Androgen excess fetal programming of female reproduction: a developmental aetiology for polycystic ovary syndrome?

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The aetiology of polycystic ovary syndrome (PCOS) remains unknown. This familial syndrome is prevalent among reproductive-aged women and its inheritance indicates a dominant regulatory gene with incomplete penetrance. However, promising candidate genes have proven unreliable as markers for the PCOS phenotype. This lack of genetic linkage may represent both extreme heterogeneity of PCOS and difficulty in establishing a universally accepted PCOS diagnosis. Nevertheless, hyperandrogenism is one of the most consistently expressed PCOS traits. Animal models that mimic fetal androgen excess may thus provide unique insight into the origins of the PCOS syndrome. Many female mammals exposed to androgen excess in utero or during early post-natal life typically show masculinized and defeminized behaviour, ovulatory dysfunction and virilized genitalia, although behavioural and ovulatory dysfunction can coexist without virilized genitalia based upon the timing of androgen excess. One animal model shows particular relevance to PCOS: the prenatally androgenized female rhesus monkey. Females exposed to androgen excess early in gestation exhibit hyperandrogenism, oligomenorrhoea and enlarged, polyfollicular ovaries, in addition to LH hypersecretion, impaired embryo development, insulin resistance accompanying abdominal obesity, impaired insulin response to glucose and hyperlipidaemia. Female monkeys exposed to androgen excess late in gestation mimic these programmed changes, except for LH and insulin secretion defects. In utero androgen excess may thus variably perturb multiple organ system programming and thereby provide a single, fetal origin for a heterogeneous adult syndrome.

Key words: anovulation/hyperandrogenism/infertility/polycystic ovary syndrome/rhesus monkey

Introduction

Polycystic ovary syndrome (PCOS) is enigmatic. Its origins and aetiology are unknown, its signs and symptoms are expressed as bewildering combinations in women from all ethnic groups and racial backgrounds (Legro, 2003; Dumesic et al., 2005); yet its phenotype is readily transmitted between generations as if regulated by a dominant gene with incomplete penetrance (Legro et al., 1998b). In this regard, it is not surprising that a rigid clinical diagnosis of PCOS remains elusive (Escobar-Morreale et al., 2005). A revised ‘consensus’ diagnosis of PCOS was agreed upon in the Netherlands during the summer of 2003 (Table I; Rotterdam ESHRE/ASRM-sponsored PCOS Consensus Workshop Group, 2004), but controversy regarding the revised versus the 1990 National Institutes of Health Consensus diagnosis of PCOS (Zawadzki and Dunaif, 1992) has already surfaced (Geisthovel, 2003).

Without agreement on a clinical definition of PCOS, the development of an animal model for the syndrome is highly problematic. Such a model has to not only reflect the broad spectrum of reproductive, metabolic and general health disorders of PCOS women, while conforming to the revised ‘consensus’ diagnosis, but must also produce individuals who exhibit differing PCOS phenotypes, as exemplified by the clinical heterogeneity in PCOS women (Legro, 2003; Adams et al., 2004; Escobar-Morreale et al., 2005; Dumesic et al., 2005). This review focuses on the relevance of animal models to PCOS, based on experimentally-induced fetal or perinatal androgen...
Table I. Diagnostic criteria for PCOS and common signs and symptoms of the syndrome

Current consensus diagnostic criteria
Two out of three of the following:
(1) Hyperandrogenism, as determined by elevated circulating levels of total or unbound testosterone or clinical signs of hirsutism
(2) Intermittent or absent menstrual cycles
(3) Polycystic ovaries (as visualized by ultrasound)
The following conditions must also be excluded: classical and non-classical congenital adrenal hyperplasia, Cushing’s syndrome and androgen secreting tumours

These criteria are extended from the previous 1990 NIH Consensus diagnosis which specified the first two criteria, alone, as a basis for PCOS diagnosis, following exclusion of conditions that mimic PCOS (listed above). Additional signs and symptoms associated with PCOS

Reproductive and endocrine
- LH hypersecretion
- Adrenal hyperandrogenism
- Ovarian endocrine hyper-responsiveness to gonadotropin stimulation for IVF
- High rates of miscarriage
- Endometrial hyperplasia and cancer
- Gestational diabetes

Metabolic
- Insulin resistance and compensatory hyperinsulinaemia
- Impaired glucose tolerance
- Type 2 diabetes
- Obesity (including abdominal adiposity)
- Pancreatic impairments in insulin responses to glucose
- Hyperlipidaemia

General health disorders
- Cardiovascular disease
- Sleep apnoea
- Acne
- Chronic inflammation

Reference sources: Zawadzki and Dunaif, 1992; Dumesic, 1995; Franks, 1995; Carmina and Lobo, 2001; Chang, 2002; Escobar-Morreale et al., 2005; Hart et al., 2004.

excess, and the proclivity of prenatally androgenized female rhesus monkeys to exhibit many of the PCOS signs that vary in their expression depending on the time of gestational exposure.

PCOS
Six to seven per cent of women in their reproductive years manifest PCOS (Diamanti-Kandarakis et al., 1999; Asuncion et al., 2000; Azziz et al., 2004). In addition, the syndrome represents the majority of young women diagnosed with type 2 diabetes (Arslanian et al., 2001; Peppard et al., 2001). PCOS combines reproductive, metabolic, cardiovascular, oncological, inflammatory and sleep abnormalities into a heterogeneous disorder that has pervasive and devastating consequences for woman’s health (Ehrmann et al., 1995; Franks, 1995; Dunaif, 1997; Fogel et al., 2001; Escobar-Morreale et al., 2004). The most recent PCOS consensus diagnosis (Table I; Rotterdam ESHRE/ASRM-sponsored PCOS Consensus Workshop Group, 2004) remains one of exclusion, and can only be reached when phenotypically similar, but mechanistically different disorders, such as classical and non-classical 21-hydroxylase deficiency, Cushing’s syndrome, and androgen secreting tumours, have been excluded (Zawadzki and Dunaif, 1992).

While the current diagnosis seems straightforward, it belies the complexity and heterogeneity of traits that can be displayed by PCOS women. Individuals commonly exhibit abnormalities beyond those encompassed by the diagnosis, as illustrated in Table I. Further complicating matters, individual PCOS women can manifest any (or none) of these additional common PCOS traits in varying combinations and degrees of severity (Ehrmann et al., 1995; Franks, 1995; Dewailly et al., 1997; Dunaif, 1997; Adams et al., 2004). Given such complex pathophysiology, together with a peripubertal onset of symptoms (Arslanian and Wittchell, 2002) that can spontaneously abate during middle age (Taylor et al., 1997), PCOS probably has a heterogeneous aetiology, arising from a variety of genetic and environmental determinants modified by sexual maturation and ageing. As such, it is unlikely that the heterogeneous PCOS phenotype has a single common origin.

