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Animal models of the polycystic ovary syndrome phenotype

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Abstract

The etiology of the polycystic ovary syndrome (PCOS) remains unclear, despite its high prevalence among infertility disorders in women of reproductive age. Although there is evidence for a genetic component of the disorder, other causes, such as prenatal insults are considered among the potential factors that may contribute to the development of the syndrome. Over the past few decades, several animal models have been developed in an attempt to understand the potential contribution of exposure to excess steroids on the development of this syndrome. The current review summarizes the phenotypes of current animal models exposed to excess steroid during the prenatal and early postnatal period and how they compare with the phenotype seen in women with PCOS.

Keywords

Infertility; PCOS; fetal programming; androgens; estrogens

1. Polycystic ovary syndrome and its origins

Globally, 8 to 12% of couples with women of childbearing age are infertile, a source of diminished health and social well-being. Genetic, physiological, social, as well as environmental causes appear to contribute to the condition. Amongst fertility disorders, polycystic ovary syndrome (PCOS) is the most common. The three main clinical criteria used for its diagnosis are NIH [1], the Rotterdam [2], and the AE-PCOSS [3] criteria. The diagnostic criteria for PCOS continue to be disputed as evident from the latest NIH workshop report [4], which states that the multiple criteria used is confusing and delays progress in understanding the syndrome. The committee recommendation was to use the inclusionary criteria defined in Rotterdam [2], but stratify them into four phenotypes: 1) androgen excess + ovulatory dysfunction, 2) androgen excess + polycystic ovarian morphology, 3) ovulatory dysfunction + polycystic ovarian morphology, and 4) androgen excess + ovulatory dysfunction + polycystic ovarian morphology.

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The etiology of PCOS is unknown and remains a topic of intense research. A large percentage of women with PCOS do not respond to ovulation induction protocols [5]. When they conceive, conception rates are low with a high percentage of pregnancies ending in spontaneous miscarriages [6, 7]. Women with PCOS are also at risk for gestational diabetes and preeclampsia [8] and show psychological disturbances [9, 10]. A metabolic component is evident in the etiology of PCOS [11–13] with about 70% of these women manifesting insulin resistance [14]. Cardiovascular disease, dyslipidemia, hypertension, diabetes mellitus, and endometrial cancer [15, 16] are some risk factors that are associated with PCOS. The increased risk of co-morbidities necessitates addressing infertility problems to ameliorate development of debilitating diseases and most importantly the transgenerational transfer of unwanted traits to the offspring.

2. Animal models to study PCOS

Increasing evidence suggests that adult dysfunctions may result from abnormal programming of developing systems during intrauterine life [17]. There is considerable evidence that androgen excess early in life may lead to manifestation of PCOS phenotype in adulthood [18, 19]. This is supported by the fact that the PCOS phenotype is often associated with conditions such as classical 21-hydroxylase deficiency in which the fetus has been exposed to high concentrations of sex steroids before birth [20]. In recent years, several animal models have evolved to investigate the impact of perinatal exposure to steroids on the development of adult reproductive and metabolic pathologies [21]. Many of these animal models that manifest the PCOS phenotype involve perinatal treatment with testosterone (T) and often referred to as androgenized models. This terminology is often misleading, since T has the ability to be aromatized to estrogen and exert its effects via estrogenic programming. Other models involve perinatal exposure to dihydrotestosterone (DHT), a non-aromatizable androgen, estrogenic compounds, or inhibitors. Comparative aspects of these animal models and the quality of the steroid responsible for inducing reproductive and metabolic deficits paralleling that seen in women with PCOS have been reviewed previously [21–23].

3. Benefits of existing animal models of PCOS

Amongst the animal models developed for studying the etiology of PCOS, mice, rats, sheep, and rhesus monkeys are the most extensively studied. Each of these models offers different benefits. It is undisputable that genealogically, rhesus monkeys are optimal, but their long developmental line (menarche occurs at ~2.5 years of age and reproductive competence between 2.5–3.5 years of age [24, 25]) and cost prohibitiveness limits their extensive usage in research. Monkeys and sheep are precocial species and complete their ovarian differentiation *in utero*, similar to humans. As such they are of better translational relevance compared to non-precocial species when the investigation focuses on ovarian function. They are also not polyovular like rodents. The larger size of both these species allows collection of sequential samples for hormonal profiling. They are also amenable for fetal manipulations. On the other hand, due to their short life span, rodents are valuable for studying transgenerational consequences of prenatal steroid excess. Mouse models also have the benefit of knocking in/out genes for addressing functionality of specific genes. As such, while translating findings from animal models to humans, the developmental trajectory of the organ system being studied needs to be taken into consideration. When prenatal insults are addressed, it is important to recognize that the impact on fetal organ systems may be mediated via changes in maternal milieu in precocial species. To compare the impact of insults on similar windows of development in altricial species, where differentiation of the organ in question occurs postnatally (e.g.: ovary), insults need to occur at corresponding

