

Maternal prenatal depression and anxiety and steroid hormones in amniotic fluid

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ABSTRACT

Maternal prenatal depression and anxiety (PDA) have been associated with increased risks of adverse birth and neurodevelopmental outcomes in children. While fetal exposure to too high or low levels of steroid hormones has been proposed as a potential biological mechanism underlying these effects, few studies have directly investigated this hypothesis using fetal tissue samples, and the existing studies have been limited to examining cortisol, cortisone or testosterone. We studied associations between PDA and steroid hormones in amniotic fluid by measuring a panel of 17 steroid hormones – including progestogens, mineralocorticoids, glucocorticoids, androgens and estrogens – and their substrate-to-product ratios in 173 women with singleton pregnancies undergoing amniocentesis during second trimester. The fetuses had no chromosomal abnormalities. We defined any PDA as meeting at least one of the following criteria: reported symptoms above clinical cut off (CES-D ≥ 20 or STAI state or trait anxiety ≥ 40) during pregnancy, lifetime diagnosis (ICD-10 codes F31–33, F41–43), and/or lifetime medication purchases (ATC-codes N06A, N05B). Elastic net regression identified two glucocorticoid metabolites, 20 α -dihydrocortisol and 5 β -tetrahydrocortisol, with lower amniotic fluid levels in fetuses of mothers with PDA compared to those without PDA (unadjusted mean difference -0.37 SD units, 95 % CI: $[-0.68, -0.07]$; and -0.40 SD units, 95 % CI: $[-0.70, -0.10]$, respectively). The model with both steroids remained significant after adjusting for maternal age, body mass index, education, smoking during pregnancy, parity, gestational age at amniocentesis and fetal sex, and in sensitivity analyses excluding mothers with diabetes and hypertensive disorders (p -values $< .05$) and was not moderated by fetal sex (p -value $> .40$). PDA was not significantly associated with any substrate-to-product ratios of the steroids, used as proxies of steroid hormone metabolizing enzymes, after correction for multiple testing. This study provides support for the prenatal programming hypothesis of PDA influencing fetal environment through suboptimal levels of steroid hormones and highlights the need to expand to a comprehensive panel of steroid metabolism.

1. Introduction

Prenatal depression and anxiety (PDA) are common. In high-income countries, 20 % of pregnant women report clinically significant symptoms, and 10–15 % of pregnant women are diagnosed with major depressive or anxiety disorder (Bennett et al., 2004; Dennis et al., 2017;

Tuovinen et al., 2021). PDA has been linked to an increased risk of preterm birth and low birth weight (Ghimire et al., 2021), suboptimal cognitive, motor and socio-emotional development, and higher risks of mental and behavioural disorders and their symptoms in children (Lahti et al., 2017; Rogers et al., 2020; Tuovinen et al., 2021). The outcomes of PDA align with the Developmental Origins of Health and Disease

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(DOHaD) framework (Barker et al., 1995), suggesting that prenatal exposure to environmental adversities has lasting effects on fetal brain development and influences later susceptibility for mental disorders.

However, the biological mechanisms linking PDA to child mental health outcomes are not fully understood. In addition to direct genetic heritability (Kendall et al., 2021), fetal exposure to too high or low levels of steroid hormones – resulting from a complex interplay between the mother, placenta and fetus – has been suggested as one key underlying biological mechanism in both preclinical and human studies (Seckl and Meaney, 2004; Noyola-Martínez et al., 2019; Solano and Arck, 2020). Whereas in preclinical studies fetal tissue is often used, studies in humans have mostly focused on maternal measures – blood, saliva, or hair – to assess steroid hormone levels during pregnancy (for a review, see Zijlmans et al., 2015), providing only indirect evidence of fetal exposure. Amniotic fluid, when collected during second trimester amniocentesis (14–22 gestational weeks), reflects fetal plasma, since during this period fetal skin is not yet fully keratinized, and fluid and solutes diffuse easily between the amniotic fluid and fetus (Underwood et al., 2005). Steroid hormones in second trimester amniotic fluid are primarily of maternal and placental origin, as the fetal endocrine organs and regulatory systems are not yet fully functional at this stage of gestation (Ishimoto and Jaffe, 2011). There are, however, sex differences in the levels of second trimester amniotic fluid androgens and estrogens (van de Beek et al., 2004), suggesting that these steroid hormones in amniotic fluid may at least partly be of fetal origin.