Nevertheless, PCOS is strongly familial and its pattern of inheritance suggests a single, autosomal dominant gene with incomplete penetrance (Legro et al., 1998b). The lack of reliable association between genotype and phenotype raises the possibility that inheritance is modified by environmental factors (Legro and Strauss, 2003), including those that occur in pregnancy (Abbott et al., 2002a). Ovarian hyperandrogenism, a key diagnostic trait, has all the hallmarks of a heritable PCOS trait (Legro et al., 1998a). Not surprisingly, enhanced functional activity of cytochrome P450 steroidogenic enzymes, 3beta-hydroxysteroid dehydrogenase, and specific kinase signalling pathways crucial for ovarian theca cell androgen biosynthesis have been identified as components of the molecular phenotype expressed in the syndrome (Wood et al., 2003, 2004; Nelson-Degreve et al., 2005; Escobar-Morreale et al., 2005). However, a genetically-defined basis for PCOS remains elusive (Legro, 1998; Escobar-Morreale et al., 2004a). Almost all initial candidate genes, including those regulating ovarian folliculogenesis and theca cell steroidogenesis, have failed to maintain linkage to a PCOS phenotype (Urbaneke et al., 2000; Gaasenbeek et al., 2004), although dysregulation of the androgen receptor gene may provide a novel mechanism to exaggerate the cellular responses of PCOS women to androgens (Hickey et al., 2002).

A heritable basis for the PCOS syndrome also may reside within genes regulating insulin action and metabolic defects. Insulin resistance, compensatory hyperinsulinaemia and accompanying abdominal obesity are frequent traits found in women with PCOS (Dunaif et al., 1985, 1987; Ehrmann et al., 1995; Dunaif, 1997). Treating PCOS women with insulin sensitizing agents [troglitazone (Dunaif et al., 1996), metformin (Nestler et al., 1998), rosiglitazone (Baillargeon et al., 2004) and pioglitazone (Bretenthaler et al., 2004)] or placing them on a calorie-restriction diet, with or without additional exercise (Kiddy et al., 1992; Huber-Buchholz et al., 1999; Stamets et al., 2004), effectivley ameliorates many of the metabolic abnormalities, reduces androgen levels and often initiates ovulatory menstrual cycles. Certainly, genomic variants related to insulin resistance, type 2 diabetes and obesity have recently been associated with PCOS (San Millan et al., 2004), with the only gene locus reliably linked with PCOS (allele 8 of D19S884) being centromeric to the insulin receptor gene located on chromosome 19 (Urbaneke et al., 1999; Tucci et al., 2001; Villuendres et al., 2003). Studies speculate that this PCOS-linked gene locus may affect signal
transduction mechanisms, causing altered expression of genes that (1) enhance theca cell steroidogenic activity, (2) regulate cell metabolic phenotype, including skeletal muscle, fat and ovarian granulosa cells and (3) stabilize mRNA (Strauss, 2003; Wood et al., 2003; Rice et al., 2005).

Thus, while familial clustering suggests a heritable aetiology for PCOS, genetic studies have yet to reliably substantiate this contention (Legro et al., 1998b). Alternatively, emerging data from animal research have associated fetal androgen excess as a potential origin for PCOS, as evidenced by the ability of discrete, experimentally-induced prenatal androgen excess of fetal rhesus monkeys, ewes and rats to programme PCOS-like phenotypes (Abbott et al., 1998, 2002a,b; Sharma et al., 2002; Birch et al., 2003; Foecking et al., 2005). By demonstrating that fetal androgen excess re-programmes multiple organ systems, PCOS animal models agree with the increased prevalence of PCOS in women with fetal androgen excess disorders, including classical congenital adrenal hyperplasia from 21-hydroxylase deficiency and congenital adrenal virilizing tumours (Barnes et al., 1994; Phocas et al., 1995; Merke and Cutler, 2001; Stikkelbroeck et al., 2003). Such commonality in phenotypes provides evidence that the intrauterine environment may alter differentiation in the female fetus, permanently re-programming its physiology and modifying its genetic susceptibility to pathology after birth.

Prenatal and perinatal exposure to androgen excess: understanding studies of genital virilization and behavioural masculinization

In mammals, including humans, fetal programming of female reproduction by androgen excess is conventionally perceived as masculinization and defeminization of the female phenotype (Wallen and Baum, 2002). Virilized or ambiguous genitalia are commonly accepted signs of female masculinization (Jost, 1970). Mediated through the action of the androgen receptor (Quigley et al., 1995), fetal androgen excess masculinizes urogenital tract development in female placental mammals by inducing the formation of both male external and internal genitalia (external: penis, scrotum; internal: Wolffian duct derivatives), while defeminizing female genital development, including suppression of mid- to lower vagina and the vaginal opening [e.g. prenatally androgenized female rhesus monkeys (Wells and van Wagenen, 1954; Goy et al., 1988b) and rats (Greene et al., 1976a,b)]. However, testes do not form since testes-determining genes, not androgens, are required for differentiation of the male gonads (Sinclair et al., 1939). Further, without testicular secretion of anti-Müllerian hormone (Josso et al., 1993; Lasala et al., 2003; Foecking et al., 2005). By demonstrating that fetal androgen excess re-programmes multiple organ systems, PCOS animal models agree with the increased prevalence of PCOS in women with fetal androgen excess disorders, including classical congenital adrenal hyperplasia from 21-hydroxylase deficiency and congenital adrenal virilizing tumours (Barnes et al., 1994; Phocas et al., 1995; Merke and Cutler, 2001; Stikkelbroeck et al., 2003). Such commonality in phenotypes provides evidence that the intrauterine environment may alter differentiation in the female fetus, permanently re-programming its physiology and modifying its genetic susceptibility to pathology after birth.

Androgen excess and polycystic ovary syndrome

Table II. Commonality of dysfunctional reproductive and metabolic traits found in PCOS women and early- and late-treated prenatally androgenized female monkeys

<table>
<thead>
<tr>
<th>Sign or symptom</th>
<th>PCOS women</th>
<th>Prenatally androgenized female monkeys</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reproductive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovarian hyperandrogenism</td>
<td>+</td>
<td>Early-treated: +, +b</td>
</tr>
<tr>
<td>Anovulation</td>
<td>+</td>
<td>Late-treated: +b, +b</td>
</tr>
<tr>
<td>Enlarged polyfollicular ovaries</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>LH hypersecretion</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Reduced steroid hormone</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>negative feedback on LH</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Impaired ovarian endocrine response to stimulation for IVF</td>
<td>- (increased response)</td>
<td>Early-treated: +d, +df</td>
</tr>
<tr>
<td>Impaired embryo development after IVF</td>
<td>? (increased miscarriages)</td>
<td>Late-treated: +d, +d</td>
</tr>
<tr>
<td>Metabolic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin resistance</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Impaired insulin secretion</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Impaired glucose tolerance</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Increased type 2 diabetes</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Increased abdominal fat</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Hyperlipidaemia</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

effects of aromatization are particularly evident in females of altricial species exposed to androgen perinatally (Wallen and Baum, 2002). Primates provide the only clear exception to this 'neural aromatization rule,' since fetal exposure to the non-aromatizable androgen, 5α-dihydrotestosterone (5α-DHT), is as effective as testosterone (an aromatizable androgen) in feminizing female sexual behaviour in adulthood (Pomerantz et al., 1985; Thornton and Goy, 1986).

