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 gonadotropic 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 Witchell, 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-Degrave *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 (Urbanek *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 (Brettenthaler *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), effectively 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 (Urbanek *et al.*, 1999; Tucci *et al.*, 2001; Villuendas *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., 1939)]. However, testes do not form since testes-determining genes, not androgens, are required for differentiation of the male gonads (Sinclair et al., 1990; Tommerup et al., 1993; Bardoni et al., 1994; Swain and Lovell-Badge, 1999). Further, without testicular secretion of anti-Mullerian hormone (Josso et al., 1993; Lee and Donahoe, 1993; Lasala et al., 2004), fetal androgen excess fails to inhibit differentiation of the ovaries, Fallopian tubes, uterus, cervix and upper vagina (Wells and van Wagenen, 1954; Goy et al., 1988b).

Primates and other precocial mammals, in which sexual differentiation and many neural systems are well-developed at birth, are only responsive to urogenital tract androgen re-programming until about mid-gestation (Clarke et al., 1976a,b; Goy et al., 1988a; Quigley et al., 1995). Altricial mammals, including many laboratory rodents, are in the midst of external genitalia differentiation and the development of many neural systems at birth, despite in utero differentiation of the gonads and much of the internal genitalia (Wallen and Baum, 2002). Not surprisingly, therefore, altricial mammal genitalia are susceptible to a limited degree of perinatal and early post-natal genital virilization, in contrast to precocial mammals. Similar to PCOS women who are not virilized, female rhesus monkeys exposed to androgen excess late in gestation (after day 90 of a 165-day pregnancy) exhibit a PCOS phenotype (Table II) without virilized genitalia (Goy et al., 1988a).

However, virilized genitalia provide only one obvious sign of female exposure to fetal or perinatal androgen excess. In terms of reproductive consequences, androgenic impact extends well beyond re-sculpturing of the genitalia, and induces masculinization and defeminization of sexual behaviour and impairment of ovulatory function. Quantified behavioural assessments provide powerful, non-invasive bioassays of androgen excess re-programming of brain function related to reproduction. As recently reviewed by Wallen and Baum (2002), androgen-induced defeminization of sexual behaviour usually depends on neuronal conversion of androgens to estrogen metabolites. Subsequent alteration of neuronal gene transcription occurs through coordinated DNA binding of estrogen to its specific receptor together with nuclear receptor coactivators, including CAMP response element binding protein (CREB)-binding protein (CBP) and steroid receptor coactivator-1 (Auger et al., 2002). Such

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

Sign or symptom	PCOS women	Prenatally androgenized female monkeys	
		Early-treated	Late-treated
Reproductive			
Ovarian hyperandrogenism	+	+[a]	+[b]
Anovulation	+	+[b]	+[b]
Enlarged polyfollicular ovaries	+	+[c]	+[c]
LH hypersecretion	+	+[d]	−[d]
Reduced steroid hormone negative feedback on LH	+	+[b,e]	+[b]
Impaired ovarian endocrine response to stimulation for IVF	− (increased response)	+[d,f]	+[d,f]
Impaired embryo development after IVF	? (increased miscarriages)	+[d]	+[d]
Metabolic			
Insulin resistance	+	+[g]	−[h]
Impaired insulin secretion	+	+[h]	−[h]
Impaired glucose tolerance	+	+[g]	+[g]
Increased type 2 diabetes	+	+[g]	−[g]
Increased abdominal fat	+	+[I]	+[j]
Hyperlipidaemia	+	+[k]	?

[a] Eisner et al., 2002.
[b] Abbott et al., 2005.
[c] Abbott et al., 2002b.
[d] Dumesic et al., 2002.
[e] Steiner et al., 1976.
[f] Dumesic et al., 2003.
[g] Abbott et al., 2003.
[h] Eisner et al., 2000.
[i] Eisner et al., 2003.
[j] Bruns CM et al., unpublished data.
[k] Abbott et al., 2002c.

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 defeminizing 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 antisense 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.*, 1994), 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 lesbianism (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 lesbianism or transexualism. 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 stigmatism (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 Dunaif, 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 III. Effects of fetal androgen exposure on the onset of female puberty, occurrence ovarian/estrus cyclicity and LH surge

Species	Steroid (given to mother)	Gestational days (TP dose and route of administration)	Puberty[a] (additional treatment)	Ovarian/estrus cycle	LH surge	Reference
Mouse (altricial)	Testosterone	13–18 (0.75 mg T s.c.)	–	Cyclic	No	Keisler *et al.*, 1991
	5α-DHT	16–18	Tends to be delayed	Acyclic	No	Sullivan and Moenter, 2004; Moenter SM, personal communication
		16–18	Flutamide as adult	Cyclic	Yes	Sullivan and Moenter, 2004
Rat (altricial)	Testosterone	Starting at 13–15 for 1–8 days	–,	Cyclic	–	Greene *et al.*, 1939
		19, 20, 21, or 22 (2.5 mg TP s.c.)	–,	Cyclic	–	Swanson and van der Werff ten Bosch, 1964b
		19, 20, 21, or 22 (10–25 mg TP s.c.)	–,	Delayed acyclic	–	Swanson and van der Werff ten Bosch, 1964b
		19, 20, 21, or 22 (20 µg TP amniotic fluid)	–,	Cyclic	–	Swanson and Werff ten Bosch, 1965
		19, 20, 21 or 22 [20 µg TP fetus]	–,	Acyclic	–	Swanson and Werff ten Bosch, 1965; Fels and Bosch, 1971
		~20 (5–45 mg TP s.c.)	–,	Cyclic	–	Fels and Bosch, 1971
		~20 (0.5–1 mg TP amniotic fluid)	–	64% Acyclic	–	Fels and Bosch, 1971
		16–19 (1 mg TP s.c.)	–	Cyclic	–	Huffman and Hendricks, 1981
		16–20	Advanced	Cyclic	–	Slob *et al.*, 1983
	5α-DHT	16–20	–	Cyclic	–	Whalen and Luttge, 1971
Guinea pig (precocial)	Testosterone	33–37	Delayed	Acyclic	–	Brown-Grant and Sherwood, 1971
Pig (altricial/precocial)	Testosterone	29–35	Delayed	Cyclic	–	Ford and Christenson, 1987
		39–45	Delayed	Cyclic	–	Ford and Christenson, 1987
Cow (precocial)	Testosterone	80–110	Normal	Cyclic	–	DeHaan *et al.*, 1988
Dog (altricial)	Testosterone	24–43	–	Cyclic	–	Beach *et al.*, 1983
Sheep–ovary intact (precocial)	Testosterone	20–term	–	Acyclic	No	Short, 1974
		30–90	Normal	Cyclic—1st year / Acyclic—2nd year	Abnormal / No	Sharma *et al.*, 2002
		30–80	–	Oligo/acyclic	Impaired	Clarke *et al.*, 1976a,b, 1977; Clarke, 1977
		60–term	–	Acyclic	No	Short, 1974
		60–90	Normal	Cyclic	Abnormal	Sharma *et al.*, 2002
		70–120	–	Oligo/acyclic	–	Clarke *et al.*, 1976a,b, 1977; Clarke, 1977
		50–100	–	Oligo/acyclic	Impaired	Clarke *et al.*, 1976a,b, 1977; Clarke, 1977
		90–140	–	Cyclic	–	Clarke *et al.*, 1976a,b, 1977; Clarke, 1977
Sheep—ovx + E₂ at birth (precocial)	Testosterone	30–90	Advanced	–	No	Wood *et al.*, 1995
	DHT	30–90	Advanced	–	Yes	Masek *et al.*, 1999
Rhesus monkey	Testosterone	40–55/65/75 (early-treated)	Delayed	Cyclic/acyclic	Yes	Dumesic *et al.*, 1997; Goy *et al.*, 1988a,b; Abbott *et al.*, 1998; Thornton, 1983
		100/115–124/139 (late-treated)	Normal	Cyclic/acyclic	Yes	Goy *et al.*, 1988a,b; Abbott *et al.*, 1998; Dumesic *et al.*, 1997
	DHT	40–95 (early-treated)	Delayed	Cyclic[b]	–	Goy *et al.*, 1988a,b
Rhesus monkey—ovx + E₂ post-natal	Testosterone	40–69/111 (early-treated)	–	–	Yes, but abnormal	Steiner *et al.*, 1976

TP = testosterone propionate; DHT = dihydrotestosterone; E₂ = 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.

Table IV. Effects of post-natal androgen treatment on attainment of puberty, ovarian/estrus cyclicity and LH surge

