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In utero Androgen Excess: A Developmental Commonality Preceding Polycystic Ovary Syndrome?

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Abstract

In utero androgen excess reliably induces polycystic ovary syndrome (PCOS)-like reproductive and metabolic traits in female monkeys, sheep, rats and mice. In humans, however, substantial technical and ethical constraints on fetal sampling have curtailed safe, pathogenic exploration during gestation. Evidence consistent with *in utero* origins for PCOS in humans has thus been slow to amass, but the balance now leans towards developmental fetal origins. Given that PCOS is familial and highly heritable, difficulty in discerning genetic contributions to PCOS pathogenesis is puzzling and, to date, accounts for <10% of PCOS presentations. Unaccounted heritability notwithstanding, molecular commonality in pathogenic mechanism is emerging, suggested by co-occurrence of replicated PCOS genetic variants and epigenetic alterations in DNA methylation at the same replicated, PCOS risk loci with bioinformatics-identified gene loci within monkey epigenetic alterations in DNA methylation array-determined gene networks from females exposed to testosterone (T) *in utero*. In addition, naturally occurring female hyperandrogenism in monkeys singles out females with PCOS-like reproductive and metabolic traits accompanied by somatic biomarkers of *in utero* T exposure. Such phenotypic and molecular convergence between highly related species, suggests not only dual genetic and epigenetic contributions to PCOS, as well as a developmental origin, but also common molecular pathogenesis extending beyond humans.

Keywords

fetal programming; developmental origins of adult disease; nonhuman primate; animal model

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Introduction

With a staggering 10–20% prevalence among premenopausal women, polycystic ovary syndrome (PCOS) is the most common endocrine and metabolic women's health disorder [1]. Its diagnosis by Rotterdam criteria requires at least two of the following three: high testosterone (T) levels or hirsutism, intermittent or absent menstrual cycles, and ultrasonography-visualized polycystic ovaries, excluding endocrine mimics such as congenital adrenal hyperplasia [1]. Luteinizing hormone (LH) hypersecretion commonly accompanies hyperandrogenism likely due to T-diminished, estradiol (E_2) induction of neural progesterone receptors in the mediobasal hypothalamus (MBH) [2,3], resulting in diminished E_2-progesterone negative feedback regulation of gonadotropin-releasing hormone (GnRH) episodic release from the MBH [4]. Elevated ovarian granulosa cell anti-mullerian hormone (AMH) commonly accompanies polycystic ovary morphology and provides an accurate biomarker for increased numbers of growing follicles [5].

Clinical sequelae for PCOS include acne, infertility, endometrial hyperplasia and malignancy, obesity, type 2 diabetes (T2D), sleep apnea, cardiovascular disease, mood disorders and sexual dysfunction [1,6,7]. PCOS is thus a uniquely challenging, multi-faceted disorder with several phenotypes, in which progressive obesity enhances severity of phenotype, diminishes wellbeing and quality of life [8]. Overt signs and symptoms of PCOS usually manifest at puberty, progressing with age from issues related to appearance and reproduction, to weight gain accompanying metabolic sequelae and diminished wellbeing [1].

Given its complexity, PCOS is highly heritable and familial, and hyperandrogenism is its most heritable trait [1]. Pathogenic mechanisms, however, have remained elusive, hindering progress towards a cure. In this regard, *in utero* findings of hyperandrogenic origins for PCOS-like traits in nonhuman primates [9,10], provided a novel, single pathogenic origin for PCOS that mimicked PCOS phenotypic diversity in women (hence PCOS-like traits), and provided a potential epigenetic amplification mechanism [11] effectively reprogramming diverse genetic backgrounds into PCOS-like individuals [12]. Confirmatory findings soon followed in sheep [13], mice [14] and rats[15], expanding into molecular insight of developmental pathogenesis [16–18]. While clinical acceptance of *in utero* hyperandrogenic origins for PCOS is not universal [19], accumulating supportive evidence from human studies is shifting the balance of evidence towards pathogenic onset for PCOS during fetal life [1,12,20]. This brief review will focus on current understanding emerging from human and animal model studies concerning *in utero* androgen excess contributing to PCOS and its potential pathogenic mechanisms, with an emphasis on nonhuman primate findings.

