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Polycystic Ovary Syndrome and its Developmental Origins

Daniel A. Dumesic[1,2,3], **David H. Abbott**[1,2], and **Vasantha Padmanabhan**[4,5]

Daniel A. Dumesic: danieldumesic@aol.com; David H. Abbott: abbott@primate.wisc.edu; Vasantha Padmanabhan: vasantha@umich.edu

[1]Wisconsin National Primate Research Center, University of Wisconsin, Madison, WI, 53715

[2]Department OB/GYN, University of Wisconsin, Madison, WI, 53792

[3]Reproductive Medicine and Infertility Associates, Woodbury, MN, 55125

[4]Department of Pediatrics, University of Michigan, Ann Arbor, MI, 48109

[5]Reproductive Sciences Program, University of Michigan, Ann Arbor, MI, 48109

Abstract

The prenatal testosterone (T)-treated adult female rhesus monkey is one animal model of polycystic ovary syndrome (PCOS) in women, with early prenatal T excess programming a permanent PCOS–like phenotype characterized by luteinizing hormone (LH) hypersecretion from reduced hypothalamic sensitivity to steroid negative feedback and relative insulin excess from increased abdominal adiposity. These combined reproductive and metabolic abnormalities are associated with ovarian hyperandrogenism and follicular arrest in adulthood, as well as premature follicle differentiation and impaired embryo development during gonadotropin therapy for in vitro fertilization (IVF). A second animal model for PCOS, the prenatal T-treated sheep also is characterized by LH hypersecretion from reduced hypothalamic sensitivity to steroid negative feedback, persistent follicles and insulin resistance, but also is associated with intrauterine growth retardation and compensatory growth after birth. The ability of prenatal T excess in both species to alter the developmental trajectory of multiple organ systems *in utero* provides evidence that the hormonal environment of intrauterine life programs target tissue differentiation, raising the possibility that T excess in human fetal development promotes PCOS in adulthood. Such a hypothesis must include data from clinical studies of PCOS women to clarify the homology between these PCOS-like animal models and PCOS *per se* in reproductive and metabolic function. Future studies should develop new clinical strategies that improve pregnancy outcome and minimize pregnancy loss in women with disorders of insulin action, including PCOS, obesity and diabetes mellitus as well as minimize transgenerational susceptibility to adult PCOS and its metabolic derangements in male close relatives.

Keywords

Prenatal androgenization; polycystic ovary syndrome; luteinizing hormone; hyperandrogenism; hyperinsulinemia; adiposity

I. Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous syndrome in women characterized by luteinizing hormone (LH) hypersecretion, ovarian hyperandrogenism, hyperinsulinemia from insulin resistance and reduced fecundity. Given a 6.6% estimated prevalence of PCOS in reproductive-aged women in the United States (i.e., at least 4 million affected women), the annual economic burden of PCOS is at least $4.4 billion, of which $1.8, $1.4 and $0.5 billion are for treating type 2 diabetes mellitus, menstrual dysfunction and infertility, respectively.

These estimates do not consider the greater frequency of pregnancy-related complications, including gestational diabetes, preeclampsia and miscarriage (1). Based upon the 1990 NIH definition of PCOS as hyperandrogenic chronic anovulation, the consequent annual health-care costs in the United States of evaluating and treating PCOS are at least 3-fold that of hepatitis C and one-third that of morbid obesity (1). These costs undoubtedly underestimate the expense of managing other PCOS phenotypes, defined by the Rotterdam criteria as any two of the three findings: clinical/biochemical hyperandrogenism, ovulatory dysfunction, polycystic ovaries (2).

While its peripubertal onset and familial clustering suggest a heritable etiology for PCOS, several candidate genes, including those regulating insulin action, androgen biosynthesis and gonadal function, have failed to fully explain its prevalence. Emerging data also implicate epigenetic changes in fetal life in the developmental origins of PCOS, with the most notable being the ability of discrete experimentally-induced prenatal testosterone (T) excess to program a permanent PCOS-like phenotype in several species. That T excess *in utero* programs multiple fetal organ systems agrees with the increased prevalence of PCOS in women with classical congenital adrenal hyperplasia (CAH) from 21 hydroxylase deficiency and with congenital adrenal virilizing tumors (3–6), confirming that the steroid milieu of intrauterine life programs differentiation of fetal target tissue.

In this regard, fetal programming of PCOS traits can be experimentally induced in several species by prenatal T excess, which permanently alters female reproductive and metabolic physiology and provides a means to assess molecular mediators involved in these perturbations. Evidence to date suggests that prenatal T-treated monkeys and sheep, like PCOS patients, manifest anovulatory infertility (7–9), adiposity-dependent compensatory hyperinsulinemia (10,11), hypergonadotropism (12–15), neuroendocrine feedback defects (11,13,14,16–20,21), functional hyperandrogenism (22–25) and polycystic ovaries (26,27). This chapter emphasizes prenatally T-treated monkey and sheep as models of PCOS because follicular differentiation in these species, as in humans and unlike rodents, is completed during fetal life. Data from these studies implicate critical times during fetal development when the steroidal status of the mother permanently alters the physiology of the fetus and modify its genetic susceptibility to disease after birth.

II. Reproductive Defects

II.A. Hyperandrogenism

Ovarian hyperandrogenism is the cardinal feature of PCOS, with *in vitro* studies of PCOS theca cells showing intrinsically increased androgen biosynthesis and augmented expression of several steroidogenic enzymes, including cytochrome P450 cholesterol side chain cleavage, 17α-hydroxylase/17–20 lyase (P450 $_{c17}$) and 3β-hydroxysteroid dehydrogenase (28,29). Hyperandrogenism is widely variable among the various PCOS phenotypes, as defined by Rotterdam criteria, and is more severe in “classic” PCOS (i.e., hyperandrogenic anovulation) than ovulatory PCOS patients (30). A similar hyperandrogenic anovulation can be induced by reprogramming adult ovarian morphology during prenatal development (11,31). Female rhesus monkeys (32), sheep (7,8,33), mice (34) and rats (35) exposed prenatally to excessive levels of T exhibit ovulatory dysfunction in adulthood Table 1). Ovaries are enlarged and polyfollicular in prenatally T-treated monkeys and sheep, and also are hyperandrogenic in prenatally T-treated monkeys and mice (26,34,36), while androgen receptor expression is upregulated in ovaries of prenatally T-treated sheep (25).