Studies investigating whether PDA is associated with amniotic fluid steroid hormones are few, comprising only four different cohorts. The majority of these studies (Sarkar et al., 2008; Glover et al., 2009; Baibazarova et al., 2013; Ghaemmaghami et al., 2014) have focused on prenatal anxiety symptoms, self-reported at the time of amniocentesis, and only one (Deuschle et al., 2018) included also depression and used diagnostic interviews. The studies have found no associations between depression or anxiety symptoms and cortisol, cortisone, or testosterone (Sarkar et al., 2008; Glover et al., 2009; Baibazarova et al., 2013; Ghaemmaghami et al., 2014; Deuschle et al., 2018). However, the only study that included diagnosis level data reported an association between depression and anxiety diagnoses during pregnancy and increased cortisol-to-cortisone metabolism (Deuschle et al., 2018). This was also the only study that used the gold standard approach of liquid chromatography with tandem mass spectrometry (LC-MS/MS) (Andrew and Homer, 2020) for steroid hormone measurement. Furthermore, the focus of these studies on cortisol, cortisone, and testosterone overlooks many other steroid hormones that are relevant to fetal neurodevelopment and in which too high or low levels may interfere with critical developmental processes (Amestoy et al., 2023; Vacher et al., 2025). These include other glucocorticoids and androgens, mineralocorticoids, progestogens and estrogens.

To address these critical knowledge gaps, we studied whether PDA – defined by symptoms, lifetime physician-diagnosed disorders and lifetime medication use – was associated with alterations in a panel of 17 steroid hormones measured in second trimester amniotic fluid. The steroid hormones included progestogens, mineralocorticoids, glucocorticoids, androgens, and estrogens, and their precursors and metabolites. Since steroid hormones are interconnected within the same biosynthesis pathway and therefore intercorrelated, we explored them collectively in relation to PDA. Additionally, we analysed substrate-to-product ratios of these steroid hormones as proxies of their metabolizing enzymes. We also investigated whether associations between PDA and amniotic fluid steroids varied by fetal sex, as maternal and fetoplacental biology and the effects of prenatal stress can vary based on fetal sex (Carpenter et al., 2017; Czamara et al., 2024).

2. Methods

2.1. Participants and procedure

Mother-child dyads came from the prospective InTraUterine sampling in early pregnancy (ITU) study (Kvist et al., 2022), that enrolled 943 pregnant mothers and their singleton children born alive in 2012–2017. Of these mothers 399 belonged to the no chromosomal testing arm because they had received a negative screen result in the national voluntary screening for fetal trisomy 21. Their recruitment, which took place in two study hospitals in Southern Finland, is described in detail in Kvist et al. (2022). The remaining 544 mothers belonged to the chromosomal testing arm. In this study, only participants from the chromosomal testing arm were included. These mothers had received a positive screen result in the national voluntary screening for fetal trisomy 21. For this reason, these mothers subsequently underwent fetal chromosomal testing at the Fetomaternal Medical Center (FMC) of the Helsinki University Hospital – including amniocentesis ($n = 179$), chorionic villus sampling (CVS; $n = 307$) or non-invasive prenatal testing (NIPT; $n = 58$) followed by trisomy PCR. Recruitment of mothers to the chromosomal testing arm took place at the FMC and included two steps: first, the mothers provided preliminary written informed consent at the time of amniocentesis / CVS / NIPT; then, the mothers whose fetuses were found not to have chromosomal abnormalities, were approached and they provided a second, final written informed consent to participate in the study. Mothers whose fetuses had chromosomal abnormalities were not included in the study, despite giving the first preliminary consent at the FMC. Of the 544 mothers in the chromosomal testing arm, 173 underwent amniocentesis between 14 + 3 and 22 + 5 gestational weeks, had an amniotic fluid sample available for analyses and thereby comprise the analytic sample of this study. The amniocenteses were performed between 7:30 AM and 3:00 PM. After amniocentesis, any extra fluid not needed for clinical purposes was stored at -80°C for later research use.

The mothers undergoing amniocentesis (and with a sample available, $n = 173$) had higher BMI ($p = .013$), were older ($p < .001$), less educated ($p < .001$), more likely to be multiparous ($p = .027$), more likely to have hypertensive disorders during pregnancy ($p = .028$) and more likely to have purchased antidepressant or anxiolytic medications during their lifetime ($p < .001$) than the mothers in the rest of the cohort. Compared to the rest of the chromosomal testing arm, the mothers undergoing amniocentesis differed only by being slightly older ($p = .026$). Characteristics for the mothers with an amniotic fluid sample available, the rest of chromosomal testing arm, and the rest of ITU participants are presented in [Supplementary material \(Table S1\)](#).

