Mechanisms of intergenerational transmission of polycystic ovary syndrome

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Abstract

Developmental origins of adult disease (DoHAD) refers to critical gestational ages during human fetal development and beyond when the endocrine metabolic status of the mother can permanently program the physiology and/or morphology of the fetus, modifying its susceptibility to disease after birth. The aim of this review is to address how DoHAD plays an important role in the phenotypic expression of polycystic ovary syndrome (PCOS), the most common endocrinopathy of women characterized by hyperandrogenism, oligo-anovulation and polycystic ovarian morphology. Clinical studies of PCOS women are integrated with findings from relevant animal models to show how intergenerational transmission of these central components of PCOS are programmed through an altered maternal endocrine–metabolic environment that adversely affects the female fetus and long-term offspring health. Prenatal testosterone treatment in monkeys and sheep have been particularly crucial in our understanding of developmental programming of PCOS because organ system differentiation in these species, as in humans, occurs during fetal life. These animal models, along with altricial rodents, produce permanent PCOS-like phenotypes variably characterized by LH hypersecretion from reduced steroid-negative feedback, hyperandrogenism, ovulatory dysfunction, increased adiposity, impaired glucose–insulin homeostasis and other metabolic abnormalities. The review concludes that DoHAD underlies the phenotypic expression of PCOS through an altered maternal endocrine–metabolic environment that can induce epigenetic modifications of fetal genetic susceptibility to PCOS after birth. It calls for improved maternal endocrine–metabolic health of PCOS women to lower their risks of pregnancy-related complications and to potentially reduce intergenerational susceptibility to PCOS and its metabolic derangements in offspring.

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Introduction

Alterations in maternal physiology can permanently program adult disease in offspring (Dumesic *et al.* 2014). Known as the developmental origins of adult disease (DoHAD), this concept originates from the Barker hypothesis, which posits how adverse influences on fetal development cause permanent changes in offspring physiology and metabolism after birth, increasing the risk for disease in adulthood (Neel 1962, Barker 2002). Accordingly, fetal stress from maternal undernutrition favors survival of offspring with the greatest capacity for energy storage to survive prolonged nutrient deprivation after birth, with low birth weight followed by infant catch-up growth predisposing individuals to chronic adult disease in today's nutrient plentiful environment (Desai *et al.* 2013). Teleologically, fetal undernutrition favors genes important for energy conservation (i.e., thrifty genotype), which would be beneficial in times of

food scarcity, but would lead to obesity and diabetes when food becomes abundant later in life (Neel 1962). Original findings (Barker & Osmond 1986) that regions of England with the highest infant mortality rates in the early 20th century subsequently had the highest mortality rates from coronary heart disease decades later were supported by later studies showing an association between low birth weight and adult development of cardiovascular disease (CVD), hypertension, insulin resistance and type 2 diabetes mellitus (Barker 2002).

Maternal overnutrition, on the other hand, also can predispose offspring to adult disease. A 'U-shaped curve' in infant birthweight relative to obesity, hypertension and insulin resistance shows that birthweight deviation from an optimal *in utero* environment, regardless of direction, increases the risk of developing metabolic syndrome in later life (Desai *et al.* 2013). Collectively, therefore, impaired fetal nutrient availability from placental insufficiency accompanies low infant birth

weight, while conversely successful fetal adaptation to maternal nutrient overabundance (i.e. obesity) favors large-for-gestational age infants (Dumesic *et al.* 2014).

These different endocrine–metabolic environments in pregnancy have important developmental implications for women with polycystic ovary syndrome (PCOS), a heterogeneous syndrome characterized by luteinizing hormone (LH) hypersecretion, hyperandrogenism, menstrual irregularity and polyfollicular ovarian morphology (Chang & Dumesic 2018). The aim of this review is to examine how an altered maternal–fetal environment can program adult disease through epigenetic modifications of fetal genetic susceptibility to PCOS after birth, as evidenced by clinical studies of PCOS women integrated with animal models using discrete experimentally induced prenatal testosterone excess to program a permanent PCOS-like phenotype (Dumesic *et al.* 2007, 2014).

Polycystic ovarian syndrome

Women with PCOS by NIH criteria (i.e., hyperandrogenism with oligo-anovulation, excluding other endocrinopathies) have insulin resistance from abnormal insulin signaling and metabolic dysfunction in insulin-responsive tissues, with increased total abdominal and visceral fat independent of obesity (Dumesic *et al.* 2016, Chang & Dumesic 2018). One-third to one-half of these PCOS women in the United States have metabolic syndrome, which is 2–3 times greater in prevalence than that of age-matched women without PCOS and worsened by obesity (Moran *et al.* 2010). The prevalence of metabolic syndrome is also lower in PCOS women from countries where obesity is less frequent (Carmina *et al.* 2006, Moran *et al.* 2010). Moreover, 40% of PCOS women develop glucose intolerance or type 2 diabetes by the fourth decade of life (Legro *et al.* 1999), increasing their risk for pregnancy complications, including diabetes, pregnancy-induced hypertension, pre-eclampsia, preterm birth, impaired endovascular trophoblast invasion and abnormal placentation, with potential fetal programming effects on the offspring (Dumesic *et al.* 2014).