A PCOS–like phenotype can be produced by injecting pregnant rhesus monkeys carrying female fetuses with 10 to 15 mg T propionate (TP) for 15 to 35 days starting on either days 40–60 (early-treated) or days 100–115 (late-treated) postconception (total gestation, 165 days),

which elevates circulating T levels in fetal females to those normally found in fetal males (37,38). These prenatal T treatments coincide with target tissue differentiation and the beginning of neuroendocrine development in early-treated females, and with ovarian follicle development and functional acquisition of hypothalamic sensitivity to hormone negative feedback in late-treated females. Early prenatally T-treated female monkeys exhibit basal hyperandrogenemia (32,39) (Figure 1), while both early (22) and late (11) prenatally T-treated females demonstrate an exaggerated T response to recombinant human (rh) chorionic gonadotropin (CG) administration. Regardless of the timing of prenatal T treatment, prenatally T-treated female monkeys have a ten-fold increase in the risk of anovulation as adults and have double the normal incidence of polyfollicular ovaries, with 33–50% of anovulatory prenatally T-treated females having such polyfollicular ovaries (36,39)(Table 1). These traits correspond with the clinical diagnosis of PCOS by either the 1990 NIH or the Rotterdam criteria (1,2). PCOS-like phenotype also can be produced in sheep by injecting pregnant sheep with 100 mg of T propionate twice per week from days 30–90 of gestation, which exposes fetal females to circulating T levels to those normally found in fetal males. (Padmanabhan V and Abbott DH, unpublished). As in monkeys, this time interval corresponds to when neuroendocrine feedback systems are established and ovarian differentiation occurs (31,40).

II.B. Abnormal follicle development

II.B.1. Increased follicle recruitment—Several morphological findings in PCOS patients implicate increased recruitment of growing follicles from the primordial follicle pool with the development of the polycystic ovaries. One of three histological studies of human ovaries with polycystic morphology shows an increased proportion of primary follicles and a reciprocally decreased proportion of primordial follicles, independent of ovulatory status or atresia (41–43). Experimental evidence for adult hyperandrogenism causing increased recruitment of ovarian follicles comes from T administration to adult female rhesus monkeys increasing the number of primary, growing preantral and small antral follicles and the proliferation of granulosa cells within them (44,45). Androgen treatment in such adult female monkeys also increases mRNA expression of follicle-stimulating hormone (FSH) receptor, insulin-like growth factor I (IGF-I) and IGF-I receptor in granulosa cells (46,47), while enhancing IGF-I and its receptor mRNA expression in primordial follicle oocytes (48). Androgens also program enhanced follicle recruitment *in utero* since prenatal T treatment of sheep from days 30 to 90 of gestation (total gestation, 147 days) increases the proportion of growing follicles (i.e., primary, preantral, and antral follicles combined), decreases the proportion of primordial follicles and induces a polyfollicular phenotype (49) (Figure 2; Table 1).

II.B.2. Impaired follicle growth—In PCOS, growth of follicles is impaired at the 6–8 mm size when granulosa cells normally begin to express aromatase and convert androgens produced by luteinizing hormone (LH)-stimulated theca cells to estradiol (E2) in the presence of FSH (50,51). An endogenous inhibitor of estrogen synthesis likely exists in small estrogen-deplete PCOS follicles with sufficient bioactive FSH (52) because cultured granulosa cells from these follicles are hyperresponsive to FSH *in vitro* (53,54). New sonographic ovarian studies in PCOS patients show that the number of 2–5 mm follicles positively correlates with serum T levels, while that of 6–9 mm follicles negatively correlates with fasting serum insulin and T levels, as well as body mass index (BMI) (55). Taken together, these findings associate hyperandrogenism with excessive early follicular growth that will not progress to the dominant stage due to androgen excess and/or hyperinsulinemia (55). In this regard, small PCOS follicles have elevated 5α-reductase activity, which increases 5α-reduced androgen levels to concentrations capable of inhibiting aromatase activity *in vitro* (56,57). Increased 5α-reductase and decreased aromatase activities also occur in estradiol (E2)-deficient follicles of early prenatally T-treated female rhesus monkeys receiving rhFSH therapy (58). Even in cycling female rhesus monkeys, dihydrotestosterone (DHT) impairs gonadotropin-stimulated E_2

secretion (59) and inhibits proliferation of cultured rat granulosa cells (60). Persistent follicular cysts in prenatally T-treated sheep further implicate impaired follicular growth as a contributing factor in developing polycystic ovaries (33) (Figure 3).

Impaired follicle growth also is associated with hyperinsulinemia from insulin resistance (61). Anovulatory PCOS patients have a greater BMI than their ovulatory sisters despite a similar degree of ovarian hyperandrogenism (62), and weight loss in obese PCOS patients reverses anovulatory infertility (63). Since insulin enhances FSH-induced upregulation of LH receptors in granulosa cells and increases their progesterone (P4) responsiveness to LH (64, 65), hyperinsulinemia presumably induces premature follicle luteinization, which arrests cell proliferation and follicle growth. Consequently small antral PCOS follicles exhibit P4 hypersecretion and overexpress LH receptors (66,67), causing an exaggerated steroidogenic shift from E2 to P4 production (68). Similarly, exaggerated follicle differentiation occurs in early prenatally T-treated female rhesus monkeys undergoing rhFSH stimulation followed by hCG administration, in which LH hypersecretion and relative insulin excess from increased abdominal adiposity accompany an exaggerated shift in intrafollicular steroidogenesis from androgen and E2 to P4 (69). Conversely, improving hyperinsulinemia in PCOS patients with insulin sensitizing agents lowers serum androgen concentration (70,71) and restores ovulation in approximately 50% of patients (72), as it does in prenatally T-treated female rhesus monkeys (9,73).

Also implicated in impaired growth of PCOS follicles are transforming growth factor-β (TGFβ) family members, including activins, inhibins, anti-mullerian hormone, growth differentiation factor 9 (GDF-9) and bone morphogenetic protein 15, which interact with each other to coordinate follicle growth and oocyte development. Activins promote follicular development by enhancing granulosa cell responsiveness to FSH, suppressing androgen synthesis and stimulating oocyte maturation, while inhibins produced by the dominant follicle stimulate theca cell androgen production for E2 synthesis (74,75). Consequently a shift from an activin-dominant to an inhibin-dominant microenvironment occurs during follicle growth (76), which is impaired in some, but not all, PCOS follicles (77–79). Moreover, low activin A levels and high follistatin levels in the circulation of some PCOS patients (80,81) correspond with diminished intrafollicular activin in prenatally T-treated sheep (26) and activin β subunit responsiveness to steroid in neonatal mice (82). These findings emphasize the further need to understand how TGFβ family members affect intraovarian paracrine signaling during fetal developmental programming.

II.C. LH hypersecretion

A neuroendocrine hallmark of PCOS is enhanced LH hypersecretion from enhanced gonadotropin-releasing hormone (GnRH) pulsatility. Consequently serum immuno- and bioactive LH levels are increased in about 70% of PCOS patients (83), with elevated LH pulse amplitude and increased LH pulse frequency causing a two- to three-fold elevation in circulating LH versus FSH levels (84). PCOS patients also show an increased LH response to GnRH stimulation (83), along with a sexually dimorphic pattern of exaggerated early LH responsiveness to GnRH analog that more closely resembles that of men and women with congenital adrenal virilizing disorders (e.g., classical CAH and adrenal virilizing carcinoma) than normal women (3,85). As further evidence of neuoendocrine dysregulation, PCOS patients show reduced hypothalamic sensitivity to P4 negative feedback on LH secretion (86, 87), which can be restored with the androgen receptor blocker, flutamide (88). Moreover, reduced hypothalamic sensitivity to P4 negative feedback on LH secretion in some girls with PCOS during adolescence (87) suggests that prepubertal hyperandrogenism may program reduced hypothalamic feedback inhibition, leading to rapid GnRH pulsatility in early development.