Species	Androgen	Post-natal days	Puberty[a]	Ovarian/estrus cycles	LH surge	Reference
Mouse (altricial)	Testosterone	1–3	–	Acyclic	–	Edwards, 1971
		1–5	–	Acyclic		Ohta and Iguchi, 1977
		5 (10 mg TP s.c.)	–	66% Acyclic		Barraclough and Leathem, 1954
		5 (20 mg TP s.c.)	–	Acyclic		Barraclough and Leathem, 1954
	DHT	1–5	–	Acyclic		Ohta and Iguchi, 1977
	Androstenedione	1–3	–	Acyclic		Edwards, 1971
Rat (altricial)	Testosterone	1	–	Acyclic	–	Swanson and van der Werff ten Bosch, 1964a,b
		1	Delayed	Acyclic	–	McDonald and Doughty, 1972
		1	Delayed	Acyclic	–	Pinilla *et al.*, 1993
		1, 2, 3, 4, 5, 6	–	Acyclic	–	Arai *et al.*, 1981
		2	–	Acyclic	–	Gorski, 1968
		2	–	Acyclic	–	Jones *et al.*, 1987
		2, 5	–	Acyclic	–	Barraclough, 1961
		2–5	–	Acyclic	–	Luttge and Whalen, 1970
		5	Advanced	Acyclic	No	Lookingland *et al.*, 1982
		5	Advanced	Acyclic	–	Grady *et al.*, 1965
		5	Advanced	Acyclic	–	McDonald and Doughty, 1972
		5	+Estrogen antagonist	Cyclic	–	McDonald and Doughty, 1973
		5	–	Acyclic	–	Arai and Gorski, 1968a,b
		5	–	Acyclic	–	Barraclough and Gorski, 1961
		5	+Day 5 pentobarbital	Cyclic	–	Arai and Gorski, 1968a,b
		5	+Adult progesterone and hypothalamic electrical stimulation	Cyclic	–	Barraclough and Gorski, 1961
		5, 6	–	Delayed anovulation syndrome	–	Gorski, 1968
		6	Advanced	Acyclic	–	Weisz and Lloyd, 1965
		8, 10	–	Cyclic	–	Gorski, 1968
		17–27	Advanced	Cyclic	–	Zarrow *et al.*, 1969
		27–30	Advanced	Cyclic	–	Knudsen and Mahesh, 1975
	5α-DHT	1	Delayed	Cyclic	–	McDonald and Doughty, 1972
		1–5	–	Acyclic	–	Arai *et al.*, 1981
		2–5	–	Cyclic	–	Luttge and Whalen, 1970
		5	Advanced	Cyclic	–	McDonald and Doughty, 1972
		27–30	Normal	Cyclic	–	Knudsen and Mahesh, 1975
	5β-DHT	1–5	–	Acyclic	–	Arai *et al.*, 1981
		27–30	Advanced	Cyclic	–	Knudsen and Mahesh, 1975
	DHEA	+3beta HSD inhibitor	Normal	Cyclic	–	Knudsen and Mahesh, 1975
	Androstenedione	2–5	–	Acyclic	–	Luttge and Whalen, 1969
Hamster (altricial)	Testosterone	2	–	Acyclic	–	Swanson, 1966
		2	–	Acyclic	–	Carter *et al.*, 1972
Dog (altricial)	Testosterone	7–89	Delayed	Cyclic/oligo-cyclic	–	Beach *et al.*, 1983
Marmoset monkey (precocial)	Testosterone	1/4–50/54	Normal	Cyclic		Abbott and Hearn, 1978
Rhesus monkey (precocial)	Testosterone	1	Normal	Cyclic	–	Treloar *et al.*, 1972
		150–365	Advanced	Cyclic	–	Van Wagenen, 1949
Human (precocial)	Testosterone	2/11–19/30	–	Cyclic		Polishuk and Anteby, 1971

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

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,

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 concomitantly with post-natal 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 hyperinsulinaemia 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 (E_2) levels (Clarke et al., 1976b, 1977; Clarke and Scaramuzzi, 1978; Buhl et al., 1978; Wood et al., 1995; Sullivan and Moenter, 2004). The ovary appears functionally unaffected since rat ovaries removed from anovulatory, adult, neonatally androgenized females and transplanted into ovariectomized, normal adult females exhibit ovulatory cycles (Ladosky et al., 1969), while ovaries removed from normal adult females and transplanted into ovariectomized, neonatally androgenized female adults or castrated adult males cease ovulatory cyclicity (Pfeiffer, 1936; 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 anovulation.

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 (Savabieasfahani 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 hypothalamo–pituitary unit to generate an LH surge in response to acute elevations in circulating estrogen levels in ovariectomized (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 gonadectomized 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 hyperinsulinaemia (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 'consenus' 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-
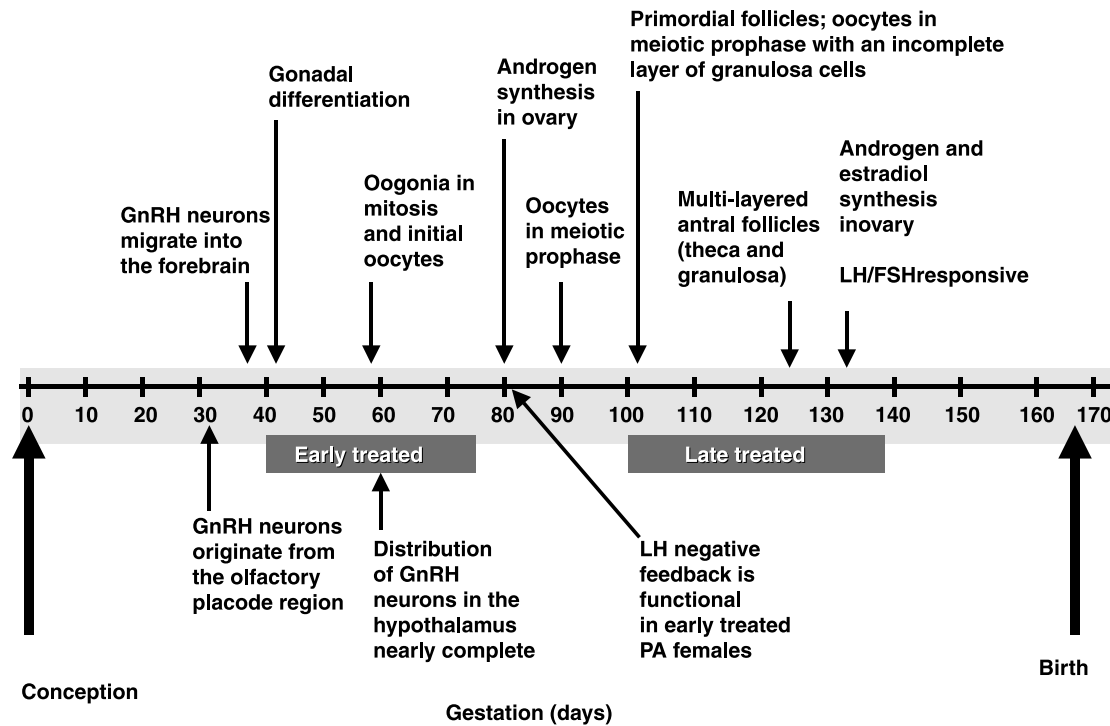

Figure 1. Gestational progression of aspects of differentiation and maturation of hypothalamic–pituitary–ovarian function, and pancreas and beta-cell function in rhesus monkeys, based upon the following publications: Baker (1966), Hoar and Monie (1981), Ellinwood *et al.* (1983), Ronnekleiv and Resko (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 polyfollicular 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, polyfollicular ovaries in prenatally androgenized female monkeys have approximately 60% greater volume than
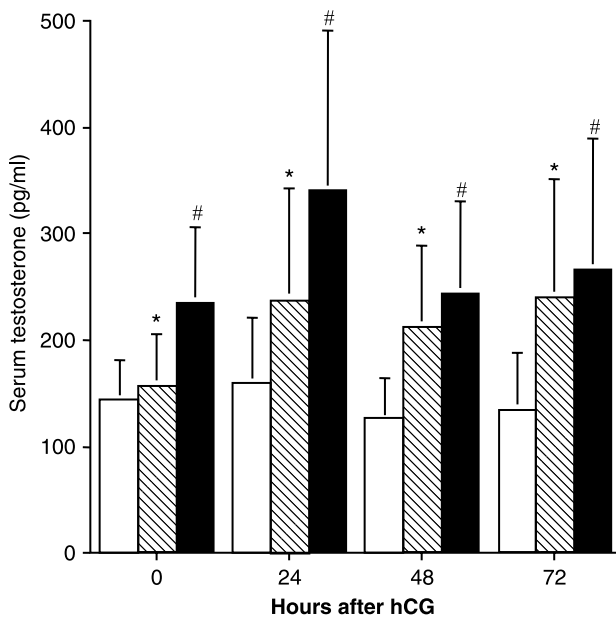

Figure 3. Mean ± SEM menstrual cycles in six early- and five late-treated prenatally androgenized (PA), and six control female rhesus monkeys during a 6 month period between the months of September–May. Menstrual cycles were determined from at least two serum progesterone values ≥1 ng/ml obtained 15 days or less before menses (Goy and Robinson, 1982; Dumesic *et al.*, 1997). Data from the months of June–August are omitted to avoid the seasonal time of increased incidence of amenorrhoea (Resko *et al.*, 1982). *$P < 0.04$ and #$P < 0.02$, early- and late-treated PA females versus controls, respectively, as determined by ANOVA. Control females = open bars; early-treated PA females = hatched bars; late-treated PA females = solid bars.

