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Steroidogenic vs. metabolic programming of reproductive neuroendocrine, ovarian, and metabolic dysfunctions

Rodolfo C. Cardoso, Muraly Puttabyatappa, and Vasantha Padmanabhan

Department of Pediatrics, University of Michigan, Ann Arbor, MI, USA

Abstract

The susceptibility of the reproductive system to early exposure to steroid hormones has become a major concern in our modern societies. Human fetuses are at risk of abnormal programming via exposure to endocrine disrupting chemicals, inadvertent use of contraceptive pills during pregnancy, as well as from excess exposure to steroids through disease states. Animal models provide an unparalleled resource to understand the developmental origin of diseases. In female sheep, prenatal exposure to testosterone (T) excess results in an array of adult reproductive disorders that recapitulate those seen in women with polycystic ovary syndrome (PCOS), including disrupted neuroendocrine feedback mechanisms, increased pituitary sensitivity to gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH) excess, functional hyperandrogenism, and multifollicular ovarian morphology culminating in early reproductive failure. Prenatal T-treatment also leads to fetal growth retardation, insulin resistance, and hypertension. Mounting evidence suggests that developmental exposure to improper steroidal as well as metabolic environment may mediate the programming of adult disorders in prenatal T-treated females and these defects are maintained or amplified by postnatal sex steroid and metabolic milieu. This review addresses the steroidal and metabolic contributions to the development and maintenance of PCOS phenotype in the prenatal T-treated sheep model, centering specifically on the effects of prenatal and postnatal treatment with androgen antagonist or insulin sensitizer as potential strategies to prevent/ameliorate these dysfunctions. Insights obtained from these intervention strategies on the mechanisms underlying these defects are likely to have translational relevance to human PCOS.

Keywords

androgens; estrogens; insulin; fertility; PCOS

Corresponding author: Vasantha Padmanabhan, Ph.D., Department of Pediatrics, University of Michigan, 300 N. Ingalls St., room 1138, Ann Arbor, MI, 48109 USA, Tel: +1 (734) 647-0276, Fax: +1 (734) 615-5441, vasantha@med.umich.edu.

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Introduction

A developing fetus is extremely susceptible to even subtle changes in the intrauterine environment. Some of the changes that the fetus is exposed to may be beneficial for healthy development and survival of the fetus while others may prove to be detrimental. Existing evidence indicates that exposure of pregnant mothers to adverse conditions via nutritional deficits/excess, stress, drugs, disease states, environmental endocrine disrupting chemicals, and/or infectious agents can have an impact on the maternal milieu culminating in adult pathologies [1–6]. Lifestyle choices made by the mother such as diet, smoking, drinking and drugs, medical interventions for treatment for pathologies, and intentional or unintentional exposure to environmental endocrine disrupting chemicals therefore pose a threat to the normal developmental trajectory of the fetus.

The concept of developmental programming is not new. Steroid hormones play a critical role during development influencing cell differentiation into organ systems [6, 7]. The reproductive and metabolic systems are especially susceptible to improper steroid exposure during development culminating in pathologies during adulthood. The developing fetus/offspring can be exposed to steroids through disease states, failed contraception and continued exposure to contraceptive steroids, maternal use of anabolic steroids, and inadvertent exposure to environmental compounds with steroidogenic potential [8]. Epidemiological studies point to developmental effects of such environmental disruptors, which act as steroid mimics, in humans. Recent increases in estrogen sensitive cancers (breast, prostate, and testis), endometriosis, male genital abnormalities, decline in semen quality, and early onset of puberty in girls all point to the looming problem [9].

It has been long known that exposure to excess testosterone (T) during fetal life induces phenotypic virilization and behavioral masculinization in the female offspring [10, 11]. In the last decade, the concept has gained momentum in the context of development of adult pathologies [1]. For instance, studies in several animal models have shown that exposure to T excess during fetal life induces reproductive neuroendocrine, ovarian, and metabolic defects in the female offspring, characteristics also seen in women with polycystic ovary syndrome (PCOS) [8]. Among the various models comprising different species (monkeys, rats, mice, and sheep), sheep is one model in which longitudinal studies focusing on multiple developmental time points in the reproductive life span have been carried out. This review focuses mainly on the prenatal T-treated sheep model with emphasis on the mediators of the reproductive neuroendocrine, ovarian, and metabolic disruptions reported in these animals drawing information from other animal models where necessary.