In utero androgen excess: human studies

During early-to-mid gestation, ~40% of girls experience elevated circulating concentrations of unbound, bioavailable, testosterone (T) levels in the fetal male range [21]. This is relevant to PCOS since comparable circulating T excursions into the fetal male range generate PCOS-like traits in gestational T-exposed female rhesus monkeys [22] and sheep [23], revealing the devasting, life-long impact on females of *in utero* androgen excess. In short-

gestation rodents, late gestation and the immediate post-partum period provide a comparable developmentally vulnerable period for females [12]. Perhaps not surprisingly, therefore, fetal male-similar T levels are found in mid-gestation amnionic fluid from daughters of women with PCOS, well exceeding those in mid-gestation daughters of women without PCOS [24]. As mid-gestation amnionic fluid T originates from the fetus [25], elevated T levels suggest hyperandrogenism in fetal daughters of women with PCOS during a crucial, developmental window when female nonhuman primates and sheep are vulnerable to PCOS-like reprogramming [12,16]. Approximately 50% of daughters born to PCOS women develop signs and symptoms of PCOS by adolescence [26], indicating the substantial risk for PCOS phenotype accompanying female *in utero* androgen excess in humans [12].

Pregnant women with PCOS retain hyperandrogenism throughout pregnancy [27], together with elevated AMH levels [28] and reduced placental aromatase expression [29]. Despite population differences [30], ~40% of PCOS women experience gestational diabetes [31] and other pregnancy complications [32], with maternal diabetes predisposing offspring to metabolic dysfunction in later life through fetal hyperinsulinemia [33]. Alternatively, a recent mouse model suggests that AMH expression in pregnancy (as seen in PCOS women) can promote both LH-mediated T excess and reduced placental aromatization of maternal androgens [28], thereby contributing to fetal hyperandrogenism in their daughters (Figure 1), although such a mechanism in humans is uncertain.

Post-natal consequences of *in utero* androgen excess are found as early as the newborn for women with PCOS. Infant daughters not only exhibit transient facial sebum [34], a biomarker of prior T exposure, but also demonstrate an elongated anogenital distance [35], a reliable biomarker for early-to-mid gestation androgen excess [36]. Newborn daughters of women with PCOS also exhibit elevated AMH levels indicative of increased numbers of ovarian antral follicles, a PCOS trait. In adulthood, women with PCOS retain an elongated anogenital distance ([37,38], typical of *in utero*, T-exposed, PCOS-like female monkeys [36] and sheep [23]. A diminished or exaggerated 2D:4D finger length ratio is also associated with both *in utero* androgen excess and PCOS, in women [39], their prepubertal daughters [24] and adult, early-to-mid gestation, T-exposed PCOS-like monkeys [36], since similar T- and E_2-regulated genes control differentiation of gonads, hands and feet. Prepubertal daughters of women with PCOS also excrete increased concentrations of dihydrotestosterone (DHT) metabolites in their urine compared to prepubertal girls of women without PCOS [40], indicating increased 5-alpha reductase activity, and perhaps amplified target tissue androgen action, well before the onset of PCOS signs and symptoms at puberty.

Mixed umbilical cord blood androgen levels from human female fetuses at term, however, have yielded inconsistent results in support of late gestation fetal hyperandrogenism in daughters of PCOS women, regardless of whether “gold standard” liquid chromatography-tandem mass spectrometry (LC-MS/MS) assays are employed. Increased T or androstenedione levels are reported in two studies [41,42], equivalent levels in one [43], and diminished levels in a further two [29,44]. Labor onset and duration, together with increasing term gestational age, however, diminish umbilical cord androgen levels and likely often confound understanding of late gestation female androgenic state [45]. Moreover, no

sex differences remain between circulating T levels in male and female human fetuses by late gestation [21], suggesting term birth is inauspicious for explore developmental hyperandrogenism.

Genetic origins: PCOS risk genes are compatible with *in utero* hyperandrogenic pathogenesis for PCOS

Family-based and extensive genome-wide association studies have yielded 17 replicated PCOS risk genes, regulating gonadotropin secretion (*FSHB*), gonadotropin action and ovarian function (*LHCGR, FSHR, DENND1A, RAB5/SUOX, HMGA2, C9orf3, YAP1, TOX3, RAD50, FBN3*), and various metabolic functions (*THADA*, *GATA4/NEIL2*, *ERBB4*, *SUMO1P1, INSR* and *KRR1*) [46–48]. Of the genes regulating gonadotropin and ovarian function, a substantial number have been proposed as enabling ovarian hyperandrogenism [46,49]. A recent alternative approach, employing rare gene variant association testing, followed by targeted resequencing of AMH in a replication cohort, identified an additional 17 PCOS-specific, rare coding and splice-site variants in AMH that diminish AMH signaling [49]. Ovarian hyperandrogenism is a potential outcome of reduced ovarian AMH inhibition of CYP17A1 expression [49]. While progress towards understanding gene variant-based PCOS heritability has clearly advanced, a heritability gap between low incidence of PCOS risk genes (~10%) and the high heritability of PCOS (~70%) [50], indicates a pressing need to identify (1) more PCOS risk genes, as each may confer a small degree of disease risk, (2) rare gene variants, as each may confer unduly large degrees of PCOS risk, and/or (3) epigenetic mechanisms altering a wide range of gene expression that confer considerable risk for PCOS. Current thinking embraces a combination of polygenic, epigenetic and developmental contributions to PCOS pathogenesis that are ameliorated or exaggerated by lifestyle [1,47].