Genetic contributions to PCOS

Twin studies estimate the heritability of PCOS as 71%, and 38% for monozygotic vs dizygotic twins, respectively (Chang & Dumesic 2018) and are supported by a recent longitudinal study of daughters born to women with PCOS and followed from infancy to post-menarche, suggesting a 62% transmission of PCOS phenotype (Crisosto *et al.* 2019). Similarly, reproductive-aged sisters of women with PCOS have an increased prevalence of a hyperandrogenic PCOS phenotype (Chang & Dumesic 2018), as do parents of PCOS women with a higher risk of metabolic dysfunction (Sir-Petermann *et al.* 2002a).

Nevertheless, genetic susceptibility to PCOS by gene-wide association studies accounts for <10% of its heritability to date, with at least 17 replicated PCOS risk genes from different human populations involving reproduction (*FSHB*, *LHCGR*, *FSHR*, *DENND1A*, *RAB5/SUOX*, *HMGA2*, *C9orf3*, *YAP1*, *TOX3*, *RAD50*, *FBN3* and *AMH*) and metabolism (*THADA*, *GATA/NEIL2*, *ERBB4*, *SUMOP11*, *INSR* and *KRR1*) (Abbott *et al.* 2019). Consequently, PCOS appears to have complex polygenic, pathogenic origins that are susceptible to endocrine–metabolic influences during pregnancy to account for its remaining heritability and variable phenotypic expression (Abbott *et al.* 2019). Shared genetic traits across all PCOS phenotypes, whether clinically diagnosed by NIH, Rotterdam criteria or self-reported, implicate common underlying pathophysiological mechanisms involving neuroendocrine, metabolic and reproductive contributions (Day *et al.* 2018).

The maternal environment in PCOS

Understanding how the maternal environment of PCOS women affects their offspring must consider obesity as a covariable since two-thirds of women in the United States are overweight or obese, as are one-half of Australian and one-third of Danish women (ASRM 2015). Maternal obesity is strongly associated with several pregnancy and perinatal complications, including gestational diabetes and hypertension, preeclampsia, preterm delivery, stillbirth, early neonatal death and small, as well as large-for-gestational age infants (ASRM 2015). Obese women also are more likely than average-weight women to have infants with heart defects, ventral wall defects, neural tube defects and multiple anomalies (ASRM 2015). From a DoHAD perspective, maternal obesity increases the risk of premature mortality from a cardiovascular event in their adult offspring, adjusting for maternal age, social class, gestational age, infant sex and birth weight, implying intergenerational programming of metabolic disease (Reynolds *et al.* 2013).

An important question is whether PCOS women are at increased risk for metabolic dysfunction in pregnancy compared to other pregnant women. Preconception hyperandrogenemia and glucose intolerance in PCOS women predict several suboptimal maternal and neonatal outcomes, including preeclampsia (Christ *et al.* 2019). During pregnancy, PCOS women continue to have greater serum androgen levels, higher fasting and 2-h post-prandial insulin values (Sir-Petermann *et al.* 2002b, Kent *et al.* 2018) and elevated anti-Müllerian hormone (AMH) levels (Kent *et al.* 2018, Tata *et al.* 2018) compared to normal women. Subsequently, the prevalence of gestational diabetes, glucose intolerance and type 2 diabetes in PCOS women is 3- to 5-fold higher than that of other women, independent of age or body mass index (BMI), and is worsened by obesity (Teede *et al.* 2018). Contributing to these events, normal

and overweight PCOS women gain more weight by mid-gestation than pregnant women without PCOS (Kent *et al.* 2018), with about 40% of PCOS women developing gestational diabetes and other pregnancy complications (Abbott *et al.* 2019). During pregnancy, moreover, PCOS versus normal women exhibit exaggerated dyslipidemia and elevated circulating inflammatory markers that predict greater risks of gestational diabetes, hypertensive disorders and adverse obstetrical/neonatal outcomes, respectively (Palomba *et al.* 2014a,b).

