HHS Public Access

Author manuscript

Mol Cell Endocrinol. Author manuscript; available in PMC 2017 November 05.

Published in final edited form as:

Mol Cell Endocrinol. 2016 November 5; 435: 29–39. doi:10.1016/j.mce.2015.11.030.

Effect of maternal PCOS and PCOS-like phenotype on the offspring's health

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Abstract

Polycystic ovary syndrome (PCOS) is a heterogeneous endocrine disorder with both reproductive and metabolic abnormalities affecting women of reproductive age. While the exact origin of PCOS is unknown, observations from clinical and animal studies suggest that maternal hyperandrogenism may be a contributing factor. Because women with PCOS manifest hyperandrogenism during pregnancy, changes in the gestational endocrine milieu may play a role in the vertical transmission of this syndrome. This review discusses the potential developmental origins of PCOS, the impact of maternal PCOS on the offspring's health and contributions of the postnatal environment, capitalizing on findings from animal models that exhibit a PCOS-like phenotype. In addition, this review highlights the scarcity of data at early gestational stages in humans and the importance of animal experimentation to better understand the cellular and molecular mechanisms involved in the programming of adult diseases, therefore, helping identify therapeutic targets for preventive and treatment strategies.

Keywords

Developmental programming; androgen; insulin; polycystic ovary syndrome; sheep

1. INTRODUCTION

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders among women of reproductive age, with prevalence ranging from 6 to 15% based on the diagnostic criteria used for clinical assessment (March et al., 2010). The clinical manifestations of PCOS are varied and include functional hyperandrogenism (clinical and biochemical), menstrual irregularities, chronic anovulation, polycystic ovaries, and reduced fertility (Conway et al., 2014, Bouchard and Fauser, 2014). In addition to reproductive manifestations, the majority of PCOS patients also exhibit metabolic disturbances, such as obesity and insulin resistance with increased risk to develop type 2 diabetes mellitus

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Disclosure statement: The authors have nothing to disclose

(T2DM) and cardiovascular diseases (Diamanti-Kandarakis and Dunaif, 2012, Moran et al., 2015). Infants born to women with PCOS are also predisposed to many adverse health outcomes. Meta-analysis studies have shown that offspring of PCOS mothers are at higher risk for preterm birth, perinatal mortality, congenital abnormalities, increased hospitalization for metabolic disorders, diseases of nervous system, and asthma (Boomsma et al., 2006, Doherty et al., 2015, Qin et al., 2013). In addition, these infants are more likely to be born by Caesarean section and have lower birth weight (Boomsma et al., 2006). Because women with PCOS are also likely to be hyperandrogenic during pregnancy (Sir-Petermann et al., 2002, Sir-Petermann et al., 2012), endocrine alterations during gestation (Sir-Petermann et al., 2007a) may contribute to increased risk for their female offspring to also develop PCOS. This premise is further supported by observations that hyperandrogenic maternal environment in patients with congenital adrenal hyperplasia (CAH) and gestational androgen treatment in experimental animal models can lead to development of PCOS phenotype in the female offspring (Abbott et al., 1998, Barnes et al., 1994, Padmanabhan and Veiga-Lopez, 2013a). This review addresses our current understanding on the contribution of maternal PCOS on the offspring's health, the proposed developmental origins of PCOS, and the impact of the postnatal environment on the expression of reproductive and metabolic dysfunctions in the female offspring, including findings from animal models that manifest a PCOS-like phenotype.

2. DEVELOPMENT OF PCOS

The heterogeneity of the phenotypic expression of PCOS has led to numerous consensus meetings aimed at defining guidelines to aid in diagnosis and clinical care of this syndrome (Fauser et al., 2012). Importantly, the underlying pathophysiology and therefore the etiology of PCOS are still not clearly understood. Multiple factors ranging from genetics to environment and their interaction may be involved in the development and manifestation of PCOS. The premise of developmental origins of this syndrome is supported by the observation that girls born either small or large for gestational age may be at increased risk to manifest PCOS during reproductive life (Melo et al., 2010, Mumm et al., 2013). However, women born appropriate for gestational age can also develop PCOS, suggesting a multifactorial etiology (de Melo et al., 2015). A genetic susceptibility combined with an insult that occurs during intrauterine or early postnatal life may lead to reorganization of the neuroendocrine, ovarian, and metabolic systems that could culminate in disruptions observed in PCOS patients during adult life (Fig. 1). This is particularly evident in patients with CAH where prenatal androgenization could lead to development of adult onset of PCOS in the offspring (Barnes et al., 1994). The prenatal organizational events alone might be insufficient to determine the expression of adult PCOS and may require subsequent insults during postnatal life (*e.g.* endocrine imbalances and/or environmental exposures) that via activational effects can unmask or amplify the underlying defects associated with the phenotypic manifestation of this syndrome (Fig. 1). For instance, obesity during early childhood, when associated with elevated androgen levels (Reinehr et al., 2005), has been shown to increase the risk for developing PCOS (Laitinen et al., 2003). Therefore, many factors including genetic susceptibility, maternal milieu, and postnatal environment are likely to synergize in the development and expression of PCOS.