Disruptions in reproductive cyclicity and fertility

Studies using different breeds of sheep (Finish-Landrace $\times$ Dorset Horn, Poll Dorset, and Suffolk) found progressive deterioration of the reproductive axis in prenatally T-treated females, though with considerable variability in the degree of severity [12–15]. These differences between distinct breeds are supportive of contribution from genetic predisposition relative to how they respond to insults. The fact that most of the gestational day (GD) 60–90-treated females cycled during the second breeding season as opposed to

GD30–90-treated animals becoming anovulatory [15] is supportive of the existence of critical windows of susceptibility and may be a function of exposure relative to when the various organ systems are differentiating.

Mating trials found that rams ignored prenatal T-treated females (GD60–90) when control females were around; however, mating success was 100% when T animals were separated from controls and bred [16]. Nevertheless, pregnancy rate in GD60–90 females was only 40% compared with controls that presented a 90% pregnancy rate [16]. Fertility testing is not possible in GD30–90 females, which are phenotypically virilized [17]. Studies carried out to determine the impact of metabolic status and adiposity found that postnatal overfeeding amplifies the severity of the reproductive phenotype in GD30–90 females with majority becoming anovulatory in the first breeding season [18]. These findings are supportive of a two-step process proposed earlier [19]: the first insult (*e.g.*, gestational T exposure) leading to organizational changes (programming) and the second (*e.g.*, obesity) amplifying the severity of the phenotype.

Steroidal vs. metabolic contributions to deficits seen in prenatal testosterone-treated sheep

Gestational T excess increases not only maternal T, but also fetal T and estradiol (E_2) concentrations in sheep [20]. Androgens and estrogens play an important role in the development of the brain [21, 22] and establishment of neuroendocrine feedback mechanisms controlling gonadotropin-releasing hormone (GnRH) and gonadotropin release [17]. Steroid hormones also act directly at the pituitary levels regulating synthesis and secretion of gonadotropins [23, 24], and at the ovarian level controlling folliculogenesis and steroidogenesis [25, 26]. Thus, improper developmental steroid exposure mediates the programming of adult reproductive disorders in prenatal T-treated females.

A second possibility is that adult defects in prenatal T-treated sheep are also facilitated by altered maternal and/or fetal metabolic milieu. Gestational T excess leads to maternal hyperinsulinemia [27] and disrupts insulin signaling in classical insulin target tissues (Lu and Padmanabhan, unpublished observations) during a period of fetal development that encompasses the time of organization of the GnRH neuronal network [28], as well as pituitary [29] and ovarian differentiation [30, 31]. Because insulin is an essential contributor of brain [32, 33] and pituitary [34] development, and establishment of ovarian reserve [35, 36], reproductive alterations may be mediated, in part, by altered insulin sensitivity. Interestingly, women with PCOS, whose characteristics prenatal T-treated sheep recapitulate, also present elevated concentrations of T and insulin during pregnancy [37].

In addition to programming during fetal development, reproductive and metabolic defects seen in prenatal T-treated sheep may be maintained or amplified by postnatal alterations in sex steroid (functional hyperandrogenism) and metabolic (hyperinsulinemia) profile. Indeed, gestational T-treatment increases androgen receptor (AR) expression in the hypothalamus [38], pituitary (Nada and Padmanabhan, unpublished observations), and granulosa cells of antral follicles [39] during adult life. Furthermore, gestational T-treatment also impairs

insulin signaling in a tissue-specific manner [40] leading to hyperinsulinemia [41] in the adult offspring.

Over the last years, our group has been investigating the effects of prenatal and postnatal treatment with androgen antagonist or insulin sensitizer as potential strategies to prevent/ameliorate dysfunctions seen in T-treated females and gain insights on the mechanisms underlying these defects. These studies found that prenatal and postnatal treatment with both androgen antagonist and insulin sensitizer prevented the advancement in onset of puberty seen in these females [42]. In addition, postnatal administration of rosiglitazone, an insulin sensitizer, was found to increase the insulin sensitivity index, decrease the number of aberrant estrous cycles during the second breeding season, and prevent further deterioration of the reproductive axis in T females [43]. As discussed below, this amelioration in reproductive function may result from improvements in 1) feedback mechanisms controlling secretion of GnRH and gonadotropins; 2) follicular development and steroidogenesis; and/or 3) general metabolic status and insulin sensitivity.

