Sex Differences in the Effect of Prenatal Testosterone Exposure on Steroid Hormone Production in Adult Rats

E. DOMONKOS[1], V. BORBÉLYOVÁ[1], L. KOLÁTOROVÁ[2], T. CHLUPÁČOVÁ[2], D. OSTATNÍKOVÁ[3], J. HODOSY[1,3], L. STÁRKA[2], P. CELEC[1,4,5]

[1]Institute of Molecular Biomedicine, Faculty of Medicine, Comenius University, Bratislava, Slovakia, [2]Institute of Endocrinology, Prague, Czech Republic, [3]Institute of Physiology, Faculty of Medicine, Comenius University, Bratislava, Slovakia, [4]Institute of Pathophysiology, Faculty of Medicine, Comenius University, Bratislava, Slovakia, [5]Department of Molecular Biology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia

Received April 24, 2017
Accepted May 26, 2017

Summary

Maternal hyperandrogenism during pregnancy might have metabolic and endocrine consequences on the offspring as shown for the polycystic ovary syndrome. Despite numerous experiments, the impact of prenatal hyperandrogenic environment on postnatal sex steroid milieu is not yet clear. In this study, we investigated the effect of prenatal testosterone excess on postnatal concentrations of luteinizing hormone, corticosterone and steroid hormones including testosterone, pregnenolone, progesterone, estradiol and 7β-hydroxy-epiandrosterone in the offspring of both sexes. Pregnant rats were injected daily with either testosterone propionate or vehicle from gestational day 14 until parturition. The hormones were evaluated in plasma of the adult offspring. As expected, females had lower testosterone and higher pregnenolone, progesterone and estradiol in comparison to males. In addition, corticosterone was higher in females than in males, and it was further elevated by prenatal testosterone treatment. In males, prenatal testosterone exposure resulted in higher 7β-hydroxy-epiandrosterone in comparison to control group. None of the other analyzed hormones were affected by prenatal testosterone. In conclusion, our results did not show major effects on sex hormone production or luteinizing hormone release in adult rats resulting from testosterone excess during their fetal development. However, maternal hyperandrogenism seems to partially affect steroid biosynthesis in sex-specific manner.

Key words

Androgen • Estrogen • Gonadotropins • Glucocorticoid • Steroidogenesis • Prenatal androgenization

Corresponding author

P. Celec, Institute of Molecular Biomedicine, Faculty of Medicine, Comenius University, Sasinkova 4, 811 08 Bratislava, Slovakia. E-mail: petercelec@gmail.com

Introduction

Exposure of female fetuses to excess testosterone during the early life may induce polycystic ovary syndrome (PCOS)-like phenotype (Stikkelbroeck *et al.* 2003). Although it has been suggested, that the time of androgen exposure during the prenatal life may have a significant role in future appearance of PCOS, the critical periods for the development of PCOS are unknown yet (Ramezani Tehrani *et al.* 2014). Animal models using rhesus monkeys (Dumesic *et al.* 2007), sheeps (Padmanabhan and Veiga-Lopez 2013) as well as rodents (Ramezani Tehrani *et al.* 2014, Roland and Moenter 2014) can mirror some of the reproductive (Rhees *et al.* 1997, Wolf *et al.* 2002, Wu *et al.* 2010, Yang *et al.* 2011, Tehrani *et al.* 2014, Padmanabhan *et al.* 2015, Veiga-Lopez *et al.* 2016) and metabolic (Yang *et al.* 2011, Sun *et al.* 2012, Abruzzese *et al.* 2016) features of PCOS observed in women. Furthermore, disturbed production of steroid hormones, including enhanced placental (Sun *et al.* 2012) and ovarian steroidogenesis (Wu *et al.* 2010, Yang *et al.* 2011, Amalfi *et al.* 2012) and, on the contrary, decreased intrafollicular steroid concentration (Veiga-Lopez *et al.* 2016) has been shown in prenatally androgenized (PA) female offspring.

© 2017 Institute of Physiology of the Czech Academy of Sciences, Prague, Czech Republic

Moreover, androgens can organize the hypothalamic-pituitary-adrenal stress response (McCormick and Mahoney 1999). Therefore it was suggested, that prenatal androgenization of female fetuses may lead to adrenal hyperandrogenism, at least to increased production of dehydroepiandrosterone (DHEA) under basal condition, and to adrenocorticotropic hormone (ACTH)-induced overproduction of DHEA, androstenedione and corticosterone (Zhou *et al.* 2005). At the neuroendocrine level, prenatal testosterone excess can result in disturbed regulation of secretion of the luteinizing hormone (LH) (Cardoso *et al.* 2016). Enhanced LH secretion may be attributed to impaired inhibitory feedback of estradiol at the hypothalamus and pituitary level (Sarma *et al.* 2005, Jackson *et al.* 2009) or to reduced peripheral sensitivity to other hormones (Cardoso *et al.* 2016). On the other hand, a low incidence of the preovulatory, estradiol-induced, LH surge was observed in testosterone-exposed females (Padmanabhan *et al.* 2015), which is probably caused by the suppressive effects of prenatal androgens on the expression of progesterone receptors in the hypothalamus (Foecking *et al.* 2005, Wu *et al.* 2010).