Behavioural masculinization is less clearly dependent on the conversion of aromatizable androgens to estrogens (Auger et al., 2002; Wallen and Baum, 2002). Simultaneous treatment of newborn female rats with testosterone propionate (TP) and CBP anti-sense oligodeoxynucleotides, a treatment that blocks estrogen receptor (ER) mediated action, fails to prevent behavioural masculinization (Auger et al., 2002). As another example of androgen-induced behavioural masculinization, fetal female rhesus monkeys exposed to 5α-DHT exhibit masculinized copulatory behaviour in adulthood (Pomerantz et al., 1986). In humans, female prenatal androgen excess via classical congenital adrenal hyperplasia enhances male-typical play and increases the incidence of bisexuality in adulthood (Dittmann et al., 1992; Hall et al., 2004; Hines et al., 2004). These behavioural effects in humans are likely androgen-mediated, since men with aromatase deficiency (Morishima et al., 1995) and functional mutations of the ER gene (Smith et al., 1994) or the aromatase cytochrome P450 gene (Carani et al., 1997) are sexually functioning, typically heterosexual men. Conversely, absent or non-functional androgen receptors in genetic men cause a female phenotype, even with a Y chromosome and undescended testes (De Bellis et al., 2000, and these men, raised as females, are sexually functioning, typically heterosexual women (Money et al., 1984; Wisniewski et al., 2000). Thus, direct fetal or perinatal androgenic re-programming of both masculinized and defeminized behaviour is clearly evident only in primates.

However, the evidence for complete absence of estrogen-related programming of behaviour in primates is not straightforward. Fetal female rhesus monkeys exposed to diethylstilboestrol (DES), an ER agonist, exhibit masculinized juvenile behaviour (Goy and Deputte, 1996). Similarly, DES-exposed women have an increased propensity for bisexuality and feminization (Ehrhardt et al., 1985; Meyer-Bahlburg and Ehrhardt, 1986). These results, together with the findings above, suggest that both androgen- and estrogen-mediated actions affect fetal programming of sexual behaviour in primates. However, the actions of DES may extend beyond ER-mediated effects since neonatal DES exposure in rodents down-regulates androgen receptor expression in the internal genitalia (Rivas et al., 2002) and enhances sensitization of tissue to estrogen action (Rivas et al., 2003). Therefore, DES treatment is not synonymous with estrogen excess and may provide a distorted representation of estrogen action during early development when administered to females during fetal or perinatal life.

Interestingly, clinical studies of PCOS women provide little evidence for masculinization and defeminization of behaviour. Women with PCOS only show a tendency to exhibit several behaviours in common with women exposed to prenatal androgen excess (i.e. classical adrenal hyperplasia), including increased prevalence of 'tomboy' play, reduced interest in dress and appearance, increased energy expenditure level and preference towards career over family life (Gorzynski and Katz, 1977). However, the two control groups of non-PCOS women used were older than the PCOS group and were specifically selected to minimize expected differences, i.e. the controls were women with strong career or athletic interests. Nevertheless, women with PCOS exceeded controls in the initiation and domination of sexual interactions (Gorzynski and Katz, 1977).

In addition, PCOS is common in lesbian women (Agrawal et al., 2004) and female-to-male transsexuals (before androgen treatment; Bosinski et al., 1997), although it is unclear whether PCOS actually increases the prevalence of feminization or transsexualism. A second study investigating psychosexual aspects in PCOS women found no evidence of masculinization before and after surgical ovarian wedge re-section for ovulatory dysfunction (Raboch et al., 1985), although it did show impaired female sexual response in PCOS women with the highest circulating testosterone levels.

Clear findings of behavioural masculinization or defeminization may be difficult to quantify in women with PCOS or established fetal androgen excess exposure (i.e. classical congenital adrenal hyperplasia) since normal ageing through and beyond reproductive life occurs simultaneously with changing masculinity and femininity (Long et al., 2004). In other words, behavioural traits reflecting fetal androgen excess diminish over a woman's lifespan. In PCOS women, behavioural assessment of previous fetal androgen exposure is further complicated by increased psychosocial stress (Lobo et al., 1983), depression (Elsenbruch et al., 2003; Weiner et al., 2004) and perceived social stigma (Kitzinger and Willmott, 2002), in addition to decreased self-esteem, social activity, romantic attachment and sexual satisfaction (Coffey and Mason, 2003; Elsenbruch et al., 2003). Even in prenatally androgenized female rhesus monkeys, changes in rearing conditions and social context diminish masculinized and defeminized behaviours (Wallen, 1996).