Neuroendocrine disruptions

Progressive reproductive deterioration seen in female sheep prenatally exposed to T excess may stem, at least in part, from tonic activation of the reproductive neuroendocrine axis [24, 44, 45]. Prenatal T-treated sheep present defects in all three steroid feedback mechanisms controlling GnRH and gonadotropin secretion (Figure 1), namely E₂ negative [17, 44], E₂ positive [17, 47, 48], and progesterone (P₄) negative feedback [45, 49]. Moreover, pituitary sensitivity to GnRH is remarkably increased in prenatal T-treated sheep [24]. The defects in steroid negative feedback and augmented pituitary responsiveness to GnRH together contribute to the luteinizing hormone (LH) excess and consequent functional hyperandrogenism seen in prenatal T-treated females (Figure 2). Further studies investigating the effects of prenatal treatment with T, dihydrotestosterone (DHT), a non-aromatizable androgen, or co-administration of the androgen antagonist, flutamide, with T have pointed to disruptions of E₂ negative feedback being programmed by androgenic action of T, with both T and DHT and not T + flutamide reducing sensitivity to E₂ [17, 45, 50]. On the other hand, disruptions in E₂ positive feedback were found in T- but not DHT-treated females suggesting that this defect is likely programmed via estrogenic actions of prenatal T [17, 45]. The observation that co-treatment with prenatal T and androgen antagonist failed to reverse the defects in E₂ positive feedback is supportive of this premise [51].

At the hypothalamic level, neurons colocalizing the neuropeptides kisspeptin, neurokinin B (NKB), and dynorphin (KNDy neurons) in the arcuate nucleus are thought to play a key role in mediating the negative feedback effects of E₂ and P₄ upon GnRH [52]. Prenatal T-treatment results in marked reduction in NKB and dynorphin in KNDy neurons with kisspeptin remaining unaltered [53]. This peptide imbalance within a single neuronal population has been proposed to underlie some of the defects in responsiveness of the GnRH system to E₂ and P₄ seen in prenatal T-treated sheep [54]. Recent findings demonstrate that exposure to T excess during fetal development decreases the number of KNDy neurons colocalizing NKB receptors (NK3R) in adult females [55]. Because NKB may act as an

autoregulatory transmitter in KNDy neurons, a combined decrease in both ligand and receptor may contribute to defects in the control of GnRH/LH secretion.

At the positive feedback level, postnatal treatment with androgen antagonist or insulin sensitizer has been shown to partially improve the neuroendocrine response, increasing the magnitude but failing to prevent the delay in LH surge response to the E₂ positive feedback challenge [51]. These results indicate that timing and magnitude of the LH surge are programmed by different neuroendocrine mechanisms with postnatal androgens and insulin determining the magnitude and estrogens likely the timing of the LH surge. The observation that prenatal T-treated females subjected to neonatal ovariectomy present improvements in E₂ positive feedback [56] further supports the hypothesis that postnatal exposure of the neuroendocrine axis to sex steroids, or other ovarian factors, is required to fully defeminize the GnRH/LH surge mechanism in T females.

The potential mediators of the alterations in hypothalamic neuropeptide (NKB and dynorphin) and receptor (NK3R) abundance are not completely elucidated but may involve androgens, estrogens, and insulin. Prenatal treatment with T and DHT, but not E₂, results in an increase in AR immunoreactivity in the arcuate nucleus and specifically in KNDy neurons in adult females and co-treatment with flutamide reverts this effect, suggesting that prenatal organization of AR distribution and expression is mediated by androgenic actions of T [38]. In contrast, prenatal T-treatment decreases the percentage of KNDy neurons that colocalize the beta subunit of insulin receptor (IR β) and co-administration of flutamide fails to prevent this change in female sheep, indicating that this alteration is programmed likely by estrogenic actions of T [57]. Because KNDy neurons are believed to mediate in part the stimulatory effects of insulin on GnRH and LH release [58, 59], alterations in IR β expression in this cell population may contribute to defects in reproductive functions seen in this animal model.

