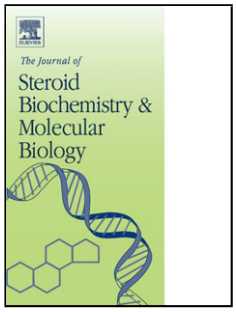


Title: Umbilical Cord Blood Androgen Levels in Girls and Boys Assessed by Gas Chromatography-Tandem Mass Spectrometry

Authors: Anna-Carin Lundell, Henrik Ryberg, Liesbeth Vandenput, Anna Rudin, Claes Ohlsson, Åsa Tivesten

PII: S0960-0760(17)30098-5
DOI: http://dx.doi.org/doi:10.1016/j.jsbmb.2017.03.022
Reference: SBMB 4922

To appear in: *Journal of Steroid Biochemistry & Molecular Biology*

Received date: 29-12-2016
Revised date: 14-2-2017
Accepted date: 30-3-2017

Please cite this article as: Anna-Carin Lundell, Henrik Ryberg, Liesbeth Vandenput, Anna Rudin, Claes Ohlsson, Åsa Tivesten, Umbilical Cord Blood Androgen Levels in Girls and Boys Assessed by Gas Chromatography-Tandem Mass Spectrometry, Journal of Steroid Biochemistry and Molecular Biologyhttp://dx.doi.org/10.1016/j.jsbmb.2017.03.022

Umbilical Cord Blood Androgen Levels in Girls and Boys Assessed by Gas Chromatography-Tandem Mass Spectrometry

Anna-Carin Lundell [a], Henrik Ryberg [b], Liesbeth Vandenput [c], Anna Rudin [a], Claes Ohlsson [c], and Åsa Tivesten [d, *]

[a] Department of Rheumatology and Inflammation Research, Institute of Medicine, University of Gothenburg, Gothenburg, Sweden; [b] Department of Clinical Chemistry, Sahlgrenska University Hospital, Gothenburg, Sweden; [c] Center for Bone and Arthritis Research (CBAR), Department of Internal Medicine and Clinical Nutrition, Institute of Medicine, University of Gothenburg, Gothenburg, Sweden; [d] Wallenberg Laboratory for Cardiovascular and Metabolic Research, Department of Internal Medicine and Clinical Nutrition, Institute of Medicine, University of Gothenburg, Gothenburg, Sweden

**Corresponding author:* Åsa Tivesten, Wallenberg Laboratory for Cardiovascular and Metabolic Research, Sahlgrenska University Hospital, Bruna Stråket 16, SE-413 45 Gothenburg, Sweden. Telephone +46 31 3422913, Fax +46 31 823732, E-mail asa.tivesten@medic.gu.se

Abbreviated title: Androgens in human cord blood by GC-MS/MS

Number of figures and tables: 2 figures, 4 tables

Disclosure statement: The authors have nothing to disclose.

Abbreviations: CV, coefficient of variation; DHT, dihydrotestosterone; DHEA, dehydroepiandrosterone; GC-MS/MS, gas chromatography-tandem mass spectrometry; LC-MS, liquid chromatography-mass spectrometry; LOD, lower limit of detection; LOQ, lower limit of quantification; SHBG, sex hormone-binding globulin

HIGHLIGHTS

- DHT levels can be estimated in human umbilical cord blood by GC-MS/MS
- The sex difference in DHT levels exceed that of testosterone
- Gestational age at delivery associates with DHT in boys and DHEA in girls
- In both sexes, DHEA, but not DHT or testosterone, associates with SHBG

ABSTRACT

Androgen exposure of the fetus during gestation plays an important role in human physiology and pathophysiology, but assessment of androgens, in particular dihydrotestosterone (DHT), in human umbilical cord blood is technically challenging. The aim of this study was to assess umbilical cord androgen levels, including DHT, at birth by a highly sensitive assay, and study their association with sex of the infant, sex-hormone-binding globulin (SHBG) levels, and gestational age at delivery. Swedish infants (27 girls, 26 boys) were recruited at maternity care clinics in Southern Sweden. Umbilical cord blood levels of dehydroepiandrosterone (DHEA), androstenedione, testosterone and DHT at delivery were assessed by a gas chromatography-tandem mass spectrometry assay. Cord blood levels of DHT were 2.4-fold higher in boys (median 27.8 pg/mL) than in girls (11.5 pg/mL), while the sex difference was less pronounced for testosterone (1.3-fold higher in boys) and non-significant for DHEA and androstenedione. Gestational age at delivery associated inversely with DHT levels in boys and with DHEA levels in girls. There was a strong inverse correlation between SHBG and DHEA in both sexes, while there were no associations between SHBG and testosterone or DHT levels. In conclusion, using state of the art technology, we report that there is a pronounced sexual dimorphism in human umbilical cord blood DHT levels. The possibility to assess a complete androgen profile in human cord blood opens up for future increased understanding of the biological impact of the fetal androgen milieu.

