriate birth weight; AGA, appropriate for gestational weight; NIH, National Institute of Health; NBW, normal birth weight; HBW, high birth weight; OR, odds ratio; ICD, International Classification of Diseases.

standardised diagnosis of PCOS; and ii) the lack of case stratification as fatal and non-fatal (De Groot *et al.* 2011). When such stratification was performed, an increased risk of non-fatal stroke was observed in menopausal PCOS women (OR, 1.94; 95% CI, 1.19–3.17) (Anderson *et al.* 2014), but no increased risk for acute myocardial infarction and/or mortality caused by cardiovascular events in women aged 61–79 years was observed during a 21-year follow-up (Schmidt *et al.* 2011). However, these studies did not provide proper external validity to allow these findings to be extrapolated to menopausal/ elderly PCOS women of other ethnic groups who might have been exposed to different environmental factors.

Although the aetiology of cardiovascular disorders is multifactorial, visceral obesity and hyperinsulinaemia play an important role in the development of cardiovascular events in women with PCOS. Visceral obesity and increased insulin levels may promote adipose tissue dysfunction and the subsequent elevation of inflammatory and metabolic markers that have an atherogenic effect in women of reproductive age (Melo *et al.* 2014). This adipose tissue dysfunction is associated with an increased prevalence of SAH, dyslipidaemia, metabolic syndrome, IR, type 2 DM and maintenance of hyperandrogenism, and it promotes endothelial dysfunction in inflammatory, oxidative and procoagulant environments, so it thus favours the occurrence of arterial thrombosis during menopause (Fig. 4; Cussons *et al.* 2006). However, regional studies are needed to determine the profile of morbidity and mortality of specific populations and to establish effective preventive interventions for PCOS and the metabolic and cardiovascular changes with which it is associated.
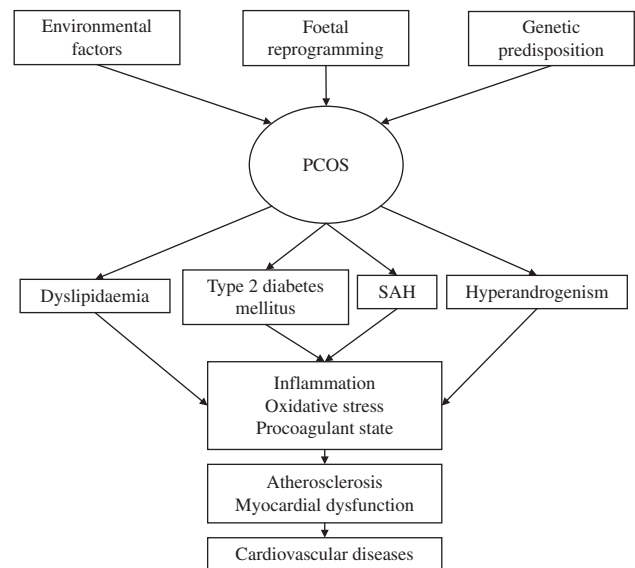
Figure 4 Pathogenesis of PCOS: evolutionary and multifactorial assessment from the reproductive age to menopause/senescence. Modified from Cussons *et al.* (2006). SAH, systemic arterial hypertension; IR, insulin resistance.

Conclusion

PCOS has a multifactorial aetiology with ethnic and regional aspects. Although being born SGA or being the child of a hyperandrogenic mother might be considered clinical markers for developmental programming by steroids, individuals who are not exposed to excess steroids and the offspring of non-hyperandrogenic mothers can also develop PCOS. This indicates that postnatal environmental factors and genetic predisposing factors also lie at the origin of this disorder. Follow-up studies that assess the same population at different stages of life will facilitate a better understanding of the interactions between environmental, genetic and intra-uterine factors in the development of PCOS and its comorbidities over a lifespan.

Declaration of interest

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

Funding

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