At the pituitary level, the increased sensitivity to GnRH seen in T females appears to be programmed via androgenic actions of T, since prenatal treatment with DHT also results in increased amplitude of LH pulses after intermittent administration of GnRH boluses under conditions in which endogenous GnRH secretion is suppressed [24]. Consistent with a pituitary effect, prenatal T-treatment increased expression of GnRH receptor (GnRHR) and decreased abundance of estrogen receptor alpha (ESR1) mRNA in the fetal pituitary [24]. These findings combined with the observation that prenatal T-excess decreases the percentage of gonadotropes colocalizing ESR1 in adult ovariectomized females [60] suggest that developmental changes in regulators of gonadotropin synthesis/secretion, including GnRHR and ESR1, may be involved in the increased pituitary responsiveness to GnRH and decreased sensitivity to E₂ seen in these animals. Other mediators involved in the increased pituitary sensitivity to GnRH are unclear, however, observations that insulin augments the effects of GnRH on LH synthesis and secretion [61, 62] suggest that insulin may play a role.

From a mechanistic perspective, future studies investigating the effects of androgen antagonist and insulin sensitizer on the neuroendocrine response to E₂ and P₄ negative feedback mechanisms may shed light on the mediators leading to these defects.

Ovarian disruptions

In addition to reproductive neuroendocrine disruptions, prenatal T-treatment results in multifollicular ovary [63, 64]. This multifollicular phenotype may stem from aberrant increase in follicular recruitment coupled with arrest in antral follicular development causing persistence.

Follicular activation/recruitment

Ovaries have a finite pool of primordial follicles from which the folliculogenesis starts through a process of activation/recruitment. Majority of the primordial follicles remain in quiescent state and only a few follicles are activated during each reproductive cycle. In addition to activation, the depletion of this pool occurs through the process called follicular atresia [65]. Follicular activation is gonadotropin-independent and is regulated by locally produced growth factors and cytokines [66] and include kit ligand (KITL), fibroblast growth factor (FGF), transforming growth factor (TGF) α , leukemia inhibitory factor (LIF), bone morphogenetic protein 4 (BMP4), anti-Müllerian hormone (AMH), and transforming growth factor (TGF) β [67, 68]. The exact mechanism of activation is not known but it is believed that the balance between activators (KITL, FGF, TGF α , LIF, and BMP4) and inhibitors (AMH and TGF β) drives the initiation process [69]. These factors activate intracellular signaling pathways such as phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) and mammalian target of rapamycin (mTOR) that promote follicle survival and primordial follicle activation [70]. Gene knockout studies suggest that PI3K/AKT-dependent phosphorylation of the transcription factor forkhead box O3a (FOXO3A) leads to premature follicular activation [71]. Gene deletion of the AKT inhibitor, phosphatase and tensin homolog (PTEN), also increases activation of follicles as a result of FOXO3A phosphorylation [72]. Similarly, gene deletion of the inhibitor of follicular activation, AMH, induces premature depletion of primordial follicles [73]. Androgen signaling is also implicated in this process; androgen through either its nuclear receptor or non-genomic mechanisms can activate the PI3K-AKT-FOXO3 signaling in oocyte [74, 75]. On the other hand, follicular atresia underlying follicular depletion is believed to be controlled by the balance between pro- and anti-apoptotic factors. These belong to the B-cell lymphoma-2 (BCL2) family that comprises anti-apoptotic BCL2 and myeloid cell leukemia-1 (MCL1), as well as pro-apoptotic BCL2-associated X protein (BAX) and BCL2-related ovarian killer (BOK) [76]. Growth factors such as KITL are known to increase BCL2 gene expression and promote early follicular survival [65]. A player receiving attention recently in the follicular transition from primordial to primary is the extracellular matrix protein fibrillin 3 (FBN3), which reduces bioavailability of TGF family members [77–79].

In terms of ovarian disruptions, prenatal T-treatment decreases AMH levels in early growing follicles, thereby reducing its inhibitory actions on early follicular growth allowing increased recruitment [80]. Moreover, prenatal T reduces BAX without changing BCL2 in early growing follicles making them resistant to atresia [81]. Prenatal T excess also increases AR protein in primordial and primary follicles [39]. All these changes are consistent with increased follicular recruitment (Figure 3A). Absence of changes in growth differentiation factor (GDF) 9 [80] in prenatal T-treated sheep ovaries and reduced GDF9

mRNA in oocytes of PCOS women [82] are inconsistent with the increased number of growing follicles, considering GDF9 knockout mice show an arrest in follicular development at the primary follicular stage [83].