Key words: Androgens, Umbilical cord blood, Sex, GC-MS/MS

1. Introduction

Androgen exposure of the fetus during gestation plays an important role in human physiology and development, such as the development of the male anatomic phenotype [1, 2]. Emerging data also suggest that androgen exposure *in utero* affects other phenotypes, such as future behavior, psychiatric disorders, metabolism and cardiovascular disease [2], although understanding in this area is incomplete.

There are inherent obstacles for gathering human samples during pregnancy, but umbilical cord blood, which is a mixture of venous and arterial components in roughly equal proportions [1], is easily accessible after delivery. Cord blood provides a snapshot of the sex hormonal milieu at late gestation, although sex hormone levels may be additionally modulated by various obstetric and other factors [1, 3]. Androgens assessed in cord blood are considered to reflect fetal production in adrenals and gonads, with a contribution from placental production and metabolism, while maternal steroid levels show weak associations with those in cord blood [1]. The main androgens and androgen precursors in humans are dehydroepiandrosterone (DHEA), androstenedione, testosterone and dihydrotestosterone (DHT). The precursors DHEA and androstenedione may be converted to active androgens, such as testosterone, in peripheral tissues and testosterone is further metabolized to DHT, a several times more potent agonist to the androgen receptor than testosterone itself [4].

Immunoassays, that have been used in most previous studies investigating umbilical cord blood androgens [1, 3], have limited accuracy and specificity for the assessment of androgens, especially at lower concentrations [5]. Assessment of DHT in the lower ranges is challenging even using liquid chromatography-mass spectrometry (LC-MS) methods due to poor ionization of this steroid [6]. Thus, while consistent data suggest higher concentrations of testosterone in umbilical cord blood of male compared to female fetuses [1], there are few similar comparisons performed regarding DHT. In fact, reliable estimations of DHT have been performed in human cord blood in only one previous study [7]

and it is currently unknown whether DHT levels at birth associates with gestational age, sex-hormone-binding globulin (SHBG) levels or other phenotypes.

In the present study, we have assessed an androgen profile in human umbilical cord blood using a high-sensitive gas chromatography-tandem mass spectrometry (GC-MS/MS) assay and studied the sexual dimorphism in androgen profile at birth. Further, we have studied its association with SHBG levels and gestational age at birth.

2. Materials and methods

2.1. Cohort and plasma samples

Families in rural areas in the Skaraborg region in South-West Sweden were enrolled at maternity care clinics, and healthy infants born at term (median gestational age at delivery 39 weeks, range 36-42 weeks) were included in the FARMFLORA study [8]. Information regarding maternal age, smoking, type of delivery (vaginal/planned section/emergency section), gestational age at delivery, weight and length at birth was gathered from a questionnaire to the parents and from birth records. The study protocol was approved by the Human Research Ethics Committee of the Medical Faculty, University of Gothenburg, Sweden.

Blood samples were obtained from the umbilical cord at the delivery; from these, heparin plasma was prepared and diluted 1:2 in PBS before storage in -80 ^{o}C. Out of the original 65 individuals included in the FARMFLORA cohort, plasma was available for sex hormone assay for 54 individuals. One sample was excluded due to technical failure of the GC-MS/MS analysis, leaving 53 samples for the present analysis (27 girls, 26 boys).

2.2. Androgen profile by GC-MS/MS

Plasma levels of testosterone, DHT, DHEA and androstenedione were measured in a single run by gas chromatography-tandem mass spectrometry (GC-MS/MS), as previously described [9]. Briefly, after the addition of isotope-labeled standards, steroids were extracted to chlorobutane, purified on a silica column, and derivatized using pentafluorobenzylhydroxylamine hydrochloride followed by pentafluorobenzoyl chloride. Steroids were analyzed in multiple reactions monitoring mode with ammonia as reagent gas using an Agilent 7000 triple quadrupole mass spectrometer equipped with a chemical ionization source. The assay for testosterone is validated by the Hormone Standardization Project at the Centers for Disease Control and Prevention (Atlanta, Georgia) using isotope-dilution LC-tandem MS [9, 10]. Assay performance, including intra-assay and inter-assay coefficients of variations (CVs), has been published previously [9]. Lower limit of detection (LOD) for the assay is

50, 4, 1.6 and 4 pg/mL and lower limit of quantification (LOQ) 400, 12, 2.5 and 8 pg/mL for DHEA, androstenedione, DHT and testosterone, respectively [9].