The pathophysiological consequences of prenatal androgen excess in males are understudied. However, some evidence shows that maternal hyperandrogenism can result in metabolic (Lazic *et al.* 2011) and reproductive dysfunctions also in the male offspring (Wolf *et al.* 2002, Ramezani Tehrani *et al.* 2013). The contradictory results observed in experiments focusing on the effects of prenatal androgen excess on postnatal steroidogenesis might be due to variable time, duration and dose of testosterone application (Ramezani Tehrani *et al.* 2013). In addition, prenatal testosterone seems to have an opposing effect on the hypothalamo-pituitary-gonadal axis at the pituitary and gonadal level. While the pituitary gland shows an enhanced responsiveness to the gonadoliberin accompanied by an overproduction of gonadotropins in both sexes (Rojas-García *et al.* 2010), testicular Leydig cells in males have reduced sensitivity to LH (Recabarren *et al.* 2012, Recabarren *et al.* 2013).

Sex differences in the production of sex steroid hormones in the adulthood might be caused by several mechanisms (Kushnir *et al.* 2010). However, whether they may be affected by prenatal testosterone is yet unexplored. Therefore, in this study, we aimed to examine the effect of prenatal testosterone exposure on the production of sex hormones and their precursors, as well as on the synthesis of selected other steroids in both sexes. In addition, the plasma concentration of LH, regulating the secretion of sex hormones production through the hypothalamo-pituitary-gonadal axis was evaluated.

Material and Methods

Animals and treatment

Lewis female and male rats were used as parental animals (10-13-week-old, Anlab, Prague, Czech Republic). Two female rats were mated with one male per cage. Detection of sperms in the vaginal smear was taken as evidence of mating considered as insemination and it served to calculate gestational days (GD). From day of insemination (GD0), dams were housed individually in polycarbonate cage (36 x 20 x 19 cm). All animals in the experiment had *ad libitum* access to food and water and were kept under 12:12 light-dark cycle in a controlled environment with 25±2 °C temperature and 55±10 % humidity. All procedures were carried out in accordance with the Slovak legislation and were approved by the local ethical committee at the Institute of Molecular Biomedicine, Comenius University, Bratislava.

From GD14 until parturition (GD21-22) pregnant dams received daily intramuscular injection of either testosterone propionate at a dose of 2 mg/kg (Sigma-Aldrich, Munich, Germany) or vehicle (olive oil; Galvex, Banská Bystrica, Slovakia), and were allowed to deliver spontaneously (Juarez *et al.* 1998). Following the weaning, the offspring was caged in groups of same-sex littermates (2-4 per cage). To analyze the hormonal profile of the offspring, 7 female and 7 male rats were used from both groups – animals prenatally exposed to testosterone excess and controls.

Hormonal measurements

At the age of 12 weeks, animals were sacrificed in deep anesthesia (ketamine 100 mg/kg + xylazine 10 mg/kg) and trunk blood was collected from the abdominal aorta. Plasma concentrations of circulating sex steroid hormones, including testosterone, estradiol, progesterone, pregnenolone and 7β-hydroxy-epiandrosterone (7β-OH-EpiA), as well as the plasma concentration of LH and corticosterone were assessed.

Testosterone and LH concentrations were measured using commercially available ELISA kits. The analytical sensitivity of the testosterone assay was 0.083 ng/ml. The inter- and intra-assay coefficients of

variations were below 5 % (DRG Diagnostic, Marburg, Germany). The sensitivity of analysis of the LH ELISA assay was 0.313 ng/ml. Inter- as well as intra-assay variation of analysis were less than 5 % (Shibayagi Co., Ltd, Ishihara, Shibukawa, Japan).

The concentrations of pregnenolone, progesterone, corticosterone, 7β-OH-EpiA and estradiol were measured using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) employing methods described elsewhere (Sosvorova *et al.* 2015, Vitku *et al.* 2015, Vitku *et al.* 2016).

Statistical analysis

To evaluate the endocrine effects of prenatal testosterone administration in the offspring, and in parallel to detect sex differences, two-way ANOVA (one factor being treatment and second being sex) with subsequent Bonferroni *post hoc* test was used. The F and t values describe the results of the ANOVA and the subsequent pairwise comparisons, respectively. The differences between groups were considered statistically significant, if $p<0.05$. Data are presented as mean + standard deviation (SD). Statistical analyses were conducted using GraphPad Prism 6 (GraphPad Software, Inc., La Jolla, CA, USA).