Epigenetic origins: developmental contribution to PCOS

T and its biopotent metabolites are highly effective regulators of DNA methylation during fetal development. They enable the majority of phenotypic sexual differentiation in multiple organ systems and tissues, including brain [51]. Increased or decreased DNA methylation can diminish or enhance, respectively, mRNA transcription of inherited gene variants [52]. Different pattern and amount of DNA methylation at any single gene locus, however, are specific to each organ system or cell type in each individual. Unlike GWAS, therefore, there is less certainty as to how genome-wide methylation studies generalize beyond an organ system or cell type. DNA is differentially methylated in a variety of organ systems in women with PCOS [53,54]. Gene-targeted DNA methylation studies of *LHCGR* have reported its hypomethylation in blood cells and subcutaneous (SC) adipose of women with PCOS, concurrent with increased *LHCGR* mRNA expression [55–57]. If comparable DNA hypomethylation of *LHCGR* occurs in PCOS ovarian theca cells, it would likely increase androgenic responses to LH pulses, causing or amplifying ovarian hyperandrogenism. GWMS and bioinformatic pathway analyses have identified clusters of differentially methylated genes in PCOS women that may alter a variety of cellular functions and processes, including immune response pathways (including autoimmunity), ovarian

steroidogenic and metabolic functions, and cancer-related pathways [56,58,59]. Notably, there are commonalties between identified PCOS risk genes and differentially methylated genes in PCOS women, including *LHCGR*, *RAB5/SUOX, AMH/AMHR2 and INSR*, suggesting convergence of molecular pathogenic mechanisms around the same critical genes. Do similar mechanistic insights emerge from animal models of *in utero* female hyperandrogenism?

Mechanistic PCOS insight from nonhuman primate models of *in utero* female hyperandrogenism

To induce fetal male levels of T in fetal female rhesus monkeys, monkey dams require daily SC injections of 10–15mg T propionate to generate circulating T levels equivalent to nighttime adult male levels of T (~20 ng/ml). Such high maternal levels are needed to exceed the primate liver's ability to produce sex hormone binding globulin (SHBG), rendering >90% of circulating T non-bioavailable, as well as the primate placenta's avid capacity to aromatize, conjugate and metabolize T, thus delivering a small fraction (1–2%) of dam T concentrations to a female monkey fetus [22], as determined by LC-MS/MS. Why start with such a challenging animal model? Until PCOS-like traits are reliably replicated in a nonhuman primate with >90% of its genome shared with humans, and with highly similar neuroendocrine, reproductive, metabolic, developmental, aging and behavioral attributes to humans [60], animal models are always open to criticism.

As illustrated in Table 1, T exposure in monkeys during early-to-mid gestation is more effective at inducing reproductive and metabolic PCOS-like signs and symptoms in female offspring than T exposure during late gestation, reinforcing the concept of a particularly vulnerable, mid-gestation developmental window for *in utero* T reprogramming of females. Mid-gestation excessive maternal weight gain and transient hyperglycemia, accompanied by fetal hyperglycemia, are all T-induced metabolic sequelae contributing additional reprogramming to exposed female fetuses [61]. As late gestation T exposure-induced PCOS-like traits demonstrate, however, a degree of fetal female monkey vulnerability to T reprogramming (and its gestational metabolic sequelae) remains beyond mid-gestation (Table 1). Increased incidence of gestational diabetes, as well as increased or diminished birthweight [54], accompanying PCOS gestations, closely emulating metabolic compromise of hyperandrogenic gestation in monkeys. Metabolically compromised gestation, alone, including obese monkeys and women [62], and women with T2D, is insufficient to cause PCOS. Increased T *in utero*, however, may reprogram female neurocircuitry controlling energy balance and increase vulnerability to *in utero* metabolic compromise [62].

Phenotypic manifestation of early-to-mid gestation T reprogramming of female monkeys begins with mid-gestation increase in fetal head size, followed in late gestation by hypolipidemia and fetal LH hypersecretion [22,61]. LH hypersecretion persists into early infancy, accompanied by modest hyperandrogenism [22], reflecting precocious development of insensitive negative feedback regulation of gonadotropin-releasing hormone (GnRH)/LH in the absence of mature ovarian hormone levels providing homeostatic constraint. While birthweight is normal, accompanying metabolic dysfunctions includes newborn

hypoglycemia, accelerated infant weight gain and relative hyperinsulinemia related to defective pancreatic beta cell compensation for insulin sensitivity and excessive beta-to-alpha cell ratio in infant pancreatic islets [61,63]. Increased fetal growth, neonatal hypoglycemia and subsequent accelerated postnatal growth are typical of human pregnancies encountering excessive maternal weight gain and hyperglycemia [64], and such gestations greatly increase the risk of developing T2D in adulthood.