2.3. Measurement of sex hormone-binding globulin (SHBG)

Human sex hormone-binding globulin (SHBG) was analyzed by a commercial sandwich ELISA (Catalog Number SHBG0B, R&D Systems, Inc.), following the instructions of the manufacturer. The mean minimum detectable dose of human SHBG of the assay is 0.006 nmol/L. At a SHBG level of 2.7 nmol/L, intra- and inter-assay CVs of the assay is 3.6% and 4.8%, respectively.

2.4. Statistical analysis

All variables were tested for normal distribution by Shapiro-Wilk normality test. For variables that were normally distributed with or without log-transformation, two-group comparisons were performed by Student's t test and multivariate associations by multiple linear regression models. Other (nonparametric) data were analyzed using a Mann-Whitney U test (two groups) or Spearman rank correlations (correlation coefficients). Frequencies were compared by chi-square test. $P<0.05$ was considered statistically significant. Statistical evaluations were performed with SPSS (version 19; SPSS, Chicago, IL, USA).

3. Results

3.1. Characteristics of the cohort

Characteristics of the cohort are presented in Table 1. The mothers of boys were on average older than mothers of girls, and boys were taller at birth compared to girls. Only one mother was smoking. The majority of children had a vaginal delivery. There were no statistically significant differences in route of delivery, gestational age or birth weight between boys and girls.

3.2. Cord blood androgen levels by GC-MS/MS according to sex of the fetus

Of the 53 samples assayed, 4 female samples were below the LOQ for DHT; these were assigned values at the LOQ for statistical analysis. All samples were above LOQ for androstenedione, DHEA and testosterone.

Cord blood levels of DHT were significantly lower in girls compared to boys (Fig. 1A); the median level in boys (27.8 pg/mL) was 2.4-fold higher than in girls (11.5 pg/mL). Testosterone levels (Fig. 1B) were also significantly higher in boys (median 216 pg/mL) than in girls (171 pg/mL), but the sex difference was less pronounced for testosterone (median 1.3-fold higher in boys than in girls) than for DHT. In accordance, the ratio between DHT and testosterone, an indicator of 5α-reductase activity, was significantly higher in boys (0.13 ± 0.06) compared to girls (0.08 ± 0.04; P = 0.001). There were no statistically significant sex differences in DHEA or androstenedione levels (Fig. 1C-D).

Levels of SHBG, assayed by immunoassay, are shown in Table 1. SHBG levels were not statistically different between boys and girls.

3.3. Correlations among cord blood androgens and SHBG

Correlations among cord blood androgens in boys and girls, respectively, are shown in a correlation matrix (Table 2). In both boys and girls, levels of DHEA and androstenedione associated with testosterone levels. In boys, DHT levels associated with testosterone levels only. In girls, DHT levels associated with both DHEA and testosterone levels.

There was a strong negative correlation between SHBG and DHEA in girls as well as boys, but none between SHBG and the other androgens (Table 2).

3.4. Association of cord blood androgens and SHBG with gestational age at birth

Because not all of the androgen levels were normally distributed even after log-transformation, we first studied associations between cord blood androgens and gestational age at birth by non-parametric statistics. In boys, DHT, but not the other androgens, associated inversely with gestational age at delivery (Table 3). In girls, DHEA, but not the other androgens, associated inversely with gestational age at delivery (Table 3). Further, SHBG levels were strongly associated with gestational age at delivery in girls, but not boys (Table 3).

To further illustrate the sex-dependent associations between gestational age at delivery and DHT, we plotted DHT against gestational age (Fig. 2). The graph illustrates that the sex difference in DHT level tends to be larger at lower gestational ages.

3.5. Association of cord blood androgens and SHBG with birth weight

We next studied the association between cord blood androgens and birth weight. In Spearman correlation analyses, there were no significant correlations between cord blood androgens or SHBG and birth weight in girls (Table 3). In boys, DHT correlated with birth weight, while there were no similar associations with DHEA, androstenedione, testosterone or SHBG levels (Table 3). As expected, birth weight associated with gestational age at delivery (in boys only; Spearman correlation coefficient $r_s = 0.69$, $P<0.001$ in boys and $r_s=0.20$, $P=0.35$ in girls), suggesting that the statistically weaker association between DHT and birth weight in boys is explained by the covariation of DHT levels with gestational age.

3.6. DHEA and SHBG; potential confounding by gestational age at delivery

We next wanted to study potential confounding of the strong association between DHEA and SHBG by gestational age, as both DHEA and SHBG associated with gestational age in girls. In multivariate regression models with DHEA as dependent variable (Table 4), SHBG remained as a

predictor for DHEA level after adjustment for gestational age at delivery. Further, adjustment for birth weight did not materially change this association. These findings suggest that the association between DHEA and SHBG is not explained by covariation with gestational age or birth weight.