From a mechanistic perspective, maternal hyperandrogenemia from PCOS is unlikely to directly program PCOS in offspring if placental aromatization is normal (Hickey *et al.* 2009), although reduced aromatase activity in term placenta from PCOS women has been reported (Maliqueo *et al.* 2013). Nevertheless, mid-gestation maternal testosterone levels positively associate with high AMH levels in adolescent female offspring, suggesting that elevations in mid-gestation maternal testosterone perturb subsequent ovarian function in daughters (Hart *et al.* 2010). More probable, however, maternal metabolic dysfunction in PCOS mothers compromises placental function of a female fetus with a genetic susceptibility to PCOS, promoting fetal hyperinsulinemia as a cause for hyperandrogenism and altered folliculogenesis *in utero* (Dumesic *et al.* 2007, 2014, Palomba *et al.* 2012, 2015, Abbott *et al.* 2019).

The fetal environment

Increased susceptibility to type 2 diabetes and obesity in offspring of type 1 diabetic mothers is metabolically determined *in utero*. Adolescent offspring of mothers with type 1 diabetes have increased body weight, dyslipidemia and insulin resistance predicted by their previous exposure to hyperinsulinemia *in utero* (Weiss *et al.* 2000). As adults, offspring of mothers with gestational or type 1 diabetes who were previously exposed to fetal hyperglycemia exhibit epigenetic changes in subcutaneous (SC) adipose, including decreased leptin promoter methylation, increased leptin gene and protein expression, decreased mitochondrial function and impaired fat storage (Hansen *et al.* 2017).

Therefore, maternal metabolic abnormalities likely affect the human fetal ovary during the second trimester of development when the primordial follicular pool gets established. Mid-trimester human fetal ovaries have several steroidogenic enzymes, genes encoding steroid-signaling pathways and receptors to steroids, insulin and insulin-like growth factors (Cole *et al.* 2006, Chang & Dumesic 2018). They also can metabolize pregnenolone sulfate to DHEA and androstenedione, and secrete DHEA, progesterone and estrone, with lesser amounts of androstenedione, estradiol and testosterone *in vitro* (Chang & Dumesic 2018). Lacking functional LH-like receptors (Wilson & Jawad 1979), however, mid-trimester human fetal ovaries are likely less responsive

than testes to the transient mid-gestational rise in fetal gonadotropins, which causes a transient sexual dimorphism in amniotic androgen levels that disappears by birth (Beck-Peccoz *et al.* 1991, Palomba *et al.* 2012, Chang & Dumesic 2018).

Instead, human fetal ovaries probably produce androgens in response to hyperinsulinemia *in vivo*, contributing to a transient mid-gestational rise of serum androgen levels into the normal male range in 40% of female fetuses that diminish below male levels in term umbilical cord blood (Beck-Peccoz *et al.* 1991). As evidence for mid-gestational fetal hyperandrogenism accompanying an altered endocrine-metabolic maternal environment, amniotic fluid testosterone levels are elevated in female fetuses of PCOS (Palomba *et al.* 2012) and diabetic (Barbieri *et al.* 1986) mothers alike, while hyperplasia of theca and pancreatic beta cells accompany ovarian theca-lutein cysts in hirsute female stillbirth offspring of diabetic women (Driscoll *et al.* 1960, Hultquist & Olding 1981). Umbilical cord testosterone levels at birth also are elevated in some (Barry *et al.* 2010), but not all (Anderson *et al.* 2010), female infants of PCOS mothers. Consequently, elongated anogenital distance, a reliable postnatal biomarker of mid-gestational fetal hyperandrogenism (Barrett *et al.* 2018), can be found in both female infants of PCOS mothers and in women with PCOS (Sanchez-Ferrer *et al.* 2017, Wu *et al.* 2017).

Animal models for PCOS

Reproduction

To understand causal mechanisms underlying these human maternal-fetal relationships, animal models have employed prenatal exposure to androgen excess in rodents, monkeys and sheep to induce metabolic and reproductive abnormalities resembling PCOS (Dumesic *et al.* 2007, Padmanabhan & Veiga-Lopez 2013, Abbott *et al.* 2019). Prenatally testosterone-treated monkeys and sheep have been essential animal models used to program a permanent PCOS-like phenotype of LH hypersecretion, ovarian/adrenal hyperandrogenism (monkeys) or functional hyperandrogenism (androgen receptor upregulation, sheep), ovulatory dysfunction and impaired glucose-insulin homeostasis (Dumesic *et al.* 2007) (Table 1). This is because tissue differentiation in these species is completed during fetal life (i.e., precocial species), as in humans (Fig. 1A, B and C), unlike mice in which only partial differentiation is completed before birth (i.e., altricial species) (Padmanabhan *et al.* 2007).