**Prenatal and perinatal androgen excess induces ovulatory dysfunction**

Anovulation or irregular menstrual cycles are hallmarks of PCOS and remain a central part of the 'consensus' diagnoses (Zawadzki and Dunai, 1992; Rotterdam ESHRE/ASRM-sponsored PCOS Consensus Workshop Group, 2004). Such ovulatory dysfunction also frequently represents fetal or perinatal exposure of females to androgen excess in many mammalian species, as exemplified by the studies illustrated in Tables III and IV. In altricial mammals, identified by their relatively delayed sexual differentiation and neural development at birth (Wallen and Baum, 2002), ovulatory dysfunction is most marked when androgen excess occurs shortly after parturition, e.g. mice, rats, hamsters and dogs (Table IV). However, androgen excess does induce ovulatory dysfunction across the perinatal period: in rats and mice, this period extends from approximately embryonic day 18 until post-natal days 6–10 (Tables II and III; Baum, 1979; McCarthy, 1994; Cooke et al., 1998), approximating developmental ages in fetal males when testicular testosterone levels are elevated (Negri-Cesi et al., 2004). Prenatal treatments in rats can be without effect unless mothers are dosed excessively (i.e. 10–25 mg/day TP; Table III), or androgens are administered on the fetal side of the placenta to the amniotic fluid or
<table>
<thead>
<tr>
<th>Species</th>
<th>Steroid (given to mother)</th>
<th>Gestational days (TP dose and route of administration)</th>
<th>Puberty (^a) (additional treatment)</th>
<th>Ovarian/estrus cycle</th>
<th>LH surge</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse (altricial)</td>
<td>Testosterone</td>
<td>13–18 (0.75 mg T s.c.)</td>
<td>Cyclic</td>
<td>No</td>
<td></td>
<td>Keisler et al., 1991</td>
</tr>
<tr>
<td></td>
<td>5α-DHT</td>
<td>16–18</td>
<td>Tends to be delayed</td>
<td>Acyclic</td>
<td>No</td>
<td>Sullivan and Moenter, 2004; Moenter SM, personal communication</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16–18</td>
<td>Flutamide as adult</td>
<td>Cyclic</td>
<td>Yes</td>
<td>Sullivan and Moenter, 2004</td>
</tr>
<tr>
<td>Rat (altricial)</td>
<td>Testosterone</td>
<td>Starting at 13–15 for 1–8 days</td>
<td>Cyclic</td>
<td>–</td>
<td></td>
<td>Greene et al., 1939</td>
</tr>
<tr>
<td></td>
<td></td>
<td>19, 20, 21, or 22 (2.5 mg TP s.c.)</td>
<td>Cyclic</td>
<td>–</td>
<td></td>
<td>Swanson and van der Werff ten Bosch, 1964b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>19, 20, 21, or 22 (10–25 mg TP s.c.)</td>
<td>Delayed</td>
<td>Acyclic</td>
<td></td>
<td>Swanson and van der Werff ten Bosch, 1964b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>19, 20, 21, or 22 (20 μg TP amniotic fluid)</td>
<td>Cyclic</td>
<td>–</td>
<td></td>
<td>Swanson and Werff ten Bosch, 1965</td>
</tr>
<tr>
<td></td>
<td></td>
<td>19, 20, 21 or 22 [20 μg TP fetus]</td>
<td>Acyclic</td>
<td>–</td>
<td></td>
<td>Fels and Bosch, 1971</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16–20</td>
<td>Advanced</td>
<td>Cyclic</td>
<td>–</td>
<td>Slob et al., 1983</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16–20</td>
<td>Cyclic</td>
<td>–</td>
<td></td>
<td>Whalen and Lutte, 1971</td>
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<td></td>
<td>5α-DHT</td>
<td>16–20</td>
<td>Cyclic</td>
<td>–</td>
<td></td>
<td>Brown-Grant and Sherwood, 1971</td>
</tr>
<tr>
<td>Guinea pig (precocial)</td>
<td>Testosterone</td>
<td>33–37</td>
<td>Acyclic</td>
<td>–</td>
<td></td>
<td>Ford and Christenson, 1971</td>
</tr>
<tr>
<td>Pig (altricial/precocial)</td>
<td>Testosterone</td>
<td>29–35</td>
<td>Cyclic</td>
<td>–</td>
<td></td>
<td>Ford and Christenson, 1971</td>
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<tr>
<td></td>
<td></td>
<td>39–45</td>
<td>Cyclic</td>
<td>–</td>
<td></td>
<td>Ford and Christenson, 1971</td>
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<tr>
<td>Cow (precocial)</td>
<td>Testosterone</td>
<td>80–110</td>
<td>Cyclic</td>
<td>–</td>
<td></td>
<td>DeHaan et al., 1988</td>
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<tr>
<td>Dog (altricial)</td>
<td>Testosterone</td>
<td>24–43</td>
<td>Cyclic</td>
<td>–</td>
<td></td>
<td>Beach et al., 1983</td>
</tr>
<tr>
<td>Sheep—ovary intact (precocial)</td>
<td>Testosterone</td>
<td>20–term</td>
<td>Acyclic</td>
<td>–</td>
<td></td>
<td>Short, 1974</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30–90</td>
<td>Normal</td>
<td>Cyclic—1st year</td>
<td>Abnormal</td>
<td>Sharma et al., 2002</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Acyclic—2nd year</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>30–80</td>
<td></td>
<td>Oligo/acyclic</td>
<td>Impaired</td>
<td>Clarke et al., 1976a,b, 1977; Clarke, 1977</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60-term</td>
<td></td>
<td>Acyclic</td>
<td>No</td>
<td>Short, 1974</td>
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<tr>
<td></td>
<td></td>
<td>60–90</td>
<td></td>
<td>Normal</td>
<td>Abnormal</td>
<td>Sharma et al., 2002</td>
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<tr>
<td></td>
<td></td>
<td>70–120</td>
<td></td>
<td>Oligo/acyclic</td>
<td>–</td>
<td>Clarke et al., 1976a,b, 1977; Clarke, 1977</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50–100</td>
<td></td>
<td>Oligo/acyclic</td>
<td>Impaired</td>
<td>Clarke et al., 1976a,b, 1977; Clarke, 1977</td>
</tr>
<tr>
<td></td>
<td></td>
<td>90–140</td>
<td></td>
<td>Cyclic</td>
<td>–</td>
<td>Clarke et al., 1976a,b, 1977; Clarke, 1977</td>
</tr>
<tr>
<td>Sheep—ovx + E(_2) at birth (precocial)</td>
<td>Testosterone</td>
<td>30–90</td>
<td>Advanced</td>
<td>–</td>
<td>No</td>
<td>Wood et al., 1995</td>
</tr>
<tr>
<td></td>
<td>DHT</td>
<td>30–90</td>
<td>Advanced</td>
<td>–</td>
<td>Yes</td>
<td>Masek et al., 1999</td>
</tr>
<tr>
<td>Rhesus monkey</td>
<td>Testosterone</td>
<td>40–55/65/75 (early-treated)</td>
<td>Delayed</td>
<td>Cyclic/acyclic</td>
<td>Yes</td>
<td>Dumesic et al., 1997; Goy et al., 1988a,b; Abbott et al., 1998; Thornton, 1983</td>
</tr>
<tr>
<td></td>
<td>DHT</td>
<td>100/115–124/139 (late-treated)</td>
<td>Normal</td>
<td>Cyclic/acyclic</td>
<td>Yes</td>
<td>Goy et al., 1988a,b; Abbott et al., 1998; Dumesic et al., 1997</td>
</tr>
<tr>
<td>Rhesus monkey—ovx + E(_2) post-natal</td>
<td>Testosterone</td>
<td>40–69/111 (early-treated)</td>
<td></td>
<td>Cyclic(^b)</td>
<td>–</td>
<td>Goy et al., 1988a,b</td>
</tr>
</tbody>
</table>

\(TP = \text{testosterone propionate; DHT = dihydrotestosterone; E}_2 = \text{estradiol; OVX = ovariectomized.}

\(^a\) Determined from a variety of measures, including first vaginal opening (rodents), menarche (menstruating primates).

\(^b\) Insufficient observations to determine whether or not periods of ovulatory dysfunction occurred in adulthood.
to the fetus directly (Table III; Swanson and Werff ten Bosch, 1965; Fels and Bosch, 1971). Precocial mammals, identified by completed sexual differentiation and well-developed neural systems at birth (Wallen and Baum, 2002), usually do not exhibit ovulatory dysfunction when exposed to androgen excess after birth (Table IV), as exemplified by marmosets, rhesus monkeys and humans. However, such mammals exhibit ovulatory dysfunction when exposed to androgen excess before birth, e.g. guinea pigs, sheep and rhesus monkeys (Table III; Figure 3). While cattle and pigs do not exhibit such anovulatory consequences (Table III), newborn piglets are intermediate between altricial and precocial mammals, in terms of development at birth.