4. Discussion

Here we employ cutting edge methodology to assess levels of androgens in umbilical cord blood of neonatal girls and boys. We report that there was a pronounced sex difference in DHT levels, exceeding the relative difference in testosterone levels between boys and girls. Gestational age at delivery was strongly negatively associated with DHT levels in boys, while DHEA levels associated with gestational age in girls. There was a strong inverse correlation between SHBG and DHEA in both sexes, while there were no associations between SHBG and testosterone or DHT levels.

Immunoassays yield unreliable results when assessing low levels of androgens [5] and even LC-MS assays may provide insufficient sensitivity, particularly for detection of low DHT levels [6]. Using an LC-MS assay, Anderson et al. reported that DHT levels were below the limit of detection (50 pg/mL) in 67% and 62% of the cord blood samples of male and female fetuses, respectively [11]. In comparison, our GC-MS/MS with a limit of detection of 1.6 pg/mL and limit of quantification of 2.5 pg/mL, provides a more than 20-fold higher sensitivity for DHT. Using this assay, we found median levels of 27.8 pg/mL in boys and 11.5 pg/mL in girls. Using similar technology, Morisset et al. reported slightly lower levels of 0.06 nmol/L (around 17 pg/mL) and 0.01 nmol/L (3 pg/mL) in umbilical blood blood of male and female fetuses, respectively [7]. Differences in assay performance and/or birth cohort may contribute to these somewhat divergent DHT levels.

While both we and others [1] found increased testosterone levels in umbilical cord blood of male compared with female fetuses, we report that the relative difference in cord levels are larger for DHT than for testosterone, which is in line with the study by Morisset et al [7]. Speculatively, this finding may support an important role of DHT for sex-specific development during fetal life. According to the "classic" pathway DHT is produced locally in the genital skin of the male fetus from the direct metabolism of testis-derived testosterone by 5α-reductase or via local production of testosterone from adrenal precursors, including DHEA and androstenedione [12]. In addition, there is a "backdoor" pathway leading to synthesis of DHT from progesterone without passing a testosterone step, which is

particularly active in the fetus [12]. DHT produced via this pathway functions as a circulating hormone. The essential role of the "backdoor" pathway was recently supported by the fact that patients with genetic deletions in enzymes participating only in this pathway display a disorder of sexual development [12]. Thus, although testosterone and DHT levels associated strongly in both girls and boys, and both testosterone and DHT are considered important for human male sexual differentiation [12], there is a recently discovered additional role of circulating DHT operating independently of testosterone production.

We found that gestational age at delivery associated with DHT levels in cord blood of boys, such that levels decreased with increased gestational age. Speculatively, DHT levels decline during pregnancy, similar to the pattern described for testosterone [13, 14]. Studies reporting that alterations in androgen levels/signaling have little impact on birth weight [15] support that the association between DHT and birth weight is explained by covariation of both with gestational age. SHBG levels were also negatively associated with gestational age at delivery, but only in girls. Further, gestational age was negatively associated with DHEA rather than DHT in girls. These results are opposite to two recent studies where weak positive correlations between gestational age and DHEA [3, 14] as well as SHBG concentrations [3] were reported; however, in contrast to us, these studies used a sex-mixed analysis and gestational age at delivery was in the range of 28-42 weeks [3, 14], as compared with 36-42 weeks in the present study.

In the present study, we could not detect a clear sexual dimorphism in cord blood SHBG level, in accordance with previous data showing either no difference [16] or that SHBG levels are modestly (11%) higher in umbilical cord blood from boys compared to girls [3]. Further, we found no association between SHBG and testosterone or DHT in either sex, in line with data from a large birth cohort showing that SHBG levels were only weakly associated with total testosterone assessed by LC-MS/MS [3].

In cord blood of both boys and girls, we detected a strong negative correlation between SHBG and DHEA, which was not explained by covariation with gestational age or birth weight. To our knowledge, this association has not been in focus previously. It is well known that bioactive androgens decrease SHBG serum concentrations [16, 17], and it is conceivable that testosterone/DHT produced locally in the liver from the precursor DHEA may influence hepatic SHBG production [17]. SHBG levels may also impact the clearance rates of bound steroids [16]; however, DHEA is only weakly bound to SHBG [18]. The physiological role of SHBG during fetal life is unknown, but both DHEA and SHBG are potentially influenced by various hormonal, nutritional and metabolic factors during gestation [18, 19]. Notably, cord blood androgens are increased in boys of insulin resistant mothers [7], and low SHBG levels are generally associated with insulin resistance [19], suggesting that the (maternal) degree of insulin sensitivity may be one such factor. Taken together, a confounder that modulates both DHEA and SHBG may be operative, and SHBG and DHEA levels in human cord blood may thus provide a shared, but reciprocal, signal of metabolic/hormonal status, which should be a focus of future studies.