During adolescence, menarche is delayed by ~6 months in both early-to-mid and late gestation T-exposed female monkeys [65], but such pubertal delay is absent when lower amounts of T are administered to monkey dams [66]. Subsequent onset of menstrual cycles in early-to-mid gestation T-exposed monkeys is accompanied by a prolonged succession of luteal insufficiency [65], demonstrating adolescent origins of ovulatory cycle dysfunction, an attribute of adolescent girls presenting with PCOS [54].

By their reproductive years, early-to-mid gestation T-exposed female monkeys become comprehensive counterparts of women with PCOS. Ovarian and adrenal hyperandrogenism co-occur with intermittent and absent menstrual cycles, as well as large, polyfollicular ovaries [9,22]. Elevated LH levels are omnipresent, driven by increased hypothalamic GnRH pulse frequency and increased pituitary gonadotrope response to GnRH, both likely resulting from diminished sensitivity to E_2- and progesterone-mediated negative feedback regulation [67], all neuroendocrine traits found in women with PCOS [68,69]. Oocyte developmental competence is compromised in these monkeys [70], and may reflect contributions from increased adiposity that accompanies diminished oocyte quality in women with PCOS [71]. Circulating AMH levels in T-exposed monkeys, however, do not exceed those of controls, and exhibit premature, aging-related decline [72]. While absence of AMH excess is not typical of women with PCOS, PCOS women over 30 years of age demonstrate a steeper decline in circulating AMH levels than their non-PCOS counterparts [73]. Early-to-mid gestation T reprogramming of ovarian follicle granulosa cells may therefore be less pronounced than ovarian theca, stroma or oocytes, and a maternal source of fetal hyperandrogenism, alone, may be insufficient to reprogram granulosa cell AMH hypersecretion or increased follicle number and proliferation.

Accompanying metabolic dysfunction is just as pronounced in adult T-exposed monkeys as in PCOS women, despite the monkeys' non-obesogenic diet. Monkey visceral fat accumulation is increased ("metabolic obesity"), likely arising from PCOS-like hyperandrogenic adipogenic constraint, limiting SC adipocyte maturation and safe lipid storage [74]. Such pro-lipotoxic traits may contribute to hyperlipidemia-associated insulin resistance, impaired pancreatic beta cell compensation and compromised islet size [63] enabling increased progression to T2D [12]. Consistent with gestational origins of PCOS-like metabolic dysfunction, increased postnatal weight gain is associated with increased risk of PCOS in women [54], as well as T2D [64]. Interestingly, DNA methylation array analysis of visceral adipose identifies transforming growth factor beta (TGF-beta) signaling as the most significantly altered pathway in adult, T-exposed female monkeys [11], implicating an influential signaling pathway regulating adipocyte catabolism (brown or beige adipose, BAT) and adipocyte accumulation of lipid (white adipose, WAT) that potentially enables positive energy balance [75,76] favoring weight gain.

When T treatment of monkey dams begins peri-pubertally (renewable SC capsules generating circulating total T levels of ~ 1.5 ng/ml), and 50% are additionally switched to a "western-style diet" (T+WSD), age at first pregnancy is later, blood flow in the primary lobe of the placenta is diminished by early gestation, and these pregnant, T-treated females demonstrate glucose intolerance, hyperinsulinemia and insulin resistance, while female fetuses harvested during late gestation exhibit increased abdominal circumference suggestive of fetal adiposity [77]. Fetal female concentrations of T and other reproduction-related hormones, as well as postnatal phenotypes, have yet to be reported for female offspring of these T-treated female monkeys.

In utero androgen excess and female behavioral reprogramming

Sexual dysfunction [7] and depression [78] co-occur with PCOS in women. In this regard, and in addition to reproductive and metabolic dysfunction, early-to-mid gestation T exposure reprograms ("organizes") prepubertal and adult female behavior. T-exposed female monkeys exhibit increased male-typical infant vocalizations, diminished intimate social grooming of mothers and interest in infants, increased mounting of peers, and diminished, but not absent, engagement in female-typical sexual interactions with males [79], all independent of circulating sex hormone levels. Such behavioral reprogramming is difficult to reconcile with traditional female gender roles in human societies, potentially leading to sexual dysfunction and depression. Recent reports from *in utero* androgen excess rodent models clearly demonstrate anxiety-like behavior in female offspring accompanied by upregulation of amygdala gene expression, including corticotropin-releasing hormone (CRH) [80], an identical neural site and neuropeptide system implicated in the pathogenesis of anxious phenotype in monkeys and humans [81], leading to depression.