Neuroendocrine dysfunction

Neuroendocrine PCOS-like traits in adult prenatally testosterone-treated female rhesus monkeys (Abbott *et al.* 2008) and sheep (Padmanabhan & Veiga-Lopez 2013)

Table 1 PCOS traits in humans compared to the prenatally androgenized monkey and sheep model.

PCOS trait	Human	Prenatally androgenized rhesus monkeys		Prenatally androgenized female sheep
		Early treated	Late treated	
Reproductive				
Ovarian hyperandrogenism	Yes	Yes	Yes	Ovarian androgen receptor upregulation
Anovulation	Yes	Yes	Yes	Yes
Enlarged polyfollicular ovaries	Yes	Yes	Yes	Yes
LH hypersecretion	Yes	Yes	No	Yes
Reduced steroid negative feedback on LH	Yes	Yes	Yes	Yes
Impaired fertility	Yes	Yes	Yes	Yes
Metabolic				
Insulin resistance	Yes	Yes	No	Yes
Beta cell impairment	With increasing BMI	Yes	No	?
Hyperglycemia	With increasing BMI	Yes	Yes	No
Increased type 2 diabetes	Yes	Yes	No	Unknown
Increased abdominal fat	Yes	Yes	With increasing BMI	?
Hypertension	Yes	Unknown	Unknown	Yes
Dyslipidemia	Yes	Yes	Unknown	Yes

are characterized by LH hypersecretion from reduced hypothalamic sensitivity to steroid negative feedback and enhanced GnRH pulsatility (Robinson *et al.* 1999, 2002, Sarma *et al.* 2005, Padmanabhan *et al.* 2006, Abbott *et al.* 2018). Adult rats exposed to prenatal testosterone also exhibit LH hypersecretion and hyperandrogenism (Tehrani *et al.* 2014). Furthermore, prenatal androgen antagonism with the antiandrogen, flutamide, restores normal LH surge characteristics in prenatally testosterone-treated sheep (Padmanabhan *et al.* 2015) and estrous cyclicity in mice (Sullivan & Moenter 2004).

Reduced hypothalamic sensitivity to progesterone negative feedback on LH secretion also is a hallmark of PCOS women (Chhabra *et al.* 2005) and is similarly restored with flutamide (Eagleson *et al.* 2000, Chhabra *et al.* 2005). Comparable neuroendocrine abnormalities are found in adolescent hyperandrogenic girls, likely representing a precursor to adult PCOS (Lundgren *et al.* 2018). Moreover, PCOS women show a sexually dimorphic pattern of exaggerated early LH responsiveness to GnRH analog that more closely resembles that of men or women with congenital adrenal virilizing disorders (e.g., classical CAH and adrenal virilizing carcinoma) than normal women (Rosenfield *et al.* 1990, Barnes *et al.* 1994). These common characteristics between prenatally testosterone-treated animal models of PCOS and PCOS women implicate androgen excess during human fetal development with permanently reduced steroid negative feedback on LH and enhanced GnRH pulsatility that persists into adult life.

Ovarian dysfunction

Developmental programming of ovarian function resembling that of PCOS has been more controversial. Prenatal testosterone treatment in both monkeys and sheep induces female subfertility, which includes

impaired quality of primate oocytes (Dumesic *et al.* 2002, Steckler *et al.* 2007b). Enlarged, polyfollicular ovaries also occur in both animal models (Abbott *et al.* 1997, Padmanabhan *et al.* 2006, 2012) as well as in prenatally testosterone-treated adult rats (Tehrani *et al.* 2014) and are accompanied by hyperandrogenism in prenatally testosterone-treated rhesus monkeys (Abbott *et al.* 2002) and rats (Tehrani *et al.* 2014) and by ovarian androgen receptor upregulation in prenatally testosterone-treated sheep (Ortega *et al.* 2009). Polyfollicular ovaries, however, do not occur in prenatally dihydrotestosterone-treated sheep (Steckler *et al.* 2007a), suggesting endocrine–metabolic actions on the fetal ovaries other than androgen. Moreover, an exaggerated age-related decline in serum AMH levels from normal values in testosterone-exposed monkeys is atypical of PCOS (Dumesic *et al.* 2009).

In contrast to these animal models, AMH overproduction is a characteristic of PCOS women. Elevated serum AMH levels in PCOS women represent the opposing effects of stimulatory reproductive (hyperandrogenism and increased antral follicle number) versus inhibitory metabolic (body fat) factors (Guedikian *et al.* 2018), although some PCOS women may have a steeper age-related decline in circulating AMH levels than normal women (Ahmad *et al.* 2018).