The research protocol of ITU has been approved by Ethics Committee of the Helsinki and Uusimaa Hospital District, and the study protocol follows the Declaration of Helsinki.

2.2. Measurement of amniotic fluid steroid hormones

The method for amniotic fluid steroid analysis was adapted from a validated method developed for plasma steroid hormones (Lahti-Pulkkinen et al., 2025) to include additional steroid metabolites and cover different concentration ranges. Samples were extracted (100 μL) using supported liquid extraction alongside a calibration curve and analysed by LC-MS/MS on a QTrap 6500 + (AB Sciex, UK) and Acquity UPLC system (Waters, UK). The data was assessed alongside calibration standards to calculate steroid concentrations in nanomolar (nM), using MultiQuant™ software (Sciex, UK).

We measured 19 steroid hormones in amniotic fluid, but excluded two (pregnenolone and 5α -dihydrotestosterone) from analyses, as their quantification was successful in less than 60 % of the participants, resulting in the following 17 steroids available for analysis: progestogens and metabolites (progesterone, 17α -hydroxyprogesterone), mineralocorticoids and precursors (11-deoxycorticosterone,

corticosterone, 11-dehydrocorticosterone, aldosterone), glucocorticoids, precursors and metabolites (11-deoxycortisol, cortisol, cortisone, 20 α -dihydrocortisol, 5 β -tetrahydrocortisol, β -cortol), androgens and precursors (androstenedione, testosterone), and estrogens, precursors and metabolites (estrone, 17 β -estradiol, estriol). Values below the limit of detection were coded as missing values. Except for aldosterone (n = 157, 9.2 % missing), 20 α -dihydrocortisol (n = 172, 0.6 % missing) and testosterone (n = 159, 8.1 % missing), the quantification of steroids was successful in the full sample (Table 1). We also calculated 17 substrate-to-product ratios of these steroids as proxies of the steroid-metabolizing enzymes (see Table S2), as previously described (Lahti-Pulkkinen et al., 2025).

2.3. Prenatal depression and anxiety

PDA was defined via (i) lifetime diagnoses of depression and/or anxiety disorders (International Statistical Classification of Diseases and Related Health Problems, 10th Revision – ICD-10, codes F31– F33, F41–F43) until amniocentesis, derived from the Care Register for Healthcare (Sund, 2012); (ii) lifetime purchases of antidepressant and/or anxiolytic prescription medications (Anatomical Therapeutic Chemical system, codes N06A, N05B) until amniocentesis, derived from The Medical Reimbursement Register (Wettermark et al., 2013); and/or (iii) self-reported depressive and anxiety symptoms using the Center for Epidemiologic Studies Depression Scale (CES-D) (Radloff, 1977) and/or the state and/or trait scales of the State-Trait Anxiety Inventory (STAI) (Spielberger et al., 1983). The participating mothers filled in CES-D and STAI questionnaires up to three times during pregnancy. In this study, the questionnaire data closest to amniocentesis (M=7.6, interquartile range=[5.0–10.4] weeks after amniocentesis) were used. The validity of the Care Register for Health Care, CES-D and STAI are reported as good (Sund, 2012; Nast et al., 2013; Lahti-Pulkkinen et al., 2020; Tuovinen et al., 2021). Since the lifetime diagnoses, lifetime medication purchases, and depressive and anxiety symptoms during pregnancy were interrelated (see Table S3), reflecting a shared vulnerability for PDA, we defined any PDA as meeting at least one of the following criteria: lifetime diagnosis, lifetime medication purchase, or reporting symptoms above clinical cut off (CES-D \geq 20 and/or state and/or trait scale of STAI \geq 40). Not having PDA was defined as filling none of the criteria.

Table 1

Descriptive statistics for steroid hormones, precursors and metabolites in amniotic fluid.