Mechanistic PCOS insight from non-primate animal models of *in utero* female hyperandrogenism

Non-primate models of *in utero* androgen excess emulate many of the reproductive and metabolic traits found in PCOS women and T-exposed monkeys (Table 1). With their relative in expense and ease of manipulation, these models have generated a plethora of incisive pharmacological and molecular manipulations that provide additional insight into pathogenic mechanisms engaged by *in utero* androgen excess (recently reviewed by [16–18]). Differential gestational timing or duration of T exposure in female sheep (Table 1), illustrate the skew in gestational vulnerability to PCOS-like reprogramming of both reproductive and metabolic traits reported in female monkeys, and suggest that longer durations of T exposure commencing before mid-gestation induce a more pronounced PCOS-like phenotype. By late gestation, following cessation of maternal early-to-late T administration, increased ovarian theca cell expression of CYP17A1 and increased release of androstenedione are already present [82]. Postnatally, however, circulating androgen levels in such T-exposed sheep are not elevated, but ovarian androgen receptor expression is increased suggesting "functional hyperandrogenism" within an androgen target organ [83]. Early-to-late DHT exposure, while recapitulating T reprogramming of reproductive traits and insulin resistance, does not disrupt maturation of regular ovarian cycles or ovarian

morphology (Table 1), suggesting limits to androgen receptor-mediated PCOS-like reprogramming. Maternal co-administration of the androgen receptor antagonist, flutamide, along with T during early-to-late gestation prevents early puberty, and likely LH hypersecretion, as well as onset of ovulatory dysfunction, PCOS-like ovarian morphology and ovarian steroidogenic abnormalities [84]. Gestational flutamide co-treatment, however, fails to prevent metabolic phenotype, including insulin resistance, adipogenic constraint, hyperlipidemia and fatty liver [16], again demonstrating limits to androgen receptor-mediated, PCOS-like reprogramming. In this regard, flutamide treatment of adult female mice previously exposed to fetal T reverses their acyclicity [14], and in some PCOS women, improves fertility, menstrual cyclicity and LH levels [85], as well as normalizing progesterone negative feedback regulation of episodic GnRH/LH release [69].

Gestational co-administration of the peroxisome proliferator-activated receptor gamma (PPARG or NR1C3), a nuclear transcription factor crucial for adipocyte maturation, along with T during early-to-late gestation, prevents insulin resistance and early puberty onset, and likely LH hypersecretion, in T-exposed female lambs, but does not prevent adipogenic dysfunction, hyperlipidemia and fatty liver [16]. In this regard, it is interesting that treatment of late gestation DHT-exposed female offspring as adults with the insulin sensitizer, metformin, restores normal cyclicity, as well as normalizing androgen and LH levels. Taken together, these findings suggest that while reprogramming of a variety of PCOS-like reproductive traits involves androgen receptor and/or insulin-mediated actions, adipogenic and lipogenic traits may involve additional reprogramming, perhaps engaging estrogenic T metabolites.

Mouse models have predominantly used the more biopotent androgenic T metabolite of DHT to induce late gestation *in utero* androgen excess and PCOS-like reprogramming (e.g., [14,17,18]). Late gestation DHT administered to female GnRH-green fluorescent protein (GFP)-transgenic mouse dams (derived from CBB6/F1 strain) produces PCOS-like female offspring exhibiting hyperandrogenism, intermittent/absent cycles, aberrant ovarian follicle morphology, LH hypersecretion derived from accelerated episodic GnRH release, fatty liver and enlarged adipocytes, without accompanying increased adiposity and insulin resistance (Table 1). Elegant use of genetic manipulation to globally delete androgen receptor (ARKO) protects fetal female mice (>98% C57BL/6J strain) from fetal DHT-induced, PCOS-like reprogramming, including absence of intermittent/absent cycles, aberrant follicle morphology and enlarged adipocytes [86]. The wild type mice from which ARKO females are derived, nevertheless, do not exhibit fetal DHT-induced hyperandrogenism or LH hypersecretion, possibly reflecting GnRH-GFP and ARKO mouse strain differences in fetal female susceptibility to DHT fetal reprogramming. Neural androgen receptor expression may be particularly crucial for DHT-mediated, PCOS-like reprogramming since selective deletion of neuronal androgen receptor expression, NeuroARKO, provides the best protection against peri-pubertal onset, DHT induction of PCOS-like traits [87]. NeuroARKO mice, however, have not yet been challenged with late gestation DHT to ascertain whether absence of neuronal androgen receptor abrogates *in utero* PCOS-like reprogramming. With less resemblance to *in utero* androgen excess in sheep, androgen receptors in *in utero* DHT-exposed female mice mediate the majority of PCOS-like reprogramming.