postnatal time points. A caveat is that consequences are direct result of the insult on the offspring and exclude impact through maternal milieu.

4. Adult reproductive phenotype of PCOS models

The developmental consequences of various steroids and inhibitors (T, DHT, estradiol valerate (EV), and letrozole) in programming postnatal development of PCOS phenotype have been studied extensively in animal models. In considering these programmers, it is important to recognize that they can be metabolized and the mediators of downstream signaling pathways are the metabolites not the parent compound. For example, T can be aromatized to estradiol and mediate effects via estrogen receptors and DHT to 3 beta-diol [26] and act through the estrogen receptor beta.

Reproductive cyclicity in animal models of PCOS

Infertility in PCOS women likely stems from their oligo- or anovulatory status [27]. All animal models exposed perinatally to T develop oligo-anovulation [22, 28], with the degree and severity of the defect reflecting the window of treatment (see Table 1). The DHT-, EV-, and letrozole-treated females also show cycle disruptions, although the nature of these defects appears to differ amongst species and treatments [29–33] (see Table 2). While all models manifest oligo- and/ anovulation, rodent models have not addressed fertility consequences. The reduced fertility of women with PCOS appears to stem from defects at the oocyte/embryonic level [34]. In the T-treated rhesus monkey, the only species where studies of oocyte competence have been undertaken [35], developmental competence of the oocyte was found to be reduced. Fertility outcome has been undertaken only with prenatal T-treated sheep (days 60–90 of gestation) and they show that successful pregnancy is achieved only in 40% of these females [36].

Neuroendocrine defects in animal models of PCOS

Hypergonadotropism (LH excess) seen in women with PCOS [27] is also a feature of prenatal T-treated rhesus monkeys [28, 37], sheep [38–40], and rats [33, 41] (see Table 1). Evidence supportive of reduced sensitivity of the neuroendocrine system to steroid negative feedback is limited to progesterone negative feedback in women with PCOS [42]. Similar findings have been found in prenatal T-treated monkeys [43] and sheep [44, 45]. Extensive investigation of impact on all three neuroendocrine feedback mechanisms (estradiol positive, estradiol negative, and progesterone negative) has been undertaken only with prenatal T-treated sheep and show that all 3 feedback systems are disrupted [23, 38, 39, 44–46]. The severity of estradiol positive feedback system appears to be a function of the window of treatment with a later window of exposure (days 60–90 of gestation) showing a less severe phenotype (delayed LH surge) than the 30–90 day exposure (delayed and severely dampened or absent LH surge) [38] model. Other animal models, such as the rat [41] and the rhesus monkey [28, 47] also manifest compromised estradiol positive feedback responses. Pituitary sensitivity has been demonstrated to be increased in PCOS subjects and a similar phenotype seen in prenatal T-treated monkeys [28, 47], sheep [40], and mice [29] but not rats [41].

Use of non-aromatizable androgen, DHT, has helped gain insights into which traits are programmed via androgens as opposed to estrogens in the development of the PCOS phenotype. Studies involving DHT in sheep [48], rats [41], and mice [29] suggest that LH excess is a trait programmed by androgens (see Table 2). Feedback tests carried out in sheep show that the estradiol negative feedback is disrupted in both T and DHT-treated females supportive of androgenic mediation. In contrast to estradiol positive feedback disruption occurring only in T, but not DHT-treated sheep [48], both prenatal T and DHT treatment

disrupted estradiol positive feedback in rats [41]. Species-specific outcomes are also evident relative to pituitary responsiveness to GnRH with an enhanced response occurring with both T and DHT in sheep [40], but not T or DHT-treated rats [41]. LH excess appears to be a feature of late gestational window letrozole-treated rats model [49, 50], but not EV-treated female rats [51]. Increased LH pulsatility is a feature of both T- and DHT-treated sheep and rats [40, 41]. In determining androgenic vs. estrogenic mediation by comparing T and DHT models, caution needs to be exercised, because DHT can be metabolized to 3-beta diol and may elicit its effects through estradiol receptor beta [26].