| Steroid hormone (nM) | n | Mean | SD |
|--|-----|--------|--------|
| <i>Progestogens and metabolites</i> | | | |
| Progesterone | 173 | 205.03 | 112.22 |
| 17 α -hydroxyprogesterone | 173 | 3.17 | 0.93 |
| <i>Mineralocorticoids and precursors</i> | | | |
| 11-deoxycorticosterone | 173 | 0.24 | 0.11 |
| Corticosterone | 173 | 1.15 | 0.53 |
| 11-dehydrocorticosterone | 173 | 3.54 | 1.24 |
| Aldosterone | 159 | 0.28 | 0.16 |
| <i>Glucocorticoids, precursors and metabolites</i> | | | |
| 11-deoxycortisol | 173 | 0.48 | 0.13 |
| Cortisol | 173 | 16.59 | 10.07 |
| Cortisone | 173 | 36.57 | 9.33 |
| 20 α -dihydrocortisol | 172 | 1.10 | 0.79 |
| 5 β -tetrahydrocortisol | 173 | 0.69 | 0.25 |
| β -cortol | 173 | 0.20 | 0.10 |
| <i>Androgens and precursors</i> | | | |
| Androstenedione | 173 | 1.60 | 1.11 |
| Testosterone | 157 | 0.43 | 0.38 |
| <i>Estrogens, precursors and metabolites</i> | | | |
| Estrone | 173 | 1.54 | 0.96 |
| 17 β -estradiol | 173 | 0.66 | 0.58 |
| Estriol | 173 | 3.49 | 2.02 |

nM=nanomolar; SD=standard deviation.

2.4. Covariates

Factors that may influence the associations between PDA and prenatal steroid hormones were chosen based on prior literature (Toriola et al., 2011; Tuovinen et al., 2021). Fetus-related factors included fetal sex (male vs female), derived from the Finnish Medical Birth Register (MBR), and gestational age (weeks) at amniocentesis, derived from medical records. From the MBR we also derived maternal factors, including pre-pregnancy body mass index (BMI; kg/m²), age at delivery (years), parity (primiparous vs multiparous), and smoking during pregnancy (no smoking vs smoked throughout pregnancy or quit during first trimester). Highest education level of the mother (secondary or lower vs tertiary) was self-reported in early pregnancy. In sensitivity analyses we accounted for maternal diabetes (ICD-10 codes E10-E14, O24) and hypertensive disorders (ICD-10 codes I10, O10-O16), derived from the MBR, the Care Register for Healthcare, or medical records.

2.5. Statistical analyses

To investigate the associations between any PDA and the 17 steroid hormones and to identify relevant steroids we applied elastic net regression (*glmnet* package; Friedman et al., 2010). Elastic net regression is a penalized regression method that combines both L1 and L2 penalties used in lasso and ridge regressions and is well suited for variable selection in a dataset with high intercorrelations (Zou and Hastie, 2005). A 10-fold cross-validation was performed at the alpha levels 0.01–0.99, and the penalty parameter value yielding the smallest prediction error was used.

Since elastic net conducts complete case analyses, we verified the results using bivariate linear mixed model (BLMM) analysis (Gao et al., 2006, 2017) – an approach that can handle missing data and accounts for intercorrelations between the steroid hormones by specifying correlations between random effects. We extended the bivariate model for use with multivariate analysis and simultaneously entered all 17 steroid hormones as the outcome and any PDA as the predictor variable.

We also applied BLMM to assess the directionality and effect sizes of the steroid hormones chosen by elastic net regression and studied whether the associations were independent of the covariates. Thus, we ran three BLMMs: unadjusted, adjusted for gestational age at amniocentesis and fetal sex, and adjusted for all covariates. Sensitivity analyses excluded women with diabetes and hypertensive disorders. We also present associations of PDA diagnoses, medications and symptoms separately with steroid hormones.

To study whether the associations between PDA and the steroid hormones selected by elastic net regression varied by fetal sex, we included an interaction term for PDA x fetal sex into the BLMM models.

To investigate the associations between any PDA and the 17 substrate-to-product ratios, we conducted linear regressions and adjusted these for covariates in a similar manner as in the BLMM. Linear regression results were corrected for multiple testing using False Discovery Rate -adjustment for p-values.

The steroid hormones and substrate-to-product ratios were square root or logarithm transformed, or rank-order normalized according to Blom's formula to achieve normal distributions for analyses. They were then standardized (M=0, SD=1) and winsorized at -3 SD and $+3$ SD. Statistical significance was set at $p < .05$. BLMM analyses, in which we used unstructured covariance structure, were performed in SAS (Version 9.4; SAS Institute Inc., Cary, NC, USA) and the other analyses in R (Version 4.3.2; R Core Team, 2021) with RStudio (Version 2024.09.0; RStudio Team, 2019).

3. Results

3.1. Descriptives

Of the 173 mothers in the study, 71 mothers (41.0 %) had any PDA; 16 mothers (9.2 %) had received a lifetime diagnosis of mood or anxiety disorder, 54 mothers (31.2 %) had purchased antidepressant or anxiolytic medications during their lifetime, and 30 mothers (44.8 % of the 67 mothers with questionnaire data) had symptom scores above the clinical cutoffs during pregnancy (26 in trait anxiety, 12 in state anxiety, 10 in depressive symptoms). The PDA variables were interrelated, with odds ratios between diagnoses, medication purchases and symptoms varying between 4.4 ($p = .09$) and 12.3 ($p < .001$) (see [Table S3](#)). The mothers with PDA were older, more likely to be multiparous, to have diabetic disorders and had amniocentesis performed at an earlier gestational age than the mothers without PDA (all $p < .05$). Characteristics of the women with and without PDA are presented in [Table 2](#).