Neuroendocrine dysregulation of LH release also occurs in prenatally T-treated females of several species, including rhesus monkeys (15), sheep (14,17,40,89,90), hamsters (91) and rodents (35) (Table 1). Early prenatally T-treated female rhesus monkeys exhibit basal LH hypersecretion ((Figure 1), increased pituitary LH responsiveness to GnRH (11,15) and reduced hypothalamic sensitivity to E2 and P4 negative feedback on LH release (11,20,21, 92). Late prenatally T-treated female rhesus monkeys show reduced hypothalamic sensitivity to P4 negative feedback on LH release alone (92). As in PCOS patients, prenatal T-treatment in primates does not abolish the E2-induced LH surge, which nevertheless can be exaggerated and delayed (15,21). Prenatal T-treatment in sheep also induces LH hypersecretion from reduced hypothalamic sensitivity to E2 and P4 negative feedback (14,17,18,24) as well as increased pituitary responsiveness to GnRH (93) and delays the onset of an otherwise truncated LH surge in Suffolk sheep (13). In Dorsett sheep, E2 fails to generate an LH surge (18,19). The collective data from both species of prenatally T-treated animals, therefore, demonstrate programming of LH hypersecretion from reduced hypothalamic sensitivity to steroid negative feedback with enhanced GnRH pulsatility (14,17,18,24) and disrupted surge mechanism (13, 18,19).

II.D. Oocyte developmental competence

Enhanced theca cell androgen biosynthesis (28,29), increased initiation of primordial follicle growth (43) and exaggerated granulosa cell responsiveness to FSH (54) are features of follicle development in PCOS patients undergoing in vitro fertilization (IVF). With more retrieved oocytes and cleaved embryos available to select for embryo transfer, PCOS patients undergoing IVF often achieve a clinical pregnancy rate comparable to that of similarly-treated normal women (94–96). Nevertheless, such PCOS patients also have increase risks of implantation failure and pregnancy loss (97) as well as impaired oocyte fertilization unrelated to gross chromosomal abnormalities or nuclear maturation (95,96,98–100). Moreover, obese PCOS patients experience low oocyte fertilization and failure of embryos to implant in their own uterus or those of their surrogates (101), implicating impaired oocyte developmental competence.

Terminally differentiated follicles of PCOS patients undergoing GnRH analog/rhFSH for IVF are hyperandrogenic with reduced intrafollicular FSH levels; they also contain meiotically-competent (metaphase II) oocytes with abnormal gene expression profiles (102,103). Based upon timing of the oocyte to T programming *in utero*, prenatally T-treated female rhesus monkeys undergoing gonadotropin stimulation for IVF also experience abnormal follicle development and impaired oocyte development (20,58). All prenatally T-treated female monkeys show abnormal intrafollicular steroidogenesis with reduced blastocyst formation (20,58), neither of which can be predicted by circulating hormone levels, or from number and maturity of oocytes collected (Figure 4). Differing from the hormonal profile of PCOS follicles, low intrafollicular E2 and androstenedione (A4) levels in late prenatally T-treated female monkeys receiving rhFSH therapy alone (58) are accompanied by a subtle impairment of blastocyst development after combined rhFSH/hCG therapy (20), consistent with E2-enhanced oocyte development in primates (104,105).

In early prenatally T-treated female monkeys, low follicle fluid E2 and A4 levels after both stimulation protocols are accompanied by an elevated P4/E2 ratio and a profound impairment of blastocyst development following combined rhFSH/hCG therapy. These findings suggest that as in humans both the E2 concentration and the P4/E2 ratio in the follicle affects oocyte development (104,106) (Figure 4). Equally important, early, but not late, prenatally T-treated female monkeys undergoing rhFSH therapy for IVF show LH hypersecretion ((Figure 1,Table 1) and relative hyperinsulinemia at oocyte retrieval (20), an important finding since insulin

together with FSH upregulates LH receptor expression in cultured murine cumulus-oocyte complexes and reduces blastocyst development (107).

Interestingly, reduced follicle fluid E2 and A4 levels in early prenatally T-treated rhesus monkeys undergoing rhFSH/hCG therapy for IVF (20) resemble those of IVF patients with diminished ovarian reserve (108) more than those of similarly-treated PCOS patients (102) and probably represent paracrine dysregulation of thecal cell P450 $_{c17}$ activity (109). Nevertheless, prenatal T treatment appears to perturb follicle growth and oocyte development by limiting the production of E2 or its action in the presence of androgen (20,58,110,111). These findings in concert with the observation that prenatally T-treated sheep also are subfertile (112) raises concern that the effects of prenatal T treatment on oocyte development ((Figure 2) might have transgenerational consequences for female offspring ((Figure 4).

III. Metabolic Defects

As major risk factors for type 2 diabetes mellitus and atherosclerosis (113), PCOS and obesity have independent and additive adverse effects on insulin action, with PCOS patients being more insulin resistant than weight-matched normal women (114). These defects appear to stem from intrinsic abnormalities of post-receptor insulin signaling (e.g. excess serine phosphorylation), abnormal insulin secretion (114,115), or polymorphic genes controlling insulin action (116–118). While several factors influence insulin sensitivity, including ethnicity, history of diabetes mellitus and BMI (114,119–121), increased abdominal adiposity is a common feature of PCOS that impairs insulin sensitivity and it is largely responsible for the increased insulin resistance observed in obese PCOS patients versus BMI-matched normal women (120). Moreover, increased abdominal adiposity is central to metabolic syndrome, a constellation of cardiovascular risk factors also including dyslipidemia, hyperglycemia and hypertension that is highly prevalent in adolescent PCOS patients (122).

Like humans, rhesus monkeys are susceptible to obesity and its glucoregulatory impairments (123). Prenatally T-treated female rhesus monkeys selectively deposit fat intra-abdominally and exhibit impaired insulin secretion or action in ways that closely resemble those of PCOS women, depending on whether the androgen excess occurred during early or late gestation (124–126) (Table 2). Detailed measures of body composition using computerized tomography with dual X-ray absorptiometry show that early T-treated females have increased visceral fat compared to control females, even when corrected for BMI and total body fat (125). Late T-treated females have increased total body and non-visceral abdominal fat compared to control females (126). Interestingly, both early and late T-treated PA females preferentially accumulate visceral fat with increasing BMI, while normal females preferentially accumulate non-visceral fat (126). Metabolic studies further show that early T-treated females have impaired insulin secretion, liberate more fatty acids than control females during a frequently sampled intravenous glucose tolerance test (73,127) and exhibit basal serum insulin levels that are positively correlated with the amounts of total body, total abdominal and visceral fat stores (126). Late T-treated females show decrements in insulin sensitivity with increasing BMI, with preservation of insulin secretory function (14). The resulting metabolic abnormalities from adiposity-related insulin resistance in prenatally T-treated female monkeys contribute to an increased risk of diabetes mellitus (27.3% and 11.1% in early-treated and late-treated females, respectively). Moreover, prenatally T-treated sheep develop impaired insulin sensitivity in early postnatal life (10), together with hypertension and hypercholesterolemia after puberty (128) as additional components of the metabolic syndrome as seen in PCOS patients (Table 1 and Table 2).