Prenatal androgen exposure has been suggested to play a role for a large number of phenotypes and diseases, for which sex differences are documented. For example, there are sex differences in verbal ability and higher cord blood testosterone levels have been associated with poorer early language development [1]. Similarly, it is conceivable that androgen exposure during the fetal period may contribute to sex differences in the development of future metabolic disturbances and cardiovascular disease, and a number of other conditions during childhood and adult life [2]. This is an exciting research area, which however has been hampered by technical restraints regarding the assessment of androgens, in particular the potent testosterone metabolite DHT, in cord blood. The fact that the sex difference in DHT levels in cord blood exceeds that of testosterone, may suggest that DHT play an important role during fetal life. Therefore, GC-based assessment of a complete androgen profile, including DHT, in fetal/cord blood of longitudinal cohort studies may yield important insight into the (patho-) physiological role of prenatal androgen exposure.

The relatively small study sample is a limitation of the present study; with increased power, we would, for example, be able to determine whether DHEA levels are higher in cord blood from girls compared to boys, as suggested by others [2, 3]. Further, it is a limitation that information about obesity, gestational diabetes and/or insulin treatment, preeclampsia or other conditions among mothers was not systematically collected. Certain obstetric factors (such as delivery after labor/no labor [3]) are potential confounders that have not been accounted for. However, only four of the children in this cohort were delivered by planned Cesarean section, and almost all of the children were thus delivered after a period of labor. We did not collect material for the estimation of activity of the enzymes involved in androgen biosynthesis, which would provide additional insight. Notably, androgen levels in umbilical cord blood reflect the fetal androgen milieu at birth, while generalizability to other time points during gestation or infancy cannot be inferred.

5. Conclusions

In conclusion, using state of the art technology to assess an androgen profile in human umbilical cord blood we report here that there is a pronounced sexual dimorphism in cord blood DHT levels, exceeding that of testosterone. Furthermore, we report a strong inverse association between DHEA and SHBG levels that should be investigated further. Estimation of a complete androgen profile, including DHT levels, in human cord blood opens up the possibility for future studies and increased understanding of the biological impact of the fetal androgen milieu.

Acknowledgments

The authors are grateful to Inger Nordström, Anna-Karin Norlén, Andreas Landin and Inger Johansson for their excellent research assistance.

This work was supported by the Swedish Research Council, the Swedish Heart-Lung Foundation, the Avtal om Läkarutbildning och Forskning research grant in Gothenburg, the Marianne and Marcus Wallenberg Foundation, AFA Insurance, and the Novo Nordisk Foundation.

References

[1] L.P. Hollier, J.A. Keelan, M. Hickey, M.T. Maybery, A.J. Whitehouse, Measurement of androgen and estrogen concentrations in cord blood: accuracy, biological interpretation, and applications to understanding human behavioral development, Front Endocrinol (Lausanne) 5 (2014) 64.

[2] E.A. Kuijper, J.C. Ket, M.R. Caanen, C.B. Lambalk, Reproductive hormone concentrations in pregnancy and neonates: a systematic review, Reprod Biomed Online 27(1) (2013) 33-63.

[3] J.A. Keelan, E. Mattes, H. Tan, A. Dinan, J.P. Newnham, A.J. Whitehouse, P. Jacoby, M. Hickey, Androgen concentrations in umbilical cord blood and their association with maternal, fetal and obstetric factors, PLoS One 7(8) (2012) e42827.

[4] P.Y. Liu, A.K. Death, D.J. Handelsman, Androgens and cardiovascular disease, Endocr Rev 24(3) (2003) 313-340.

[5] A.W. Hsing, F.Z. Stanczyk, A. Belanger, P. Schroeder, L. Chang, R.T. Falk, T.R. Fears, Reproducibility of serum sex steroid assays in men by RIA and mass spectrometry, Cancer Epidemiol Biomarkers Prev 16(5) (2007) 1004-1008.

[6] D.T. Harwood, D.J. Handelsman, Development and validation of a sensitive liquid chromatography-tandem mass spectrometry assay to simultaneously measure androgens and estrogens in serum without derivatization, Clin Chim Acta 409(1-2) (2009) 78-84.

[7] A.S. Morisset, M.C. Dube, R. Drolet, M. Pelletier, F. Labrie, V. Luu-The, Y. Tremblay, J. Robitaille, S. John Weisnagel, A. Tchernof, Androgens in the maternal and fetal circulation: association with insulin resistance, J Matern Fetal Neona 26(5) (2013) 513-519.