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

Figure 2. Serum testosterone levels following a 200 IU i.m. injection of rHCG at 0 h in five early- and five late-treated prenatally androgenized (PA), and six control female rhesus monkeys. Data are shown as backtransformed means + 95% confidence intervals. *$P < 0.05$ and #$P < 0.01$, early- and late-treated PA females versus controls for all times combined, respectively, as determined by ANOVA. Data from early-treated PA females and control females are modified from Eisner *et al.*, 2002. Control females = open bars; early-treated PA females = hatched bars; late-treated PA females = solid bars.
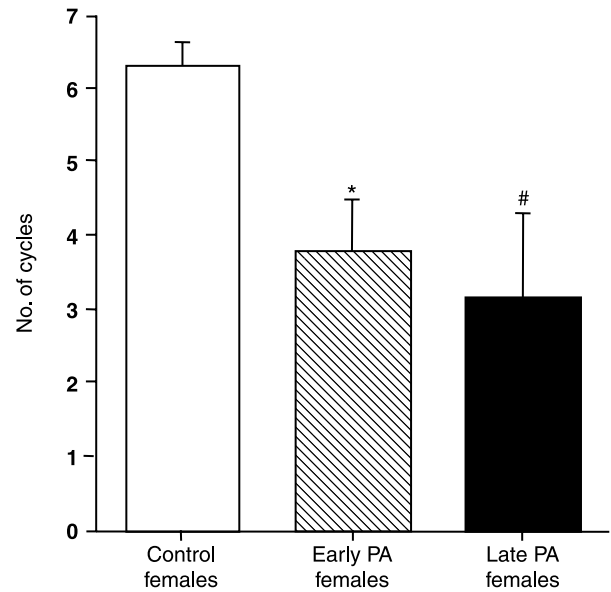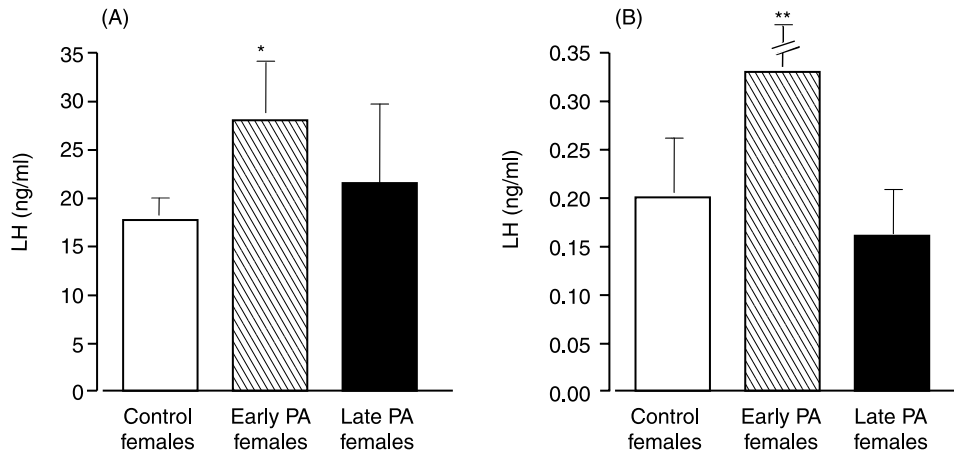
Figure 4. Elevated circulating LH levels in early-treated prenatally androgenized (PA) and control rhesus monkeys after (**A**) immuno-determination from control ($n = 24$), early-treated PA ($n = 10$) and late-treated PA ($n = 3$) females; *$P < 0.001$ versus controls by ANOVA, data modified from Dumesic *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.

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 hypothalamic–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 E_2 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 E_2 (Figure 6).

Steiner *et al.* (1976) achieved similar results when treating gonadectomized rhesus monkeys with exogenous E_2 replacement. Ovariectomized, early-treated androgenized females and orchidectomized males required E_2 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 E_2 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 E_2 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
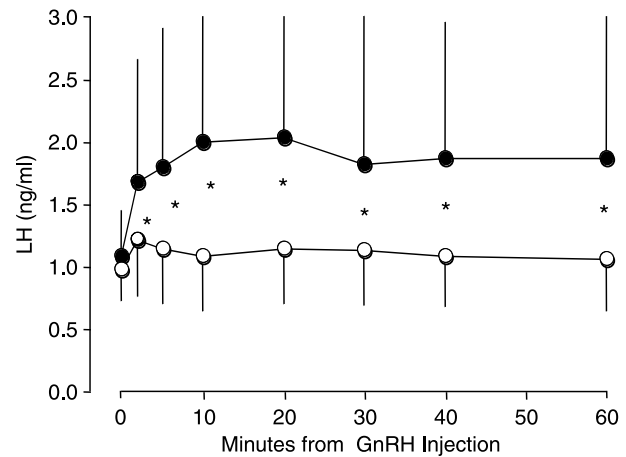

Figure 5. Increased pituitary gonadotrope immuno-LH responsiveness in six early-treated prenatally androgenized (PA) female rhesus monkeys at all timepoints (*$P < 0.05$ versus 0 min by ANOVA) after i.v. injection of GnRH compared to six control females. Data are shown as backtransformed means + 95% confidence intervals. Control females = open circles; early-treated PA females = solid circles.
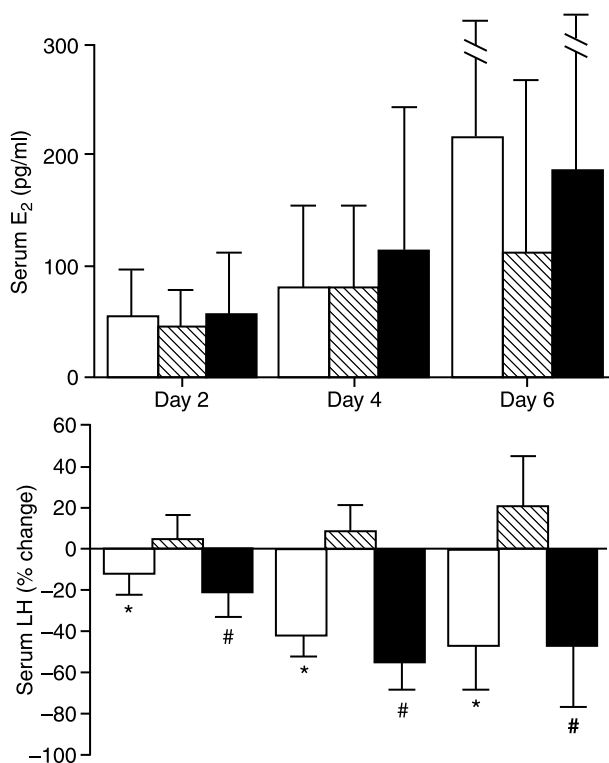
Figure 6. Normal serum E_2 response and reduced ovarian-mediated negative feedback of LH in five early-treated prenatally androgenized (PA) female rhesus monkeys (hatched bars) during rhFSH induced ovarian stimulation for IVF, as compared to five control (open bars) and four late-treated prenatally androgenized (solid bars) females. *$P < 0.03$ versus early-treated PA females, all times combined, #$P < 0.02$ versus early-treated PA females, all times combined, as determined by ANOVA. Data are shown as backtransformed means $+$ 95% confidence intervals.

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 E_2 and androstenedione occur following rhFSH therapy alone, with the follicular fluid progesterone/E_2 ratio being normal in response to rhFSH therapy followed by rHCG administration (Dumesic et al., 2002, 2003). Consistent with the ability of E_2 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 E_2 and androstenedione occur after both types of stimulation protocol, and are further accompanied by an elevated progesterone/E_2 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 blastocoels. Such impaired embryonic development may be related to both the absolute amount of E_2 and the progesterone to E_2 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 steroiodogenesis in follicles containing mature oocytes that fertilize and develop to blastocysts. In these follicles, either the ratio of progesterone to E_2 (Figure 7) is increased in early-treated females, or the E_2 level relative to the progesterone/E_2 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 E_2 ratio versus E_2 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 andogen 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 E_2 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).
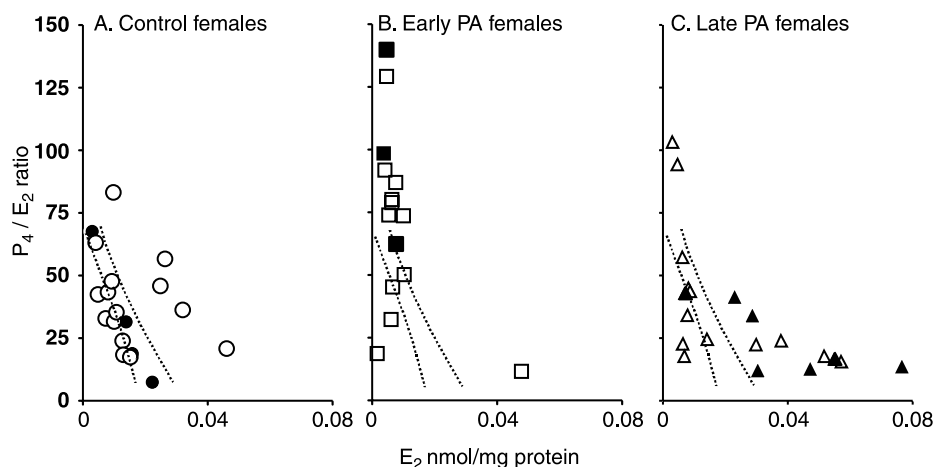
Figure 7. Negative associations between follicular fluid progesterone to E_2 (P_4/E_2) ratio and E_2 levels (nmol/mg protein) related to embryo/oocyte development in (**A**) 19 follicles from control female rhesus monkeys, (**B**) 14 follicles from early-treated prenatally androgenized females, (**C**) 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.

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.

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 experimentally-induced prenatal androgen programming in monkeys to entrain variable organ systems *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 primate 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.

Acknowledgements

References

Abbott DH and Hearn JP (1978) The effects of neonatal exposure to testosterone on the development of behaviour in female marmoset monkeys. Ciba Found Symp,299–327.

Abbott DH, Dumesic DA, Eisner JW, Kemnitz JW and Goy RW (1997) The prenatally androgenized female rhesus monkey as a model for polycystic ovarian syndrome. In Azziz R, Nestler JE, and Dewailly D (eds) Androgen Excess Disorders in Women. Lippencott-Raven Press, Philadelphia, pp 369–382.

Abbott DH, Dumesic DA, Eisner JR, Colman RJ and Kemnitz JW (1998) Insights into the development of PCOS from studies of prenatally androgenized female rhesus monkeys. Trends Endocrinol Metab 9,62–67.

Abbott DH, Dumesic DA and Franks S (2002a) Developmental origin of polycystic ovary syndrome—a hypothesis. J Endocrinol 174,1–5.

Abbott DH, Eisner JR, Colman RJ, Kemnitz JW and Dumesic DA (2002b) Prenatal androgen excess programs for PCOS in female rhesus monkeys. In Chang RJ, Dunaif A, and Hiendel J (eds) Polycystic Ovary Syndrome. Marcel Dekker, Inc., New York, pp 119–133.

Abbott DH, Eisner JR, Goodfriend TL, Medley RD, Peterson EJ, Colman Ricki J, Kemnitz JW and Dumesic DA (2002c) Leptin and total free fatty acids are elevated in the circulation of prenatally androgenized female rhesus monkeys. 84th Annual Meeting of the Endocrine Society, San Francisco, CA, Abstract #P2-329.

Abbott DH, Bruns CM, Barnett DK, Zhou R, Colman RJ, Kemnitz JW, Padmanabhan V, Goodfriend TL and Dumesic DA (2003) Metabolic and reproductive consequences of prenatal testosterone exposure. 85th Annual Meeting of the Endocrine Society, Philadelphia, PA, Abstract #S34-1.