2.1. Origins of PCOS

2.1.1. Genetic basis—Available evidence points to a genetic susceptibility for the development of PCOS (Franks et al., 1997). There is high familial aggregation with about 20–40% of first-degree female relatives of women with PCOS being affected by this syndrome (Legro et al., 1998). Observations among Dutch twins that heritability for PCOS is 0.79 suggest that over 70% of pathogenesis of PCOS has a genetic basis (Vink et al., 2006). Considering the heterogeneity of this syndrome, it is possible that more than one gene is involved in the PCOS etiology (Franks et al., 1997, Franks et al., 2006). To date, mutations or polymorphism affecting multiple genes have been implicated in the development of PCOS (Table 1). Of these genes, replication studies have shown *FBN3, HSD17B6, POMC, ACRR2A, FEM1B, SGTA, LHCGR*, and *DENNDIA* to have good association between PCOS and variants of these genes (Ewens et al., 2010). Such association was not evident with other gene loci either due to small sample size, varied diagnostic criteria, or phenotypic heterogeneity (Goodarzi et al., 2011a, McAllister et al., 2015). Therefore, it appears that PCOS inheritance occurs in a complex manner similar to T2DM (Smushkin and Vella, 2010), with variations in several genes leading to accumulation of the moderate effect of individual genes (Franks et al., 1997, Franks et al., 2006). Consequently, genetics in combination with risk-increasing lifestyle (*e.g.* overnutrition and sedentarism) and environmental factors, as discussed below, might drive the development and manifestation of PCOS traits (Huber-Buchholz et al., 1999, Franks et al., 2006).

2.1.2. Prenatal exposure to steroid excess—Mounting evidence from experiments in animal models and clinical cases has substantiated the hypothesis that the origins of PCOS occur before adolescence and could take place as early as intrauterine life (Franks et al., 1997). It is apparent from these that an abnormal uterine milieu may cause reprogramming of key processes that lead to adult onset of PCOS. Development of PCOS phenotype in female offspring subjected to prenatal androgen excess resulting from maternal CAH (Hague et al., 1990, Barnes et al., 1994), congenital virilizing tumors (Barnes et al., 1994), and loss of function mutations in aromatase (Morishima et al., 1995) or sex hormone-binding globulin gene (SHBG) (Hogeveen et al., 2002) have clearly implicated hyperandrogenism as one of the main causal factors. Findings that prenatal exposure to excess androgens leads to the development of a PCOS-like phenotype during adulthood in several animal models (discussed below) (Padmanabhan and Veiga-Lopez, 2013a, Padmanabhan and Veiga-Lopez, 2013b, Abbott et al., 1998) also substantiate this premise. A possibility to consider is that hyperandrogenism may be involved in the vertical transmission of PCOS in humans, as PCOS women exhibit increased levels of androgens during pregnancy (Sir-Petermann et al., 2002, Sir-Petermann et al., 2012).

Environmental endocrine disrupting chemicals (EDCs) that disrupt ovarian and metabolic function are also being investigated as contributors in the development of PCOS phenotype (Barrett and Sobolewski, 2014). Bisphenol A (BPA), an ubiquitously present estrogenic EDC, is receiving considerable attention in this regard (Kandaraki et al., 2011, Peretz et al., 2014). One mode through which BPA may promote the development of PCOS is by its ability to stimulate ovarian theca-interstitial cells to overproduce androgens by regulating 17alpha-hydroxylase (Zhou et al., 2008). Levels of BPA have also been found to correlate

with androgen levels in women (Takeuchi et al., 2004). Animal studies have shown that prenatal exposure to BPA can lead to the development of PCOS-like symptoms (Peretz et al., 2014). While epidemiological studies reveal a close correlation between PCOS and circulating BPA levels in humans (Kandaraki et al., 2011, Takeuchi et al., 2004), a causal link is yet to be established.

Inappropriate exposure during development to steroids or EDCs can also induce epigenetic alterations and influence the development and severity of many adult onset diseases (Li and Huang, 2008, Xita and Tsatsoulis, 2010). Epigenetic alterations have been observed as non-random X-chromosome inactivation that may modulate the androgen receptor gene in PCOS women (Hickey et al., 2002, Hickey et al., 2006, Shah et al., 2008). Although global methylation of peripheral blood DNA was not significantly altered in women with PCOS (Xu et al., 2010), this does not rule out tissue-specific changes as observed in the adipose tissue of prenatally androgenized rhesus macaques (Xu et al., 2011) and women with PCOS (Jones et al., 2015). Similar epigenetic DNA alterations have also been observed in rats that received prenatal testosterone treatment (Xia et al., 2015, Zhang et al., 2014). These data suggest that prenatal exposure to steroid excess or EDCs may induce changes in epigenetic mechanisms leading to the development of PCOS phenotype later in life.

3. ANIMAL MODELS

Considering that the diagnosis of PCOS is made postpubertally and our inability to procure fetal samples (other than cord blood at term), it is virtually impossible to determine the early events that contribute to development of this pathology in humans. Animal models provide a valuable tool to overcome these predicaments. In addition to being a means to address causal and mechanistic events that lead to the development of PCOS phenotype, animal models allow preventive and therapeutic strategies to be tested. Among the various animal models, rats, mice, sheep, and rhesus macaques are the most commonly used in PCOS research with each offering different benefits (Maliqueo et al., 2014, van Houten and Visser, 2014, Padmanabhan and Veiga-Lopez, 2013b, Abbott et al., 2008b, Padmanabhan and Veiga-Lopez, 2013a). Rhesus macaques are genealogically closer to humans and thus can be a good translational model. However, its use is very limited because of the longer developmental timeline and associated prohibitive costs. Sheep, which are precocial species with organ developmental trajectory similar to humans (Padmanabhan and Veiga-Lopez, 2013b), have the advantage of relatively short time line from birth to adulthood (puberty achieved at ~28 weeks) and are amenable for repeated sampling and fetal manipulations. Rodents can be useful for transgenerational studies because of their shorter lifespan but unlike humans, they are polyovular and organ differentiation continues during the postnatal period.