Ovarian defects in animal models of PCOS

Women with PCOS are reported to have increased ovarian volume and/or a multifollicular morphology [52]. However, the inclusion of the polycystic ovarian morphology as diagnostic criteria is highly controversial [4]. Relative to various animal models developed, prenatal T treatment leads to a multifollicular ovarian phenotype (accumulation of multiple antral follicles) in most species studied, although in rodent models the ovarian phenotype is cystic [33, 53, 54] (see Table 3). Detailed ovarian morphometric studies carried out only in sheep across the reproductive life span have documented increased follicular recruitment and depletion [55]. Serial ultrasonographies carried out in sheep have also provided evidence in support of follicular persistence in prenatal T-treated sheep [56]. At the granulosa cell level, follicles in prenatal T-treated sheep manifest an imbalance in relative balance of activins, inhibins, and follistatin expression [54], features also evident in women with PCOS [57, 58]. Other key players of ovarian function are also disrupted in prenatal T-treated female sheep [59, 60, 61]. Changes in androgen and estrogen receptors are evident in large preantral and antral follicular stages in adult females, while only a defect in androgen receptor is evident in primordial, primary and preantral follicles during fetal life. A decline in adiponectin, a mediator of preovulatory follicle modeling [60], and caspase-3, an effector of apoptosis, are evident in antral follicles of prenatal T-treated adult females, while a reduction in BAX expression, a pro-apoptotic factor, was evident during fetal life in early follicles [61]. These changes are consistent with the enhanced follicular recruitment and arrest evident in these animals [55, 56].

Relative to the quality of steroid responsible for programming the ovarian phenotype, prenatal T-treated sheep develop a multifollicular ovarian morphology, but not prenatal DHT-treated sheep [54, 55]. In contrast, the ovarian morphology is affected in DHT-treated rat models [30, 33] (see Table 4), supportive of species-specific outcomes. The EV- and letrozole-treated models also develop a polycystic ovarian morphology [30, 49, 51]. To dissect out androgenic and estrogenic contributions, it is important to employ approaches to selectively antagonize androgenic and estrogenic actions. Similarly, when translating findings of ovarian function from rodent models to human, it is imperative to take into account the polyovular nature and the postnatal differentiation of the rodent ovary into consideration. Precocial species such as rhesus monkeys and sheep may be better models in terms of translating ovarian findings to human.

5. Adult metabolic phenotype of PCOS models

Women with PCOS have several accompanying metabolic derangements, which include insulin resistance [14], visceral adiposity [62], elevations in triglycerides, total cholesterol, free fatty acids, and the atherogenic index [63]. These women are at risk for beta cell dysfunction [64] and hypertension [12]. Insulin resistance is also a feature of prenatal T-treated sheep [65, 66], monkeys [67], and postnatal-T-treated rats [68] (see Table 5). Similar findings are also evident in prenatal DHT-treated sheep [66] and postnatal DHT-treated rats [30, 68]. Glucose intolerance has been described in prenatal DHT-treated mice although

insulin resistance was not evident [32] (see Table 6). Early T-treated monkeys also display pancreatic beta cell dysfunction near the end of their reproductive life [67].

Visceral adiposity has been reported in prenatal T-treated monkeys [69] and rats [70]. Interestingly, the impact of postnatal T-treatment on fat deposition in rats appears to be a function of the strain used with some showing an effect and others not [68, 71]. Visceral adiposity does not appear to be a feature of prenatal T-treated sheep during their postpubertal life [72]; in contrast these females manifest reduced visceral adiposity similar to that of lean women with PCOS [73]. An imbalance in the plasma lipid profile is evident in prenatal and postnatal T-treated rat models reflected as an increase in triglycerides and cholesterol [68, 70]. An imbalance in free fatty acid is also found in monkeys [74] and sheep [72]. No metabolic disruptions have been reported in the letrozole-treated rat model [30]. Hypertension is a feature of prenatal T-treated sheep [75], a characteristic not studied in other species. It is essential to recognize that inconsistencies across animal models may stem not only from differences in window of treatment relative to the timing of critical organ/tissue differentiation, but also from the timing of the study relative to their reproductive life span.