Placental aromatization

Whether maternal testosterone can serve as a source of fetal hyperandrogenism or a programming mechanism for a PCOS-like phenotype in female offspring remains unclear. Gestational AMH excess in mice, however, can induce LH-mediated maternal testosterone excess with reduced placental aromatization of maternal androgens, potentially programming a PCOS-like phenotype in female offspring (Tata *et al.* 2018).

Figure 1 Timing of developmental programming of critical target tissues in (A) rhesus monkeys, (B) sheep and (C) humans as precocial species (Abbott *et al.* 2005, Padmanabhan & Veiga-Lopez 2013).

Reduced aromatase activity in term placenta from PCOS women also has been reported (Maliqueo *et al.* 2013), potentially increasing exposure of female fetuses to androgen excess. PCOS-related enhancement of fetal hyperandrogenism through placental dysfunction agrees with metabolically compromised PCOS placenta exhibiting features related to hypoxia, a condition known to downregulate placental aromatase (Koster *et al.* 2015, Kumar *et al.* 2018). Maternal androgens of PCOS mothers, however, may not substantially contribute to programming PCOS in their offspring due to sufficient placental aromatization (Hickey *et al.* 2009). More likely, an altered maternal endocrine–metabolic environment accompanying both PCOS and placental compromise (Koster *et al.* 2015, Palomba *et al.* 2015) probably promotes fetal ovarian hyperandrogenism via

hyperinsulinemia acting as a secretagogue *in utero*, with complex androgen–insulin interactions reprogramming sensitive target tissues in susceptible female offspring (Dumesic *et al.* 2007, 2014, Abbott *et al.* 2019).

Metabolism

Prenatally testosterone-treated monkeys and sheep as precocial species provide mechanistic links between endocrine–metabolic dysfunction in pregnancy and its long-term reproductive and metabolic consequences in offspring. In prenatally testosterone-treated monkeys and sheep, maternal glucose intolerance causes transient hyperinsulinemia in their female fetuses. Specifically, prenatal testosterone treatment in rhesus monkeys impairs maternal glucose tolerance and

stimulates fetal insulin release, which then potentiates insulin action within the fetus (Abbott *et al.* 2010). Prenatal testosterone treatment in sheep reduces progesterone levels and induces hyperinsulinemia (Abi Salloom *et al.* 2015). But when prenatally testosterone-treated sheep are co-treated simultaneously with either flutamide or rosiglitazone, maternal progesterone levels are improved, while juvenile insulin resistance and early adult hyperleptinemia are prevented (Cardoso *et al.* 2016).

In addition, mid-gestation fetal sheep ovaries express LH receptors with expression levels responsive to the prevailing androgen environment (Hogg *et al.* 2011). These endocrine–metabolic interactions during pregnancy are also influenced by placental function, which further modifies the *in utero* environment to program offspring in different ways.

Metabolic dysfunction

Mid-gestational prenatal testosterone treatment in rhesus monkeys and sheep programs adipose dysfunction and insulin resistance in adult offspring (Dumesic *et al.* 2007, Padmanabhan *et al.* 2010). Prenatally testosterone-treated female rhesus monkeys develop a PCOS-like phenotype characterized by progressive metabolic dysfunction with age, insulin resistance and preferential abdominal adiposity accompanied by hyperlipidemia (Eisner *et al.* 2000, 2003, Bruns *et al.* 2007, Zhou *et al.* 2007). Early-to-mid-gestation testosterone-treated female rhesus monkeys exhibit increased visceral fat (Eisner *et al.* 2003), adipose insulin resistance and impaired insulin secretion (Zhou *et al.* 2007, Abbott *et al.* 2019), while late gestation testosterone-treated females have increased total body (non-visceral) fat (Bruns *et al.* 2007), impaired insulin sensitivity and intact pancreatic insulin secretion, contributing to 27.3 and 11.1% rates of type 2 diabetes in the former and latter females, respectively. A TGF- β signaling pathway involving altered DNA methylation patterns of visceral fat in early-to-mid-gestation testosterone-treated female rhesus monkeys implies an epigenetic basis for reprogramming of adipose (Xu *et al.* 2011).

Adult prenatally testosterone-treated sheep also develop impaired insulin sensitivity (Recabarren *et al.* 2005, Padmanabhan *et al.* 2010, Cardoso *et al.* 2016), along with hypertension and hypercholesterolemia after puberty (King *et al.* 2007) as important metabolic abnormalities, although their long-term risks of developing increased adiposity and diabetes with age remain unclear since studies were performed through only 2 years of life (i.e. prime reproductive life). Interestingly, adult rats exposed to prenatal testosterone also exhibit insulin resistance in the absence of overt glucose intolerance (Noroozzadeh *et al.* 2015).