Many of the animal models developed to study PCOS involve perinatal treatment with testosterone or other steroids such as dihydrotestosterone, estradiol valerate and steroid synthesis inhibitors (Abbott et al., 2008b, Padmanabhan et al., 2006, Maliqueo et al., 2014, Kauffman et al., 2015). The comparative aspects, advantages, and limitations of these models have been extensively reviewed elsewhere (Padmanabhan and Veiga-Lopez, 2013a). Treatment with testosterone during the prenatal period in macaques and sheep, and late

prenatal and early postnatal periods in rodents results in the adult onset of PCOS-like traits. Particularly in female sheep, prenatal exposure to testosterone results in an array of adult reproductive disorders that include disrupted neuroendocrine feedback mechanisms, increased pituitary sensitivity to gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH) hypersecretion, functional hyperandrogenism, and multifollicular ovarian morphology culminating in early reproductive failure (Padmanabhan and Veiga-Lopez, 2013b). Additionally, prenatal testosterone treatment leads to fetal growth retardation, insulin resistance, and hypertension in the female sheep (Padmanabhan et al., 2006, Cardoso et al., 2015). The adult phenotype of many of these animal models meets the established guidelines for diagnosis of PCOS and therefore can aid in understanding early developmental events associated with this syndrome. Importantly, the fetal levels achieved with gestational testosterone treatment in sheep are similar to those reported in the male offspring and are within a physiological range (Veiga-Lopez et al., 2011, Abi Salloum et al., 2015). Of interest, serum testosterone levels in 40% of human female fetuses during the second trimester are found to be within the range observed in male fetuses (Beck-Peccoz et al., 1991). However, the fetal levels of androgens achieved during early gestation in PCOS women, when organ differentiation occurs, are not known. Nevertheless, the prevalence of PCOS in females from opposite-sex twin pairs (where the male fetus can produce androgens that can potentially be transferred to the female co-twin) is not different compared to females from same-sex twin pairs or singletons (Kuijper et al., 2009). However, females with opposite sex co-twin show inconsistent expression of masculization traits (Ahrenfeldt et al., 2015, Tul et al., 2012, Loehlin and Martin, 2000). As such, an absence of PCOS prevalence among women with male co-twins is not a strong evidence for lack of fetal testosterone exposure before PCOS onset. This inconsistency could be due to protection measures that are present in the fetus and placenta that reduce free androgen levels through SHBG or aromatization (Xita and Tsatsoulis, 2010). Such protection mechanisms appear to be compromised in PCOS women (Maliqueo et al., 2013), thus allowing androgens to have an influence in the development of PCOS.

4. MATERNAL AND FETAL ALTERATIONS IN PCOS PHENOTYPES

4.1. Placental changes

The placenta is a transient association of maternal and fetal tissue that allows for physiological exchange between the mother and fetus during gestation (Gude et al., 2004). Pregnancy complications arising from failure in placentation commonly occur due to failure of trophoblastic invasion into the musculoelastic coat of the spiral arteries, resulting in incomplete vascular transformation and increased impedance in the uterine arteries (Jindal et al., 2007), thus compromising fetal growth (Prefumo et al., 2004). An increase in blood flow resistance in the uterine arteries and a reduction in subendometrial and endometrial vascularity have been observed in young non-pregnant women with PCOS indicating endometrial derangements that could impair implantation and placentation (Palomba et al., 2006). In pregnant patients with PCOS, ultrasound examination has shown that uterine artery Doppler indices are frequently altered indicating a defect in trophoblast invasion (Palomba et al., 2010). Macroscopic observations demonstrate that placentae from PCOS women have irregular shape, reduced weight, thickness/volume and density with higher

fetal-placental weight ratio and cord coiling index (Palomba et al., 2012b, Palomba et al., 2013). Microscopic alterations were also observed and include utero-placental vascular lesions, chronic villitis and intervillositis, and abnormal villus maturity along with absence of physiologic change of spiral vessels (Palomba et al., 2013). Interestingly, these changes are reported to be correlated with free androgens and indices of insulin resistance (Palomba et al., 2013). Changes in placentation could arise due to low-grade chronic inflammation present in non-pregnant women with PCOS (Orio et al., 2005), which could lead to abnormal immune regulation during implantation and subsequent pregnancy. In addition to ovarian and adipose tissue contribution to hyperandrogenism, placental tissue could also contribute to increased androgen levels as placentae from women with PCOS have higher HSD3B and lower aromatase expression resulting in increased androgen levels during pregnancy in PCOS women of Spanish descent (Maliqueo et al., 2013).

In the sheep model, gestational testosterone excess advanced placental differentiation leading to maintenance of placental efficiency early during fetal life but not near term, when placental efficiency was found to be reduced (Beckett et al., 2014). Similarly, down-regulation of amino acid transporters following gestational testosterone treatment possibly leading to reduced placental efficiency has also been observed in rats (Sathishkumar et al., 2011). Moreover, prenatal testosterone treatment in rats increased placental HSD17B2, estrogen and androgen receptor expression (Sun et al., 2012), and reduced placental weight (Slob et al., 1983), suggesting altered steroid synthesis and regulation similar to the observations in pregnant women with PCOS (Maliqueo et al., 2013). All together, these findings indicate that androgen excess during pregnancy leads to alterations in placenta that may affect the fetal endocrine milieu and growth.

4.2. Maternal and fetal hormonal milieu

Adverse factors that compromise the maternal environment may influence fetal development resulting in the adult onset of disease (Fowden et al., 2006). These detrimental conditions can originate from maternal nutritional deficiency, exposure to environmental EDCs, and/or preexisting diseases conditions such as pregestational diabetes or PCOS. Studies in a cohort of Spanish descent indicate that pregnant PCOS patients continue to be hyperandrogenic during pregnancy (Sir-Petermann et al., 2002), thus making it possible for vertical transmission. Some studies have found newborns of mothers with PCOS to have elevated umbilical cord levels of androgens (Barry et al., 2010, Mehrabian and Kelishadi, 2012), while other studies reported no change or low levels (Anderson et al., 2010, Hickey et al., 2009). It needs to be recognized that these studies investigated samples collected at term, long past the period of critical organ differentiation. One study found elevated second trimester amniotic fluid concentrations of testosterone in pregnant PCOS women (Palomba et al., 2012a). The fetus is normally protected from maternal androgens through placental aromatization or high levels of SHBG (Xita and Tsatsoulis, 2010). While placental aromatase activity appears to be reduced in PCOS women at term (Maliqueo et al., 2013), the activity levels during the first and second trimester of pregnancy are unknown. Animal studies have found that estradiol generated from aromatization of testosterone can itself program some of the defects (Padmanabhan and Veiga-Lopez, 2011).