Developmentally, intrauterine growth restriction (IUGR), a risk factor for adult onset metabolic diseases [76], is a phenotypic characteristic manifested in prenatal T-treated sheep [77] and rats [78]. Since IUGR has only been reported in a Spanish cohort of PCOS offspring [79, 80] and not in a U.S. cohort [81], its contribution to the etiology of the syndrome remains to be resolved. One study reported subsequent catch-up growth in PCOS women [82], a feature also evident in prenatal T-treated sheep [77]. Prenatal T-treated monkeys prior to menarche also manifest accelerated growth trajectory, although they fail to manifest IUGR [83].

6. Translational relevance of animal models to PCOS and future directions

The finding that PCOS phenotype is associated with conditions such as classical 21-hydroxylase-deficiency in which the fetus has been exposed to high amounts of sex steroids before birth [20] suggest that steroid excess early in life may lead to manifestation of PCOS phenotype in adulthood [84]. Early studies measuring T in cord blood samples have found that 40% of human female fetuses are exposed to elevated levels of T, comparable to that seen in male fetuses at 19–25 weeks of gestation [85]. It remains to be determined whether the female fetuses exposed to higher T *in utero* are susceptible to develop PCOS later in life (gene by environment interaction). In translating findings from animal models of steroid excess to humans, it is important to know fetal exposure levels during critical windows of development so appropriate conclusions can be drawn. Such studies are hard to undertake in rodent models. In the gestational T-treated sheep model, female fetuses are exposed to T at levels found in the male fetuses [86].

Because investigations involving tissue resources from human subjects are difficult, animal models that manifest PCOS phenotype provide valuable tissue resources to target mechanistic questions. These models can provide a platform for developing effective early prevention/treatment strategies to prevent/overcome reproductive/metabolic dysfunctions. The findings from these animal models may also have public health implication in the context of environmental exposures to steroid mimics. Human fetuses are at risk of abnormal programming via exposure to endocrine disrupting chemicals that serve as steroid modulators from various domains in the environment (Figure 1) [87], as well as from excess exposure to steroids through disease states [88].

Future studies with animal models should capitalize on the identified strengths of the various models to dissect out the early causal signals involved in the developmental progression of

PCOS. Studies should target time points during development that are comparable to time points of organ differentiation in humans and strive to dissect out the relative fetal and maternal contributions in programming the human PCOS phenotype. Recently, a new animal model in which T is delivered directly into the fetus has been developed [89]. Unfortunately, in addition to lack of information on whether a PCOS phenotype develops during adult life, the internal exposure level of T achieved by this approach is far above the normal endogenous male range. Refinement of this model holds promise in helping dissect out the fetal vs. maternal contribution. Because of the potential for such PCOS traits to be carried forward to subsequent generations, transgenerational studies that focus on causal mechanisms are very much needed to help segregate genetic/epigenetic interactions and differences in individual susceptibility.

Required are also studies in humans mapping the developmental changes in fetal and maternal steroidal and metabolic environment. Clinical studies should target early gestational stages and gain information on developmental changes at the maternal level and when possible capitalize on amniocentesis and postmortem samples to assess fetal contribution. Such studies should include normal pregnancies as well as PCOS pregnancies. Since much of the programming on the ovary and brain may have occurred early during gestation in humans, studies with term cord blood samples are not appropriate media for these investigations. Human studies need to be expanded to dissect out the relative contribution of both androgens and estrogens and the reference to T-treated models as androgenized models should be avoided.

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Highlights

- Perinatal steroid excess can program PCOS phenotypic expression.
- Perinatal steroid excess can occur through many environmental sources.
- Translation of animal studies to findings in PCOS women has strengths and weaknesses.

Figure 1.

Sources and domains of exposure of environmental endocrine disruptors. Intentional exposures refer to exposures that occur while the individual is aware of the exposure. Unintentional exposures refer to exposures that occur without the individual's awareness of the exposure. * Represent exposures that occur during early pregnancy states without maternal awareness of pregnancy. PCBs: Polychlorinated biphenyls.

Table 1

Reproductive cyclicity and neuroendocrine attributes of prenatal T-treated monkeys, sheep and rats and postnatal T-treated rats relative to those of women with PCOS.