GnRH-GFP transgenic mice have also enabled neuro-immunohistochemical delineation of hypothalamic changes that may underlie PCOS-like reprogramming of GnRH release and its negative feedback regulation. Late gestation DHT increases anatomical and functional gamma-aminobutyric acid (GABA) neuronal connectivity to GnRH neurons, generating increased episodic release of GnRH, as well as LH hypersecretion, related to diminished progesterone negative feedback regulation [14,88]. Such enhanced GABA excitatory connectivity, originating from the hypothalamic arcuate nucleus, is established well before puberty, when circulating androgen levels are low [88]. Since GABA neurons express progesterone, estrogen and androgen receptors, and GnRH neurons do not express the former, aberrant GABA excitatory connectivity may mediate diminished progesterone (and E_2) negative feedback regulation demonstrated by *in utero* androgen excess female mice, and potentially rats, sheep and monkeys.

Unexpectedly, long-term administration of flutamide to adult, DHT-treated female mice normalizes neuronal connectivity between GABA and GnRH neurons, as well as episodic release of GnRH, circulating LH levels, negative feedback regulation of GnRH/LH, ovarian cyclicity and follicle morphology [88], suggesting extant, extra-ovarian androgen excess is crucial to maintain neuronal reprogramming and its PCOS-like sequelae. Figure 2 illustrates hypothetical sites, implicated by animal models, for specific, androgen receptor-mediated, *in utero* programming of PCOS-like traits. The implication, emphasized by sheep and transgenic mouse studies, is that abrogation of androgen production and action in specific organ systems may effectively normalize PCOS traits.

Naturally occurring *in utero* androgen excess and female hyperandrogenism: Origins of PCOS beyond humans?

Naturally occurring high levels of T confer athletic advantage in women [89] and accompany female behavioral dominance in some, but not all, nonhuman primates [90,91]. About 15–25% of women in their reproductive years exhibit androgen excess, but most have PCOS [92]. Naturally occurring, hyperandrogenic (high T) female macaque monkeys emulate PCOS women in the co-occurrence of PCOS-like traits [93,94]. In a multi-Primate Research Center study, adult female macaques with average BMI and in prime reproductive years, were identified with high levels of T, 1SD above each population mean, in three separate laboratory populations of macaques. In the Wisconsin rhesus monkey population [94], PCOS-like traits of high T females exhibited generalized hyperandrogenism and increased steroidogenesis, including elevated circulating levels of unbound, “free” testosterone (unpublished results), hypersecretion of both LH and AMH, as well as uterine endometrial hyperplasia and infertility (Table 1). Intriguingly, a predominance of infertility and insulin resistance are found among high T monkeys with the most extreme elevations of T, 2SD above the monkey population mean (unpublished results), and equivalent to hyperandrogenic criteria required for peer-reviewed clinical studies of PCOS [95].

Thus, within limits, high T may not impair either fertility or metabolic function. Subtle changes in anatomical biomarkers of prior T exposure suggest fetal origins for naturally hyperandrogenic female rhesus monkeys [94], analogous to findings in behaviorally

dominant female Malagasy lemurs [91], a more ancient branch of nonhuman primates. Interestingly, mild-to-moderate PCOS-like phenotypes formed the majority of those identified among naturally hyperandrogenic female monkeys, emulating PCOS phenotype prevalence in studies of women recruited from local populations, and not from clinical referrals (Table 2). The contrast of predominantly mild-to-moderate phenotypes in naturally occurring PCOS-like monkeys and PCOS women recruited from local human populations to the predominantly severe and classic phenotypes of early-to-mid gestation T-exposed monkeys and clinically referred PCOS women suggests commonality in PCOS phenotype may include duration or degree of gestational T exposure, and age and BMI (younger age and normal BMI with more mild-to-moderate phenotype). Naturally occurring PCOS-like phenotypes beyond humans certainly supports increasing speculation of survival and reproductive advantages from hyperandrogenic, energy-conserving, insulin resistant, delayed fecundity, female phenotypes [1,96].

In utero Androgen Excess and Androgen Receptor: Developmental Commonality and Molecular Gateway to PCOS?

Mounting evidence from human and animal studies repeatedly implicates appropriately timed *in utero* androgen excess, from either maternal and/or fetal sources, as high risk for PCOS emerging at adolescence. Figure 1 provides a diagrammatic representation of maternal and fetal sources of gestational androgen excess, together with relevant PCOS risk genes, programming for ovarian androgen excess. Figure 2 illustrates hypothetical sites for female reprogramming mediated by androgen receptor, as identified by genetically manipulated mouse studies [17,18]. Such a unified hypothetical model is compatible with postnatal androgen activating reprogrammed function, such that anti-androgens or androgen-diminishing consequences of weight loss interventions, including lifestyle, diet, bariatric surgery, and insulin sensitizing treatments, ameliorate PCOS traits in adulthood. Increasing sophistication of bioinformatics to assess risk for functional outcome of genomic and epigenomic variants vulnerable to *in utero* androgen excess, hold promise for identification of PCOS risk in newborn, enabling early intervention.