Fetal hyperandrogenism can also result from increased fetal production of androgens. Because pregnant PCOS women are at increased risk for developing gestational diabetes (Boomsma et al., 2006, Kjerulff et al., 2011), the fetus may become hyperglycemic and subsequently hyperinsulinemic, which in turn can stimulate ovarian androgen hypersecretion (Dumesic et al., 2014). Although maternal testosterone and insulin levels are elevated in PCOS patients (Sir-Petermann et al., 2002, Sir-Petermann et al., 2007a), whether they are also increased in the fetus during critical periods of development is not known. In addition to being at risk for developing gestational diabetes, pregnant PCOS patients are also at increased risk for developing hypertension, preeclampsia, preterm birth, and abnormal endothelial function and placentation, all of which can alter the maternal milieu and thereby influence fetal development (Boomsma et al., 2006, Kjerulff et al., 2011, Altieri et al., 2010, van der Spuy and Dyer, 2004, Paradisi et al., 2001).

Animal studies provide the opportunity to collect blood samples and fetal tissue to investigate endocrine, molecular, and cellular modifications at specific stages of fetal development. In both rhesus macaques and sheep, gestational treatment with testosterone leads to not only an increase in maternal but also in fetal concentrations of androgens, while an increase in fetal estradiol was evident only in sheep (Veiga-Lopez et al., 2011, Abi Salloum et al., 2015, Abbott et al., 2008a). Gestational testosterone treatment reduced progesterone, increased insulin, and decreased medium chain acylcarnitines in the mothers while disrupting maternal-fetal associations for many metabolites including amino acids in sheep (Abi Salloum et al., 2015). Similar disruptions may also occur in rats, as placental amino acid transporters are reduced with testosterone treatment in these animals (Sathishkumar et al., 2011). In sheep, prenatal testosterone also reduced the bioavailability of growth factors, such as activin B and insulin like growth factor 1 (IGF1) (Crespi et al., 2006, Veiga-Lopez et al., 2011). Reduced levels of bioavailable IGF1 likely result from downregulation of hepatic mRNA expression as well as from increased expression of its binding protein (Crespi et al., 2006). The altered maternal and fetal levels of gonadal steroids and associated reduction in amino acids and bioavailability of growth factors have implications on fetal organ formation and growth and could contribute to fetal growth restriction.

4.3. Intra-uterine Growth Restriction

Intra-uterine growth restriction (IUGR) is associated with adult onset of several disorders including cardiovascular and metabolic diseases (Barker, 2004). In addition, it is now recognized that low birth weight may also lead to reproductive disorders, such as PCOS (Ibanez et al., 1998, de Bruin et al., 1998, Rhind et al., 2001). Pregnant Spanish descent women with PCOS are also reported to have newborns born small for gestational age (Sir-Petermann et al., 2005).

In the sheep model, prenatal testosterone treatment results in IUGR during late gestation (Steckler et al., 2005, Veiga-Lopez et al., 2011) and low birth weight in the female offspring (Manikkam et al., 2004). In marmosets, maternal androgen levels during the first trimester negatively correlate with offspring's body weight, length, and girth measurements (Smith et al., 2010). Rodents also show associated reduction in birth weight with prenatal testosterone

treatment (Sathishkumar et al., 2011). The weight of many of the fetal organs such as kidney, liver, spleen, and uterus in sheep were also proportionately reduced with prenatal testosterone (Steckler et al., 2005). Taken together, these data suggest that overall fetal development is affected by prenatal testosterone exposure.

5. CONTRIBUTIONS OF THE POSTNATAL ENVIRONMENT

Along with alterations during fetal development, changes in the endocrine milieu during early postnatal life may play an important role in maintaining/amplifying the reproductive and metabolic defects programmed *in utero*. In the last years, a "two-hit hypothesis" has been proposed to explain the pathogenesis of neurodegenerative diseases and cancer, suggesting that a series of insults that alone are insufficient to lead to disease may be necessary for the development, manifestation, and progression of these disorders (Bayer et al., 1999, Tang et al., 2008). A two-hit hypothesis in the context of PCOS in women (Bremer, 2010) has also been proposed. This premise by Bremer suggests that preexisting hyperandrogenism (first hit) subsequently disturbs the hypothalamic–pituitary–ovarian axis (second hit), resulting in ovulatory dysfunction and sustained hyperandrogenism. Pathogenesis of PCOS-like phenotype in the prenatally testosterone-treated macaque model has also been proposed to occur through a two event process involving prenatal testosterone exposure and postnatal adiposity (Abbott et al., 1998). Studies in prenatal testosterone-treated sheep provide experimental confirmation for the involvement of sequential insults (two-hit) in the manifestation and severity of PCOS-like traits (Steckler et al., 2009). The sequence of organizational and activational events leading to the origin and manifestation of PCOS phenotype (Fig. 1) along with phenotypic expression of developmental changes in human, monkeys and sheep (Fig. 2) are discussed below.