Ameliorating impaired insulin action has beneficial glucoregulatory effects in both PCOS patients and prenatally T-treated female monkeys, as evidenced by the abilities of metformin

and thiazolidinediones to improve insulin action in PCOS patients (70,129,130) and of pioglitazone to improve insulin action in both early and late prenatally T-treated monkeys (73). Such parallels in metabolic dysfunction between PCOS patients and prenatally T-treated female monkeys as well as sheep provide additional evidence of fetal androgen excess programming of metabolic function. Studies in sheep suggest programming of insulin resistance is facilitated by androgenic action of T (131).

IV. Barker Hypothesis

According to the developmental origins of adult disease hypothesis (i.e., the Barker hypothesis), adverse influences in early development lead to permanent changes in physiology and metabolism, resulting in increased disease risk in adulthood (Table 2). Original observations that regions of England having the highest rates of infant mortality in the early 20th century also had the highest rates of mortality from coronary heart disease decades later have been further supported by subsequent studies showing an association between low birth weight and adult development of cardiovascular disease (CVD), hypertension, insulin resistance and type 2 diabetes mellitus (132). Teleologically, fetal undernutrition would favor genes important for energy conservation (i.e., thrifty genotype), which would be beneficial in times of food scarcity, but would lead to obesity and diabetes when food becomes abundant later in life (133). Alternatively, fetal undernutrition might lead to an organized process in which fetal brain development is spared to the detriment of other organ systems (i.e., thrifty phenotype), perhaps as an adaptive response for postnatal survival in a nutrient-deplete environment (134). Evidence for such a phenomenon in PCOS can be found in poor intrauterine growth and low birth weight accompanying precocious puberty and PCOS in northern Spanish women (135) and PCOS pregnancies in Chilean women (136), but not in larger groups of Finnish (137) and Dutch individuals (138). Theoretically, maternal T excess could reduce fetal growth and birth weight through impaired placental function since experimentally-induced maternal T excess decreases rodent and sheep offspring birthweight (24,139,140); impaired placental aromatization in women also accompanies diminished uteroplacental perfusion and low infant birthweight (141,142). Advanced placental differentiation to reduce intrauterine growth restriction also is a feature of T-treated pregnant sheep (143).

While prenatally T-treated female sheep (49,140,144) and rats (145) exhibit intrauterine growth retardation (IUGR) and low birth weight, prenatal T-treated rhesus monkeys do not (39,146) (Table 2). Furthermore the IUGR in prenatally T-treated fetal sheep near term is characterized by an increased head to fetal weight ratio (49) corresponding with a brain-sparing effect (147) and is followed by postnatal weight gain (or catch-up growth) (140) and with insulin resistance in adulthood (24) (Table 2). Early prenatally T-treated female rhesus monkeys (39), on the other hand, show an increase in body weight during early infancy (Abbott and Tarantal, unpublished results), as well as during late adolescence/early adulthood and undergo delayed puberty in a manner similar to that of male puberty. Therefore, prenatally T-treated sheep and rats may be suitable models for PCOS with placental insufficiency, particularly since the former have enlarged left cardiac ventricles, kidneys and adrenals suggestive of CVD (24), while the latter have increased mortality (148).

V. Adrenal

Resembling the 25–60% prevalence of adrenal hyperandrogenism in PCOS (149,150), early prenatally T-treated female monkeys show adrenal hyperandrogenism, presumably from enhanced 17α-hydroxylase/17,20 lyase activity of the zona reticularis in adulthood (23). Basal and ACTH-stimulated cortisol levels, however, are normal in prenatally T-treated monkeys. Basal and ACTH-stimulated cortisol levels are also normal in prenatally T-treated adult sheep (Padmanabhan, unpublished). Moreover, prenatally T-treated sheep demonstrate a

proportionate increase in fetal adrenal weight with IUGR near term, demonstrating an altered trajectory of adrenal development accompanying fetal growth retardation (49).

VI. Alternate mechanisms of developmental programming

During the second trimester of human development, serum T levels are elevated into the male range in 40% of female fetuses (151). Therefore the wide variation in androgenic exposure that normally occurs during human development could certainly influence developmental programming of the fetus. Mechanisms beyond T-induced developmental programming, however, also may exist since close male relatives of PCOS patients exhibit metabolic dysfunction similar to that of their female kin (152–154). In support of this, prenatally T-treated male monkeys exhibit insulin resistance and diminished insulin response to glucose in adulthood (155), despite normal male levels of circulating T during fetal life (38). Therefore the combination of steroid and metabolic abnormalities *in utero* might perturb development of several fetal organ systems and increase the risk of developing reproductive and metabolic diseases in later life. Consistent with this hypothesis, genes for receptors to insulin, IGF-I and IGF-II and protein for P450c17 enzyme exist in second trimester human fetal ovaries (156, 157). In addition, female stillbirth offspring of diabetic mothers have increased birth weight and pancreatic beta cell hyperplasia with hirsutism, ovarian theca-lutein cysts and thecal cell hyperplasia (158,159), while elevated amniotic fluid levels of the β-hCG and T occur in pregnant diabetic mothers receiving insulin (160).

Moreover, while direct androgen action in the female fetus may account for some aspects of adult reproductive function, some elements of fetal developmental programming may be mediated by conversion of T to E2 through placental or fetal gonadal aromatization. Cancer and infertility have long been recognized in women exposed prenatally to diethylstilbestrol (DES) (161,162), as have paraovarian cysts and infertility in rodents exposed perinatally to DES, allyl E2, or E2-17β or E2 benzoate (163–165). More recently, persistent follicular cysts have been noted in sheep exposed prenatally to T, but not to DHT (33,166), while IUGR and LH surge defects occur in similarly-treated sheep exposed to bisphenol, an estrogenic endocrine-disrupting compound (167). At the ovarian level, reduced primordial follicle numbers occur in ovaries of late gestational fetal baboons following maternal exposure to an aromatase inhibitor (168), while decreased oocyte-granulosa cell microvilli, and presumably perturbed oocyte-granulosa cell signaling, characterize the maturing fetal ovary following diminished estrogen exposure (169,170).