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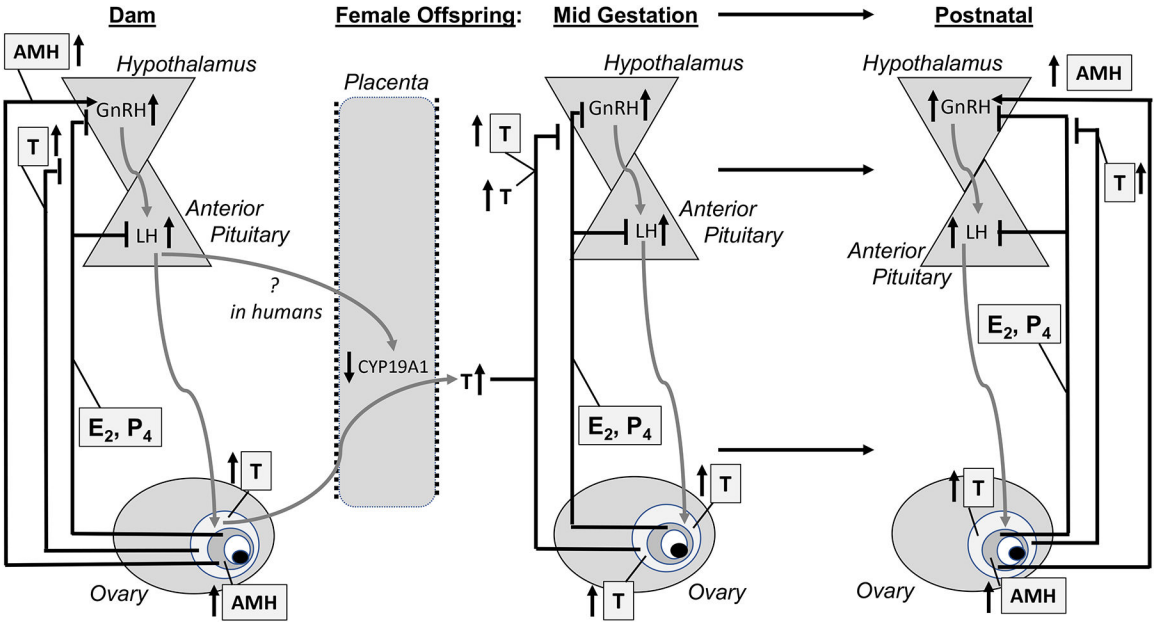

Figure 1.

Hypothetical maternal and fetal contributions to *in utero* female androgen excess reprogramming for a hyperandrogenic female offspring.