To better understand the mechanism of increased follicular recruitment in prenatal T-treated females, PI3K/AKT and FOXO family of transcription factors together with FBN3 (dinucleotide repeat marker in intron 55 of this gene has been linked to PCOS [84]) and other apoptotic genes that seem to be central players need to be investigated.

Follicular arrest and persistence

With progression of follicular development, pre-antral/secondary follicles with acquisition of multiple layers of granulosa cells are formed. The somatic cells of these follicles become less sensitive to oocyte-secreted growth factors changing from heretofore gonadotropin-independent to gonadotropin-dependent. Follicle-stimulating hormone (FSH) is the main gonadotropin required by these follicles and its actions at the gonadal level are controlled by activin, which promotes FSH actions, as well as inhibins and follistatin, which inhibit [85]. FOXO1 has also been shown to interact with activin to promote follicular growth [86]. FSH actions are also mediated by insulin-like growth factor (IGF) I or II [87]. As follicles grow, the amount of AMH produced by these follicles reduces, thus increasing sensitivity of granulosa cells to FSH [88].

As follicles develop further transitioning into antral follicles the following factors promote preovulatory follicle formation. In granulosa cells, FSH and activins stimulate aromatase (CYP19A1) expression while LH and inhibins stimulate thecal CYP17A1. Activins produced by granulosa cells suppress CYP17A1 expression and are blocked by follistatin binding of activin [89]. Insulin can act through either its receptor or through IGF receptors and insulin action is greatly enhanced by the adipocytokine, adiponectin [90]. Insulin together with adiponectin promotes gonadotropin action and granulosa cell steroidogenesis [91]. Non-dominant follicles that do not develop into preovulatory follicles undergo atresia and pro-apoptotic factors such as tumor necrosis factor (TNF) α , prohibitin, Fas, p53 and others are implicated [65]. In addition to secreting growth factors such as GDF9, oocytes also acquire developmental competence with the ability to complete meiosis and undergo fertilization [92]. Other factors such as WNT/Frizzled signaling pathway, IGF and IGF binding proteins (IGFBP), and vascular endothelial growth factor (VEGF) can also influence follicular growth and maturation [93–95].

In the context of ovarian defects, prenatal T-treatment alters the ratio of FSH regulatory proteins with increased mRNA levels of follistatin and reduced activin β B [64]. In addition, antral follicles have greater AMH protein expression, which reduces sensitivity to FSH [80]. These likely create a net negative FSH milieu in the follicle and contribute to follicular arrest in development (Figure 3B). The increased level of follistatin observed in prenatal T-treated females [64] could increase ovarian androgen production by negating activin action. The thecal androgen production could also be influenced by LH hypersecretion [44, 45, 96]. Although the reduced expression of the enzyme CYP17A1 in the theca interna of antral follicles in prenatal T-treated sheep [97] is inconsistent with hyperandrogenism, this may be offset by an increase in the activity of this enzyme, compensatory induction of another

isoform, or autocrine/paracrine feedback inhibition [97, 98]. However, gestational T increases AR in these follicles supporting functional hyperandrogenism [39]. The reduced adiponectin in granulosa cells of prenatal T sheep may affect follicular insulin sensitivity and compromise growth [99]. Findings that prenatal T-treatment reduces anti-apoptotic protein BCL2 and the apoptosis effector protein caspase-3 in granulosa cells of antral follicles [81] support a shift in the balance of the pro- versus anti-apoptotic genes that likely arrest the follicle from undergoing atresia or developing further. While oocyte fertilization has not been examined directly in prenatal T-treated sheep, mating studies showed a reduced pregnancy rate [16]. This is consistent with impaired oocyte fertilization observed in PCOS women and prenatal T-treated macaques [96, 100, 101].