Results

Plasma testosterone concentrations differed between the experimental groups (Fig. 1A, F=30.8, $p<0.001$). Females had in comparison to males 6.5- and 7-fold lower concentration of testosterone in plasma in the control (females: 0.29±0.12 ng/ml vs. males: 1.93±0.52 ng/ml, t=3.04, $p<0.05$) and in the testosterone group (females: 0.43±0.35 ng/ml vs. males: 3.02± 1.91 ng/ml, t=4.81, p<0.001), respectively. Prenatal testosterone treatment did not affect plasma testosterone concentrations in the adulthood (F=2.62, $p=0.12$). The interaction between sex and treatment was not significant (F=1.57, $p=0.22$). The groups did not differ in LH concentration. Neither gender (F=0.32, $p=0.58$) nor treatment (F=0.08, $p=0.78$) had a significant effect on LH in plasma, and no interaction between sex and treatment was found (Fig. 1B, F=0.36, $p=0.55$).

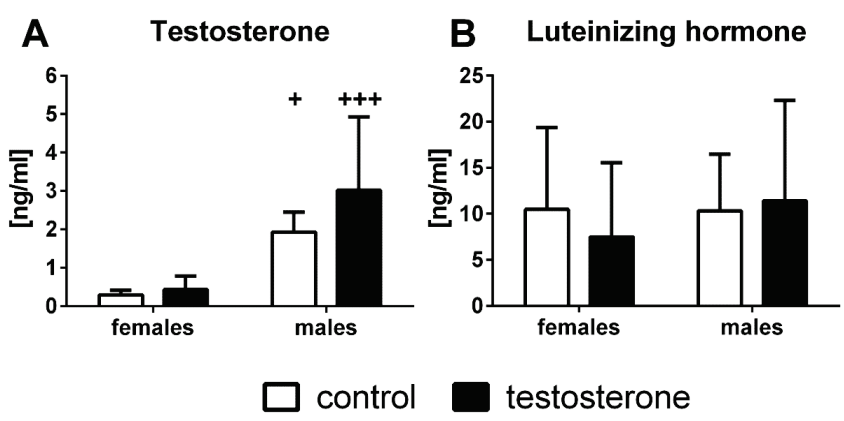

Fig. 1. Testosterone (**A**) and luteinizing hormone (**B**) concentration in rat plasma. Males have significantly higher concentration of testosterone in plasma than females in both the control (p<0.05) and in testosterone exposed group (p<0.001). Data are presented as mean + SD. + p<0.05, +++ p<0.001, + signs sex differences.

Significant sex differences were found in pregnenolone (Fig. 2A, F=44.7, $p<0.001$), progesterone (Fig. 2B, F=12.8, $p<0.01$) and estradiol concentration (Fig. 2C, F=17.5, $p<0.001$). In females, statistical analysis revealed higher progesterone (females: 2.73±2.18 ng/ml vs. males: 0.72±0.29 ng/ml, t=2.97, $p<0.05$) and estradiol (females: 0.02±0.01 ng/ml vs. males: 0.007±0.002 ng/ml, t=3.51, $p<0.001$) concentration in comparison to males only in animals that were prenatally exposed to testosterone. In the particular control groups, there was a non-significant trend to higher concentrations of these hormones in females compared to males ($p=0.09$ and $p=0.05$, respectively). Plasma pregnenolone was by 85 % higher in females than in males, regardless of the treatment (in controls: females: 1.82±0.87 ng/ml vs. males: 0.26±0.12 ng/ml, t=5.51, $p<0.01$ and in groups prenatally treated with testosterone: females: 2.79±1.25 ng/ml vs. males: 0.32±0.17 ng/ml, t=6.07, $p<0.001$). The effect of prenatal treatment on plasma concentrations of pregnenolone (F=2.85, $p=0.11$), progesterone (F=0.99, $p=0.33$) or estradiol (F=0.63, $p=0.44$) in the adulthood was not significant. Similarly, no significant sex x treatment interaction was found (pregnenolone: F=2.27, $p=0.15$; progesterone: F=0.38, $p=0.54$; estradiol: F=0.61, $p=0.45$).

Plasma concentration of 7β-OH-EpiA was significantly affected by prenatal testosterone (Fig. 2D, F=6.64, $p<0.05$). The Bonferroni *post hoc* test revealed

a significant difference with 66.7 % higher concentration of 7β-OH-EpiA in males prenatally exposed to testosterone excess in comparison to control males (testosterone group: 0.06±0.03 ng/ml vs. control group: 0.02±0.02 ng/ml, t=2.58, p<0.05). Such effect of treatment was not observed in females (testosterone group: 0.06±0.03 ng/ml vs. control group: 0.04±0.02 ng/ml, t=1.06, p=0.60). Neither the effect of sex (F=1.26, p=0.27), nor the interaction between sex and treatment (F=1.16, p=0.29) were significant statistically.