Intercorrelations between the amniotic fluid steroid hormones varied in effect size from small to large, with negative correlations ranging from -0.01 to -0.25 and positive correlations from 0.01 to 0.85 (see [Figure S1](#)). The steroid hormone raw values in nanomolar units are presented in [Table 1](#). Descriptives for the substrate-to-product ratios are presented in [Table S2](#).

Table 2

Characteristics for the mothers with and without any prenatal depression or anxiety.

| | Maternal prenatal depression or anxiety | | p-value for group difference |
|--|---|-------------------|------------------------------|
| | Yes (n = 71) | No (n = 102) | |
| | N (%) / Mean (SD) | N (%) / Mean (SD) | |
| Maternal age (years) | 37.29 (5.06) | 35.63 (5.53) | .043 |
| Pre-pregnancy BMI (kg/m ²) | 25.70 (5.41) | 24.41 (4.39) | .123 |
| Gestational week at amniocentesis | 16.15 (1.73) | 16.77 (2.18) | .038 |
| Maternal education | | | .291 |
| Secondary or lower | 26 (36.6) | 26 (25.5) | |
| Tertiary | 35 (49.3) | 59 (57.8) | |
| Missing | 10 (14.1) | 17 (16.7) | |
| Smoking during pregnancy | | | .643 |
| Yes | 7 (9.9) | 8 (7.8) | |
| No | 64 (90.1) | 94 (92.2) | |
| Parity | | | .007 |
| Primiparous | 21 (29.6) | 51 (50.0) | |
| Multiparous | 50 (70.4) | 51 (50.0) | |
| Diabetic disorders | | | .010 |
| Yes | 22 (31.0) | 15 (14.7) | |
| No | 49 (69.0) | 87 (85.3) | |
| Hypertensive disorders | | | .693 |
| Yes | 7 (9.9) | 12 (11.8) | |
| No | 64 (90.1) | 90 (88.2) | |
| Fetal sex | | | .549 |
| Male | 35 (49.3) | 55 (53.9) | |
| Female | 36 (50.7) | 47 (46.1) | |

SD=standard deviation; BMI=body mass index

Prenatal depression or anxiety is defined via symptoms (Center for Epidemiologic Studies Depression Scale; ≥ 20 , State-Trait Anxiety Inventory, state scale; ≥ 40 , and/or State-Trait Anxiety Inventory, trait scale; ≥ 40) during pregnancy), lifetime mood or anxiety disorders (ICD-10 codes F31–33, F41–43) and/or lifetime antidepressant / anxiolytic medication purchases (ATC-codes N06A, N05B).

Diabetic disorders: ICD-10 codes E10-E14, O24; Hypertensive disorders: ICD-10 codes I10, O10-O16

Group differences were examined with χ^2 - and independent samples *t*-tests.

3.2. PDA and steroid hormones

From the panel of 17 steroid hormones available for 142 amniotic fluid samples, elastic net regression selected two glucocorticoid metabolites, 20α -dihydrocortisol and 5β -tetrahydrocortisol, collectively sharing 6.87 % of variance with PDA. These results were verified in the full 173 amniotic fluid samples by BLMM, which showed that these two glucocorticoid metabolites were the only steroids significantly associated with PDA ($p < .05$; see [Figure S2](#)). BLMM analyses showed that the amniotic fluid levels of these two glucocorticoid metabolites were lower in fetuses of mothers with PDA compared with mothers with no PDA (20α -dihydrocortisol: mean difference -0.37 SD units, 95 % CI= $[-0.68; -0.07]$; 5β -tetrahydrocortisol: mean difference -0.40 SD units, 95 % CI= $[-0.70; -0.10]$ in the unadjusted models) (see [Fig. 1](#)). While the levels of 20α -dihydrocortisol and 5β -tetrahydrocortisol in amniotic fluid were higher in the fetuses of mothers undergoing amniocentesis later in gestation ($r = 0.64$, $p < .001$; $r = 0.43$, $p < .001$; for gestational week and level of 20α -dihydrocortisol and 5β -tetrahydrocortisol) and 20α -dihydrocortisol was also higher in the fetuses of primiparous than multiparous mothers (mean difference 0.58 SD units, 95 % CI= $[0.28; 0.88]$, $p < .001$), the adjusted BLMM models remained significant (see [Fig. 1](#)). The model also remained significant when we excluded women with diabetes and hypertensive disorders (combined effect of glucocorticoid metabolites, $p = .002$).