5.1. Gonadal steroid hormones

Altered steroid action on organ system development and function may result from changes in circulating concentrations of steroid hormones (*e.g.* ovarian and adrenal hyperandrogenism), changes in the expression of steroid receptors, and/or via exposure to environmental compounds with steroid-like structure (*e.g.* phytoestrogens, industrial byproducts, and pesticides) (Gore, 2008, Bahrke et al., 1998, Smithells, 1981, Levin et al., 1991, New, 2006, Jefferson et al., 2012). Studies in a cohort of Spanish descent have reported that in addition to androgen levels being elevated during pregnancy in women with PCOS (Sir-Petermann et al., 2002), their daughters also present hyperandrogenism around puberty (Sir-Petermann et al., 2009). Nevertheless, because the diagnosis of PCOS in women is often made only after the establishment of reproductive competence, the role of gonadal steroids during early postnatal life on the development and progression of PCOS is unknown. Moreover, while alterations in the prenatal (Sir-Petermann et al., 2002) and postnatal environments (Sir-Petermann et al., 2009, Sir-Petermann et al., 2007b, Sir-Petermann et al., 2007a) have been reported in daughters of PCOS women, the scarcity of longitudinal studies in these individuals prevents closer examination of the putative interactions between *in utero* ("first-hit) and postnatal ("second-hit") insults. In this regard, studies utilizing surgical and pharmacological interventions have started to elucidate the

contributions of postnatal steroid hormones to the development of PCOS-like traits in animal models of prenatal exposure to androgen excess.

Prenatally testosterone treated female rhesus monkeys have been shown to manifest hyperandrogenism and LH hypersecretion during the infantile development, suggesting that these may be endocrine antecedents of the adult PCOS-like phenotype (Abbott et al., 2005, Eisner et al., 2002). Observations that exogenous administration of androgen excess induces accelerated growth and weight gain as well as premature menarche in prepubertal female rhesus monkeys further suggest that early endocrine alterations may be involved in the expression of reproductive and metabolic dysfunctions in these animals (Van Wagenen, 1949).

In female sheep, prenatal testosterone treatment alters the steroid receptor balance increasing the expression of androgen receptor in the hypothalamus (Cernea et al., 2011), pituitary gland (Cardoso & Padmanabhan, unpublished observations), and ovaries (Ortega et al., 2009). The impact of postnatal steroids in modifying the prenatally programmed outcomes in the sheep model appears to be organ-specific. At the neuroendocrine level, the severity of the effects of prenatal testosterone-treatment on disrupting some of the feedback mechanisms appears to depend on the pattern of steroid exposure during postnatal life. For instance, neonatal ovariectomy partially prevents the deleterious effect of prenatal testosterone treatment on the estradiol positive feedback, indicating that postnatal exposure to sex steroids (and/or other ovarian-derived factors) is necessary to completely defeminize the GnRH/gonadotropin surge mechanism (Jackson et al., 2013). Furthermore, postnatal treatment with flutamide, an androgen antagonist, increased the total LH secreted in response to the estradiol positive feedback challenge in prenatal testosterone treated sheep, indicating that postnatal androgens play a role in determining the magnitude of the LH surge (Abi Salloum et al., 2012). Postnatal treatment with flutamide also prevented the advancement of puberty seen in prenatal testosterone treated sheep, suggesting that activation of the androgenic pathway during postnatal development is required for unmasking/amplifying potential alterations programmed by prenatal exposure to excess testosterone (Padmanabhan et al., 2015). While women with PCOS have been reported to present normal preovulatory LH surge in response to estradiol administration (Baird et al., 1977), the inhibitory effects of estradiol and progesterone on LH pulsatile secretion appear to be compromised in these individuals (Eagleson et al., 2000). Interestingly, similar to observed in the sheep model, treatment of PCOS women with flutamide improves the sensitivity of the neuroendocrine axis to the negative feedback of gonadal steroids (Eagleson et al., 2000).

Postnatal exposure to estradiol has been shown to amplify the luteal defects induced by prenatal exposure to testosterone excess in sheep (Veiga-Lopez et al., 2014), however, the impact of increased postnatal androgen action on ovarian function remains to be determined. Findings that prenatal testosterone treated sheep show progressive decline in cyclicity (Birch et al., 2003, Manikkam et al., 2006) may indicate that an imbalance in the peripubertal steroid milieu may serve as a “second hit” leading to gradual deterioration in ovarian function.

In addition to reproductive alterations, postnatal changes in steroid and insulin actions may also contribute to the development and progression of metabolic dysfunctions in females. Recent studies point to a reciprocal role of androgens and insulin in the maintenance of hyperandrogenism and insulin resistance in women with PCOS, with insulin sensitizers reducing hyperandrogenism (Brettenthaler et al., 2004, Pasquali and Gambineri, 2013), while attenuation of androgen actions improves peripheral insulin sensitivity (Moghetti et al., 1996). Nonetheless, in the sheep model, postnatal treatment with flutamide failed to restore normal insulin activation (phosphorylation) of AKT in the muscle and liver of prenatal testosterone treated females, indicating that insulin resistance in metabolic tissues is not overcome (Lu & Padmanabhan, unpublished observations). The impact of postnatal treatment with flutamide on peripheral insulin resistance in these females remains to be determined.

5.2. Nutrition and early postnatal growth

Nutritional status and rate of growth are also likely to modulate the impact of prenatal insults on the adult phenotype. Childhood obesity, when associated with increased androgen levels (Reinehr et al., 2005), is widely believed to contribute to the PCOS pathogenesis and to negatively influence its clinical manifestation (Anderson et al., 2014, Franks, 2008). A longitudinal, population-based study indicated that obesity at 14 years of age is associated with a 61% higher risk of manifesting symptoms of PCOS at 31 years of age (Laitinen et al., 2003). Although the mechanisms linking childhood obesity and adult PCOS phenotype are unknown, the observations that testosterone and dehydroepiandrosterone sulfate (DHEAS) levels are markedly increased in obese prepubertal girls (Reinehr et al., 2005) suggest that early alterations in the steroid milieu may be involved. This could arise because adipose tissue increases androgen and suppresses SHBG production leading to hyperandrogenism (Yildiz et al., 2008). Additionally, obesity and associated hyperglycemia can increase adipose tissue oxidative stress, mononuclear cell infiltration, and promote chronic low-grade inflammation with increased cytokines production (Gonzalez, 2012, Gonzalez et al., 2005). These cytokines can also stimulate theca cell proliferation and thereby increase ovarian androgen production leading to hyperandrogenism (Spaczynski et al., 1999). Adipose tissue can also store lipid soluble steroids and EDCs in addition to being steroidogenic (Li et al., 2014) wherein it can convert androgen precursors to active androgens such as androstenedione and testosterone (Pantasri and Norman, 2014). Because weight loss is accompanied by a reduction in circulating concentrations of androgens in prepubertal girls (Reinehr et al., 2005), weight management represents an important preventive strategy for obese and overweight patients.