PCOS phenotype	PCOS women	Prenatal					Postnatal	
		Monkey		Sheep		Rat ^{SD}	Rat ^{SD/W}	
		T ^P		T ^P		T ^F		
		GD (40–60) to (55–120)	GD (110–115) to 139	GD 30–90	GD 60–90	GD 16–19	3h PN	
Oligo- anovulation	yes	yes	yes	yes	no	yes	----	
LH excess	yes	yes	no	yes	no	yes	----	
Disrupted E ₂ positive feedback	?	no	----	yes	yes	yes	----	
Reduced E ₂ negative feedback	?	yes	----	yes	----	----	----	
Reduced P ₄ negative feedback	yes	yes	yes	yes	----	----	----	
↑ GnRH sensitivity	yes	yes	----	yes	----	no	----	

Abbreviations: E₂: estradiol, GD: gestational hours, P₄: progesterone, and †: increased. Superscripts: F^{Free}, P^{Propionate}, SD^{Sprague-Dawley}, W^{Wistar}. For details of specific references see reference # 22.

Table 2

Reproductive cyclicity and neuroendocrine attributes of prenatal DHT-treated sheep, rats, and mice and postnatal DHT, letrozole, and EV-treated rats relative to those of women with PCOS.

PCOS phenotype	PCOS women	Prenatal			Postnatal				
		Sheep	Rat ^{SD}	Mouse	Rat ^W	Rat ^W	Rat ^W	Rat ^W	Rat ^W
		DHT ^P	DHT ^F	DHT ^P	DHT ^P	DHT ^P	Letrozole	Letrozole	EV
		GD 30–90	GD 16–19	GD 16–18	3h PN	21d PN (90d)	21d PN (90d)	42d PN (21d)	14 d PN
Oligo- anovulation	yes	no	yes	yes	---	yes	yes	yes	yes
LH excess	yes	yes	yes	yes	---	---	---	yes	no
Disrupted E ₂ positive feedback	?	no	yes	---	---	---	---	---	---
Reduced E ₂ negative feedback	?	yes	---	---	---	---	---	---	---
Reduced P ₄ negative feedback	yes	---	---	---	---	---	---	---	---
↑ GnRH sensitivity	yes	yes	no	---	---	---	---	---	---

Abbreviations: d: day, E₂: estradiol, EV: estradiol valerate, h: hours, GD: gestational day, P₄: progesterone, PN: postnatal, and ↑: increased. Superscripts: F_{free}, P_{propionate}, SD_{Sprague-Dawley}, W_{Wistar}. For details of specific references see reference # 22.

Table 3

Ovarian attributes of prenatal T-treated monkeys, sheep and rats and postnatal T-treated rats relative to those of women with PCOS.

PCOS phenotype	PCOS women	Prenatal				Postnatal	
		Monkey		Sheep		Rat ^{SD/W}	
		T ^P		T ^P		T ^P	
		GD (40–60) to (55–120)	GD (110–115) to 139	GD 30–90	GD 60–90	GD 16–19	3h PN
Hyperandrogenism	yes	yes ^{func}	yes ^{func}	yes ^{func}	---	no, yes	no
Infertility	yes	not tested	yes	not tested	yes	not tested	---
PCO morphology	yes	yes	yes	yes	---	yes	---
↑ ovary weight/vol	yes	yes	yes	yes	---	---	---
Follicular persistence	?	---	---	yes	---	---	---
Enhanced follicular recruitment	yes	---	---	yes	---	---	---
↑ intrafollicular androgen	yes	no	no	---	---	---	---
Reduced oocyte competence	yes	yes	yes	---	---	---	---

Abbreviations: h: hours, GD: gestational day, PN: postnatal, and ↑: increased. Superscripts: F^{free}, func^{functional}, P^{propiionate}, SD^{Sprague-Dawley}, W^{Wistar}. For details of specific references see reference # 22.

Table 4

Ovarian attributes of prenatal DHT-treated sheep, rats, and mice and postnatal DHT, letrozole, and EV-treated rats relative to those of women with PCOS.