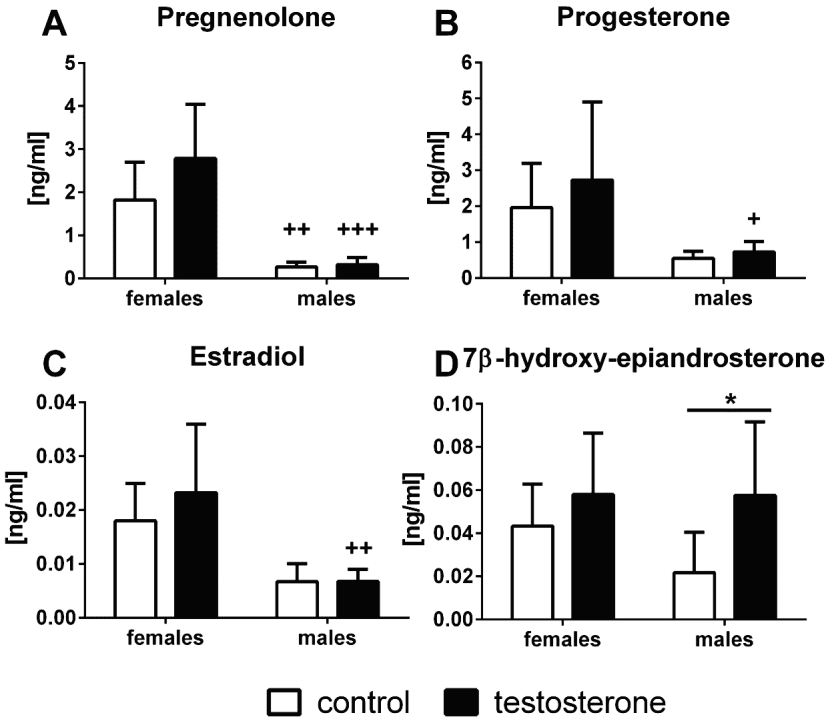

Fig. 2. Pregnenolone (**A**), progesterone (**B**), estradiol (**C**) and 7β-hydroxy-epiandrosterone (**D**) concentration in rat plasma. Males have significantly lower concentration of pregnenolone in plasma than females in both the control (p<0.001) and in testosterone exposed group (p<0.001). Prenatally testosterone exposed males have lower concentration of progesterone (p<0.05) and estradiol (p<0.01) in comparison with prenatally testosterone exposed females. Testosterone exposed males have higher 7β-hydroxy-epiandrosterone concentration than control males. Data are presented as mean + SD. + p<0.05, ++ p<0.01, +++ p<0.001, * p<0.05, + signs sex differences, * signs the treatment effect.

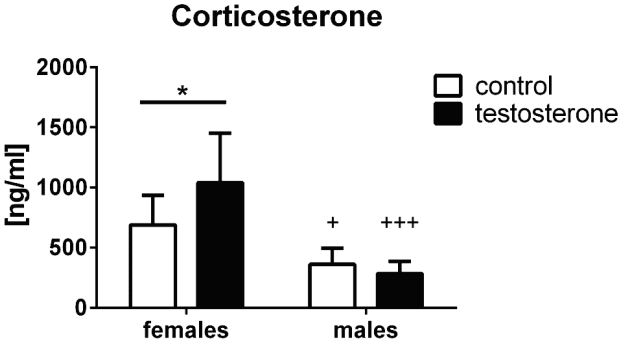

Fig. 3. Corticosterone concentration in rat plasma. Males have significantly lower concentration of corticosterone in plasma than females in both the control (p<0.05) and in testosterone exposed group (p<0.001). Prenatally testosterone exposed females display higher concentration of corticosterone (p<0.05) than control females. Data are presented as mean + SD. + p<0.05, +++ p<0.001, * p<0.05, + signs sex differences, * signs the treatment effect.

Figure 3 shows a significant sex difference in plasma corticosterone (F=31.8, p<0.001). Females had higher corticosterone concentration in both, the control (by 47.6 %, females: 689±246 ng/ml vs. males: 361±134 ng/ml, t=2.42, p<0.05) and testosterone-exposed groups (by 72.7 %, females: 1039±412 ng/ml vs. males: 284±103 ng/ml, t=5.56, p<0.001). Prenatal testosterone treatment had no effect on the concentration of corticosterone (F=2.00, p=0.17). However, there was a significant interaction between gender and treatment (F=4.95, p<0.05). While prenatal testosterone did not affect corticosterone in males (t=0.57, p>0.99), females prenatally treated with testosterone had 1.5-fold higher corticosterone concentration than control females (t=2.57, p<0.05).