Conclusions

- Prenatal T-treatment in monkeys and sheep programs a permanent PCOS-like phenotype characterized by LH hypersecretion from reduced hypothalamic sensitivity to sex steroid negative feedback, functional hyperandrogenism, ovulatory dysfunction, polycystic ovaries and impaired glucose-insulin homeostasis.
- Prenatal T-treatment in both species induces female subfertility, which in part represents the impaired developmental competence of primate oocytes.
- Mechanisms beyond T-induced developmental programming likely exist since exposure of monkeys to prenatal T excess also impairs glucose-insulin homeostasis without affecting body weight in both adult sexes, while similarly-treated fetal sheep show intrauterine growth retardation with compensatory growth after birth.
- Critical times exist during fetal development when the steroidal status of the mother permanently alter the physiology of the fetus and modify its susceptibility to disease after birth.

- Optimizing the effects of the maternal diet and hormonal environment on fetal growth and development might minimize transgenerational susceptibility to PCOS and to its metabolic derangements in male close relatives and could improve the fertility of PCOS women while reducing their risk of pregnancy-related complications.

Key unanswered questions

The prenatally T-treated animal models of PCOS implicate hyperandrogenism or hyperestrogenism during critical times of fetal development in the pathogenesis of PCOS and of its metabolic derangements in male close relatives. They agree with the increased prevalence of PCOS in women exposed to fetal T excess, including CAH from 21-hydroxylase deficiency and congenital adrenal virilizing tumors (3–6) and of insulin resistance in men with 21-hydroxylase deficiency (171). As such, T excess programming probably leads to at least two abnormalities, namely reproductive and metabolic, which may interact to increase susceptibility to an adult PCOS phenotype (Figure 5). Equally important and evident in prenatally T-treated animal models of PCOS, variation in gestational timing of T excess programming *in utero*, along with differences in target tissue sensitivity to steroid action, also may contribute to heterogeneity in the adult phenotype.

Unfortunately, experimental constraints on the use of human fetal tissue for biomedical research limit our knowledge of the relationships between the human fetus and its maternal environment. Consequently, understanding how developmental programming affects human growth and development continues to require animal models to pioneer the probable fetal origins of adult disease. In doing so, future animal studies need to clarify the neuroendocrine mechanisms governing hypothalamic sensitivity to hormone negative feedback and the endocrine/paracrine signaling and their effects on follicle growth and oocyte development. At the ovarian level, knowledge of how developmentally relevant endocrine/paracrine factors and genes interact to promote optimal gene expression in the fetal oocyte for later fertilization and successful preimplantation embryogenesis also is necessary. With such information, new clinical strategies targeting long-term correction of follicle growth and development could improve fertility, optimize ovarian responsiveness to gonadotropin therapy and enhance pregnancy outcome by IVF, thereby promoting the transfer of fewer embryos into the uterus and decreasing the risk of multiple gestation and its adverse consequences on maternal-fetal health.

Also important is recognizing how the maternal environment affects fetal growth and development. With obesity the fastest-growing medical problem in America and two-thirds of American adults being overweight, impaired glucose-insulin homeostasis in pregnancy from insulin-resistance diseases, including obesity, PCOS and diabetes mellitus, also have implications on fetal developmental programming. Whether such programming events are secondary to altered abdominal adiposity or additional pancreatic or insulin receptor-mediated events, the implications are that genetically-determined hyperandrogenism can be modified by both maternal and environmental factors to program an adult PCOS phenotype and its male equivalent. In support of this, nutrient-deficient diets also can adversely affect long-term physiology of the offspring (172,173) and alter DNA methylation in the human placenta (174), suggesting detrimental outcomes from epigenetic and metabolic abnormalities. Therefore additional clinical strategies that optimize the effects of the maternal diet and environment on fetal growth and development may be able to minimize transgenerational susceptibility to acquiring the adult PCOS phenotype and its metabolic derangements in male close relatives.

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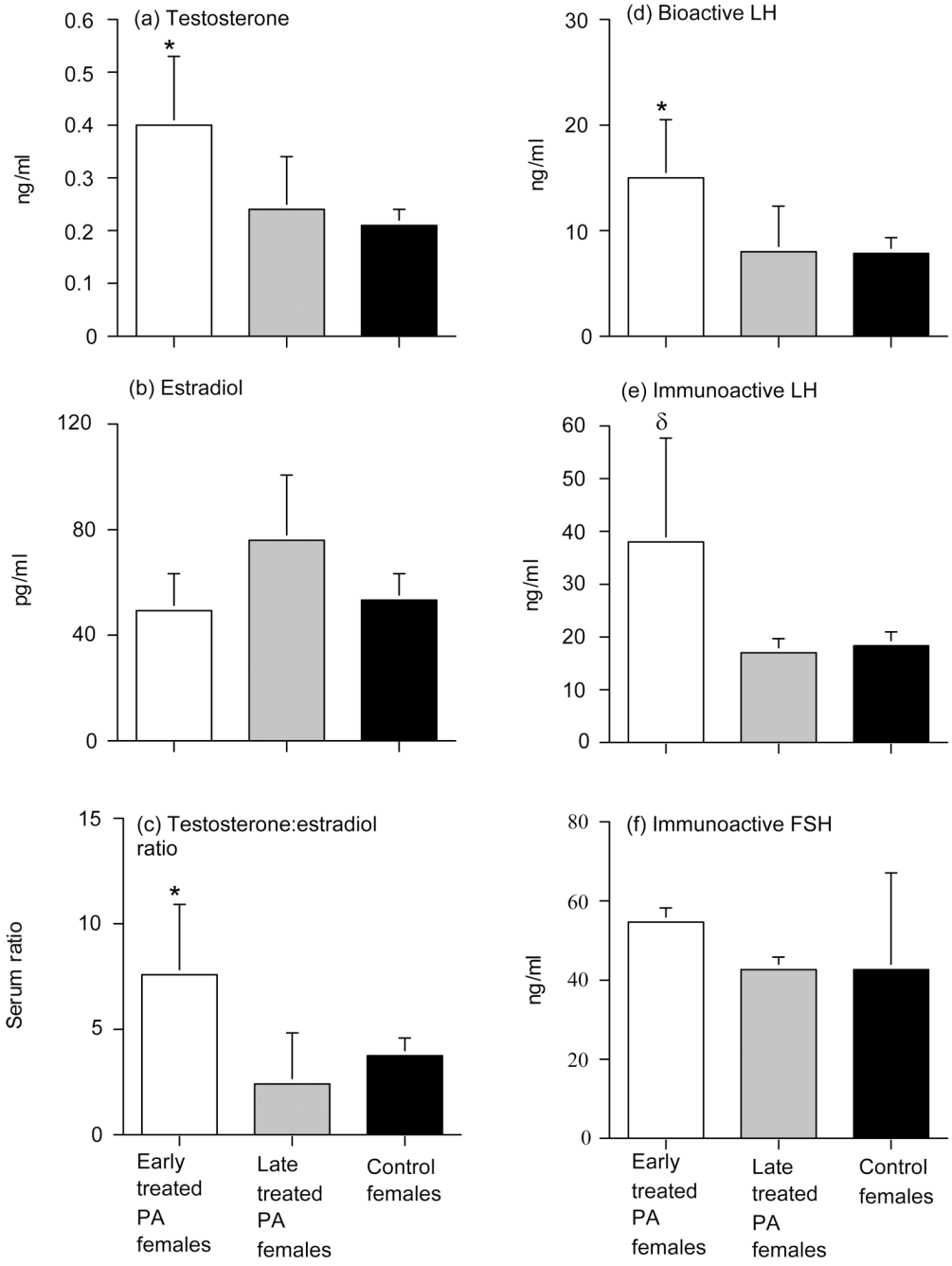

Figure 1.