[8] A.C. Lundell, B. Hesselmar, I. Nordstrom, I. Adlerberth, A.E. Wold, A. Rudin, Higher B-cell activating factor levels at birth are positively associated with maternal dairy farm exposure and negatively related to allergy development, J Allergy Clin Immunol 136(4) (2015) 1074-1082 e1073.

[9] M.E. Nilsson, L. Vandenput, A. Tivesten, A.K. Norlen, M.K. Lagerquist, S.H. Windahl, A.E. Borjesson, H.H. Farman, M. Poutanen, A. Benrick, M. Maliqueo, E. Stener-Victorin, H. Ryberg, C. Ohlsson, Measurement of a Comprehensive Sex Steroid Profile in Rodent Serum by High-Sensitive Gas Chromatography-Tandem Mass Spectrometry, Endocrinology 156(7) (2015) 2492-2502.

[10] J.C. Botelho, C. Shacklady, H.C. Cooper, S.S. Tai, K. Van Uytfanghe, L.M. Thienpont, H.W. Vesper, Isotope-dilution liquid chromatography-tandem mass spectrometry candidate reference method for total testosterone in human serum, Clin Chem 59(2) (2013) 372-380.

[11] H. Anderson, N. Fogel, S.K. Grebe, R.J. Singh, R.L. Taylor, A. Dunaif, Infants of women with polycystic ovary syndrome have lower cord blood androstenedione and estradiol levels, J Clin Endocrinol Metab 95(5) (2010) 2180-2186.

[12] A. Biason-Lauber, W.L. Miller, A.V. Pandey, C.E. Fluck, Of marsupials and men: "Backdoor" dihydrotestosterone synthesis in male sexual differentiation, Mol Cell Endocrinol 371(1-2) (2013) 124-132.

[13] R.P. Grinspon, N. Loreti, D. Braslavsky, C. Valeri, H. Schteingart, M.G. Ballerini, P. Bedecarras, V. Ambao, S. Gottlieb, M.G. Ropelato, I. Bergada, S.M. Campo, R.A. Rey, Spreading the clinical window for diagnosing fetal-onset hypogonadism in boys, Front Endocrinol (Lausanne) 5 (2014) 51.

[14] M. Hill, A. Paskova, R. Kanceva, M. Velikova, J. Kubatova, L. Kancheva, K. Adamcova, M. Mikesova, Z. Zizka, M. Koucky, H. Sarapatkova, V. Kacer, P. Matucha, M. Meloun, A. Parizek, Steroid profiling in pregnancy: a focus on the human fetus, J Steroid Biochem Mol Biol 139 (2014) 201-222.

[15] H.L. Miles, S. Gidlof, A. Nordenstrom, K.K. Ong, I.A. Hughes, The role of androgens in fetal growth: observational study in two genetic models of disordered androgen signalling, Arch Dis Child Fetal Neonatal Ed 95(6) (2010) F435-438.

[16] G.L. Hammond, Diverse roles for sex hormone-binding globulin in reproduction, Biol Reprod 85(3) (2011) 431-441.

[17] M.A. Thaler, V. Seifert-Klauss, P.B. Luppa, The biomarker sex hormone-binding globulin - from established applications to emerging trends in clinical medicine, Best Pract Res Clin Endocrinol Metab 29(5) (2015) 749-760.

[18] W. Somboonporn, S.R. Davis, Testosterone effects on the breast: implications for testosterone therapy for women, Endocr Rev 25(3) (2004) 374-388.

[19] B. Aydin, S.J. Winters, Sex Hormone-Binding Globulin in Children and Adolescents, J Clin Res Pediatr Endocrinol 8(1) (2016) 1-12.

Figure legends

Figure 1. Levels of androgens in umbilical cord blood according to sex. Androgens were determined by GC-MS/MS in a cohort of Swedish children (26 boys, 27 girls) born at term. A, dihydrotestosterone levels (DHT); B, testosterone; C, dehydroepiandrosterone (DHEA); D, androstenedione. **P<0.01, ****P<0.0001 in girls versus boys (Mann-Whitney U test). ns, not statistically significant.