Discussion

In the present study, sex differences in plasma steroid hormones, such as pregnenolone, progesterone, corticosterone, testosterone and estradiol were shown in adult rats regardless of the prenatal testosterone treatment. While prenatal testosterone increased the secretion of corticosterone in females and the production of 7β-OH-EpiA in males, prenatal testosterone exposure affected the concentrations of other examined steroid hormones neither in females, nor in males.

Production of pregnenolone by the conversion of cholesterol catalyzed by the cytochrome P450 cholesterol side-chain cleavage enzyme (P450scc) is the first committed step in the biosynthetic pathway of all steroid hormones (Hu *et al.* 2010). Our results indicate that prenatal testosterone does not disturb initiation of steroidogenesis, but might partially affect the metabolism of several precursors in sex-specific manner. Higher corticosterone concentrations in females treated prenatally with testosterone, in comparison to control females, suggest that in the female offspring prenatal testosterone can lead to enhanced secretion of adrenal steroids. In PA female rhesus monkeys ACTH injection induced adrenal hyperandrogenism accompanied by increased corticosterone production. However, in these animals the most of adrenocortical steroids, including glucocorticoids, were not elevated when they were evaluated under basal condition. On the other hand, dexamethasone-induced negative feedback on the ACTH secretion was not able to suppress corticosterone concentration in these PA females (Zhou *et al.* 2005). In accordance with that, enhanced basal ACTH secretion or higher sensitivity of the adrenal gland, mainly in zona fasciculata, to its regulators could be assumed in our paradigm of prenatal androgenization of female offspring. Furthermore, an enhanced enzymatic activity of 3β-hydroxysteroid dehydrogenase in PA female monkeys has been suspected to increase conversion of pregnenolone to progesterone, which is a key substrate for corticosterone production, instead of the metabolism of pregnenolone towards androgens through 17α-hydroxypregnenolone (Zhou *et al.* 2005). This hypothetical mechanism is supported by higher progesterone observed in female rats prenatally exposed to testosterone or dihydrotestosterone on GD16-19 (Wu *et al.* 2010, Amalfi *et al.* 2012). In our study, no differences between testosterone-treated and control females were observed in plasma progesterone. On the other hand, there is evidence of reduced 3β-hydroxysteroid dehydrogenase gene expression in the granulose cells resulting in reduced progesterone production capacity in PCOS women (Doldi *et al.* 2000). Another published experiment in rats showed that prenatal testosterone exposure does not affect corticosterone in adult female offspring (Sun *et al.* 2012). Lower dose of testosterone, different vehicle and older age at corticosterone evaluation in our experiment in comparison with mentioned study could explain the contradictory results. Further studies are surely needed to investigate the mechanisms underlying the finding of higher corticosterone concentrations in females treated prenatally with testosterone.

Androgens can be synthesized from pregnenolone *via* two different pathways: 1) involving production of progesterone, which is converted to androstenedione *via* 17α-hydroxyprogesterone, 2) involving production of DHEA, which is further converted to either androstenedione or androstenediol. Besides its importance as the most abundant circulating androgen in males, testosterone is also the main precursor of estrogens in women. Testosterone is partially aromatized to estradiol also in males. Unlike most of the recent studies, in our experiment neither testosterone, nor estradiol concentration were affected by prenatal testosterone administration. However, the results of the studies examining the effect of prenatal testosterone on adult sex hormone concentrations are inconsistent. While subcutaneous injection of 3 mg testosterone per day on GD16-19 resulted in lower testosterone concentration in adult male rats, single injection of 20 mg testosterone on GD20 had no effect when compared to control offspring (Ramezani Tehrani *et al.* 2013). A dose-response study showed that although a lower dose of prenatal testosterone treatment (0.5 mg daily from GD14-19) is sufficient to induce a 10-fold elevation of maternal circulating testosterone and masculinization of reproductive organs of the female offspring, to markedly increase fetal testosterone concentration a higher dose (at least 1 mg) is needed (Wolf *et al.* 2002). On the contrary, increased secretion of LH-stimulated androstenedione was found *in vitro* in the thecal cells of follicles of PA female sheep. Interestingly, these PA females did neither exhibit increased plasma concentration of androgens, estrogens or gonadotropins (Hogg *et al.* 2012). On the other hand, adult male offspring of ewes exposed to testosterone propionate injections exhibited higher plasma testosterone then control males (Recabarren *et al.* 2013). In several published experiments on rats, PA females had higher plasma testosterone (Wu *et al.* 2010, Yang *et al.* 2011, Amalfi *et al.* 2012) and its precursors, 17α-hydroxyprogesterone and androstenedione (Yang *et al.* 2011), as well as higher basal estradiol concentration (Wu *et al.* 2010) in the adulthood. Lower estradiol was found in PA female rats in proestrus, in the stage of follicular development, that usually fails in PCOS (Amalfi *et al.* 2012). We were able to induce higher LH production neither in PA females, nor in males, which

is in contrast to other studies mimicking PCOS in animal models (Sarma *et al.* 2005, Jackson *et al.* 2009, Cardoso *et al.* 2016) or examining the effect of prenatal testosterone in males (Recabarren *et al.* 2012, Recabarren *et al.* 2013). Discrepancies between the mentioned studies, as well as the inconsistency of our results with the previously reported findings can be caused by numerous technical reasons related to the design of the experiments, but also by the use of different strains/species of the experimental animals. In addition, the evaluation of the results should take into account the factors known to influence the endocrine milieu including age and the biological cyclicity, of several hormones, both ultradian and infradian.