<table>
<thead>
<tr>
<th>Species (altricial)</th>
<th>Androgen</th>
<th>Post-natal days</th>
<th>Puberty*</th>
<th>Ovarian/estrus cycles</th>
<th>LH surge</th>
<th>Reference</th>
</tr>
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<tr>
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<td>Testosterone</td>
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<td>Edwards, 1971</td>
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<td></td>
<td></td>
<td>1–5</td>
<td>Acyclic</td>
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<td>Ohta and Iguchi, 1977</td>
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<td></td>
<td>5 (10 mg TP s.c.)</td>
<td>–</td>
<td>Acyclic</td>
<td>66%</td>
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<td></td>
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<td></td>
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<td>Acyclic</td>
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<td>1, 2, 3, 4, 5, 6</td>
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<td>Acyclic</td>
<td>–</td>
<td>Arai et al., 1981</td>
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<td></td>
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<td></td>
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<td>Acyclic</td>
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<td></td>
<td>5</td>
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<td>Acyclic</td>
<td>–</td>
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<td>McDonald and Doughty, 1972</td>
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<tr>
<td></td>
<td>5</td>
<td>+ Estrogen antagonist</td>
<td>Cyclic</td>
<td>–</td>
<td>Arai and Gorski, 1968a,b</td>
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<td></td>
<td>5</td>
<td>+ Day 5 pentobarbital</td>
<td>Cyclic</td>
<td>–</td>
<td>Arai and Gorski, 1968a,b</td>
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<td></td>
<td>5</td>
<td>+ Adult progesterone and hypothalamic electrical stimulation</td>
<td>Cyclic</td>
<td>–</td>
<td>Barralough and Gorski, 1961</td>
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<td>6, 6</td>
<td>Delayed</td>
<td>Acyclic</td>
<td>–</td>
<td>Lutte and Whalen, 1970</td>
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<td>–</td>
<td>Cyclic</td>
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<td>Gorski, 1968</td>
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<td></td>
<td>17–27</td>
<td>Advanced</td>
<td>Cyclic</td>
<td>–</td>
<td>Zarrow et al., 1969</td>
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<tr>
<td></td>
<td>27–30</td>
<td>Advanced</td>
<td>Cyclic</td>
<td>–</td>
<td>Knudsen and Mahesh, 1975</td>
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<tr>
<td>5α-DHT</td>
<td>1</td>
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<td>Cyclic</td>
<td>–</td>
<td>Arai et al., 1981</td>
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<td></td>
<td>1–5</td>
<td>Acyclic</td>
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<td>Knudsen and Mahesh, 1975</td>
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<td>Acyclic</td>
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<td>Advanced</td>
<td>Cyclic</td>
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<td></td>
<td>27–30</td>
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<td>Cyclic</td>
<td>–</td>
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<td>5β-DHT</td>
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<td>–</td>
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<td></td>
<td>27–30</td>
<td>Advanced</td>
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<td>–</td>
<td>Knudsen and Mahesh, 1975</td>
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<tr>
<td>DHEA</td>
<td>+ 3beta HSD inhibitor</td>
<td>Normal</td>
<td>Cyclic</td>
<td>–</td>
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<td>1/4–50/54</td>
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<td>–</td>
<td>Polishuk and Antebay, 1971</td>
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</table>

DHEA = dehydroepiandrosterone; HSD = hydroxysteroid dehydrogenase.
*a Determined from a variety of measures, including first vaginal opening (rodents), menarche (menstruating primates).

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and thus may be less susceptible to fetal exposure to androgen excess.

Neuronal uptake of steroid hormone appears necessary to induce androgen-induced anovulation, at least in rats, since androgen-induced anovulation can be prevented by pentobarbital-induced anaesthesia (Table IV; Arai and Gorski, 1968a,b). Further, since aromatizable (testosterone, androstenedione and dehydroepiandrosterone) or non-aromatizable (5α-DHT and 5β-DHT) androgens can induce ovulatory dysfunction (Tables III and IV), estrogen and androgen receptors may both be involved in the mechanism. Evidence for an estrogen-mediated action comes from the administration of an estrogen antagonist concurrently with postnatal testosterone treatment of female rats. The dual treatment prevents androgen excess induction of persistent estrus (Table IV; McDonald and Doughty, 1973) and implicates aromatization of androgen to estrogen in the mechanism. In ewes, testosterone, but not 5α-DHT, abolishes the estrogen-induced LH surge and induces polyfollicular ovaries (West et al., 2001; Foster et al., 2002). Conversely, evidence supporting a direct androgen-mediated action comes from the use of non-aromatizable androgen to induce anovulation, and the ability of the androgen antagonist, flutamide, to restore normal ovulatory cycles in adult, prenatally androgenized female mice with acyclicity (Table III; Sullivan and Moenter, 2004), with the latter implicating γ-aminobutyric acid-mediated inhibition of gonadotrophin-releasing hormone (GnRH) neurons as a possible mechanism of fetal androgen excess induced anovulation.

Hypothalamus or ovary: where is the major site for androgen excess induced ovulatory dysfunction?

There are clear differences between non-primate mammals and primates in the mechanism of anovulation induced by fetal or perinatal androgen excess. Whereas androgen excess in non-primates primarily disengages the ability of the hypothalamus–pituitary to generate an ovulation-inducing LH surge, this is not the case in primates. In the latter, altered steroid negative feedback regulation of LH and compensatory hyperinsulinemia from insulin resistance may disrupt ovulatory function, causing anovulation (Abbott et al., 2002a).

In non-primates, the hypothalamic–pituitary unit from the androgenized female, like the normal male, becomes incapable of generating a GnRH-induced LH surge in response to sharply rising estradiol (E2) levels (Clarke et al., 1976b, 1977; Clarke and Scaramuzzi, 1978; Buhl et al., 1978; Wood et al., 1995; Sullivan and Moenter, 2004). The ovaries appear functionally unaffected since rat ovaries removed from anovulatory, adult, neonatally androgenized females and transplanted into ovariec-tomized, normal adult females exhibit ovulatory cycles (Ladosky et al., 1969), while ovaries removed from normal adult females and transplanted into ovariec-tomized, neonatally androgenized female adults or castrated adult males cease ovulatory cyclicity (Pfeiffer, 1956; Ladosky et al., 1969). The pituitary also appears functionally unaffected because pituitaries removed from adult male rats and transplanted beneath the median eminence of hypophysectomized, ovary intact, normal females restore regular ovarian cycles (Harris and Jacobsohn, 1951; Martinez and Bittner, 1956). Moreover electrical stimulation of the hypothalamus above the pituitary in the progesterone-primed, neonatally androgenized female rats induces ovulation (Table IV; Barraclough and Gorski, 1961). Collectively, these studies identify the non-primate hypothalamus as the key site for androgen excess programming of ovulation.

In ewes exposed to androgen excess during early gestation, ovarian cycles and the LH surge are abolished by the second breeding season (Foster et al., 2002; Birch et al., 2003). However, in ewes exposed to androgen excess during mid-gestation a form of the estrogen-induced LH surge is present. In comparison to normal females, the LH surge is delayed and reduced (Savabeasafahani et al., 2005): it is not abolished probably because such androgen excess occurs mostly beyond a fetal age at which the hypothalamus is susceptible to re-programming. As in neonatally androgenized rats, there appears to be a finite fetal developmental period in ewes during which androgen excess programming can abolish the hypothalamic GnRH surge mechanism. Recent data from immature prenatally androgenized ewes suggest that ovarian function in such females may also be compromised since increased expression of ovarian follistatin, along with reduced expression of ovarian activin beta-B mRNA, may indicate compromised intra-follicular activin availability in most follicles, thereby impairing follicle development (West et al., 2001). These ovarian abnormalities may reflect excessive LH and insulin stimulation from fetal androgen induced hypersecretion of LH and insulin, respectively (Birch et al., 2003; Manikkam et al., 2004), and indicate programmed ovarian dysfunction beyond a disrupted ovulatory mechanism.