Mean (±SEM) serum values in early (open bars) and late (grey bars) treated prenatally T-treated and control (black bars) adult female rhesus monkeys reflecting (a) testosterone, (b) estradiol, (c) testosterone:estradiol ratio, (d) bioactive LH, (e) immunoactive LH and (f) immunoactive FSH during either the early follicular phase of the menstrual cycle or equivalent time during a 30-day anovulatory period. * $p<0.05$, versus controls, δ $p<0.08$ versus controls (Data from reference 39).

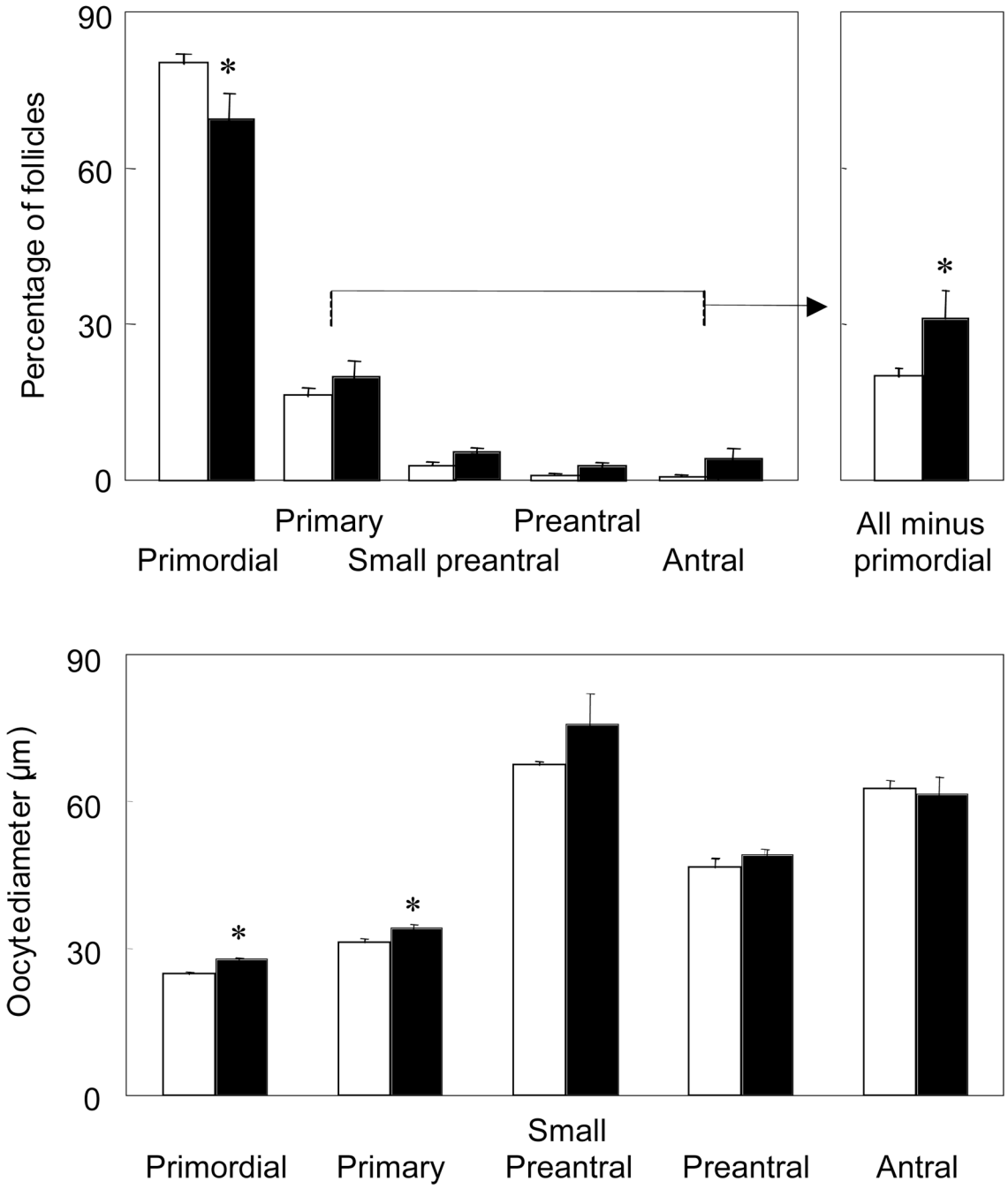

Figure 2.

Effect of prenatal T treatment from days 30 to 90 of gestation on the distribution of follicles (top panel) and oocyte diameter (bottom panel) in fetal ovine ovaries at 140 d of gestation. Each bar represents mean ± SEM. Asterisks indicate significant differences ($P < 0.05$) (Data from reference 31).