Findings in the sheep model also support the premise that nutrition and early postnatal growth may modulate the expression of adult PCOS-like traits. In these females, prenatal testosterone treatment results in fetal growth retardation and early postnatal catch-up growth (Manikkam et al., 2004), which are considered early markers of future adult diseases (Ong et al., 2000, Cianfarani et al., 1999, Barker, 2004). Furthermore, excessive weight gain during early life amplifies the deleterious effects of prenatal exposure to excess testosterone on reproductive function in sheep (Steckler et al., 2009). More specifically, postnatal overfeeding markedly reduced the number of females presenting a LH surge after estrous

synchronization with prostaglandin $F_{2\alpha}$, thereby disrupting normal reproductive cyclicity (Steckler et al., 2009). In rhesus monkeys, although prenatal testosterone treated females have normal birth weight (Abbott et al., 2008a), they manifest a greater body weight gain during the infantile and juvenile development (Abbott et al., 2009). Together, these observations indicate that accelerated rate of growth and overnutrition may represent postnatal insults ("second-hit") that facilitate the manifestation and exacerbates the severity of PCOS-like dysfunctions in females prenatally exposed to testosterone excess.

5.3. Metabolic status

Considering the reciprocal interplay between androgens and insulin in PCOS after the establishment of disease (Brettenthaler et al., 2004, Moghetti et al., 1996, Diamanti-Kandarakis and Dunaif, 2012), it is possible that early alterations in the metabolic status may also contribute to the development and expression of PCOS traits during adulthood. In fact, studies in two PCOS populations of Spanish descent reported that daughters of PCOS women manifest metabolic alterations, including increased levels of insulin, before the onset of hyperandrogenism (Sir-Petermann et al., 2007b, Sir-Petermann et al., 2009). Although these girls have an increased propensity to develop PCOS (Battaglia et al., 2002, Sir-Petermann et al., 2009), a causal relationship between early hyperinsulinemia and adult PCOS phenotype is yet to be determined.

Observations in animal models further indicate that hyperinsulinemia may indeed contribute to the development of PCOS-like reproductive dysfunctions. In female rhesus monkeys, prenatal testosterone-treatment results in juvenile insulin hypersecretion (Abbott et al., 2009), and insulin sensitizer treatment initiated during adulthood normalizes menstrual cycles in the majority of females (Zhou et al., 2007). In sheep, early postnatal treatment with rosiglitazone, an insulin sensitizer, prevented the advancement of puberty seen in prenatal testosterone treated females, suggesting that increased insulin actions during postnatal development may contribute to this reproductive alteration (Abi Salloum et al., 2015). Moreover, rosiglitazone treatment initiated after puberty increased the total LH secretion during the estradiol positive feedback test (Abi Salloum et al., 2012) and prevented further reproductive deterioration in prenatal testosterone treated sheep (Veiga-Lopez et al., 2010). However, rosiglitazone treatment initiated after the onset of puberty, a time point when reproductive deterioration is already established, did not completely restore reproductive function to control levels (Veiga-Lopez et al., 2010). It remains to be ascertained whether treatment with an insulin sensitizer initiated during the juvenile development, when insulin homeostasis defects but not reproductive defects are evident, completely restores reproductive function in prenatal testosterone treated sheep.

6. CONCLUSIONS AND FUTURE DIRECTIONS

In conclusion, epidemiological studies in humans and observations in prenatal testosterone treated animal models indicate that alterations programmed *in utero* may play an important role on the development and manifestation of PCOS traits during adult life. Nevertheless, the lack of information on the maternal and fetal endocrine environments during gestation and the scarcity of longitudinal studies in humans limit our understanding of the relationships

between the human fetus and its maternal environment in PCOS pregnancy. Therefore, animal models of fetal programming represent an important research tool to examine the impact of the intrauterine environment on the offspring's health and to delineate the cellular and molecular mechanisms involved.

Although the characterization of the endocrine status at term may help identify a possible link between maternal hyperandrogenism and the development of adult diseases in the offspring, much of the programming of reproductive and metabolic functions/dysfunctions likely occurs early during gestation. Therefore, future clinical studies characterizing longitudinal changes in the maternal/fetal steroid and metabolic milieus during early stages of pregnancy are required. Such studies should capitalize on advanced fetal surveillance technologies and should include normal pregnancies as well as the several states that can lead to maternal hyperandrogenism (*e.g.* CAH and PCOS).

From an animal model perspective, pharmacological and transgenic approaches may help to further elucidate the cellular and molecular pathways involved in the prenatal/early postnatal programming of adult diseases, thus identifying therapeutic targets for preventive and treatment strategies. Moreover, because of the potential for PCOS traits to be carried forward to subsequent generations, transgenerational studies can establish the impact of the uterine environment in females manifesting a PCOS-like phenotype on the offspring's health (instead of the direct impact of gestational treatment with testosterone). In addition, these studies may help to identify potential epigenetic and vertical transfer mechanisms underlying these reproductive and metabolic dysfunctions.