In contrast to non-primate mammals, fetal androgen excess in primates does not abolish the ability of the hypothalamic–pituitary unit to generate an LH surge in response to acute elevations in circulating estrogen levels in ovariec-tomized (Steiner et al., 1976) or ovary intact (Dumesic et al., 1997) prenatally androgenized females, and in castrated (Karsch et al., 1973) or testis intact (Hodges and Hearn, 1978) males. These findings are reinforced by the ability of normal ovaries to induce LH surges and exhibit ovulation when transplanted into gonad-ectomized adult males (Norman and Spies, 1986). However, the LH surge mechanism is not entirely normal following fetal androgen exposure, and appears exaggerated (Steiner et al., 1976; Dumesic et al., 1997) and delayed (Steiner et al., 1976) in adult, prenatally androgenized females. Such ovulatory function in prenatally androgenized females may thus be disrupted by androgen excess programming of LH hypersecretion and hyperinsulinemia (Abbott et al., 2002a, 2004), reflecting a more subtle form of ovulatory dysfunction than that of similarly exposed non-primates, akin to the ovulatory abnormality found in PCOS women (Barnett and Abbott, 2003). Interestingly, primates may not be susceptible to androgen excess-induced anovulation from abolition of the hypothalamic GnRH surge because their pituitary can mount an estrogen-induced LH surge without a concomitant increase in hypothalamic GnRH activity (Knobil and Hotchkiss, 1994; Abbott et al., 2004).

PCOS in female rhesus monkeys exposed to fetal androgen excess

The population of prenatally androgenized female rhesus monkeys at the National Primate Research Center of the University of Wisconsin, Madison have proved remarkable, experimentally-induced
phenotypic mimics of PCOS signs and symptoms (Table II; Abbott et al., 1997, 1998, 2002b; Dumesic et al., 2005) that may well have a genetic basis in women (Legro et al., 1998b; Strauss, 2003; Escobar-Morreale et al., 2005; Franks and McCarthy, 2004). Since the origins for PCOS in women are still unknown, and only one, as yet undefined, gene candidate has been reliably associated with a PCOS phenotype (Urbanek et al., 1999; Tucci et al., 2001; Villuendas et al., 2003), this may be a highly relevant time for an animal model to provide unique insight into origin and mechanism that have proved elusive from study of patients, alone.

The prenatally androgenized female rhesus monkeys at Wisconsin were originally produced for studies of fetal programming of psychosexual behaviour by androgen excess (Goy and Resko, 1972). Mothers were injected daily with 5–15 mg TP s.c. for 15–88 consecutive days starting at various gestational ages (26–110 days; Goy and Robinson, 1982; Goy and Kemnitz, 1983). Female fetuses of mothers treated with TP experienced circulating levels of testosterone equivalent to those found in fetal males (Resko et al., 1987). Of these treated females, 20 prenatally androgenized females experienced fetal androgen excess when their mothers were injected s.c. with only 10 mg TP for 15–35 consecutive days beginning on gestational days 40–44 (early treated; n = 11) or between days 100–115 for 15–25 consecutive days (late-treated; n = 9), in a total gestation period of 165 days (Eisner et al., 2000). Early-treated prenatally androgenized female rhesus monkeys are exposed to androgen excess at a gestational age when many organ systems, including those regulating reproduction and metabolism, are still in the early stages of differentiation (Figure 1). Late-treated females, on the other hand, experience androgen excess when most organ systems have completed differentiation, but are undergoing functional maturation. Therefore, these two gestational exposures to androgen excess should yield different fetal programming outcomes, with early gestational treatment altering structure and multiple aspects of function, and late gestational treatment limited to modifying functional components still in the process of maturation (Figure 1).

Not surprisingly, early-treated prenatally androgenized females exhibit both virilized genitalia, and enhanced male and diminished female components of behaviour, while late-treated females exhibit aspects of the behavioural traits, alone (Goy et al., 1988a). Using control females unexposed to fetal androgen excess and matched for age, body weight and BMI (Eisner et al., 2000, 2002, 2003; Dumesic et al., 2002, 2003), these prenatally androgenized females represent a core study group of monkeys that illustrate how differential timing of fetal androgen excess is important for the production of two variants of a PCOS phenotype (Table II). The finding that both early- and late-treated prenatally androgenized female rhesus monkeys exhibit PCOS is highly significant since neither late-treated monkeys nor PCOS women exhibit virilized genitalia, while early-treated monkeys do. Androgen excess programming of disrupted female reproduction can occur regardless of whether or not the genitalia are virilized.

Prenatally androgenized female rhesus monkeys comply with the ‘consensus’ diagnoses for PCOS

As shown in Table I, a diagnosis of PCOS requires androgen excess, intermittent or absent menstrual cycles and polycystic ovaries, or at least two of these criteria. Both early- and late-

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Gestational progression of aspects of differentiation and maturation of hypothalamic–pituitary–ovarian function, and pancreas and beta-cell function in rhesus monkeys, based upon the following publications: Baker (1966), Hoar and Monie (1981), Ellinwood et al. (1983), Ronnekleiv and Resko (1990), Sperling (1994), Quanbeck et al. (1997), Fischer (2003). The timing of exposures of females to androgen excess (early- and late-treated) is indicated in relation to fetal developmental progress.
treated prenatally androgenized female rhesus monkeys readily meet the first requirement since both exhibit ovarian hyperandrogenism when compared with normal females of similar age, weight and BMI. As illustrated in Figure 2, immediately before and in the 24–72 h time interval following a 200 IU i.m. injection of recombinant HCG (rHCG) during the early follicular phase or anovulatory period (Eisner et al., 2002), both types of androgenized female exhibit higher circulating testosterone levels than controls. In terms of basal serum testosterone levels (Abbott et al., 1997), values are approximately 50% greater in prenatally androgenized females compared to normal females.

Regarding the diagnostic requirement of intermittent or absent menstrual cycles, both early- and late-treated prenatally androgenized females again demonstrate ovulatory dysfunction. The androgenized females exhibit approximately 40–50% fewer menstrual cycles than normal females (Figure 3), with normal females undergoing 5–8 menstrual cycles during the 6 month period illustrated, while early-treated prenatally androgenized females range from 0–5 cycles and late-treated females range from 0–7. This longer interval of time for monitoring compared to the shorter times used in previous studies (1–3 months; Abbott et al., 1998) better illustrates the significant degree of menstrual dysfunction in prenatally androgenized females as an entire group, and indicates that anovulation is pronounced in these females regardless of BMI.

Forty percent of prenatally androgenized females, compared to ~14% of controls, have polycystic ovaries (Abbott et al., 1997, 2002b) that resemble the morphology of polycystic ovaries found in PCOS women (Adams et al., 1986; Franks, 1995). In addition, polycystic ovaries in prenatally androgenized female monkeys have approximately 60% greater volume than polyfollicular or normal ovaries in control females (Abbott et al., 2002b), suggestive of enlarged stromal volume, another PCOS ovarian trait (Dewailly et al., 1997). Similar numbers of early- and late-treated prenatally androgenized females contribute to the ovarian abnormality (Abbott et al., 1998).

Regarding exclusion of other disorders that mimic PCOS (Table I), prenatally androgenized female monkeys neither express the abnormalities of cortisol biosynthesis found in congenital adrenal hyperplasia and Cushing’s syndrome (Zhou R and Abbott DH, unpublished results) nor do they harbour androgen-secreting tumours. Consequently, their collective reproductive defects, without obvious PCOS-mimic disorders, qualify prenatally androgenized female rhesus monkeys for a PCOS diagnosis. The qualification of late-treated prenatally androgenized females for a PCOS diagnosis is particularly noteworthy, since these females do not possess virilized genitalia and thus resemble PCOS more closely clinically than early-treated females.