Abbott DH, Foong SC, Barnett DK and Dumesic DA (2004) Nonhuman primates contribute unique understanding to anovulatory infertility in women. ILAR J 45,116–131.

Abbott DH, Dobbert MJW, Levine JE, Dumesic DA, Tarantal AF (2005) Androgen excess induces fetal programming of LH hypersecretion in a female rhesus monkey model for polycystic ovary syndrome (PCOS). Abstract #OR28-2. Presented at the 87th Annual Meeting to the Endocrine Society, San Diego, June 4–7, 2005

Adams J, Polson DW and Franks S (1986) Prevalence of polycystic ovaries in women with anovulation and idiopathic hirsutism. Br Med J 293, 355–359.

Adams JM, Taylor AE, Crowley WF Jr and Hall JE (2004) Polycystic ovarian morphology with regular ovulatory cycles: insights into the pathophysiology of polycystic ovarian syndrome. J Clin Endocrinol Metab 89,4343–4350.

Agrawal R, Sharma S, Bekir J, Conway G, Bailey J, Balen AH and Prelevic G (2004) Prevalence of polycystic ovaries and polycystic ovary syndrome in lesbian women compared with heterosexual women. Fertil Steril 82,1352–1357.

Arai Y and Gorski RA (1968a) Critical exposure time for androgenization of the developing hypothalamus in the female rat. Endocrinology 82, 1010–1014.

Arai Y and Gorski RA (1968b) Protection against the neural organizing effect of exogenous androgen in the neonatal female rat. Endocrinology 82,1005–1009.

Arai Y, Yamanouchi K, Mizukami S, Yanai R, Shibata K and Nagasawa H (1981) Induction of anovulatory sterility by neonatal treatment with 5 beta-dihydrotestosterone in female rats. Acta Endocrinol (Copenh) 96, 439–443.

Arslanian SA and Witchell S (2002) Premature pubarche, insulin resistance, and adolescent polycystic ovary syndrome. In Chang RJ, Heindel JJ, and Dunaif A (eds) Polycystic Ovary Syndrome. Marcel Dekker, Inc., New York, pp 37–53.

Arslanian SA, Lewy VD and Danadian K (2001) Glucose intolerance in obese adolescents with polycystic ovary syndrome: roles of insulin resistance and beta-cell dysfunction and risk of cardiovascular disease. J Clin Endocrinol Metab 86,66–71.

Asuncion M, Calvo RM, San Millan JL, Sancho J, Avila S and Escobar-Morreale HF (2000) A prospective study of the prevalence of the polycystic ovary syndrome in unselected Caucasian women from Spain. J Clin Endocrinol Metab 85,2434–2438.

Auger AP, Perrot-Sinal TS, Auger CJ, Ekas LA, Tetel MJ and McCarthy MM (2002) Expression of the nuclear receptor coactivator, cAMP response element-binding protein, is sexually dimorphic and modulates sexual differentiation of neonatal rat brain. Endocrinology 143, 3009–3016.

Azziz R, Black V, Hines GA, Fox LM and Boots LR (1998) Adrenal androgen excess in the polycystic ovary syndrome: sensitivity and responsivity of the hypothalamic–pituitary–adrenal axis. J Clin Endocrinol Metab 83,2317–2323.

Azziz R, Woods KS, Reyna R, Key TJ, Knochenhauer ES and Yildiz BO (2004) The prevalence and features of the polycystic ovary syndrome in an unselected population. J Clin Endocrinol Metab 89,2745–2749.

Baillargeon JP, Jakubowicz DJ, Iuorno MJ, Jakubowicz S and Nestler JE (2004) Effects of metformin and rosiglitazone, alone and in combination, in nonobese women with polycystic ovary syndrome and normal indices of insulin sensitivity. Fertil Steril 82,893–902.

Baird DT, Corker CS, Davidson DW, Hunter WM, Michie EA and Van Look PF (1977) Pituitary–ovarian relationships in polycystic ovary syndrome. J Clin Endocrinol Metab 45,798–801.

Baker TG (1966) A quantitative and cytological study of oogenesis in the rhesus monkey. J Anat 100,761–776.

Barbieri RL, Saltzman DH, Torday JS, Randall RW, Frigoletto FD and Ryan KJ (1986) Elevated concentrations of the beta-subunit of human chorionic gonadotropin and testosterone in the amniotic fluid of gestations of diabetic mothers. Am J Obstet Gynecol 154, 1039–1043.

Bardoni B, Zanaria E, Guioli S, Floridia G, Worley KC, Tonini G, Ferrante E, Chiumello G, McCabe ER, Fraccaro M et al. (1994) A dosage sensitive locus at chromosome Xp21 is involved in male to female sex reversal. Nat Genet 7,497–501.

Barnes RB, Rosenfield RL, Ehrmann DA, Cara JF, Cuttler L, Levitsky LL and Rosenthal IM (1994) Ovarian hyperandrogynism as a result of congenital adrenal virilizing disorders: evidence for perinatal masculinization of neuroendocrine function in women. J Clin Endocrinol Metab 79,1328–1333.

Barnett DK and Abbott DH (2003) Reproductive adaptations to a large-brained fetus open a vulnerability to anovulation similar to polycystic ovary syndrome. Am J Hum Biol 15,296–319.

Barraclough CA (1961) Production of anovulatory, sterile rats by single injections of testosterone propionate. Endocrinology 68,62–67.

Barraclough CA and Gorski RA (1961) Evidence that the hypothalamus is responsible for androgen-induced sterility in the female rat. Endocrinology 68,68–79.

Barraclough CA and Leathem JH (1954) Infertility induced in mice by a single injection of testosterone propionate. Proc Soc Exp Biol Med 85,673–674.

Baum MJ (1979) Differentiation of coital behavior in mammals: a comparative analysis. Neurosci Biobehav Rev 3,265–284.

Beach FA, Buehler MG and Dunbar IF (1983) Sexual cycles in female dogs treated with androgen during development. Behav Neural Biol 38,1–31.

Beck-Peccoz P, Padmanabhan V, Baggiani AM, Cortelazzi D, Buscaglia M, Medri G, Marconi AM, Pardi G and Beitins IZ (1991) Maturation of hypothalamic–pituitary–gonadal function in normal human fetuses: circulating levels of gonadotropins, their common alpha-subunit and free testosterone, and discrepancy between immunological and biological activities of circulating follicle-stimulating hormone. J Clin Endocrinol Metab 73,525–532.

Ben-Chetrit A and Greenblatt EM (1995) Recurrent maternal virilization during pregnancy associated with polycystic ovarian syndrome: a case report and review of the literature. Hum Reprod 10,3057–3060.

Bilowus M, Abbassi V and Gibbons (1986) Female pseudohermaphroditism in a neonate born to a mother with polycystic ovarian disease. J Urol 136,1098–1100.

Birch RA, Padmanabhan V, Foster DL, Unsworth WP and Robinson JE (2003) Prenatal programming of reproductive neuroendocrine function: fetal androgen exposure produces progressive disruption of reproductive cycles in sheep. Endocrinology 144,1426–1434.

Bonser J, Walker J, Purohit A, Reed MJ, Potter BV, Willis DS, Franks S and Mason HD (2000) Human granulosa cells are a site of sulphatase activity and are able to utilize dehydroepiandrosterone sulphate as a precursor for oestradiol production. J Endocrinol 167,465–471.

Bosinski HA, Peter M, Bonatz G, Arndt R, Heidenreich M, Sippell WG and Wille R (1997) A higher rate of hyperandrogenic disorders in female-to-male transsexuals. Psychoneuroendocrinology 22,361–380.

Brettenthaler N, De Geyter C, Huber PR and Keller U (2004) Effect of the insulin sensitizer pioglitazone on insulin resistance, hyperandrogenism, and ovulatory dysfunction in women with polycystic ovary syndrome. J Clin Endocrinol Metab 89,3835–3840.

Brown-Grant K and Sherwood MR (1971) The "early androgen syndrome" in the guinea-pig. J Endocrinol 49,277–291.

Buhl AE, Norman RL and Resko JA (1978) Sex differences in estrogen-induced gonadotropin release in hamsters. Biol Reprod 18,592–597.

Carani C, Qin K, Simoni M, Faustini-Fustini M, Serpente S, Boyd J, Korach KS and Simpson ER (1997) Effect of testosterone and estradiol in a man with aromatase deficiency. N Engl J Med 337,91–95.

Carmina E and Lobo RA (2001) Polycystic ovaries in Hirsute women with normal menses. Am J Med 111,602–606.

Carter CS, Clemens LG and Hoekema DJ (1972) Neonatal androgen and adult sexual behavior in the golden hamster. Physiol Behav 9,89–95.

Chang RJ (2002) Polycystic ovary syndrome: diagnostic criteria. In Chang RJ, Heindel JJ, and Dunaif A (eds) Polycystic Ovary Syndrome. Marcel Dekker, Inc., New York, pp 361–365.

Clarke IJ (1977) The sexual behaviour of prenatally androgenized ewes observed in the field. J Reprod Fertil 49,311–315.

Clarke IJ and Scaramuzzi RJ (1978) Sexual behaviour and LH secretion in spayed androgenized ewes after a single injection of testosterone or oestradiol-17beta. J Reprod Fertil 52,313–320.

Clarke IJ, Scaramuzzi RJ and Short RV (1976a) Effects of testosterone implants in pregnant ewes on their female offspring. J Embryol Exp Morphol 36,87–99.

Clarke IJ, Scaramuzzi RJ and Short RV (1976b) Sexual differentiation of the brain: endocrine and behavioural responses of androgenized ewes to oestrogen. J Endocrinol 71,175–176.

Clarke IJ, Scaramuzzi RJ and Short RV (1977) Ovulation in prenatally androgenized ewes. J Endocrinol 73,385–389.