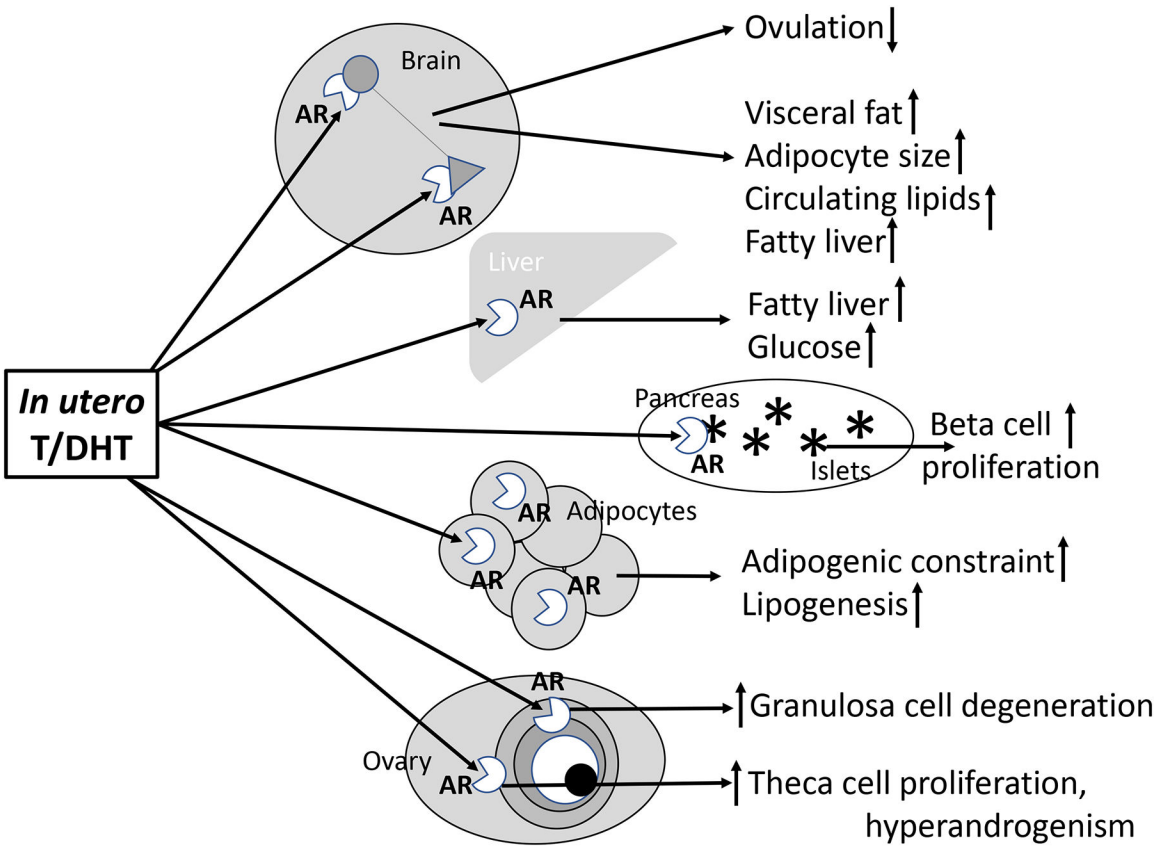

Figure 2.

Hypothetical target sites for androgen receptor mediated *in utero* androgen excess female fetal reprogramming for reproductive and metabolic PCOS-like traits.

Table 1.

PCOS-like reproductive and metabolic traits exhibited by *in utero* androgen excess animal models in adulthood, and by naturally occurring hyperandrogenic adult female monkeys.

Species	Gestation staae	Route, treatment	Hyper-androaenism	Intermittent, absent cycles	Polycystic ovaries	Diminished fertility	Increased LH	Increased adiposity	Insulin resistance	Pancreatic defect
Monkey	Early-to-mid	M, TP	+	+	+	+	+	+	+	+
	Late	M, TP	+	+	?	−	−	+	−	−
	Late	M, letrozole	?	+	?	?	−	−	?	?
	Naturally occurring	None	+	−	+	+	+	?	+	?
Sheep	Early-to-late	M, T	+/−	+	+	+	+	+	+	?
	Early-to-late	M, DHT	−	−	+	?	+	?	+	?
	Mid	M, T	−	+	?	+	−	?	?	?
	Mid-to-late	M, T	−	+	−	+	+	−	+	?
	Mid-to-late	F, T	−	?	?	?	?	−	+	?
Rat	Late	M, T	+	+	−	+	+	+	−	?
	Late	M, DHT	+	+	Cystic	+	+	+	?	?
Mouse	Late	M, T	?	−	−	?	?	?	?	?
	Late	M, DHT	+	+	−	+	+	−	−	+
	Late	M, AMH	+	+	Cystic	+	+	−	−	−
	All	ARKO	−	+	−	+	−	?	?	?
	All	NeuroArko	?	+	+	−	+	?	?	?
	All	AromKO	+	+	Cystic	+	+	+	−	?
	All	ERaKO	+	+	Cystic	+	+	?	?	?
	All	LHR excess	+	+	Cystic	+	+	?	?	?

M, administered to dam; F, administered to fetus; P, propionate; DHT, dihydrotestosterone; letrozole, aromatase inhibitor; ARKO, androgen receptor knockout; AromKO, aromatase knockout; ERaKO, estrogen receptor alpha knockout; LHR, LH receptor; Cystic, large follicle cysts, not more numerous, non-dominant antral follicles; ?, unknown.

Table 2.

Classification of PCOS-Like Phenotypes in PCOS-like Adult Female Rhesus Monkeys and in women with PCOS

	PCOS-Like/PCOS Phenotype[1] (% of PCOS-like and PCOS individuals)			
Female Population	**Type A**	**Type B**	**Type C**	**Type D**
Early-to-mid gestation T-exposed female monkeys[2]	38	25	12	25
PCOS women[3] (from clinical referrals)	49	13	14	17
Naturally occurring PCOS-like female monkeys[4]	25	8	42	25
PCOS women[5] (from unselected human populations)	25	19	35	20

[1]Type A: hyperandrogenism or hirsutism (HA) + intermittent/absent cycles (OD) + (polycystic ovary morphology) (PCOM); Type B: HA + OD; Type C: HA + PCOM; Type D: OD + PCOM, as described in Abbott, 2017

[2]Derived from Abbott et al., 2013

[3]Derived from Norman et al., 2007 *Lancet* 370:685; Wild et al., 2010 *J Clin Endocrinol Metab* 95:2038; Dumesic et al., 2015 *Endocr Rev*. 36:487

[4,5]Derived from Abbott, 2017