To the best of our knowledge, the effect of prenatal testosterone exposure on the production of 7β-OH-EpiA, an androgenic derivate of DHEA, has been examined neither in females, nor in males, yet. Since 7β-OH-EpiA has been shown to exert anti-inflammatory (Hennebert *et al.* 2008) and cytoprotective effects (Davidson *et al.* 2008, Le Mee *et al.* 2008), including neuroprotection in neurodegenerative disorder such as Alzheimer's disease (Dudas *et al.* 2004), we considered the investigation of the effects of prenatal testosterone on production of 7β-OH-EpiA in the adulthood as important. The finding of higher concentration of 7β-OH-EpiA in the plasma of prenatally testosterone exposed males could be of interest for further studies focusing on its potential role in the pathogenesis of both, neurodegenerative and neurodevelopmental disorders associated with prenatal hyperandrogenism, such as the autism spectrum disorder (Xu *et al.* 2015).

One of the major limitations of our study is that we have not analyzed the estrous cycle phase in the female offspring at sacrifice. We were not able to quantify or detect other important steroid hormones, including the main precursors of testosterone, such as DHEA, androstenedione and androstenediol, or the 5α-reduced metabolite of testosterone and strong androgen – dihydrotestosterone. Similarly, the analysis of the expression and/or activity of the enzymes important for the metabolism of steroids, including StAR, P450scc, 3β-hydroxysteroid dehydrogenase, 17α-hydroxylase, 17/20-lyase, aromatase and 5α-reductase in various tissues, is missing in our study and would be valuable for the interpretation of the results.

In conclusion, our results show that prenatal testosterone exposure can partially affect the biosynthetic pathway of steroid hormones in sex-specific manner, in the absence of any changes in plasma concentration of pregnenolone, progesterone, testosterone, estradiol or LH. The mechanisms through which prenatal testosterone led to higher corticosterone in females and 7β-OH-EpiA in males, as well as the potential causal role of these hormones in several disorders associated with prenatal androgen excess remain to be elucidated.

Conflict of Interest

There is no conflict of interest.

Acknowledgement

The study was supported by the MEYS CR (OP RDE, Excellent research – ENDO.CZ), by MH CZ – DRO (Institute of Endocrinology – EÚ, 00023761), by the Slovak Research and Development Agency (APVV-0753-10, APVV-0539-12) and the Comenius University (UK/308/2016).

References

ABRUZZESE GA, HEBER MF, FERREIRA SR, VELEZ LM, REYNOSO R, PIGNATARO OP, MOTTA AB: Prenatal hyperandrogenism induces alterations that affect liver lipid metabolism. *J Endocrinol* **230**: 67-79, 2016.

AMALFI S, VELEZ LM, HEBER MF, VIGHI S, FERREIRA SR, OROZCO AV, PIGNATARO O, MOTTA AB: Prenatal hyperandrogenization induces metabolic and endocrine alterations which depend on the levels of testosterone exposure. *PLoS One* **7**: e37658, 2012.

CARDOSO RC, BURNS A, MOELLER J, SKINNER DC, PADMANABHAN V: Developmental programming: insulin sensitizer prevents the GnRH-stimulated LH hypersecretion in a sheep model of PCOS. *Endocrinology* **157**: 4641-4653, 2016.

DAVIDSON J, WULFERT E, ROTONDO D: 7beta-hydroxy-epiandrosterone modulation of 15-deoxy-delta12, 14-prostaglandin J2, prostaglandin D2 and prostaglandin E2 production from human mononuclear cells. *J Steroid Biochem Mol Biol* **112**: 220-227, 2008.

DOLDI N, GROSSI D, DESTEFANI A, GESSI A, FERRARI A: Polycystic ovary syndrome: evidence for reduced 3 beta-hydroxysteroid dehydrogenase gene expression in human luteinizing granulosa cells. *Gynecol Endocrinol* **14**: 32-37, 2000.

DUDAS B, HANIN I, ROSE M, WÜLFERT E: Protection against inflammatory neurodegeneration and glial cell death by 7β-hydroxy epiandrosterone, a novel neurosteroid. *Neurobiol Dis* **15**: 262-268, 2004.