We also tested associations of maternal PDA symptoms, diagnosed disorders, and medication purchases individually with the two glucocorticoids in amniotic fluid identified by elastic net regression. We found that PDA symptoms above clinical cut off were associated with significantly lower levels of amniotic fluid 20α -dihydrocortisol and 5β -tetrahydrocortisol ($p < .05$ for unadjusted and adjusted models), but diagnoses and medication purchases were not. However, the associations of each PDA type with these glucocorticoids were in the same direction (see [Table S4](#)).

3.3. PDA and steroid hormone metabolizing enzymes

[Supplementary Table S5](#) shows that any PDA was significantly associated with the assumed activation of the following steroid hormone metabolizing enzymes in amniotic fluid: 11β -hydroxysteroid dehydrogenase (mean difference= -0.19 SD units, 95 % CI= $[-0.34; -0.05]$, $p = .011$) and 17β -hydroxysteroid dehydrogenase (mean difference= -0.16 SD units, 95 % CI= $[-0.31; -0.01]$, $p = .034$), represented by corticosterone-to-11-dehydrocorticosterone and estrone-to- 17β -estradiol ratios, respectively. Neither of these associations remained significant when adjusted for covariates (both $p > .10$). After adjusting for covariates, the association of any PDA with the assumed activation of aromatase (represented by testosterone-to- 17β -estradiol ratio) became significant (mean difference= -0.12 SD units, 95 % CI= $[-0.22; -0.02]$, $p = .025$). These enzymes, however, were not significant when represented by ratios of cortisol to cortisone, androstenedione to testosterone and androstenedione to estrone (all $p > .05$; see [Table S5](#)). None of the associations between PDA and steroid hormone ratios survived correction for multiple testing (all $p > .10$).

3.4. Sex differences

The interactions of fetal sex and PDA were not significant for 20α -dihydrocortisol or 5β -tetrahydrocortisol or for the BLMM model (all $p > .40$). The levels of these two steroid hormones did not differ between male and female fetuses (both $p > .20$).

4. Discussion

This study found that out of 17 second trimester amniotic fluid steroid hormones, including two progestogens, four mineralocorticoids, six glucocorticoids, two androgens, and three estrogens, maternal PDA –