Coffey S and Mason H (2003) The effect of polycystic ovary syndrome on health-related quality of life. Gynecol Endocrinol 17,379–386.

Cooke B, Hegstrom CD, Villeneuve LS and Breedlove SM (1998) Sexual differentiation of the vertebrate brain: principles and mechanisms. Front Neuroendocrinol 19,323–362.

Cresswell JL, Barker DJ, Osmond C, Egger P, Phillips DI and Fraser RB (1997) Fetal growth, length of gestation, and polycystic ovaries in adult life. Lancet 350,1131–1135.

De Bellis A, Quigley CA, Marschke KB, el-Awady MK, Lane MV, Smith EP, Sar M, Wilson EM and French FS (1994) Characterization of mutant androgen receptors causing partial androgen insensitivity syndrome. J Clin Endocrinol Metab 78,513–522.

DeFronzo RA (1992) Pathogenesis of type 2 (non-insulin dependent) diabetes mellitus: a balanced overview. Diabetologia 35,389–397.

DeHaan KC, Berger LL, Kesler DJ, McKeith FK, Faulkner DB and Cmarik GF (1988) Effect of prenatal androgenization on growth performance and carcass characteristics of steers and heifers. J Anim Sci 66, 1864–1870.

Dewailly D, Cortet-Rudelli C and Deroubaix-Allard D (1997) Definition, clinical manifestations, and prevalence of PCOS. In Azziz R, Nestler JE, and Dewailly D (eds) Androgen Excess Disorders in Women. Lippincott-Raven Publishers, Philadelphia, PA, pp 259–268.

Diamanti-Kandarakis E, Kouli CR, Bergiele AT, Filandra FA, Tsianateli TC, Spina GG, Zapanti ED and Bartzis MI (1999) A survey of the polycystic ovary syndrome in the Greek island of Lesbos: hormonal and metabolic profile. J Clin Endocrinol Metab 84,4006–4011.

Dittmann RW, Kappes ME and Kappes MH (1992) Sexual behavior in adolescent and adult females with congenital adrenal hyperplasia. Psychoneuroendocrinology 17,153–170.

Dor J, Shulman A, Levran D, Ben-Rafael Z, Rudak E and Mashiach S (1990) The treatment of patients with polycystic ovarian syndrome by in-vitro fertilization and embryo transfer: a comparison of results with those of patients with tubal infertility. Hum Reprod 5,816–818.

Dumesic DA (1995) Hyperandrogenic anovulation: a new view of polycystic ovary syndrome. Postgrad Obstet Gynecol 15,1–6.

Dumesic DA, Abbott DH, Eisner JR and Goy RW (1997) Prenatal exposure of female rhesus monkeys to testosterone propionate increases serum luteinizing hormone levels in adulthood. Fertil Steril 67,155–163.

Dumesic DA, Schramm RD, Peterson E, Paprocki AM, Zhou R and Abbott DH (2002) Impaired developmental competence of oocytes in adult prenatally androgenized female rhesus monkeys undergoing gonadotropin stimulation for in vitro fertilization. J Clin Endocrinol Metab 87, 1111–1119.

Dumesic DA, Schramm RD, Bird IM, Peterson E, Paprocki AM, Zhou R and Abbott DH (2003) Reduced intrafollicular androstenedione and estradiol levels in early-treated prenatally androgenized female rhesus monkeys receiving FSH therapy for in vitro fertilization. Biol Reprod 69, 1213–1219.

Dumesic DA, Schramm RD and Abbott DH (2005) Early origins of polycystic ovary syndrome (PCOS). Reprod Fertil Dev 17,349–360.

Dunaif A (1997) Insulin resistance and the polycystic ovary syndrome: mechanism and implications for pathogenesis. Endocr Rev 18,774–800.

Dunaif A, Hoffman AR, Scully RE, Flier JS, Longcope C, Levy LJ and Crowley WF Jr (1985) Clinical, biochemical, and ovarian morphologic features in women with acanthosis nigricans and masculinization. Obstet Gynecol 66,545–552.

Dunaif A, Graf M, Mandeli J, Laumas V and Dobrjansky A (1987) Characterization of groups of hyperandrogenic women with acanthosis nigricans, impaired glucose tolerance, and/or hyperinsulinemia. J Clin Endocrinol Metab 65,499–507.

Dunaif A, Scott D, Finegood D, Quintana B and Whitcomb R (1996) The insulin-sensitizing agent troglitazone improves metabolic and reproductive abnormalities in the polycystic ovary syndrome. J Clin Endocrinol Metab 81,3299–3306.

Eagleson CA, Bellows AB, Hu K, Gingrich MB and Marshall JC (2003) Obese patients with polycystic ovary syndrome: evidence that metformin does not restore sensitivity of the gonadotropin-releasing hormone pulse generator to inhibition by ovarian steroids. J Clin Endocrinol Metab 88,5158–5162.

Edwards DA (1971) Neonatal administration of androstenedione, testosterone or testosterone propionate: effects on ovulation, sexual receptivity and aggressive behavior in female mice. Physiol Behav 6,223–228.

Ehrhardt AA, Meyer-Bahlburg HF, Rosen LR, Feldman JF, Veridiano NP, Zimmerman I and McEwen BS (1985) Sexual orientation after prenatal exposure to exogenous estrogen. Arch Sex Behav 14,57–77.

Ehrmann DA, Barnes RB and Rosenfield RL (1995) Polycystic ovary syndrome as a form of functional ovarian hyperandrogenism due to dysregulation of androgen secretion. Endocr Rev 16,322–353.

Eisner JR, Dumesic DA, Kemnitz JW and Abbott DH (2000) Timing of prenatal androgen excess determines differential impairment in insulin secrection and action in adult female rhesus monkeys. J Clin Endocrinol Metab 85,1206–1210.

Eisner JR, Barnett MA, Dumesic DA and Abbott DH (2002) Ovarian hyperandrogenism in adult female rhesus monkeys exposed to prenatal androgen excess. Fertil Steril 77,167–172.

Eisner JR, Dumesic DA, Kemnitz JW, Colman RJ and Abbott DH (2003) Increased adiposity in female rhesus monkeys exposed to androgen excess during early gestation. Obes Res 11,279–286.

Ellinwood WE, McClellan MC, Brenner RM and Resko JA (1983) Estradiol synthesis by fetal monkey ovaries correlates with antral follicle formation. Biol Reprod 28,505–516.

Elsenbruch S, Hahn S, Kowalsky D, Offner AH, Schedlowski M, Mann K and Janssen OE (2003) Quality of life, psychosocial well-being, and sexual satisfaction in women with polycystic ovary syndrome. J Clin Endocrinol Metab 88,5801–5807.

Eppig JJ, O'Brien MJ, Pendola FL and Watanabe S (1998) Factors affecting the developmental competence of mouse oocytes grown in vitro: follicle stimulating hormone and insulin. Biol Reprod 59,1445–1453.

Escobar-Morreale HF, Luque-Ramirez M and San Millan JL (2005) The molecular-genetic basis of functional hyperandrogenism and the polycystic ovary syndrome. Endocr Rev 26,252–282.

Escobar-Morreale HF, Botella-Carretero JI, Villuendas G, Sancho J and San Millan JL (2004) Serum interleukin-18 concentrations are increased in the polycystic ovary syndrome: relationship to insulin resistance and to obesity. J Clin Endocrinol Metab 89,806–811.

Fayez JA, Bunch TR and Miller GL (1974) Virilization in pregnancy associated with polycystic ovary disease. Obstet Gynecol 44,511–521.

Fels E and Bosch LR (1971) Effect of prenatal administration of testosterone on ovarian function in rats. Am J Obstet Gynecol 111,964–969.

Fischer DA (2003) Endocrinology of fetal development. In Larsen PR, Kronenberg HM, Melmed S, and Polonsky KS (eds) Williams Textbook of Endocrinology. 10th edn. WB Saunders, Philadelphia, PA, pp 811–841.

Foecking EM, Szabo M, Schwartz NB, Levine JE (2004) Neuroendocrine consequences of prenatal androgen exposure in the female rat; absence of luteinizing hormone surges, suppression of progesterone receptor gene expression, and acceleration of the gonadotropin-releasing hormone pulse generator. Biol Reprod 72,1475–1483.

Fogel RB, Malhotra A, Pillar G, Pittman SD, Dunaif A and White DP (2001) Increased prevalence of obstructive sleep apnea syndrome in obese women with polycystic ovary syndrome. J Clin Endocrinol Metab 86, 1175–1180.

Ford JJ and Christenson RK (1987) Influences of pre- and postnatal testosterone treatment on defeminization of sexual receptivity in pigs. Biol Reprod 36,581–587.

Foster DL, Padmanabhan V, Wood RI and Robinson JE (2002) Sexual differentiation of the neuroendocrine control of gonadotrophin secretion: concepts derived from sheep models. Reprod Suppl 59,83–99.

Franks S (1995) Polycystic ovary syndrome. N Engl J Med 333,853–861.

Franks S and McCarthy M (2004) Genetics of ovarian disorders: polycystic ovary syndrome. Rev Endocr Metab Disord 5,69–76.

Gaasenbeek M, Powell BL, Sovio U, Haddad L, Gharani N, Bennett A, Groves CJ, Rush K, Goh MJ, Conway GS et al. (2004) Large-scale analysis of the relationship between CYP11A promoter variation, polycystic ovarian syndrome, and serum testosterone. J Clin Endocrinol Metab 89, 2408–2413.

Geisthovel F (2003) A comment on the European Society of Human Reproduction and Embryology/American Society for Reproductive Medicine consensus of the polycystic ovarian syndrome. Reprod Biomed Online 7,602–605.

Gorski RA (1968) Influence of age on the response to paranatal administration of a low dose of androgen. Endocrinology 82,1001–1004.

Gorzynski G and Katz JL (1977) The polycystic ovary syndrome:psychosexual correlates. Arch Sex Behav 6,215–222.