Adipogenic dysfunction

Subcutaneous (SC) adipose normally increases lipid storage capacity through adipocyte enlargement and new adipocyte formation to buffer fatty acid influx when caloric intake exceeds energy utilization (Romacho *et al.* 2014). Prenatally, testosterone-treated adult rhesus monkeys have an impaired ability to increase SC lipid storage relative to BMI (Bruns *et al.* 2007), suggesting abnormal adipogenesis, whereby multipotent adipose stem cells (ASCs) first undergo commitment to preadipocytes and then differentiate into newly formed adipocytes (Chazenbalk *et al.* 2013). Using adipogenic gene markers, SC abdominal adipose of aging early testosterone-treated females shows impaired preadipocyte differentiation into adipocytes (i.e. decreased *C/EBP α*) accompanying hyperandrogenemia and enhanced earlier ASC commitment to preadipocytes (i.e. increased *ZFP423*) possibly compensating for subsequent impaired preadipocyte differentiation (Keller *et al.* 2014). In agreement, prenatal *Zfp423* knockout in mice fed a high-fat diet exaggerates diet-induced obesity, ectopic fat deposition and insulin resistance (Shao *et al.* 2017). In support of developmental programming as a contributor to such events, studies of prenatally testosterone-treated juvenile female sheep show increased ASC commitment to preadipocytes and decreased preadipocyte differentiation of visceral adipocytes, with the later prevented by dual prenatal flutamide/rosiglitazone co-treatment (Puttabyatta *et al.* 2018).

In normal human SC abdominal adipose, androgen inhibits early-stage human SC abdominal adipogenesis, induces insulin resistance and impairs catecholamine-stimulated lipolysis through reduced protein expression of β_2 -adrenergic receptor and hormone-sensitive lipase (HSL) independent of BMI or age (Dicker *et al.* 2004, Arner 2005, Corbould 2007, Chazenbalk *et al.* 2013) (Fig. 2). Similarly, in nonobese insulin-resistant PCOS women, SC abdominal adipose exhibits lipolytic catecholamine resistance from diminished protein levels of β_2 -adrenergic receptor, HSL and protein kinase A regulatory-II β component (PKA-RegII β) (Ek *et al.* 1997, Faulds *et al.* 2003). Such androgen-related events *in vivo* would likely constrain SC fat storage and cause lipotoxicity from ectopic lipid accumulation in non-adipose tissues (Chazenbalk *et al.* 2013), agreeing with findings in normal-weight PCOS women of altered SC abdominal ASC gene expression of adipogenic/angiogenic functions involving testosterone through TGF- β signaling (Dumesic *et al.* 2019).

In the absence of systemic testosterone, however, cultured SC abdominal ASCs from normal-weight PCOS women show exaggerated ASC commitment to preadipocytes followed by enhanced adipocyte lipid content during mature adipocyte formation that

Figure 2 Hypothetical model of androgen actions on subcutaneous abdominal adipogenesis in normal-weight PCOS women. In this model, androgen (1) inhibits preadipocyte differentiation to mature adipocytes, (2) blocks catecholamine-induced lipolysis and (3) impairs insulin inhibition of lipolysis. Exaggerated adipose stem cell (ASC) development to adipocytes also occurs via androgen-independent mechanisms. Theoretically, exaggerated ASC lipid accumulation within a hyperandrogenic environment could predispose PCOS women to lipotoxicity. A: Commitment; B: Differentiation/lipid accumulation; C: Lipolysis.

negatively and positively correlate with circulating fasting glucose and androgen levels, respectively (Fisch *et al.* 2018). Such exaggerated ASC commitment to preadipocytes in cultured SC abdominal ASCs from these PCOS women, possibly accompanying intra-adipocyte hyperandrogenism (O'Reilly *et al.* 2017), likely represents a regulatory mechanism to maintain glucose-insulin homeostasis during accelerated fat accretion (Fisch *et al.* 2018), as evident in nonobese PCOS women by an altered gene expression favoring SC abdominal lipid metabolism (Chazenbalk *et al.* 2012).

In contrast, human visceral fat normally shows increased lipolytic activity and resistance to testosterone inhibition of catecholamine-induced lipolysis compared to SC abdominal fat, despite both fat depots expressing androgen receptors (Dicker *et al.* 2004). In addition, visceral adipose of nonobese PCOS women shows exaggerated catecholamine-induced lipolysis accompanying normal antilipolytic insulin action (Ek *et al.* 2002, Arner 2005). This suggests that upregulation of visceral fat lipolysis from a functional increase in the PKA-HSL complex could promote insulin resistance secondary to elevated portal free fatty acids (Ek *et al.* 2002, Arner 2005). In normal-weight PCOS women, therefore, such a phenomenon could promote lipolysis for fatty acid oxidation through insulin resistance to curtail fat accretion over time, as previously shown in nondiabetic Pima Indians with a proclivity toward excess weight gain (Swinburn *et al.* 1991).