Acknowledgments

Funding support: NIH: P01 HD044232

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Highlights

1. Pathogenesis of PCOS can be explained by the two-hit hypothesis.
2. The two hits involve perinatal organizational and postnatal activational events.
3. PCOS in women disrupts maternal milieu allowing vertical transmission.
4. Data from women with PCOS and animal models relative to the two-hit are compared.
5. Lack of human data and use of animal models relative to PCOS onset are discussed.

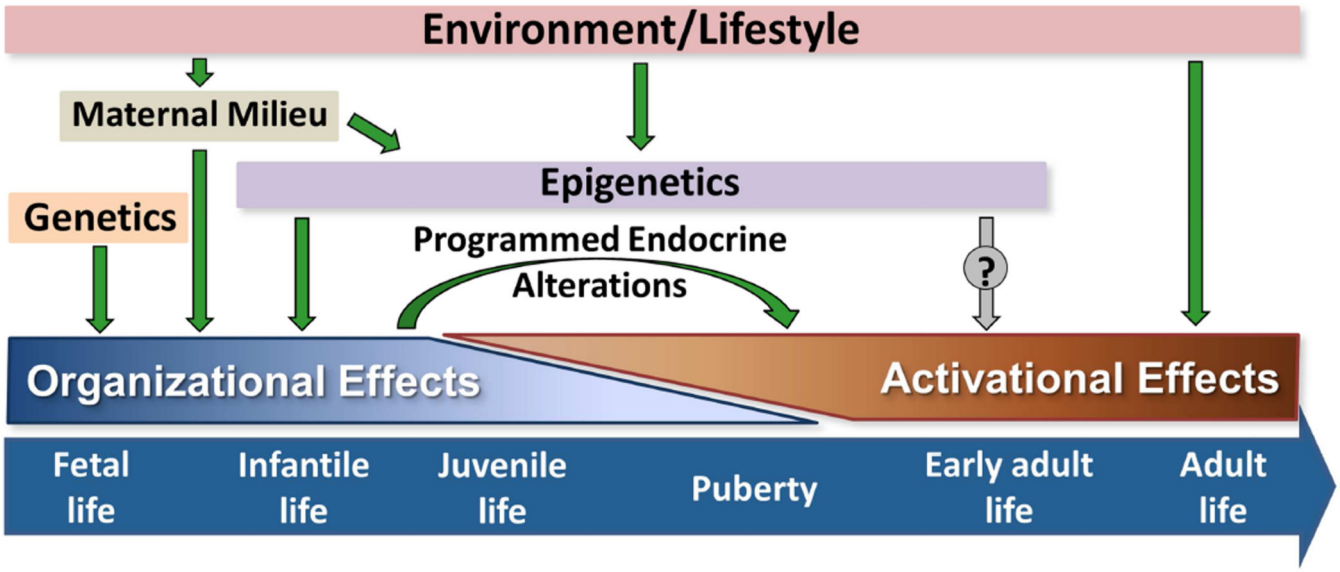

Figure 1.

Schematic showing the timeline of the organizational and activational effects that program adult onset disorders. In addition to genetic susceptibility, environmental changes and/or lifestyle choices have the potential to elicit changes in the maternal milieu and lead to epigenetic alterations that contribute to organizational changes during early development. Once organizational events are completed, programmed alterations in endocrine system, environmental and lifestyle factors can then influence the manifestation and severity of adult onset diseases through activational changes that may or may not involve epigenetic alterations.