Goy RW and Deputte BL (1996) The effects of diethylstilbestrol (DES) before birth on the development of masculine behavior in juvenile female rhesus monkeys. Horm Behav 30,379–386.

Goy RW and Kemnitz JW (1983) Early, persistent, and delayed effects of virilizing substances delivered transplacentally to female rhesus fetuses. In Zbinden G, Cuomo V, Racagni G, and Weiss B (eds) Application of Behavioral Pharmacology in Toxicology. Raven Press, New York, pp 303–314.

Goy RW and Resko JA (1972) Gonadal hormones and behavior of normal and pseudohermaphroditic nonhuman female primates. Recent Prog Horm Res 28,707–733.

Goy RW and Robinson JA (1982) Prenatal exposure of rhesus monkeys to patent androgens: morphological, behavioral, and physiological consequences. In Banbury Report II: Environmental Factors in Human Growth and Development. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp 355–378.

Goy RW, Bercovitch FB and McBrair MC (1988a) Behavioral masculinization is independent of genital masculinization in prenatally androgenized female rhesus macaques. Horm Behav 22, 552–571.

Goy RW, Uno H and Sholl SA (1988b) Psychological and anatomical consequences of prenatal exposure to androgens in female rhesus. In Mori T and Nagasawa H (eds) Toxicity of Hormones in Perinatal Life. CRC Press, Inc., Boca Raton, Florida, pp 127–142.

Grady KL, Phoenix CH and Young WC (1965) Role of the developing rat testis in differentiation of the neural tissues mediating mating behavior. J Comp Physiol Psychol 59,176–182.

Greene RR, Burrell MW and Ivy AC (1939) Experimental intersexuality. The effect of antenatal androgens on sexual development in female rats. Am J Anat 65,415–469.

Grumbach MM and Ducharme, Jr (1960) The effects of androgens on fetal sexual development: androgen-induced female pseudohermaphrodism. Fertil Steril 11,157–180.

Hall CM, Jones JA, Meyer-Bahlburg HF, Dolezal C, Coleman M, Foster P, Price DA and Clayton PE (2004) Behavioral and physical masculinization are related to genotype in girls with congenital adrenal hyperplasia. J Clin Endocrinol Metab 89,419–424.

Harris GW and Jacobsohn D (1951) Functional grafts of the anterior pituitary gland. J Physiol 113,35–36.

Hart R, Hickey M and Franks S (2004) Definitions, prevalence and symptoms of polycystic ovaries and polycystic ovary syndrome. Best Pract Res Clin Obstet Gynaecol 18,671–683.

Hickey T, Chandy A and Norman RJ (2002) The androgen receptor CAG repeat polymorphism and X-chromosome inactivation in Australian Caucasian women with infertility related to polycystic ovary syndrome. J Clin Endocrinol Metab 87,161–165.

Hines M, Brook C and Conway GS (2004) Androgen and psychosexual development: core gender identity, sexual orientation and recalled childhood gender role behavior in women and men with congenital adrenal hyperplasia (CAH). J Sex Res 41,75–81.

Hoar RM and Monie IW (1981) Comparative development of specific organ systems. In Kimmel CA and Buelke-Sam J (eds) Developmental Toxicology. Raven Press, New York, pp 13–33.

Hodges JK and Hearn JP (1978) A positive feedback effect of oestradiol on LH release in the male marmoset monkey, *Callithrix jacchus*. J Reprod Fertil 52,83–86.

Homburg R, Armar NA, Eshel A, Adams J and Jacobs HS (1988) Influence of serum luteinising hormone concentrations on ovulation, conception, and early pregnancy loss in polycystic ovary syndrome. Br Med J 297,1024–1026.

Huber-Buchholz MM, Carey DG and Norman RJ (1999) Restoration of reproductive potential by lifestyle modification in obese polycystic ovary syndrome: role of insulin sensitivity and luteinizing hormone. J Clin Endocrinol Metab 84,1470–1474.

Huffman L and Hendricks SE (1981) Prenatally injected testosterone propionate and sexual behavior of female rats. Physiol Behav 26,773–778.

Jones HM, Vernon MW and Rush ME (1987) Systematic studies invalidate the neonatally androgenized rat as a model for polycystic ovary disease. Biol Reprod 36,1253–1265.

Josso N, Cate RL, Picard JY, Vigier B, di Clemente N, Wilson C, Imbeaud S, Pepinsky RB, Guerrier D, Boussin L et al. (1993) Anti-mullerian hormone: the Jost factor. Recent Prog Horm Res 48,1–59.

Jost A (1970) Hormonal factors in the sex differentiation of the mammalian foetus. Philos Trans R Soc Lond B Biol Sci 259,119–130.

Karsch FJ, Dierschke DJ and Knobil E (1973) Sexual differentiation of pituitary function: apparent difference between primates and rodents. Science 179,484–486.

Katz M and Carr PJ (1976) Abnormal luteinizing hormone response patterns to synthetic gonadotrophin releasing hormone in patients with polycystic ovarian syndrome. J Endocrinol 70,163–171.

Keisler LW, Vom Saal FS, Keisler DH, Walker SE (1991) Hormonal manipulation of the prenatal environment alters reproductive morphology and increases longevity in autoimmune NZB/W mice. Biol Reprod 44,707–716.

Kiddy DS, Hamilton-Fairley D, Bush A, Short F, Anyaoku V, Reed MJ and Franks S (1992) Improvement in endocrine and ovarian function during dietary treatment of obese women with polycystic ovary syndrome. Clin Endocrinol (Oxf) 36,105–111.

Kitzinger C and Willmott J (2002) 'The thief of womanhood: women's experience of polycystic ovarian syndrome. Soc Sci Med 54,349–361.

Knobil E and Hotchkiss J (1994) The menstrual cycle and its neuroendocrine control. In Knobil E, Neill J, Ewing LL, Greenwald GS, Markert CL, and Pfaff DW (eds) The Physiology of Reproduction, Vol 2. Raven Press, New York, pp 1971–1994.

Knudsen JF and Mahesh VB (1975) Initiation of precocious sexual maturation in the immature rat treated with dehydroepiandrosterone. Endocrinology 97,458–468.

Kreiner D, Liu HC, Itskovitz J, Veeck L and Rosenwaks Z (1987) Follicular fluid estradiol and progesterone are markers of preovulatory oocyte quality. Fertil Steril 48,991–994.

Ladosky W, Noronha JG and Gaziri IF (1969) Luteinization of ovarian grafts in rats related to treatment with testosterone in the neonatal period. J Endocrinol 43,253–258.

Lasala C, Carre-Eusebe D, Picard JY and Rey R (2004) Subcellular and molecular mechanisms regulating anti-Mullerian hormone gene expression in mammalian and nonmammalian species. DNA Cell Biol 23,572–585.

Lee MM and Donahoe PK (1993) Mullerian inhibiting substance: a gonadal hormone with multiple functions. Endocr Rev 14,152–164.

Legro RS (1998) Insulin resistance in polycystic ovary syndrome: treating a phenotype without a genotype. Mol Cell Endocrinol 145,103–110.

Legro RS (2003) Diagnostic criteria in polycystic ovary syndrome. Semin Reprod Med 21,267–275.

Legro RS and Strauss JF (2003) Molecular progress in infertility: polycystic ovary syndrome. Fertil Steril 78,569–576.

Legro RS, Driscoll D, Strauss JF, III, Fox J and Dunaif A (1998a) Evidence for a genetic basis for hyperandrogenemia in polycystic ovary syndrome. Proc Natl Acad Sci USA 95,14956–14960.

Legro RS, Spielman R, Urbanek M, Driscoll D, Strauss JF, III and Dunaif A (1998b) Phenotype and genotype in polycystic ovary syndrome. Recent Prog Horm Res 53,217–256.

Legro RS, Kunselman AR, Dodson WC and Dunaif A (1999) Prevalence and predictors of risk for type 2 diabetes mellitus and impaired glucose tolerance in polycystic ovary syndrome: a prospective, controlled study in 254 affected women. J Clin Endocrinol Metab 84,165–169.

Levine JE, Terasawa E, Hoffmann SM, Dobbert MJW, Foecking E, Abbott DH (2005) Luteinizing hormone (LH) hypersecretion and diminished LH Responses to RU486 in a nonhuman primate model for polycystic ovary syndrome (PCOS). Abstract #P1-85 presented at the 87th Annual Meeting to the Endocrine Society, San Diego, June 4–7, 2005.

Lobo RA, Granger LR, Paul WL, Goebelsmann U and Mishell DR Jr (1983) Psychological stress and increases in urinary norepinephrine metabolites, platelet serotonin, and adrenal androgens in women with polycystic ovary syndrome. Am J Obstet Gynecol 145,496–503.

Long DN, Wisniewski AB and Migeon CJ (2004) Gender role across development in adult women with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. J Pediatr Endocrinol Metab 17,1367–1373.

Lookingland KJ, Wise PM and Barraclough CA (1982) Failure of the hypothalamic noradrenergic system to function in adult androgen-sterilized rats. Biol Reprod 27,268–281.

Luttge WG and Whalen RE (1969) Partial defeminization by administration of androstenedione to neonatal female rats. Life Sci 8,1003–1008.

Luttge WG and Whalen RE (1970) Dihydrotestosterone, androstenedione, testosterone. Comparative effectiveness in masculinizing and defeminizing reproductive systems in male and female rats. Hormones Behav 2,1–10.

Magendantz HG, Jones DE and Schomberg DW (1972) Virilization during pregnancy associated with polycystic ovary disease. Obstet Gynecol 40,156–162.

Manikkam M, Crespi EJ, Doop DD, Herkimer C, Lee JS, Yu S, Brown MB, Foster DL and Padmanabhan V (2004) Fetal programming: prenatal testosterone excess leads to fetal growth retardation and postnatal catch-up growth in sheep. Endocrinology 145,790–798.

Marshall JC and Eagleson CA (1999) Neuroendocrine aspects of polycystic ovary syndrome. Endocrinol Metab Clin North Am 28,295–324.