Altered SC abdominal adipocyte size

The size distribution of adipocytes within an adipose depot represents a balance between adipocyte enlargement and new adipocyte formation to buffer fatty acid influx (Romacho *et al.* 2014). An increased proportion of small SC abdominal adipocytes occurs in prenatally testosterone-treated adult rhesus monkeys and

sheep (Veiga-Lopez *et al.* 2013, Keller *et al.* 2014). This altered adipocyte morphology in early adult, prenatally testosterone-treated sheep occurs when visceral adiposity and insulin sensitivity are still normal and precedes metabolic dysfunction in later life, perhaps as a compensatory adaptation to the perturbed intrauterine environment (Cardoso *et al.* 2016). Interestingly, reduced adipocyte size in prenatally testosterone-treated sheep is not reversed with flutamide co-treatment, implying endocrine-metabolic mechanisms apart from androgen, including possible estrogen action in this species (Cardoso *et al.* 2016, Puttabyatappa *et al.* 2018).

An increased proportion of small SC abdominal adipocytes also occurs in normal-weight PCOS women (Dumesic *et al.* 2016) and precedes the development of enlarged mature adipocytes in overweight PCOS women (Mannaras-Holm *et al.* 2011), explaining why some, but not all, such individuals maintain normal glucose-insulin homeostasis (Chang & Dumesic 2018). In this regard, enhanced small adipocyte formation accompanying *ZFP423* upregulation and epigenetic changes in the *ZFP423* promoter region protect against insulin resistance in humans (Longo *et al.* 2018). That regional fat depots develop in the human fetus between 14 and 28 weeks of gestation supports the hypothesis that abdominal fat can be developmentally programmed during early-to-mid-gestation (Poissonnet *et al.* 1988) (Fig. 1).

Maternal-fetal interactions

Altered endocrine-metabolic interactions in pregnancy also can influence fetal growth. In this regard, prenatally testosterone-treated female sheep (Manikkam *et al.* 2004, Steckler *et al.* 2005, Crespi *et al.* 2006) and rats (Slob *et al.* 1983, McGivern 1989) exhibit intrauterine growth retardation and low birth weight near term (McGivern 1989, Manikkam *et al.* 2004,

Padmanabhan *et al.* 2006). In prenatally testosterone-treated fetal sheep, these events accompany advanced placental differentiation (Beckett *et al.* 2014) that is insufficient to maintain placental function during late gestation, leading to intrauterine growth retardation, mainly in female offspring (Beckett *et al.* 2014), followed by postnatal weight gain (or catch-up growth) (Manikkam *et al.* 2004) and insulin resistance in adulthood (Cardoso *et al.* 2016). Moreover, precocious puberty occurs in prenatally testosterone-treated sheep independent of body weight and is prevented by prenatal co-treatment with flutamide or rosiglitazone, confirming pubertal timing as a programmed androgen-insulin-mediated event (Padmanabhan *et al.* 2015). Therefore, prenatally testosterone-treated sheep and rats may be suitable models for placental insufficiency, particularly since these sheep also have hypertension and myocardial changes associated with left ventricular hypertrophy (King *et al.* 2007, Vyas *et al.* 2016), while the rodents have increased mortality (Wolf *et al.* 2004).

In contrast, prenatally testosterone-treated rats have a normal birth weight, with exaggerated weight gain by 30 days of age (Tehrani *et al.* 2014). Similarly, early prenatally testosterone-treated female monkeys have a normal birth weight, with an increase in body weight beginning in early infancy (Herman *et al.* 2000, Abbott *et al.* 2007, 2008, 2010) followed by delayed puberty as seen in males and an adult PCOS-like phenotype. Mechanisms beyond testosterone-induced developmental programming likely exist since exposure of monkeys to prenatal testosterone excess impairs glucose–insulin homeostasis without affecting body weight in both adult sexes (Abbott *et al.* 2007).

From a human perspective, macrosomic infants of obese glucose-intolerant mothers represent successful fetal adaptation to maternal nutrient overabundance (Mane *et al.* 2017). On the other hand, low infant birthweight in women with impaired placental aromatization and diminished uteroplacental perfusion represents impaired fetal nutrient availability from placental insufficiency (Tanguy *et al.* 1981, Thomsen *et al.* 1982). Both large and small for gestational age infants have been reported in pregnant women with PCOS (Palomba *et al.* 2014a,b), with birth weight and maternal mid-gestational BMI positively correlated with newborn adiposity as determined by cord blood leptin levels (Maliqueo *et al.* 2009).