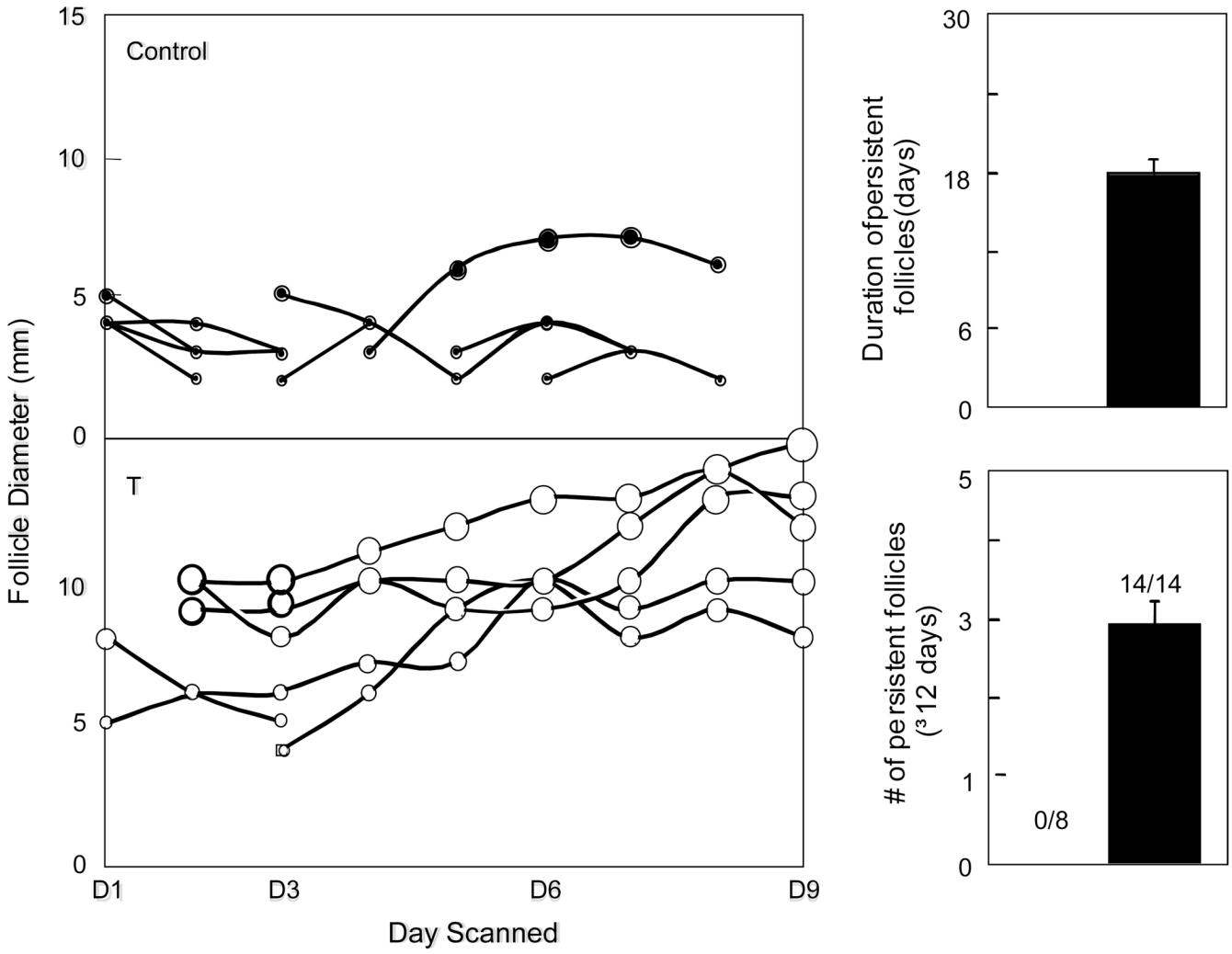

Figure 3.

Ovarian follicular dynamics determined by ultrasonography in both ovaries of a representative control and prenatal T-treated sheep are shown in the left panel. Each line represents one follicle. Only follicles that reached a size of 3 mm and persisted for at least 2 days are shown. Note the increase in maximum size and duration of the larger follicles on the ovary in prenatal T-treated sheep. Mean number (bottom right) and duration (top right) of persistent follicles in ovaries of control (n=8) and prenatal T-treated (n=14) sheep. Numbers within bottom histogram indicate number of animals in each group showing persistent follicles (Data from reference 31).

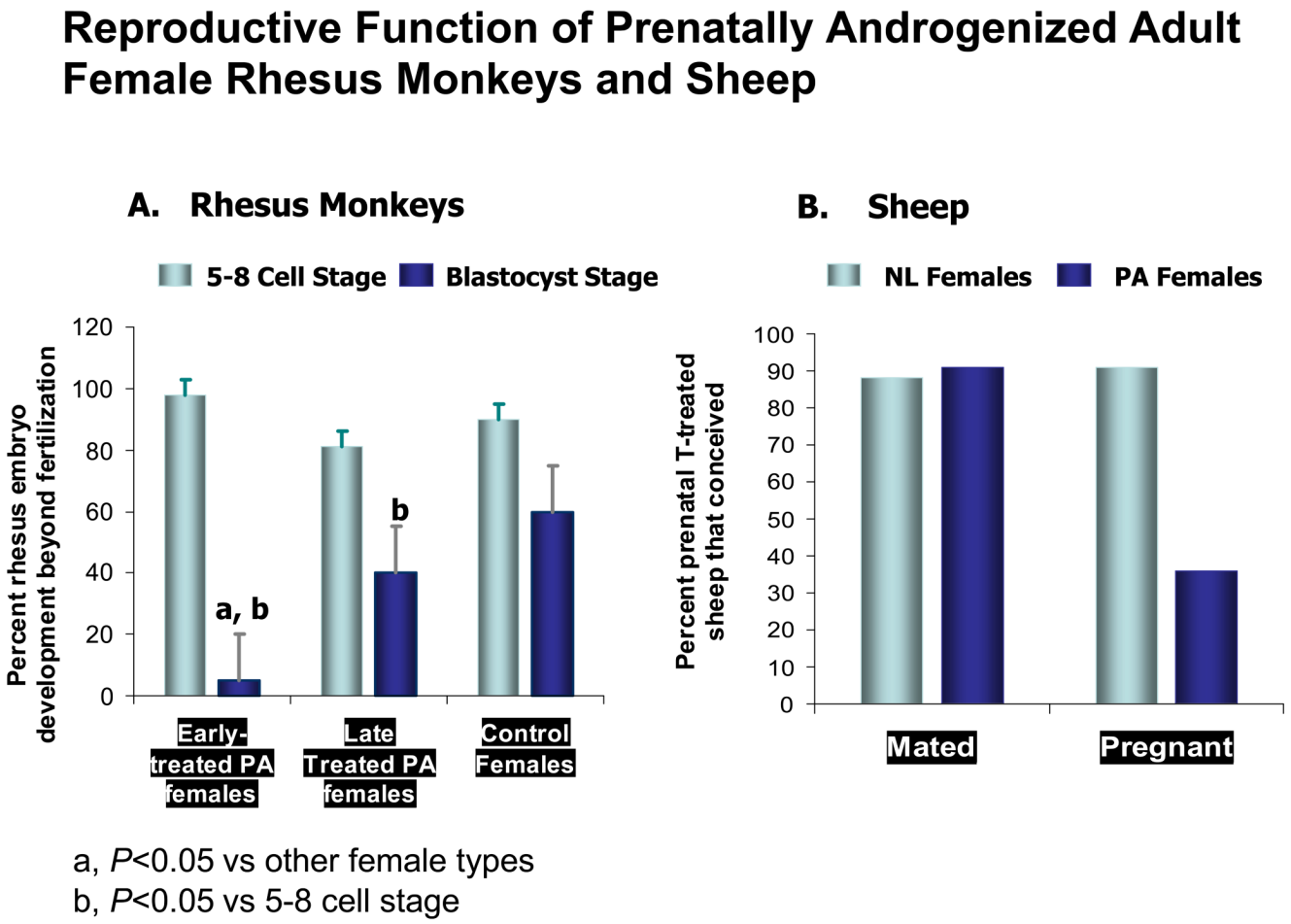

Figure 4.

A). Mean (upper 95% confidence limit) percentage of zygotes developing to the 5–8 cell (open bars) and blastocyst (solid bars) stages in 5 early prenatally T-treated, 5 late prenatally T-treated and 5 control adult female rhesus monkeys following ovarian hyperstimulation for IVF. a: $p<0.05$ versus control and late prenatally T-treated females at the same stage; b: $p<0.05$ versus 5–8 cell stage (Data modified from reference 20). B) Histograms on the right shows percentage of prenatal T-treated sheep (n=12) that successfully mated or conceived following estrus synchronization with two injections of PGF2α administered 11 days apart. To overcome mating preference, ram access was limited to only prenatal T-treated females. First service mating and pregnancy results for the breeding herd (n=109; hatched bar) bred during the same time are provided for comparison (Data modified from reference 112).