Additional PCOS signs and symptoms expressed in prenatally androgenized female monkeys

Reproductive endocrine defects in addition to PCOS diagnostic criteria

Unlike their unified qualification for a PCOS diagnosis, early- and late-treated prenatally androgenized female monkeys differ in their expression of PCOS traits beyond the diagnostic criteria, in analogous fashion to the heterogeneity in signs and symptoms expressed in PCOS women (Table I). LH hypersecretion is
found only in early-treated females (Figure 4). Throughout early and late middle-age, both before and during controlled ovarian stimulation cycle for IVF, elevations in circulating LH levels by immuno- versus bio-active determination (Figure 4) are restricted to these early gestation exposed females. This LH elevation appears to involve at least two alterations in hypothalamo-pituitary function. Pituitary gonadotrope LH responsiveness to a 20 μg i.v. injection of GnRH is increased in early-treated females (Figure 5), suggestive of an underlying increase in endogenous GnRH priming. Ovarian hormone mediated negative feedback regulation of LH is also diminished in early-treated females during the first 6 days of a FSH-induced, controlled ovarian stimulation cycle for IVF, in comparison to either late-treated or control females (Figure 6). While FSH treatment equally elevates serum E2 levels in all females, serum LH levels diminish in late-treated androgenized and control females, alone. In contrast, serum LH levels in early-treated androgenized females remain unchanged despite the large increases in circulating E2 (Figure 6).

Steiner et al. (1976) achieved similar results when treating gonadectomized rhesus monkeys with exogenous E2 replacement. Ovariectomized, early-treated androgenized females and orchidectomized males required E2 replacement levels equivalent to those found in the normal late follicular phase (~100 pg/ml) to exhibit suppression of their circulating LH levels. Control ovariectomized females, on the other hand, only required E2 replacement levels equivalent to those found in the early follicular phase (~20 pg/ml) to induce suppression of their post-ovariectomy LH levels (Steiner et al., 1976). Both early- and late-treated prenatally androgenized females exhibit reduced progesterone-mediated LH negative feedback (Levine et al., 2005). Similar defects in LH negative feedback regulation are found in PCOS women since such individuals exhibit enhanced pituitary gonadotrope LH responsiveness to exogenous GnRH (Katz and Carr, 1976; Rebar et al., 1976; Baird et al., 1977) and diminished combined E2 and progesterone mediated negative feedback regulation of LH (Pastor et al., 1998; Marshall and Eagleson, 1999; Eagleson et al., 2003).

Fertility defects found beyond the PCOS diagnostic criteria

Early- and late-treated prenatally androgenized female rhesus monkeys also differ in their follicle and oocyte responses to controlled ovarian stimulation for IVF (Dumesic et al., 2002, 2003, 2005). Reduced oocyte quality, which contributes to increased rates of implantation failure and pregnancy loss after IVF in PCOS patients (Homburg et al., 1988; Sagle et al., 1988; Dor et al., 1990; Tarlatzis et al., 1995), occurs to varying degrees in female rhesus macaques exposed prenatally to excess androgens. While early gestation androgen treatment produces a greater degree of oocyte developmental impairment in vitro after controlled ovarian stimulation for IVF, both early- and late-treated prenatally androgenized females demonstrate reduced blastocyst development and abnormal follicular fluid steroid levels (Dumesic et al., 2002, 2003), suggesting that development of their follicles and enclosed oocytes is compromised following

Figure 4. Elevated circulating LH levels in early-treated prenatally androgenized (PA) and control rhesus monkeys after (A) immuno-determination from control (n = 24), early-treated PA (n = 10) and late-treated PA (n = 3) females; *P < 0.001 versus controls by ANOVA, data modified from Dumesic et al. (1997) and (B) bio-active determination from five control, early-treated and five late-treated PA females; **P < 0.03 versus controls by ANOVA, data modified from Dumesic et al., 2002. Control females = open bars; early-treated PA females = hatched bars; late-treated PA females = solid bars.

Figure 5. Increased pituitary gonadotrope immuno-LH responsiveness in six early-treated prenatally androgenized (PA) female rhesus monkeys at all timepoints (*P < 0.05 versus 0 min by ANOVA) after i.v. injection of GnRH compared to six control females. Data are shown as backtransformed means ± 95% confidence intervals. Control females = open circles; early-treated PA females = solid circles.

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fetal androgen excess. None of the intrafollicular steroid hormone abnormalities and embryo development failures accompanying controlled ovarian stimulation for IVF are observed in the circulating steroid hormone levels, or in the number and maturity of oocytes collected, respectively.

Abnormalities in follicular steroid hormone function occur when early- and late-treated prenatally androgenized females are stimulated with either recombinant human (rh) FSH alone for 7–9 days (Dumesic et al., 2003) or with combined rhFSH/rHCG stimulation [rhFSH for 7–9 days followed by rHCG administration 27 h before oocyte retrieval (Dumesic et al., 2002)]. In late-treated prenatally androgenized females, low follicular fluid levels of E2 and androstenedione occur following rhFSH therapy alone, with the follicular fluid progesterone/E2 ratio being normal in response to rhFSH therapy followed by rHCG administration (Dumesic et al., 2002, 2003). Consistent with the ability of E2 to enhance the fertilization and cleavage rates of in vitro matured rhesus and human oocytes (Tesarik and Mendoza, 1995; Zheng et al., 2003), subtle embryonic developmental impairment is evident by the blastocyst stage following combined rhFSH/rHCG treatment in these late gestation exposed females (Dumesic et al., 2002).

In early-treated prenatally androgenized females, low follicular fluid levels of E2 and androstenedione occur after both types of stimulation protocol, and are further accompanied by an elevated progesterone/E2 ratio in the follicular fluid following combined rhFSH/rHCG therapy. Early-treated females also exhibit a pronounced impairment in embryonic development after the fetal genome activation stage (5–8 cells), with approximately 68% of the embryos failing to compact to morulae, and ~96% failing to form blastocysts. Such impaired embryonic development may be related to both the absolute amount of E2 and the progesterone to E2 ratio in the follicle because intermediate follicular progesterone levels in humans are associated with oocytes that develop into dividing embryos after fertilization, with lower and higher intrafollicular progesterone levels predicting failure to conceive (Rom et al., 1987) and reduced pregnancy outcome (Kreiner et al., 1987).

Equally important, all prenatally androgenized females receiving combined rhFSH/rHCG therapy for IVF exhibit abnormal steroidogenesis in follicles containing mature oocytes that fertilize and develop to blastocysts. In these follicles, either the ratio of progesterone to E2 (Figure 7) is increased in early-treated females, or the E2 level relative to the progesterone/E2 ratio is elevated in late-treated females. Successful oocytes (that develop to blastocyst after fertilization) from early- and late-treated prenatally androgenized animals arise from follicles that lie completely outside the 95% confidence interval for the relationship between follicular fluid progesterone to E2 ratio versus E2 that exists for successful oocytes in control females (Figure 7). These two patterns of distinctly different abnormal intrafollicular steroidogenesis, based upon the timing of prenatal androgen exposure, raise concerns regarding the potential adverse effects of any prenatal androgen exposure on oocyte developmental competence beyond the blastocyst stage.