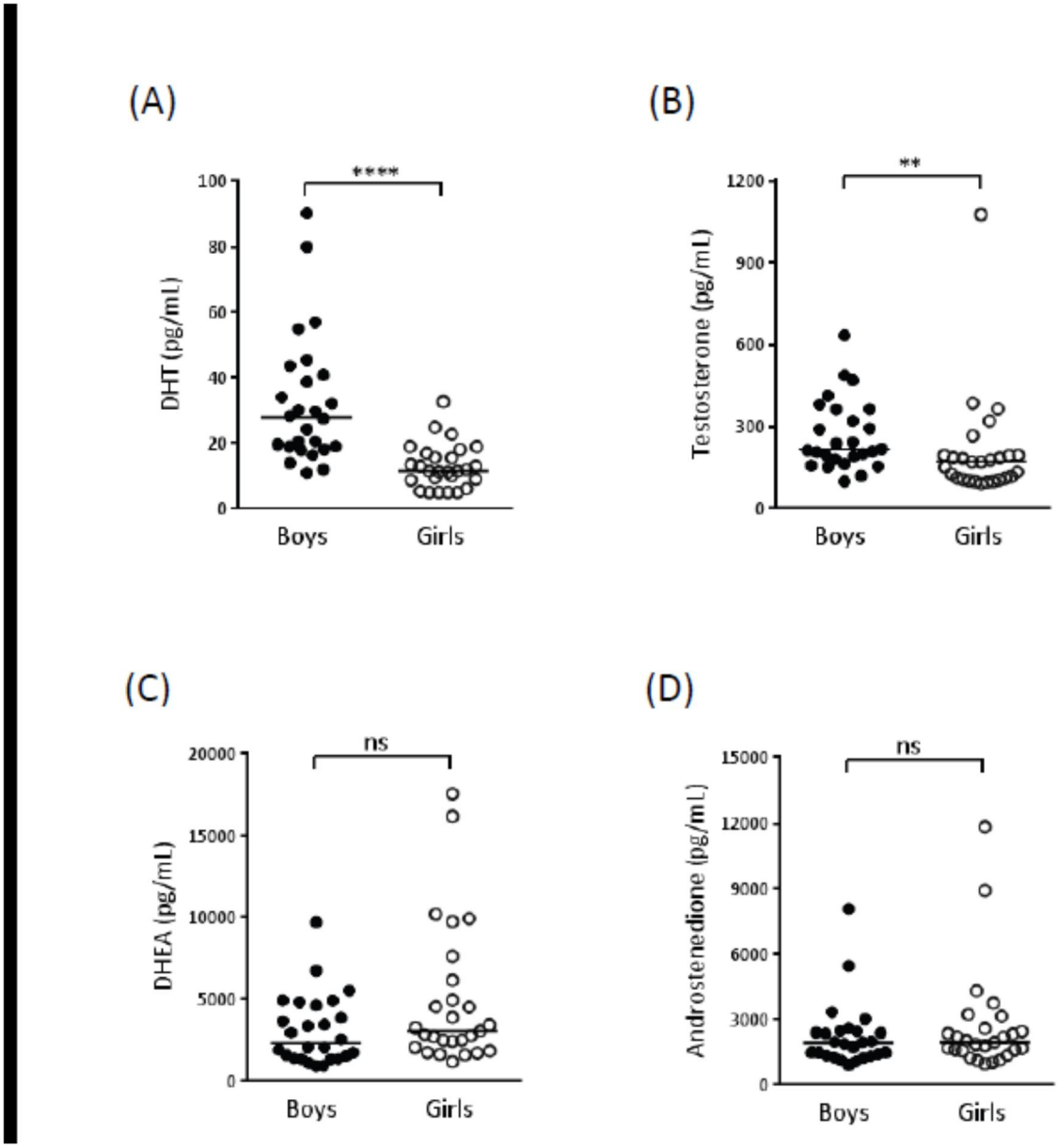
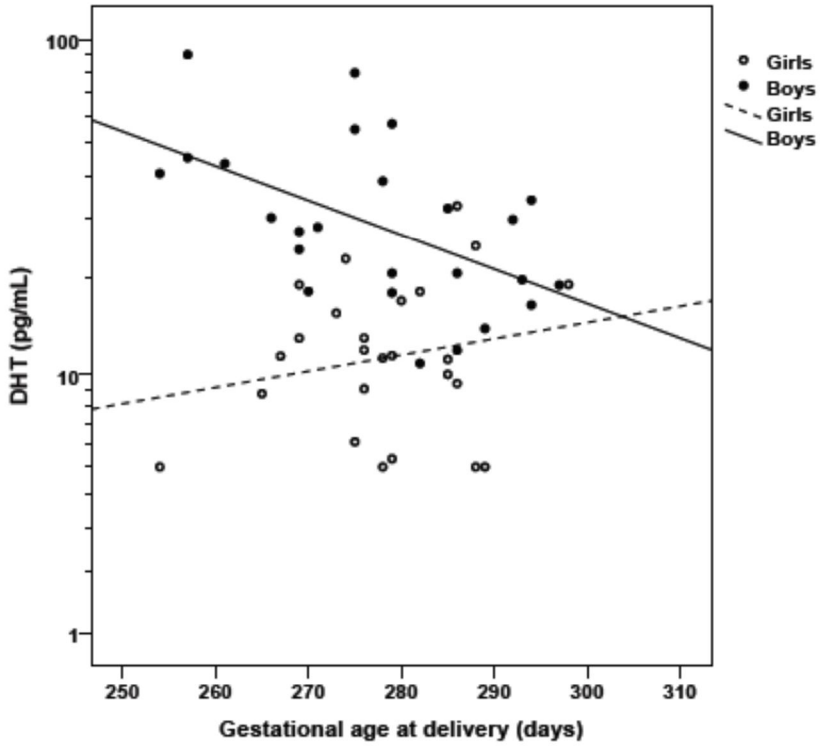