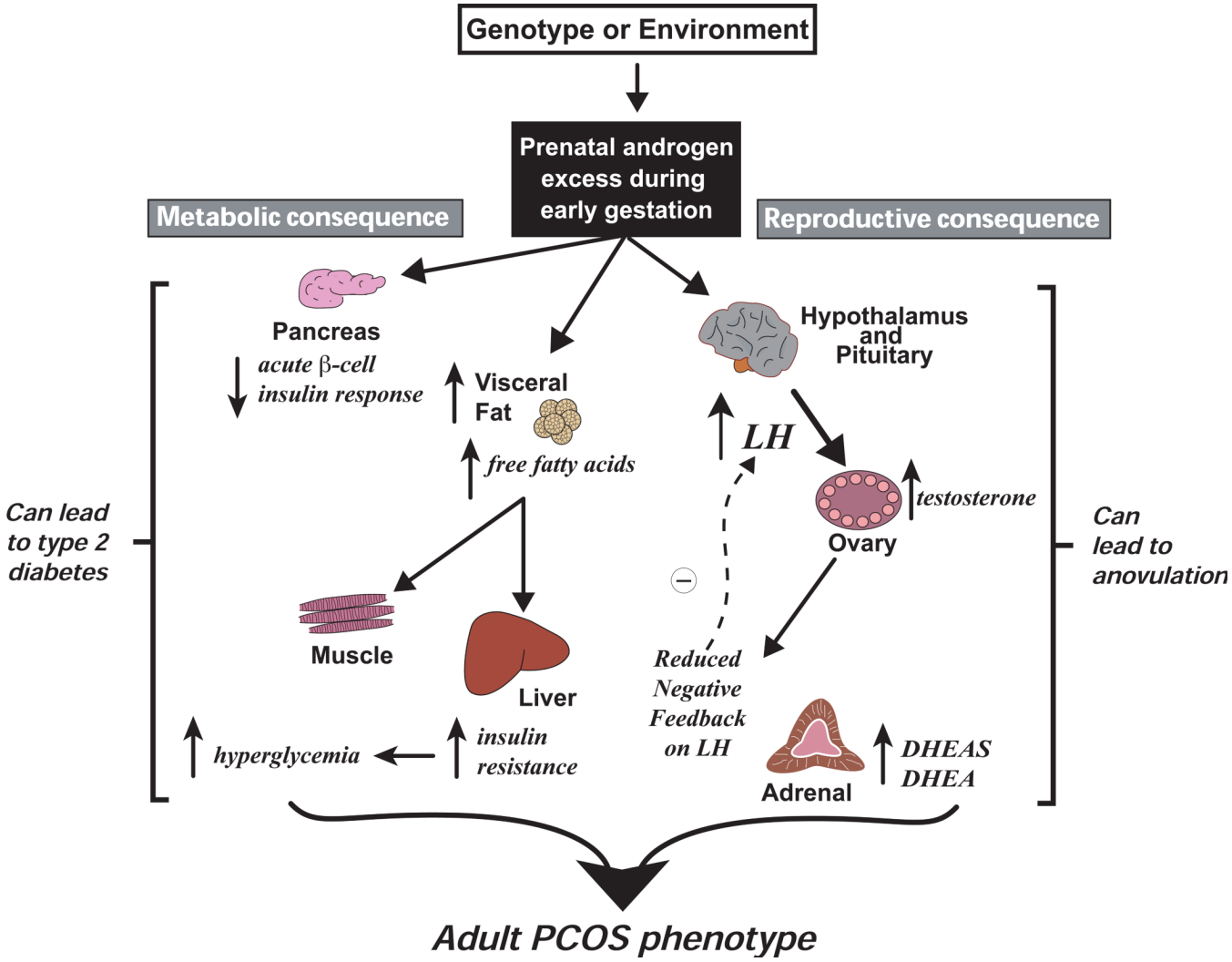

Figure 5.

Diagrammatic representation of possible early gestation, fetal androgen excess programming of adult PCOS traits. Genetic or environmental mechanisms induce fetal hyperandrogenism causing permanent changes in reproductive and metabolic function. Reproductive consequences include: (1) altered hypothalamic-pituitary function causing LH hypersecretion, (2) ovarian hyperandrogenism with or without LH hypersecretion, (3) reduced steroid hormone negative feedback regulation of LH, (4) adrenal hyperandrogenism, and (5) ovulatory dysfunction. Metabolic consequences include: (1) increased abdominal adiposity with elevated circulating total free fatty acid levels, (2) impaired pancreatic insulin secretory response to glucose, (3) impaired insulin action and compensatory hyperinsulinemia, (4) hyperglycemia, and (5) increased incidence of type 2 diabetes. Insulin resistance and compensatory hyperinsulinemia may be functionally implicated in the anovulatory mechanism (Data from reference 39).

Table 1

Reproductive and metabolic PCOS-like abnormalities in prenatally androgenized female rhesus monkeys and sheep. Details of the traits are discussed in the text. ?: trait yet to be assessed.

PCOS trait[1]	Prenatally Androgenized Female Rhesus Monkeys		Prenatally Androgenized Female Sheep
	Early treated	Late treated	
Reproductive			
Ovarian hyperandrogenism	Yes	Yes	Ovarian androgen receptor upregulation
Anovulation	Yes	Yes	Yes
Enlarged polyfollicular ovaries	Yes	Yes	Yes
LH hypersecretion	Yes	No	Yes
Reduced steroid negative feedback on LH	Yes	Yes	Yes
Impaired embryonic development	Yes	Yes	Impaired fertility
Metabolic			
Insulin resistance	Yes	No	Yes
Beta cell impairment	Yes	No	**?**
Hyperglycemia	Yes	Yes	No
Increased type 2 diabetes	Yes	No	Unknown
Increased abdominal fat	Yes	With increasing BMI	Unknown
Hypertension	Unknown	Unknown	Yes
Hyperlipidemia	Yes	Unknown	Yes

[1] Details provided in the text

Table 2

Prediction of abnormal traits expected in prenatally androgenized female rhesus monkeys and sheep from the Barker hypothesis. Details of the traits are discussed in the text. +: trait present, −: trait absent, ?: trait yet to be assessed.

Abnormal traits	Barker hypothesis prediction	Early treated PA female observation	Late treated PA female observation	PA female sheep observation
Low birthweight	+	−	−	+
Catch-up growth	+	−	?	+
Visceral obesity	+	+	+ with high BMI	?
Insulin resistance	+	+	−	+
Beta cell impairment	+	+	−	?
Glucose intolerance	+	+	+	?
Hypertension	+	?	?	+