Martinez C and Bittner JJ (1956) A nonhypophyseal sex difference in estrous behaviour of mice bearing pituitary grafts. Proc Soc Exp Biol Med 91, 506–509.

Masek KS, Wood RI and Foster DL (1999) Prenatal dihydrotestosterone differentially masculinizes tonic and surge modes of luteinizing hormone secretion in sheep. Endocrinology 140,3459–3466.

McCarthy MM (1994) Molecular aspects of sexual differentiation of the rodent brain. Psychoneuroendocrinology 19,415–427.

McClamrock HD and Adashi EY (1992) Gestational hyperandrogenism. Fertil Steril 57,257–274.

McDonald PG and Doughty C (1972) Comparison of the effect of neonatal administration of testosterone and dihydrotestosterone in the female rat. J Reprod Fertil 30,55–62.

McDonald PG and Doughty C (1973) Androgen sterilization in the neonatal female rat and its inhibition by an estrogen antagonist. Neuroendocrinology 13,182–188.

Merke DP and Cutler GB, Jr (2001) New ideas for medical treatment of congenital adrenal hyperplasia. Endocrinol Metab Clin North Am 30, 121–135.

Meyer-Bahlburg HF and Ehrhardt AA (1986) Prenatal diethylstilbestrol exposure: behavioral consequences in humans. Monogr Neural Sci 12, 90–95.

Money J, Schwartz M and Lewis VG (1984) Adult erotosexual status and fetal hormonal masculinization and demasculinization: 46,XX congenital virilizing adrenal hyperplasia and 46,XY androgen-insensitivity syndrome compared. Psychoneuroendocrinology 9,405–414.

Moran C, Reyna R, Boots LS and Azziz R (2004) Adrenocortical hyperresponsiveness to corticotropin in polycystic ovary syndrome patients with adrenal androgen excess. Fertil Steril 81,126–131.

Morishima A, Grumbach MM, Simpson ER, Fisher C and Qin K (1995) Aromatase deficiency in male and female siblings caused by a novel mutation and the physiological role of estrogens. J Clin Endocrinol Metab 80,3689–3698.

Negri-Cesi P, Colciago A, Celotti F and Motta M (2004) Sexual differentiation of the brain: role of testosterone and its active metabolites. J Endocrinol Invest 27 (Suppl 6),120–127.

Nelson-Degrave VL, Wickenheisser JK, Hendricks KL, Asano T, Fujishiro M, Legro RS, Kimball SR, Strauss JF 3rd and McAllister JM (2005) Alterations in MEK and ERK signaling in theca cells contribute to excessive androgen production in polycystic ovary syndrome (PCOS). Mol Endocrinol 19,379–390.

Nestler JE, Jakubowicz DJ, Evans WS and Pasquali R (1998) Effects of metformin on spontaneous and clomiphene-induced ovulation in the polycystic ovary syndrome. N Engl J Med 338,1876–1880.

New MI (1992) Female pseudohermaphroditism. Semin Perinatol 16, 299–318.

Norman RL and Spies HG (1986) Cyclic ovarian function in a male macaque: additional evidence for a lack of sexual differentiation in the physiological mechanisms that regulate the cyclic release of gonadotropins in primates. Endocrinology 118,2608–2610.

Ohta Y and Iguchi T (1977) Development of the vaginal epithelium showing estrogen-independent proliferation and cornification in neonatally androgenized mice. Endocrinol Jpn 23,333–340.

Pastor CL, Griffin-Korf ML, Aloi JA, Evans WS and Marshall JC (1998) Polycystic ovary syndrome: evidence for reduced sensitivity of the gonadotropin-releasing hormone pulse generator to inhibition by estradiol and progesterone. J Clin Endocrinol Metab 83,582–590.

Payne AH and Jaffe RB (1974) Androgen formation from pregnenolone sulfate by the human fetal ovary. J Clin Endocrinol Metab 39,300–304.

Peppard HR, Marfori J, Iuorno MJ and Nestler JE (2001) Prevalence of polycystic ovary syndrome among premenopausal women with type 2 diabetes. Diabetes Care 24,1050–1052.

Pfeiffer CA (1936) Sexual differences of the hypophyses and their determination by the gonads. Am J Anat 58,195–225.

Phocas I, Chryssikopoulos A, Sarandakou A, Rizos D and Trakakis E (1995) A contribution to the classification of cases of non-classic 21-hydroxylase-deficient congenital adrenal hyperplasia. Gynecol Endocrinol 9, 229–238.

Pinilla L, Trimino E, Garnelo P, Bellido C, Aguilar R, Gaytan F and Aguilar E (1993) Changes in pituitary secretion during the early postnatal period and anovulatory syndrome induced by neonatal oestrogen or androgen in rats. J Reprod Fertil 97,13–20.

Polishuk WZ and Anteby S (1971) Testosterone in premature babies and its effect on sexual development. Lancet 1,344.

Pomerantz SM, Roy MM, Thornton JE and Goy RW (1985) Expression of adult female patterns of sexual behavior by male, female, and pseudo-hermaphroditic female rhesus monkeys. Biol Reprod 33,878–889.

Pomerantz SM, Goy RW and Roy MM (1986) Expression of male-typical behavior in adult female pseudohermaphroditic rhesus: comparisons with normal males and neonatally gonadectomized males and females. Horm Behav 20,483–500.

Quanbeck C, Sherwood NM, Millar RP and Terasawa E (1997) Two populations of luteinizing hormone-releasing hormone neurons in the forebrain of the rhesus macaque during embryonic development. J Comp Neurol 380,293–309.

Quigley CA, De Bellis A, Marschke KB, el-Awady MK, Wilson EM and French FS (1995) Androgen receptor defects: historical, clinical, and molecular perspectives. Endocr Rev 16,271–321.

Raboch J, Kobilkova J and Starka L (1985) Sexual life of women with the Stein-Leventhal syndrome. Arch Sex Behav 14,263–270.

Rebar R, Judd HL, Yen SS, Rakoff J, Vandenberg G and Naftolin F (1976) Characterization of the inappropriate gonadotropin secretion in polycystic ovary syndrome. J Clin Invest 57,1320–1329.

Resko JA, Goy RW, Robinson JA and Norman RL (1982) The pubescent rhesus monkey: some characteristics of the menstrual cycle. Biol Reprod 27,354–361.

Resko JA, Buhl AE and Phoenix CH (1987) Treatment of pregnant rhesus macaques with testosterone propionate: observations on its fate in the fetus. Biol Reprod 37,1185–1191.

Rice S, Christoforidis N, Gadd C, Nikolaou D, Seyani L, Donaldson A, Margara R, Hardy K and Franks S (2005) Impaired insulin-dependent glucose metabolism in granulosa-lutein cells from anovulatory women with polycystic ovaries. Hum Reprod 20,373–382.

Rivas A, Fisher JS, McKinnell C, Atanassova N and Sharpe RM (2002) Induction of reproductive tract developmental abnormalities in the male rat by lowering androgen production or action in combination with a low dose of diethylstilbestrol: evidence for importance of the androgen–estrogen balance. Endocrinology 143,4797–4808.

Rivas A, McKinnell C, Fisher JS, Atanassova N, Williams K and Sharpe RM (2003) Neonatal coadministration of testosterone with diethylstilbestrol prevents diethylstilbestrol induction of most reproductive tract abnormalities in male rats. J Androl 24,557–567.

Rom E, Reich R, Laufer N, Lewin A, Rabinowitz R, Pevsner B, Lancet M, Shenker JG, Miskin R, Adelmann-Grill BC et al. (1987) Follicular fluid contents as predictors of success of in-vitro fertilization-embryo transfer. Hum Reprod 2,505–510.

Ronnekleiv OK and Resko JA (1990) Ontogeny of gonadotropin-releasing hormone-containing neurons in early fetal development of rhesus macaques. Endocrinology 126,498–511.

Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group (2004) Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod 19,41–47.

Sagle M, Bishop K, Ridley N, Alexander FM, Michel M, Bonney RC, Beard RW and Franks S (1988) Recurrent early miscarriage and polycystic ovaries. Br Med J 297,1027–1028.

San Millan JL, Corton M, Villuendas G, Sancho J, Peral B and Escobar-Morreale HF (2004) Association of the polycystic ovary syndrome with genomic variants related to insulin resistance, type 2 diabetes mellitus, and obesity. J Clin Endocrinol Metab 89,2640–2646.

Sarlis NJ, Weil SJ and Nelson LM (1999) Administration of metformin to a diabetic woman with extreme hyperandrogenemia of nontumoral origin: management of infertility and prevention of inadvertent masculinization of a female fetus. J Clin Endocrinol Metab 84,1510–1512.

Savabieasfahani M, Lee JS, Herkimer C, Sharma TP, Foster DL and Padmanabhan V (2005) Fetal programming: testosterone exposure of the female sheep during mid-gestation disrupts the dynamics of its adult gonadotropin secretion during the periovulatory period. Biol Reprod 72,221–229.

Sharma TP, Herkimer C, West C, Ye W, Birch R, Robinson JE, Foster DL and Padmanabhan V (2002) Fetal programming: prenatal androgen disrupts positive feedback actions of estradiol but does not affect timing of puberty in female sheep. Biol Reprod 66,924–933.

Short RV (1974) Sexual differentiation of the brain of the sheep. In Forest MG and Bertrand J (eds) Sexual endocrinology of the perinatal period, Colloque International INSERM, Lyon. INSERM, Paris, France pp. 121–142.

Simpson ER, Zhao Y, Agarwal VR, Michael MD, Bulun SE, Hinshelwood MM, Graham-Lorence S, Sun T, Fisher CR, Qin K and Mendelson CR (1997) Aromatase expression in health and disease. Recent Prog Horm Res 52,185–213.

Sinclair AH, Berta P, Palmer MS, Hawkins JR, Griffiths BL, Smith MJ, Foster JW, Frischauf AM, Lovell-Badge R and Goodfellow PN (1990) A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. Nature 346,240–244.