PCOS phenotype	PCOS women	Prenatal			Postnatal				
		Sheep	Rat ^{SD}	Mouse	Rat ^W	Rat ^W	Rat ^W	Rat ^W	Rat ^W
		DHT ^P	DHT ^F	DHT ^P	DHT ^P	DHT ^P	Letrozole	Letrozole	EV
		GD 30-90	GD 16-19	GD 16-18	3h PN	21d PN (90d)	21d PN (90d)	42d PN (21d)	14 d PN
Hyperandrogenism	yes	no ^{func}	no, yes	yes, no	no	no	yes	yes	no
Infertility	yes	not tested	not tested	---	---	---	---	---	---
PCO morphology	yes	no	yes	---	---	yes	yes	yes	yes
↑ ovary weight/vol	yes	yes, fetal	---	---	---	no	yes	no	no
Follicular persistence	?	no	---	---	---	---	---	---	---
Enhanced follicular recruitment	yes ^A	yes, fetal	---	---	---	---	---	---	---
↑ intrafollicular androgen	yes	---	---	---	---	---	---	---	---
Reduced oocyte competence	yes	---	---	---	---	---	---	---	---

Abbreviations: h: hours, d: day, EV: estradiol valerate, GD: gestational day, PN: postnatal, and ↑: increased. Superscripts: ^Abased on cortical biopsies ^Ffree, ^{func}functional, ^Ppropionate, ^{SD}Sprague-Dawley, ^WWistar. For details of specific references see reference # 22.

Table 5

Metabolic attributes of prenatal T-treated monkeys, sheep and rats and postnatal T-treated rats relative to those of women with PCOS.

PCOS phenotype		Prenatal					Postnatal
	PCOS women	Monkey		Sheep		Rat ^{SD}	Rat ^{SD/W}
		T ^P		T ^P		T ^F	T ^P
		GD (40–60) to (55–120)	GD (110–115) to 139	GD 30–90	GD 60–90	GD 16–19	3h PN
Reduced insulin sensitivity	yes	no	yes		yes	no	yes
Pancreatic β-cell dysfunction	at risk	yes	no				
IUGR	yes ^A	no	no		yes	yes	
Catch-up growth	yes ^A	yes ^C			yes	no	
↑ visceral fat	yes ^B	yes					yes, no
↑ serum triglycerides	yes ^B						yes
↑ total cholesterol	yes ^B						yes
↑ free fatty acids	yes ^B	yes					
↑ atherogenic index	yes ^B						yes
Hypertension	at risk			yes			

Abbreviations: h: hours, GD: gestational day, PN: postnatal, and ↑: increased. Superscripts: ^ASpanish cohort, ^Bin obese PCOS women, ^Cprior to menarche, ^Ffree, ^Ppropionate, ^{SD}Sprague-Dawley, ^WWistar. For details of specific references see reference # 22.

Table 6

Metabolic attributes of prenatal DHT-treated sheep, rats, and mice and postnatal DHT, letrozole, and EV-treated rats relative to those women with PCOS.

PCOS phenotype	PCOS women	Prenatal			Postnatal			
		Sheep	Rat ^{SD}	Mouse	Rat ^W	Rat ^W	Rat ^W	Rat ^W
		DHT ^P	DHT ^F	DHT ^P	DHT ^P	Letrozole	Letrozole	EV
		GD 30–90	GD 16–19	GD 16–18	21d PN (90d)	21d PN (90d)	42d PN (21d)	14 d PN
Reduced insulin sensitivity	yes	yes	---	no ^A	yes	no	---	---
Pancreatic β -cell dysfunction	at risk	---	---	yes	---	---	---	---
IUGR	yes ^B	---	---	---	---	---	---	---
Catch-up growth	yes ^B	---	---	---	---	---	---	---
→ visceral fat	yes ^C	---	---	no	no	no	---	---
→ serum triglycerides	yes ^C	---	---	---	no	no	---	---
→ total cholesterol	yes ^C	---	---	---	no	no	---	---
→ free fatty acids	yes ^C	---	---	---	no	no	---	---
→ atherogenic index	yes ^C	---	---	---	no	no	---	---
Hypertension	at risk	---	---	---	---	---	---	---

Abbreviations: EV: estradiol valerate, d: day, GD: gestational day, h: hours, and $\uparrow$: increased. Superscripts: ^A prenatal treatment GD16–20, ^B Spanish cohort, ^C in obese PCOS women, ^F free, ^P propionate, ^{SD} Sprague-Dawley, ^W Wistar. For details of specific references see reference # 22.