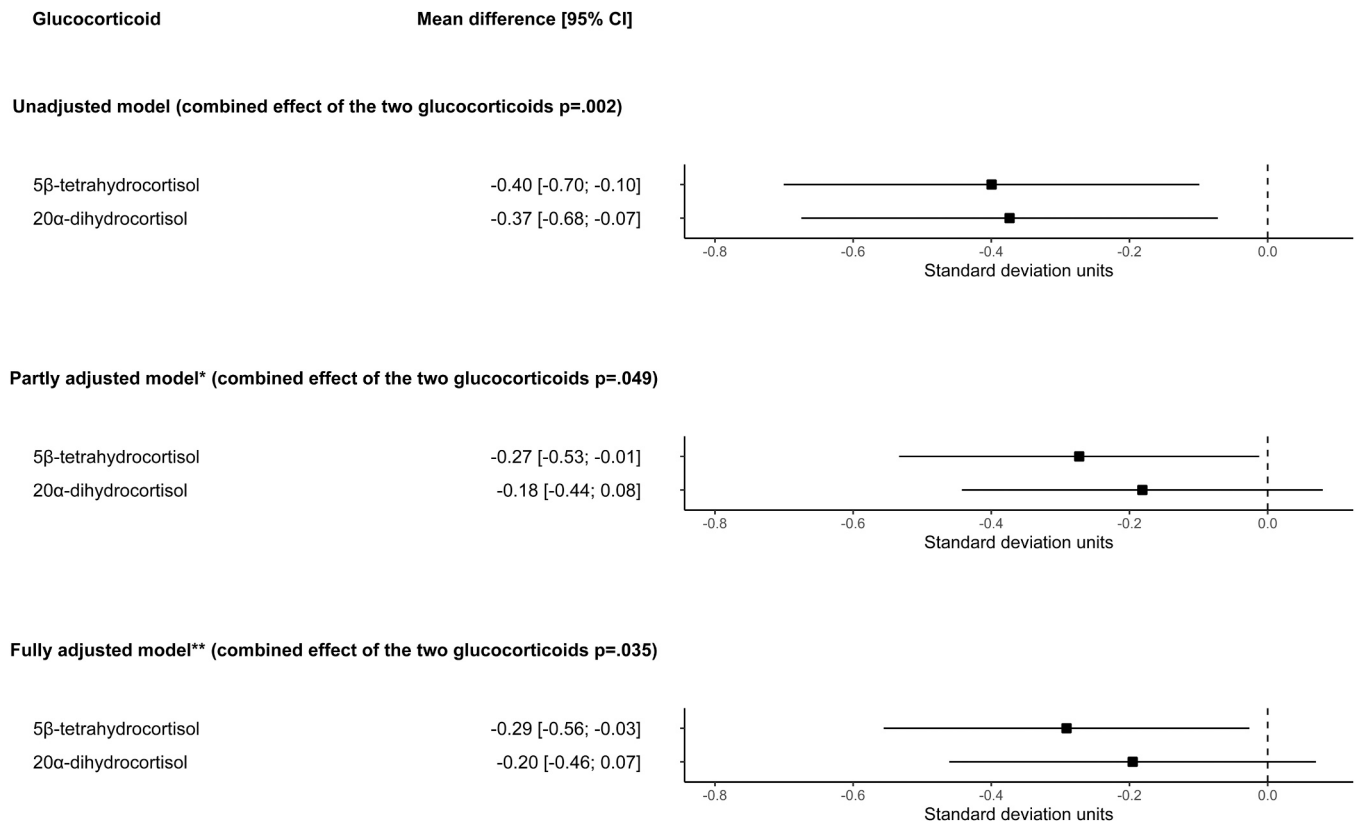


Fig. 1. Unadjusted and adjusted mean differences of amniotic fluid 5 β -tetrahydrocortisol and 20 α -dihydrocortisol in fetuses of mothers with any prenatal depression or anxiety compared with mothers with no prenatal depression or anxiety. Forest plots display mean differences (squares) and their 95 % confidence intervals (error bars). *Model is adjusted for gestational week at amniocentesis and fetal sex. **Model is adjusted for gestational week at amniocentesis, fetal sex, maternal age (years), maternal pre-pregnancy BMI (kg/m²), parity, smoking and education.

defined through reported symptoms during pregnancy, lifetime depression and anxiety diagnoses and lifetime antidepressant and anxiolytic medication purchases until amniocentesis – was associated with two glucocorticoid metabolites, 20 α -dihydrocortisol and 5 β -tetrahydrocortisol. The levels of these metabolites in amniotic fluid were 0.37 and 0.40 SDs lower in the fetuses of mothers with PDA compared to mothers with no PDA. Together they shared 6.87 % of variance with PDA, and their combined association with PDA was not explained by mother- or fetus-related factors, namely maternal age, body mass index, education, smoking, parity, gestational age at amniocentesis and fetal sex, remained significant in sensitivity analyses excluding mothers with common pregnancy complications, and did not differ by fetal sex.

To the best of our knowledge, most of the amniotic fluid steroid hormones investigated here, including 20 α -dihydrocortisol and 5 β -tetrahydrocortisol, have not been previously studied in relation to maternal PDA. Furthermore, 20 α -dihydrocortisol and 5 β -tetrahydrocortisol have overall been subject to much less research than the major glucocorticoids, cortisol and cortisone, and there is a limited understanding of their role during pregnancy. While increased cortisol metabolism into tetrahydrocortisol metabolites has been found in women with preeclampsia (Kosicka et al., 2018), and a recent study (Miranda et al., 2025) found that cortisol metabolism was shifted towards 5 α -tetrahydrocortisol in the term amniotic fluid of small-for-gestational age neonates, our findings suggest that further research is warranted for better understanding of the roles of these glucocorticoid metabolites in human fetal development.