DUMESIC DA, ABBOTT DH, PADMANABHAN V: Polycystic ovary syndrome and its developmental origins. *Rev Endocr Metab Disord* **8**: 127-141, 2007.

FOECKING EM, SZABO M, SCHWARTZ NB, LEVINE JE: Neuroendocrine consequences of prenatal androgen exposure in the female rat: absence of luteinizing hormone surges, suppression of progesterone receptor gene expression, and acceleration of the gonadotropin-releasing hormone pulse generator. *Biol Reprod* **72**: 1475-1483, 2005.

HENNEBERT O, PELISSIER MA, LE MEE S, WÜLFERT E, MORFIN R: Anti-inflammatory effects and changes in prostaglandin patterns induced by 7β-hydroxy-epiandrosterone in rats with colitis. *J Steroid Biochem Mol Biol* **110**: 255-262, 2008.

HOGG K, YOUNG JM, OLIVER EM, SOUZA CJ, MCNEILLY AS, DUNCAN WC: Enhanced thecal androgen production is prenatally programmed in an ovine model of polycystic ovary syndrome. *Endocrinology* **153**: 450-461, 2012.

HU J, ZHANG Z, SHEN W-J, AZHAR S: Cellular cholesterol delivery, intracellular processing and utilization for biosynthesis of steroid hormones. *Nutr Metab (Lond)* **7**: 47, 2010.

JACKSON LM, TIMMER KM, FOSTER DL: Organizational actions of postnatal estradiol in female sheep treated prenatally with testosterone: programming of prepubertal neuroendocrine function and the onset of puberty. *Endocrinology* **150**: 2317-2324, 2009.

JUAREZ J, DEL RIO-PORTILLA I, CORSI-CABRERA M: Effects of prenatal testosterone on sex and age differences in behavior elicited by stimulus pups in the rat. *Dev Psychobiol* **32**: 121-129, 1998.

KUSHNIR MM, BLAMIRES T, ROCKWOOD AL, ROBERTS WL, YUE B, ERDOGAN E, BUNKER AM, MEIKLE AW: Liquid chromatography-tandem mass spectrometry assay for androstenedione, dehydroepiandrosterone, and testosterone with pediatric and adult reference intervals. *Clin Chem* **56**: 1138-1147, 2010.

LAZIC M, AIRD F, LEVINE EJ, DUNAIF A: Prenatal androgen treatment alters body composition and glucose homeostasis in male rats. *J Endocrinol* **208**: 293-300, 2011.

LE MEE S, HENNEBERT O, FERREC C, WULFERT E, MORFIN R: 7beta-hydroxy-epiandrosterone-mediated regulation of the prostaglandin synthesis pathway in human peripheral blood monocytes. *Steroids* **73**: 1148-1159, 2008.

MCCORMICK CM, MAHONEY E: Persistent effects of prenatal, neonatal, or adult treatment with flutamide on the hypothalamic-pituitary-adrenal stress response of adult male rats. *Horm Behav* **35**: 90-101, 1999.

PADMANABHAN V, VEIGA-LOPEZ A: Sheep models of polycystic ovary syndrome phenotype. *Mol Cell Endocrinol* **373**: 8-20, 2013.

PADMANABHAN V, VEIGA-LOPEZ A, HERKIMER C, ABI SALLOUM B, MOELLER J, BECKETT E, SREEDHARAN R: Developmental programming: prenatal and postnatal androgen antagonist and insulin sensitizer interventions prevent advancement of puberty and improve LH surge dynamics in prenatal testosterone-treated sheep. *Endocrinology* **156**: 2678-2692, 2015.

RAMEZANI TEHRANI F, NOROOZZADEH M, ZAHEDIASL S, GHASEMI A, PIRYAEI A, AZIZI F: Prenatal testosterone exposure worsen the reproductive performance of male rat at adulthood. *PLoS One* **8**: e71705, 2013.

RAMEZANI TEHRANI F, NOROOZZADEH M, ZAHEDIASL S, PIRYAEI A, HASHEMI S, AZIZI F: The time of prenatal androgen exposure affects development of polycystic ovary syndrome-like phenotype in adulthood in female rats. *Int J Endocrinol Metab* **12**: e16502, 2014.

RECABARREN MP, ROJAS-GARCIA PP, EINSPANIER R, PADMANABHAN V, SIR-PETERMANN T, RECABARREN SE: Pituitary and testis responsiveness of young male sheep exposed to testosterone excess during fetal development. *Reproduction* **145**: 567-576, 2013.

RECABARREN SE, RECABARREN M, ROJAS-GARCIA PP, CORDERO M, REYES C, SIR-PETERMANN T: Prenatal exposure to androgen excess increases LH pulse amplitude during postnatal life in male sheep. *Horm Metab Res* **44**: 688-693, 2012.