Figure 2. Cord blood androgens and gestational age at birth in girls and boys. Association between gestational age at delivery and DHT (log scale). Open circles indicate girls and filled circles boys. Corresponding Pearson correlation coefficients are r= 0.20 (P=0.35) for girls and r= -0.53 (P=0.006) for boys.



TABLES

Table 1

Characteristics of the cohort

	Boys	Girls	*p* [a]
N	26	27	
Maternal age, years	34.3 ± 4.4	31.7 ± 4.5	0.037
Maternal smoking, n	0	1	ND
Vaginal delivery, n (%)	21 (84)	23 (92)	0.38
Planned sectio, n (%)	3 (12)	1 (4)	ND
Emergency sectio, n (%)	1 (4)	1 (4)	ND
Gestational age at delivery, d	278 ± 13	278 ± 9	0.94
Weight at birth, g	3634 ± 514	3484 ± 342	0.23

Length at birth, cm	51.2 ± 2.0	50.1 ± 1.4	0.045
SHBG, nmol/L	23.4 ± 18.7	16.7 ± 11.7	0.19

Values are mean ± SD, unless otherwise specified. ND, not determined.

[a] *p*-values are from t-test, but SHBG levels were compared by Mann-Whitney U test and frequencies were compared by Chi-square test.

Table 2

Correlations among cord blood androgens and SHBG

	DHEA	Androstenedione	Testosterone	DHT
Boys (n=26)				
Androstenedione	0.32 (p = 0.11)			
Testosterone	0.40 (p = 0.043)	0.49 (p = 0.011)		
DHT	-0.15 (p = 0.45)	0.02 (p = 0.91)	0.46 (p = 0.020)	
SHBG	-0.68 (p < 0.001)	-0.20 (p = 0.32)	-0.22 (p = 0.28)	0.23 (p = 0.25)
Girls (n=27)				
Androstenedione	0.24 (p = 0.23)			

Testosterone	0.60 (p = 0.001)	0.60 (p = 0.001)		
DHT	0.43 (p = 0.026)	0.08 (p = 0.69)	0.60 (p = 0.001)	
SHBG	-0.67 (p < 0.001)	-0.10 (p = 0.63)	-0.18 (p = 0.37)	0.04 (p = 0.85)

Correlation coefficients from Spearman correlation analysis are shown with 2-tailed significance P-values within brackets.

Table 3

Correlations between cord blood androgens and SHBG and gestational age at birth and birth weight in boys and girls

	DHEA	Androstenedione	Testosterone	DHT	SHBG
Gestational age at birth					
Boys	-0.01 (p = 0.95)	0.18 (p = 0.40)	-0.28 (p = 0.17)	-0.54 (p = 0.006)	-0.05 (p = 0.82)
Girls	-0.46 (p = 0.022)	-0.03 (p = 0.91)	-0.09 (p = 0.67)	0.08 (p = 0.70)	-0.69 (p < 0.001)
Birth weight					
Boys	0.01 (p = 0.97)	0.11 (p = 0.62)	-0.25 (p = 0.22)	-0.41 (p = 0.041)	-0.13 (p = 0.53)

Girls	-0.26 (p = 0.20)	-0.02 (p = 0.92)	-0.11 (p =0.61)	-0.12 (p = 0.57)	0.32 (p =0.11)

Correlation coefficients from Spearman correlation analysis are shown with 2-tailed significance P-values within brackets.

N = 26 (boys) and 27 (girls).

Table 4

Associations between cord blood DHEA level and SHBG in girls, with and without adjustment for gestational age and birth weight

	Standardized β-coefficient (P-value)
Univariate	-0.65 (p < 0.001)
Adjusted for gestational age at birth	-0.51 (p = 0.023)
Adjusted for gestational age and birth weight	-0.52 (p = 0.029)

Multiple linear regression analysis in girls with DHEA (log-transformed) as the dependent variable and SHBG as independent variable, with our without addition of gestational age and birth weight in the model.