Poor intrauterine growth and low birth weight accompany precocious puberty and PCOS in northern Spanish women (Ibanez *et al.* 1996, 1998) and in PCOS pregnancies in Chilean and Iranian women (Sir-Petermann *et al.* 2005, Mehrabian & Kelishadi 2012), but not in larger groups of Finnish (Laitinen *et al.* 2003) and Dutch individuals (Sadrzadeh *et al.* 2003, Koster *et al.* 2015). Low or normal birth weight in some infants of pregnant women with PCOS can accompany placental abnormalities, including chronic villitis/intervillositis,

impaired decidual trophoblast invasion and reduced placental size, that limit nutrient delivery to the fetus and may (Palomba *et al.* 2014c) or may not (Koster *et al.* 2015) reduce the ratio of fetal to placental weight.

Since infants of PCOS mothers also can have normal birth weights (Laitinen *et al.* 2003, Dumesic *et al.* 2014), developmental programming of adiposity can occur in the human fetus (Poissonnet *et al.* 1988) without altering infant birth weight, yet still increase the risk of excessive postnatal weight gain and onset of PCOS in susceptible offspring. Mechanisms independent of testosterone-induced developmental programming are supported by findings that male relatives of PCOS women exhibit metabolic dysfunction similar to that of their female relatives (Dumesic *et al.* 2007, Yilmaz *et al.* 2018). Additional transgenerational transmission of PCOS through the male line is also supported by a strong association of genetic variants previously linked to male-pattern balding with PCOS phenotypes (Day *et al.* 2018).

Endocrine antecedents of PCOS

Endocrine antecedents of PCOS occur in female infants born to PCOS mothers and further emphasize that interactions between genes of the fetus and the endocrine–metabolic environment of pregnancy may program offspring after birth. Infant girls born to PCOS versus non-PCOS mothers exhibit increased sebum production by the pilosebaceous unit as a marker of prenatal androgen excess (Homburg *et al.* 2017). They also exhibit AMH overproduction as a marker of growing follicles, which persists in prepubertal life and improves, along with exaggerated ovarian responsiveness to GnRH analog administration, when PCOS mothers receive metformin in pregnancy, beginning at or before conception (Sir-Petermann *et al.* 2006, Crisosto *et al.* 2012). Consistent with these findings, female children of PCOS women have enlarged ovaries and hyperinsulinemia that precede onset of LH hypersecretion and androgen excess during puberty in some (Crisosto *et al.* 2007, Kent *et al.* 2008, Sir-Petermann *et al.* 2009), but not all (Legro *et al.* 2017) studies.

From a metabolic perspective, adiposity of infants from PCOS women positively correlates with birth weight and maternal mid-gestational BMI (Maliqueo *et al.* 2009), the latter of which increases disproportionately in normal and overweight PCOS women compared to other pregnant women (Kent *et al.* 2018). Infant males born to PCOS mothers exhibit higher body weight and develop insulin resistance in adulthood as a risk factor for acquiring type 2 diabetes (Recabarren *et al.* 2008). Although metformin use in pregnancy appears to improve some ovarian characteristics in female offspring of PCOS mothers (Sir-Petermann *et al.* 2006, Crisosto *et al.* 2012), increased childhood adiposity, insulin resistance and increased newborn head size following gestational treatment of

PCOS women with metformin demonstrates the need to minimize gestational interventions in humans (Hanem *et al.* 2019). In addition, interactions between genes of the susceptible fetus and maternal PCOS endocrine–metabolic environment are likely further altered by postnatal environmental inputs, since widespread epigenomic remodeling occurs throughout human aging, being inherited across generations through non-genetic mechanisms (Benayoun *et al.* 2015, Simpkin *et al.* 2016).

Conclusion

The collective data suggest that epigenetic changes in fetal life may impact the developmental origins of PCOS, assuming a critical time interval of fetal susceptibility beginning in early-to-mid-gestation when developmental programming occurs (Dumesic *et al.* 2007, Abbott *et al.* 2019). By inducing permanent PCOS-like phenotypes characterized by LH hypersecretion from reduced steroid negative feedback, hyperandrogenism, ovulatory dysfunction and impaired glucose–insulin homeostasis, animal models of prenatal testosterone treatment support clinical studies of PCOS women in providing insights into when developmental programming occurs during human development, how placental function alters the maternal–fetal relationship to affect fetal growth, and perhaps why birth weights of PCOS women differ throughout the world. Improved maternal endocrine–metabolic health of PCOS women to lower their risk of pregnancy-related complications could potentially reduce intergenerational susceptibility to PCOS and its metabolic derangements in offspring.

Declaration of interest

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

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