Together, these findings suggest that follicular arrest in prenatal T-treated models results from failure at multiple levels involving coordinated effect of several factors. Analysis of other factors such as IGFs, VEGFs, WNTs and other pro-apoptotic proteins and interventional studies negating actions of these mediators are required to completely elucidate how follicular arrest and persistence develop in prenatal T-treated models.

Metabolic dysfunctions

In addition to reproductive disruptions, prenatal T-treatment leads to intrauterine growth restriction (IUGR), low birth weight and postnatal catch-up growth [102], risk factors for adult well-being [103, 104]. Developmental changes in the IGF/IGFBP system in prenatal T-treated sheep are consistent with changes in growth trajectory with a reduction in IGF bioavailability evident during IUGR and an increase during postnatal catch-up growth [102, 105]. Radiotelemetric studies found gestational T excess to also increase arterial and diastolic blood pressure [106]. Metabolic disruptions have also been reported in other prenatal T-treated animal models [107, 108].

Gestational T also reduces peripheral insulin sensitivity leading to hyperinsulinemic status indicative of insulin resistance in sheep [41, 109–111]. Comparative studies with T- and DHT-treated females indicate that the programming of insulin sensitivity defects occurs via androgenic actions of T [41]. Importantly, the window of susceptibility for developing insulin resistance was found to be confined to a shorter programming window, namely 60–90 days of gestation [41]. Interestingly, at postpubertal time points (~16 mo of age), insulin sensitivity is increased, visceral adiposity and adipocyte size are reduced, and circulating palmitic acid is increased in prenatal T females [112]. Relative to earlier observations of reduced insulin sensitivity during early life and adulthood, these findings of increased insulin sensitivity and reduced adiposity postpubertally are suggestive of a period of developmental adaptation.

Investigation of the expression of IR and members of its signaling pathway in adult females revealed that T excess leads to a general down-regulation of many members of the insulin signaling cascade in liver and muscle consistent with them being insulin resistant [40]. In contrast, prenatal T excess upregulated many members of the insulin signaling cascade in the adipose tissue, supportive of increased insulin sensitivity [40]. These findings parallel changes reported in women with PCOS [113–117].

In addition to programming by steroids and insulin, dysfunctions may also be facilitated by deficits in nutrient transfer to the fetus. Gestational T-treatment advances placental differentiation, evident as early as day 65 of gestation [118]. Advanced placental differentiation was sufficient to maintain placental efficiency during early stages of gestation, but not at later stages, culminating in low birth weight [118]. The observations that DHT also advanced placental differentiation, but not T + androgen antagonist, support programming via androgenic actions of T [118]. Because alterations in fetal nutrition result in developmental adaptations that affect adult health [119], compromised placental differentiation and function likely play a role in the phenotype seen in this sheep model.

It is well-established that obesity, a condition associated with systemic inflammation, is a risk factor for insulin resistance and diabetes [120, 121]. Adipokines, oxidative stress, and pro-inflammatory cytokines, such as interleukin 4 (IL4), IL6, and TNF α have been identified as important factors linking obesity and insulin resistance [121–123]. Whether these factors also mediate insulin resistance in females prenatally exposed to T excess remains to be determined. Nevertheless, it is known that postnatal overfeeding leading to increased body weight gain exacerbates the insulin resistance in prenatal T females [41].

Human translation

The phenotypic attributes of prenatal T sheep are similar to features seen in women with PCOS, a major reproductive disorder affecting women of reproductive age [124]; as such, prenatal T sheep may serve as a valuable model for understanding the developmental origin of the PCOS phenotype. This is especially so because the organization of GnRH neuronal network, completion of ovarian differentiation and pancreatic islet differentiation in sheep occurs *in utero* as is the case in humans [125]. Other benefits of sheep for studying developmental origin of reproductive and metabolic disorders include the wealth of available normative information, feasibility of performing studies in natural environment, availability of hypophyseal-portal approaches to gain an understanding of neural secretory dynamics, ability to non-invasively monitor follicular dynamics longitudinally, feasibility to tap into fetal circulation and the relatively short time line from birth to adulthood (28 wk to puberty).