Asynchronous follicle differentiation and oocyte maturation in prenatally androgenized females might also be associated with hyperinsulinaemia and LH hypersecretion because insulin enhances FSH-induced up-regulation of LH receptors in granulosa cells, thereby increasing their ability to produce progesterone in response to LH (Willis and Franks, 1995; Willis et al., 1996; Eppig et al., 1998). Such metabolic and reproductive abnormalities are difficult to detect in late-treated prenatally androgenized females receiving rhFSH therapy followed by rHCG, except for the inability to normally increase the serum glucose/insulin ratio with rising serum E2 levels (Dumesic et al., 2002). Early-treated prenatally androgenized females undergoing the same controlled ovarian stimulation for IVF, however, show both LH hypersecretion and the inability to normally suppress serum insulin levels between day 1 of rhFSH treatment and the day of oocyte retrieval (Dumesic et al., 2002). These data raise the possibility that timing of prenatal androgen excess influences susceptibility of the oocyte to androgen programming in utero by altering follicle differentiation through metabolic and/or neuroendocrine dysfunction.

**Metabolic defects found beyond the PCOS diagnostic criteria**

In addition to the presentation of reproductive defects, PCOS is the leading cause of type 2 diabetes in premenopausal women (Legro et al., 1999). Insulin resistance and impaired insulin secretion in response to glucose are the metabolic deficits central to the development of type 2 diabetes mellitus in PCOS women (DeFronzo, 1992). The risk is further compounded by the presence of obesity, particularly when present in the abdominal compartment (Wagenknecht et al., 2003).
Prenatally androgenized female rhesus monkeys harbour similar metabolic problems. Early-treated females exhibit impaired insulin secretion while late-treated females show decrements in insulin sensitivity with increasing adiposity estimated by BMI, with preservation of insulin secretory function (Eisner et al., 2000). Detailed measures of body composition in late-treated females have not been elucidated, though early treated females have increased visceral fat compared to control females as measured by computerized tomography combined with dual X-ray absorptiometry, even when corrected for BMI and total body fat (Eisner et al., 2003). Consistent with visceral adiposity, early-treated females liberate fatty acids to a greater extent than control females during a frequently sampled i.v. glucose tolerance test (Abbott et al., 2002c). Similar to PCOS women, the metabolic deficits and altered body composition induced by prenatal androgen exposure in early gestation result in an increased prevalence of diabetes in female rhesus monkeys, while late-treated females develop a more attenuated metabolic phenotype (Abbott et al., 2003; Abbott DH et al., unpublished data). These findings support the notion that fetal androgen excess can induce metabolic deficits similar to those expressed in PCOS women (Table I) and that the timing of fetal exposure is again important in determining phenotypic presentation.

Potential mechanisms of fetal androgen excess leading to PCOS in humans

Clearly, fetal androgen excess in animal models produces adult traits that mimic PCOS. In experimentally induced fetal androgen excess, induction of maternal hyperandrogenism is commonly used to effectively deliver excess androgen to the fetus. A similar, but naturally occurring, situation may arise in pregnant PCOS women suggesting an analogous androgen excess delivery mechanism to that experimentally employed. Circulating levels of total and unbound or ‘free’ testosterone are elevated above normal values during pregnancy in PCOS women (Sir-Petermann et al., 2002). Indeed, in some PCOS pregnancies, maternal androgen elevations are sufficiently excessive to cause virilization of the mother (Magendantz et al., 1972; Fayez et al., 1974; Bilowus et al., 1986; McClamrock and Adashi, 1992; Sarlis et al., 1999) and, occasionally, a female fetus (Bilowus et al., 1986; Ben-Chetrit and Greenblatt, 1995). Nevertheless, while genital virilization is uncommon among women with PCOS, the mid- to late gestational timing of maternal androgen excess in pregnant PCOS women (22–28 weeks of gestation; Sir-Petermann et al., 2002) may provide a potential mechanism for delivery of maternal androgen excess at a fetal age when urogenital development is no longer responsive to such hormonal disruption (>13 weeks of gestation; Grumbach and Ducharme, 1960; New, 1992). Normally, transmission of androgen excess from mother to a female fetus does not occur unless circumstances arise that compromise placental function, such as placental aromatase deficiency (Simpson et al., 1997) or undernutrition (Cresswell et al., 1997).

An additional source of human female fetal hyperandrogenism can arise from a hyperandrogenic fetal ovary (Barbieri et al., 1986; Beck-Peccoz et al., 1991), hyperandrogenic fetal adrenal cortex (Barnes et al., 1994) or both. Maternal hyperinsulinaemia, also found in PCOS compared to normal pregnancies (Sir-Petermann et al., 2002), induces excessive placental HCG secretion leading to fetal ovarian hyperplasia and hyperandrogenism (Barbieri et al., 1986). Further, women with adrenal hyperandrogenism due to either 21-hydroxylase deficiency (Barnes et al., 1994) or 17,20 lyase excess (Azziz et al., 1998; Moran et al., 2004), also demonstrate PCOS. Adrenal androgens may thus serve as substrates for ovarian androgen production since fetal ovaries are able to convert steroid precursors, including dehydroepiandrosterone sulfate, to functional ovarian androgens (Payne and Jaffe, 1974; Bonser et al., 2000).

Thus, both maternal and fetal hyperandrogenism in humans can provide plausible mechanisms for the induction of female fetal androgen excess and fetal programming of PCOS. Such hyperandrogenism may be genetically or environmentally determined, and additional genetic (e.g. genes regulating insulin secretion and action) and environmental (e.g. dietary) factors may interact with androgen excess reprogramming to produce the heterogeneous phenotypes observed in women with PCOS.

Figure 7. Negative associations between follicular fluid progesterone to E2 (P4/E2) ratio and E2 levels (nmol/mg protein) related to embryo/oocyte development in (A) 19 follicles from control female rhesus monkeys, (B) 14 follicles from early-treated prenatally androgenized females, (C) 19 follicles from late-treated PA females. Control females = circles; late-treated PA females = squares; early-treated PA females = triangles; solid symbols = progression to blastocyst; open symbols = progression to at least metaphase II but not beyond morula.
Importance of gestational timing of fetal androgen excess for programming of heterogeneous PCOS phenotypes

The ability of prenatal androgen excess in fetal rhesus monkeys to programme target tissue differentiation in utero provides unique insight into the mechanisms by which androgen excess fetal programming in primates perturbs adult reproduction. Prenatally androgenized female rhesus monkeys develop permanent, yet heterogeneous, PCOS-like abnormalities, including a combination of reproductive and metabolic abnormalities accompanied by disordered folliculogenesis, impaired oocyte development and ovarian hyperandrogenism (Table II). This ability of discrete androgen excess programs to programme target tissue differentiation in utero towards one of many adult PCOS phenotypes depends upon the timing of prenatal androgenization relative to gonadal differentiation, pancreatic organogenesis and neuroendocrine development (Figure 1). As such, the prenatally androgenized female rhesus monkey as a model of PCOS implicates hyperandrogenism during critical exposure times of prime fetal development in the pathogenesis of the adult PCOS phenotype. By suggesting that genetically-determined hyperandrogenism beginning in intrauterine life programmes human fetal development for PCOS in adulthood, such a hypothesis opens new directions in the treatment of PCOS through greater understanding of the hormonal environment during intrauterine life and how it programmes target tissue differentiation in the fetus.

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References


Androgen excess and polycystic ovary syndrome


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