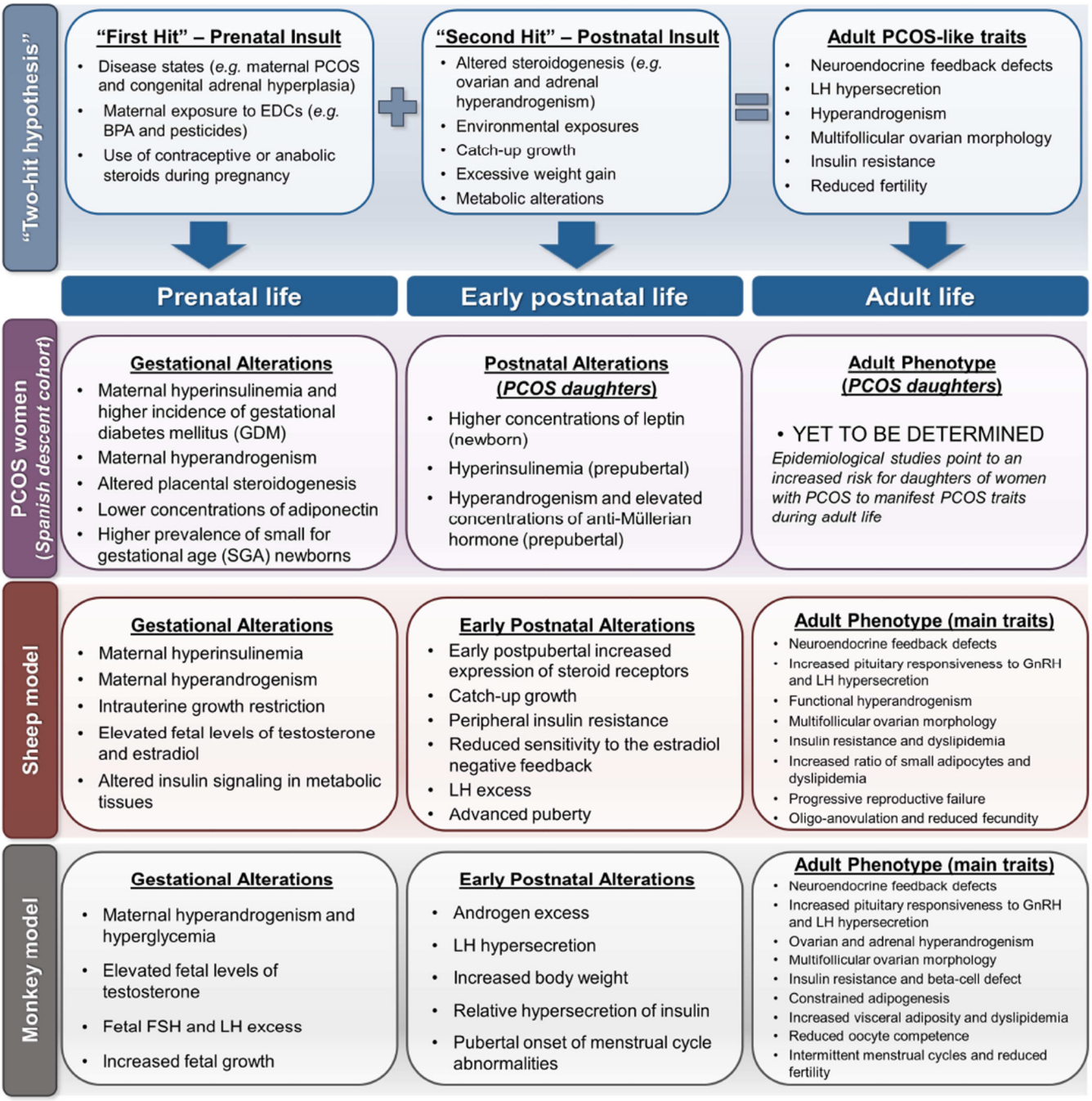

Figure 2.

Schematic of the "two-hit hypothesis" for development and manifestation of PCOS-like traits in females. Known changes in prenatal, early postnatal, and adult life in offspring of PCOS women, as well as in the monkey and sheep models for PCOS are shown. Prenatal insults ("first-hit") in conjunction with postnatal insults ("second-hit") may be involved in the pathogenesis of PCOS. Observations in prenatal testosterone treated sheep and rhesus

monkeys support the premise that postnatal endocrine and metabolic alterations play a role in maintaining/amplifying the PCOS-like traits in these females.

Table 1

Mutations or polymorphisms identified in genes associated with polycystic ovary syndrome (PCOS)

Gene Name	Gene Symbol	Cohort	Approach	Reference
Follistatin	*FST*	European	Radiation Hybrid (RH) Mapping	Urbanek et al., 1999
DENN/MADD domain containing 1A	*DENND1A*	Han Chinese	genome-wide association study (GWAS)	Chen et al., 2011
		American Caucasian	Genotyping	Goodarzi et al., 2012
		North European	Genotyping	Louwers et al., 2013
		Danish	Genotyping	Eriksen et al., 2013
		Hui Chinese	GWAS	Ha et al., 2015
Luteinizing hormone receptor	*LHCGR*	Han Chinese	GWAS	Chen et al., 2011
		Sardinian	Genotyping	Capalbo et al., 2012
		North European	Genotyping	Louwers et al., 2013
		Egyptian	Genotyping	Bassiouny et al., 2014
		Hui Chinese	GWAS	Ha et al., 2015
Follicle-stimulating hormone receptor	*FSHR*	Han Chinese	GWAS	Shi et al., 2012
		North European	Genotyping	Louwers et al., 2013
Thyroid adenoma associated	*THADA*	Han Chinese	GWAS	Chen et al., 2011
		North European	Genotyping	Louwers et al., 2013
		American Caucasian	GWAS	Day et al., 2015
		Hui Chinese		Ha et al., 2015
Yes-associated protein 1	*YAP1*	Han Chinese	GWAS	Li et al., 2012
		North European	Genotyping	Louwers et al., 2013
Follicle-stimulating hormone beta	*FSHB*	American Caucasian	GWAS	Hayes et al., 2015
				Day et al., 2015
Fibrillin 3	*FBN3*	Majority of European Origin	Genotyping	Ewens et al., 2010,
				Urbanek et al., 2005
Chromosome 9 open reading frame 3	*C9ORF3*	Han Chinese	GWAS	Zhao et al., 2015
		North European	Genotyping	Louwers et al., 2013
erb-b2 receptor tyrosine kinase 3	*ERBB3*	Han Chinese	GWAS	Shi et al., 2012
		American Caucasian	GWAS	Day et al., 2015
erb-b2 receptor tyrosine kinase 4	*ERBB4*	American Caucasian	GWAS	Day et al., 2015
DNA helicase RAD5	*RAD5*	American Caucasian	GWAS	Day et al., 2015
KRR1, small subunit (SSU) processome component, homolog	*KRR1*	American Caucasian	GWAS	Day et al., 2015
Activin A receptor type IIA	*ACVR2A*	American Caucasian	Genotyping	Ewens et al., 2010
Pro-opiomelanocortin	*POMC*	American Caucasian	Genotyping	Ewens et al., 2010
Fem-1 homolog B	*FEM1B*	American Caucasian	Genotyping	Ewens et al., 2010

Gene Name	Gene Symbol	Cohort	Approach	Reference
Small Glutamine-rich Tetratricopeptide-containing protein Alpha	*SGTA*	American Caucasian	Genotyping	Ewens et al., 2010
RAB5B, member RAS oncogene family	*RAB5B*	Han Chinese	GWAS	Shi et al., 2012
High mobility group AT-hook 2	*HMGA2*	Han Chinese	GWAS	Shi et al., 2012
TOX high mobility group box family member 3	*TOX3*	Han Chinese	GWAS	Zhao et al., 2015
SUMO1 pseudogene 1	*SUMO1P1*	Han Chinese	GWAS	Shi et al., 2012
		North European	Genotyping	Louwers et al., 2013
Sulfite oxidase	*SUOX*	Han Chinese	GWAS	Shi et al., 2012
Zinc finger protein 217	*ZNF217*	Han Chinese	GWAS	Shi et al., 2012