RHEES RW, KIRK BA, SEPHTON S, LEPHART ED: Effects of prenatal testosterone on sexual behavior, reproductive morphology and LH secretion in the female rat. *Dev Neurosci* **19**: 430-437, 1997.

ROJAS-GARCÍA PP, RECABARREN MP, SARABIA L, SCHÖN J, GABLER C, EINSPANIER R, MALIQUEO M, SIR-PETERMANN T, REY R, RECABARREN SE: Prenatal testosterone excess alters Sertoli and germ cell number and testicular FSH receptor expression in rams. *Am J Physiol Endocrinol Metab* **299**: E998-E1005, 2010.

ROLAND AV, MOENTER SM: Reproductive neuroendocrine dysfunction in polycystic ovary syndrome: insight from animal models. *Front Neuroendocr* **35**: 494-511, 2014.

SARMA HN, MANIKKAM M, HERKIMER C, DELL'ORCO J, WELCH KB, FOSTER DL, PADMANABHAN V: Fetal programming: excess prenatal testosterone reduces postnatal luteinizing hormone, but not follicle-stimulating hormone responsiveness, to estradiol negative feedback in the female. *Endocrinology* **146**: 4281-4291, 2005.

SOSVOROVA L, VITKU J, CHLUPACOVA T, MOHAPL M, HAMPL R: Determination of seven selected neuro- and immunomodulatory steroids in human cerebrospinal fluid and plasma using LC-MS/MS. *Steroids* **98**: 1-8, 2015.

STIKKELBROECK NM, HERMUS AR, BRAAT DD, OTTEN BJ: Fertility in women with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Obstet Gynecol Surv* **58**: 275-284, 2003.

SUN M, MALIQUEO M, BENRICK A, JOHANSSON J, SHAO R, HOU L, JANSSON T, WU X, STENER-VICTORIN E: Maternal androgen excess reduces placental and fetal weights, increases placental steroidogenesis and leads to long-term health effects in their female offspring. *Am J Physiol Endocrinol Metab* **303**: E1373-E1385, 2012.

TEHRANI FR, NOROOZZADEH M, ZAHEDIASL S, PIRYAEI A, AZIZI F: Introducing a rat model of prenatal androgen-induced polycystic ovary syndrome in adulthood. *Exp Physiol* **99**: 792-801, 2014.

VEIGA-LOPEZ A, MOELLER J, ABBOTT DH, PADMANABHAN V: Developmental programming: rescuing disruptions in preovulatory follicle growth and steroidogenesis from prenatal testosterone disruption. *J Ovarian Res* **9**: 39, 2016.

VITKU J, CHLUPACOVA T, SOSVOROVA L, HAMPL R, HILL M, HERACEK J, BICIKOVA M, STARKA L: Development and validation of LC-MS/MS method for quantification of bisphenol A and estrogens in human plasma and seminal fluid. *Talanta* **140**: 62-67, 2015.

VITKU J, HERACEK J, SOSVOROVA L, HAMPL R, CHLUPACOVA T, HILL M, SOBOTKA V, BICIKOVA M, STARKA L: Associations of bisphenol A and polychlorinated biphenyls with spermatogenesis and steroidogenesis in two biological fluids from men attending an infertility clinic. *Environ Int* **89-90**: 166-173, 2016.

WOLF CJ, HOTCHKISS A, OSTBY JS, LEBLANC GA, GRAY LEJ: Effects of prenatal testosterone propionate on the sexual development of male and female rats: a dose-response study. *Toxicol Sci* **65**: 71-86, 2002.

WU X-Y, LI Z-L, WU C-Y, LIU Y-M, LIN H, WANG S-H, XIAO W-F: Endocrine traits of polycystic ovary syndrome in prenatally androgenized female Sprague-Dawley rats. *Endocr J* **57**: 201-209, 2010.

XU X-J, ZHANG H-F, SHOU X-J, LI J, JING W-L, ZHOU Y, QIAN Y, HAN S-P, ZHANG R, HAN J-S: Prenatal hyperandrogenic environment induced autistic-like behavior in rat offspring. *Physiol Behav* **138**: 13-20, 2015.

YANG X, ZHANG Y, WU X, BAE CS, HOU L, KUANG H, WANG Y, STENER-VICTORIN E: Cryptotanshinone reverses reproductive and metabolic disturbances in prenatally androgenized rats via regulation of ovarian signaling mechanisms and androgen synthesis. *Am J Physiol Regul Integr Comp Physiol* **300**: R869-R875, 2011.

ZHOU R, BIRD IM, DUMESIC DA, ABBOTT DH: Adrenal hyperandrogenism is induced by fetal androgen excess in a rhesus monkey model of polycystic ovary syndrome. *J Clin Endocrinol Metab* **90**: 6630-6637, 2005.