The attributes of prenatal T treated sheep recapitulate both the reproductive and metabolic phenotype of PCOS women (oligo/anovulation, functional hyperandrogenism, multifollicular ovarian morphology, and insulin resistance) and meet the NIH, Rotterdam, and AE-PCOSS criteria (125). One limitation of the sheep model is that their longer life span compared to rats and mice makes it challenging to study transgenerational effects of prenatal steroid excess. The benefit of knocking in/out genes, which provide a powerful tool for addressing functionality of specific genes in mice, are also difficult to perform in sheep. On the other hand, rats and mice are polyovular and altricial, thus making it difficult to translate some findings to humans. The long developmental timeline and elevated costs limit extensive use in research of prenatal T-treated rhesus monkeys that bear developmental and genealogical similarity to humans. Therefore, it is important to take into account the developmental time line of organ systems of each model while probing mechanisms to enable human translation.

Conclusions

Studies discussed in this review centering on sheep as a model system highlight the concerns inappropriate exposure to steroid hormones/steroid mimics pose to the well-being of the developing offspring. Importantly, these studies point to the coordinated impact of several systems (Figure 4) in establishing or disrupting the final phenotype and thereby emphasizing the need for developing integrative approaches to overcome pathology. Our studies in the prenatal T model clearly point to disruptions at the neuroendocrine, ovarian and metabolic level each impinging on the other. This highlights the need for interventions targeting at multiple levels for achieving optimal success.

From a mechanistic perspective, future studies should focus on further elucidating the mediators involved in programming and maintaining the dysfunctions seen in this animal model. Moreover, because of the potential for these alterations to be carried forward to subsequent generations, transgenerational studies are needed to help elucidate potential epigenetic mechanisms implicated in the reprogramming of reproductive and metabolic systems.

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Fig. 1.

Schematic of neuroendocrine feedback systems involved in the control of GnRH/LH secretion that are reprogrammed by prenatal T excess. Upper panel: Pattern of secretion of GnRH/LH in control female sheep; lower panel: Pattern of secretion of GnRH/LH in prenatal T-treated female sheep. (1) E₂ negative feedback: GnRH/LH release is under the control of negative feedback action of E₂, which is predominant during the prepubertal and anestrus period. Prenatal T-treatment decreases the sensitivity of the neuroendocrine axis to E₂, resulting in increased LH pulse frequency. (2) E₂ positive feedback: Positive feedback actions of E₂ responsible for generation of the preovulatory GnRH/LH surge and onset of cyclicity. Prenatal T-treated females present delayed and dampened LH surge. (3) P₄ negative feedback: After puberty (right panels), elevated concentrations of P₄ reduce secretion of GnRH/LH pulses preventing ovulation to occur during the luteal phase. Prenatal T-treatment decreases the sensitivity of the neuroendocrine axis to P₄, leading to increased LH pulsatile release. Panels illustrating the hormonal profile in control females have been modified from [46].

Fig. 2.

Impact of prenatal exposure to T excess on the reproductive neuroendocrine axis in female sheep. Prenatal T-excess (middle panel) results in impaired steroid feedback mechanisms and increased pituitary responsiveness to GnRH, leading to increased frequency and amplitude of LH pulses (LH hypersecretion). Prenatal intervention with androgen antagonist (left panel) prevents the organizational alterations programmed by T excess during fetal life. Postnatal interventions with androgen antagonist and insulin sensitizer agents (right panel) improve neuroendocrine functions and prevent further deterioration of the reproductive system. These interventions are believed to revert/ameliorate (red X) organizational modifications programmed prenatally by T excess.

Fig. 3.

Schematic showing the impact of prenatal T-treatment on the ovary. Prenatal T-treatment causes multifollicular ovarian phenotype by increasing primordial follicular activation/recruitment (A) and follicular arrest/persistence (B). Increased follicular activation may result from increased phosphorylation of FOXO transcription factors, decrease in inhibitory AMH levels, or follicular atresia. Follicular arrest may stem from reduced gonadotropin sensitivity through increased follistatin and AMH, reduced insulin sensitivity due to decreased adiponectin, or lack of follicular atresia. Green lines indicate activation, red lines indicate inhibition, and the thickness of the lines represents the intensity of activation or inhibition. The factors in blue blocks have not been investigated in prenatal T-treated sheep but have been reported in other models of T treatment.

Fig. 4. Self-perpetuating vicious cycle involving the three systems (neuroendocrine, ovarian, and metabolic) and main alterations observed in females exposed to prenatal T excess.