Sir-Petermann T, Maliqueo M, Angel B, Lara HE, Perez-Bravo F and Recabarren SE (2002) Maternal serum androgens in pregnant women with polycystic ovarian syndrome: possible implications in prenatal androgenization. Hum Reprod 17,2573–2579.

Slob AK, den Hamer R, Woutersen PJ and van der Werff ten Bosch JJ (1983) Prenatal testosterone propionate and postnatal ovarian activity in the rat. Acta Endocrinol (Copenh) 103,420–427.

Smith EP, Boyd J, Frank GR, Takahashi H, Cohen RM, Specker B, Williams TC, Lubahn DB and Korach KS (1994) Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man. N Engl J Med 331, 1056–1061.

Sperling MA (1994) Carbohydrate metabolism: insulin and glucagons. In Tulchinsky D and Little AB (eds) Maternal–Fetal Endocrinology. 2nd edn. WB Saunders, Philadelphia, PA, pp 380–400.

Stamets K, Taylor DS, Kunselman A, Demers LM, Pelkman CL and Legro RS (2004) A randomized trial of the effects of two types of short-term hypocaloric diets on weight loss in women with polycystic ovary syndrome. Fertil Steril 81,630–637.

Steiner RA, Clifton DK, Spies HG and Resko JA (1976) Sexual differentiation and feedback control of luteinizing hormone secretion in the rhesus monkey. Biol Reprod 15,206–212.

Stikkelbroeck NM, Hermus AR, Braat DD and Otten BJ (2003) Fertility in women with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Obstet Gynecol Surv 58,275–284.

Strauss JF III (2003) Some new thoughts on the pathophysiology and genetics of polycystic ovary syndrome. Ann NY Acad Sci 997,42–48.

Sullivan SD and Moenter SM (2004) Prenatal androgens alter GABAergic drive to gonadotropin-releasing hormone neurons: implications for a common fertility disorder. Proc Natl Acad Sci USA 101,7129–7134.

Swain A and Lovell-Badge R (1999) Mammalian sex determination: a molecular drama. Genes Dev 13,755–767.

Swanson HH (1966) Modification of the reproductive tract of hamsters of both sexes by neonatal administration of androgen or oestrogen. J Endocrinol 36,327–328.

Swanson HE and van der Werff ten Bosch JJ (1964a) The "early-androgen" syndrome; its development and the response to hemi-spaying. Acta Endocrinol (Copenh) 45,1–12.

Swanson HE and van der Werff ten Bosch JJ (1964b) The "early-androgen" syndrome; differences in response to pre-natal and post-natal administration of various doses of testosterone propionate in female and male rats. Acta Endocrinol (Copenh) 47,37–50.

Swanson HE and Werff ten Bosch JJ (1965) The "early-androgen" syndrome; effects of pre-natal testosterone propionate. Acta Endocrinol (Copenh) 50,379–390.

Tarlatzis BC, Grimbizis G, Pournaropoulos F, Bontis J, Lagos S, Spanos E and Mantalenakis S (1995) The prognostic value of basal luteinizing hormone:follicle-stimulating hormone ratio in the treatment of patients with polycystic ovarian syndrome by assisted reproduction techniques. Hum Reprod 10,2545–2549.

Taylor AE, McCourt B, Martin KA, Anderson EJ, Adams JM, Schoenfeld D and Hall JE (1997) Determinants of abnormal gonadotropin secretion in clinically defined women with polycystic ovary syndrome. J Clin Endocrinol Metab 82,2248–2256.

Tesarik J and Mendoza C (1995) Nongenomic effects of 17β-estradiol on maturing human oocytes: relationship to oocyte developmental potential. J Clin Endocrinol Metab 80,1438–1443.

Thornton JE (1983) Effects of Prenatal Androgens on Adult Ovarian Cyclicity and Female Sexual Behavior in the Rhesus Monkey. University of Wisconsin, Madison.

Thornton J and Goy RW (1986) Female-typical sexual behavior of rhesus and defeminization by androgens given prenatally. Horm Behav 20, 129–147.

Tommerup N, Schempp W, Meinecke P, Pedersen S, Bolund L, Brandt C, Goodpasture C, Guldberg P, Held KR, Reinwein H et al. (1993) Assignment of an autosomal sex reversal locus (SRA1) and campomelic dysplasia (CMPD1) to 17q24.3-q25.1. Nat Genet 4,170–174.

Treloar OL, Wolf RC and Meyer RK (1972) Failure of a single neonatal dose of testosterone to alter ovarian function in the Rhesus monkey. Endocrinology 90,281–284.

Tucci S, Futterweit W, Concepcion ES, Greenberg DA, Villanueva R, Davies TF and Tomer Y (2001) Evidence for association of polycystic ovary syndrome in caucasian women with a marker at the insulin receptor gene locus. J Clin Endocrinol Metab 86,446–449.

Urbanek M, Legro RS, Driscoll DA, Azziz R, Ehrmann DA, Norman RJ, Strauss JF, III, Spielman RS and Dunaif A (1999) Thirty-seven candidate genes for polycystic ovary syndrome: strongest evidence for linkage is with follistatin. Proc Natl Acad Sci USA 96,8573–8578.

Urbanek M, Wu X, Vickery KR, Kao LC, Christenson LK, Schneyer A, Legro RS, Driscoll DA, Strauss JF, III, Dunaif A and Spielman RS (2000) Allelic variants of the follistatin gene in polycystic ovary syndrome. J Clin Endocrinol Metab 85,4455–4461.

van Wagenen G (1949) Accelerated growth with sexual precocity in female monkeys receiving testosterone propionate. Endocrinology 45,544–546.

Villuendas G, Escobar-Morreale HF, Tosi F, Sancho J, Moghetti P and San Millan JL (2003) Association between the D19S884 marker at the insulin receptor gene locus and polycystic ovary syndrome. Fertil Steril 79,219–220.

Wagenknecht LE, Langefeld CD, Scherzinger AL, Norris JM, Haffner SM, Saad MF and Bergman RN (2003) Insulin sensitivity, insulin secretion, and abdominal fat: The Insulin Resistance Atherosclerosis Study (IRAS) Family Study. Diabetes 52,2490–2496.

Wallen K (1996) Nature needs nurture: the interaction of hormonal and social influences on the development of behavioral sex differences in rhesus monkeys. Horm Behav 30,364–378.

Wallen K and Baum MJ (2002) Masculinization and defeminization in altricial and precocial mammals: comparative aspects of steroid hormone action. In Pfaff DW, Arnold AP, Etgen AM, Fahrbach SE, and Rubin RT (eds) Hormones, Brain and Behavior, Vol 4. Academic Press, San Diego, pp 385–423.

Weiner CL, Primeau M and Ehrmann DA (2004) Androgens and mood dysfunction in women: comparison of women with polycystic ovarian syndrome to healthy controls. Psychosom Med 66,356–362.

Weisz J, Lloyd CW (1965) Estrogen and androgen production in vitro from 7.3-H-progesterone by normal and polycystic rat ovaries. Endocrinology 77,735–744.

Wells LJ and van Wagenen G (1954) Androgen-induced female pseudohermaphroditism in the monkey (*Macaca mulatta*): anatomy of the reproductive organs. Contrib Embryol 235,95–106.

West C, Foster DL, Evans NP, Robinson J and Padmanabhan V (2001) Intrafollicular activin availability is altered in prenatally-androgenized lambs. Mol Cell Endocrinol 185,51–59.

Whalen RE and Luttge WG (1971) Perinatal administration of dihydrotestosterone to female rats and the development of reproductive function. Endocrinology 89,1320–1322.

Willis D and Franks S (1995) Insulin action in human granulosa cells from normal and polycystic ovaries is mediated by the insulin receptor and not the type-1 insulin-like growth factor receptor. J Clin Endocrinol Metab 80,3788–3790.

Willis D, Mason H, Gilling-Smith C and Franks S (1996) Modulation by insulin of follicle-stimulating hormone and luteinizing hormone actions in human granulosa cells of normal and polycystic ovaries. J Clin Endocrinol Metab 81,302–309.

Wisniewski AB, Migeon CJ, Meyer-Bahlburg HF, Gearhart JP, Berkovitz GD, Brown TR and Money J (2000) Complete androgen insensitivity syndrome: long-term medical, surgical, and psychosexual outcome. J Clin Endocrinol Metab 85,2664–2669.

Wood RI, Mehta V, Herbosa CG and Foster DL (1995) Prenatal testosterone differentially masculinizes tonic and surge modes of luteinizing hormone secretion in the developing sheep. Neuroendocrinology 62,238–247.

Wood JR, Nelson VL, Ho C, Jansen E, Wang CY, Urbanek M, McAllister JM, Mosselman S and Strauss JF, III (2003) The molecular phenotype of polycystic ovary syndrome (PCOS) theca cells and new candidate PCOS genes defined by microarray analysis. J Biol Chem 278,26380–26390.

Wood JR, Ho CK, Nelson-Degrave VL, McAllister JM, Strauss JF 3rd. (2004) The molecular signature of polycystic ovary syndrome (PCOS) theca cells defined by gene expression profiling. J Reprod Immunol 63, 51–60.

Zarrow MX, Naqvi RH and Denenberg VH (1969) Androgen-induced precocious puberty in the female rat and its inhibition by hippocampal lesions. Endocrinology 84,14–19.

Zawadzki JA and Dunaif A (1992) Diagnostic criteria for polycystic ovary syndrome: towards a rational approach. In Dunaif A, Givens JR, Haseltine FP, and Merriam GR (eds) Polycystic Ovary Syndrome. Blackwell Scientific, Boston, pp 377–384.

Zheng P, Wei S, Bavister BD, Yang J, Ding C and Ji W (2003) 17β-estradiol and progesterone improve in-vitro cytoplasmic maturation of oocytes from unstimulated prepubertal and adult rhesus monkeys. Hum Reprod 18,2137–2144.