In addition to genetic transmission (Kendall et al., 2021), it has generally been assumed that within the endocrine system higher cortisol levels and lower cortisol-to-cortisone metabolism in the mother during pregnancy are responsible for the transmission of PDA effects on the fetus (Reynolds et al., 2013; Zijlmans et al., 2015). However, there

are also findings that challenge this assumption (Reynolds et al., 2013; Zijlmans et al., 2015). Consistent with these latter findings, our results demonstrate lower glucocorticoid metabolites in the amniotic fluid of mothers with PDA, calling into question the notion that increased prenatal glucocorticoid exposure is the primary mechanism. Importantly, insufficient levels of prenatal glucocorticoids may also be detrimental for healthy progress of the pregnancy and fetal development (Ilia et al., 2024). In addition, the normative rise in glucocorticoids during pregnancy (Lahti-Pulkkinen et al., 2025), together with the fact that prolonged psychological stress may lead to blunted cortisol reactivity and lower HPA-axis activity (Miller et al., 2007), could elicit an endocrine response that is not as straightforward as has been suggested. The stressors induced by the pregnancy with a risk for fetal chromosomal abnormalities and going through amniocentesis may lead to higher cortisol secretion in healthy (no PDA) mothers (Ventura et al., 2012; Ghaemmaghami et al., 2014). On the contrary, mothers with PDA may react with a blunted stress-response and hence have lower levels of glucocorticoids available for placental transfer to the fetus. Therefore, it may be that not only too high levels, but also too low levels of steroid hormones can be considered suboptimal for fetal development (Noyola-Martínez et al., 2019; Solano and Arck, 2020).

Another possible explanation might be in altered metabolism of glucocorticoids. Although the associations of PDA with activity of 20 α -hydroxysteroid dehydrogenase, 5 β -reductase and 3 α -hydroxysteroid dehydrogenase in amniotic fluid – enzymes proposed to metabolize cortisol into 20 α -dihydrocortisol and 5 β -tetrahydrocortisol – were not statistically significant in our sample, the direction of the associations suggested suppressed enzyme activity in amniotic fluid of mothers with PDA. Even though speculative, this may suggest that PDA could be linked to relatively higher cortisol accumulation in amniotic fluid, an effect that might have been detectable with a larger sample size.

Besides the two glucocorticoid metabolites, in our study no other steroid hormones were associated with PDA. Although, when we entered all the steroid hormones in one model, there was a non-significant trend towards lower levels for 14 of the 17 steroids in amniotic fluid of mothers with PDA (Supplementary Figure S2). This may reflect a broader pattern in which PDA is associated with lower levels of steroid hormones in amniotic fluid, a relationship that a larger sample size might replicate and reveal as statistically significant.

Most of the previous studies of PDA effects on second trimester amniotic fluid glucocorticoids have focused on cortisol and its inactive metabolite cortisone. Although most of these studies (Sarkar et al., 2008; Baibazarova et al., 2013; Ghaemmaghami et al., 2014; Deuschle et al., 2018) have included fewer participants than our study and, apart from one study (Deuschle et al., 2018), measured only self-reported anxiety symptoms at the time of amniocentesis, their findings of no association between PDA and levels of cortisol and cortisone are in line with our results. In addition, our results expand on these earlier findings by suggesting that it is not necessarily the level of amniotic fluid cortisol itself but the ways it is metabolized and the overall glucocorticoid balance that is affected by PDA. Similar evidence for this hypothesis was found in the study by Deuschle et al. (2018), reporting that PDA, defined by a diagnostic interview, was associated with increased activity of 11 β -hydroxysteroid dehydrogenase type 2 – an enzyme metabolizing cortisol to cortisone – but not with cortisol or cortisone levels themselves.

Sex steroids could have a role in transmitting PDA effects to the fetus, as androgens and estrogens measured in maternal blood during pregnancy have been linked to maternal perceived stress (Colicino et al., 2023) and estradiol fluctuations to depression susceptibility (Amiel Castro et al., 2021). However, our results of amniotic fluid testosterone not being associated with PDA are consistent with the previous findings (Sarkar et al., 2008), and we extend these findings by reporting that PDA was not associated with testosterone's precursor androgen, androstenedione, or any of the three estrogens either. We did find an association between PDA and activation of aromatase, represented by testosterone-to-17 β -estradiol ratio, although it did not survive the correction for multiple testing. Also, PDA was not associated with aromatase when represented by androstenedione-to-estrone ratio, which reflects the downstream conversion of androgens to estrogens. In addition, it should be noted that the male fetus' own androgen production (van de Beek et al., 2004) might make it challenging to detect differences in androgen levels caused by PDA.

To our knowledge there are no prior studies investigating the effect of PDA on amniotic fluid progestogens or mineralocorticoids, but it has been suggested that there exists a crucial equilibrium between maternal glucocorticoids and progesterone during pregnancy (Solano and Arck, 2020). However, we did not observe significant associations of PDA either with the levels of progestogens or mineralocorticoids or the ratios of progestogens to glucocorticoids.

Sex differences in the programming effects of prenatal stress have been observed both in animal and human research, with evidence of varying vulnerabilities for both sexes (Eriksson et al., 2010; Carpenter et al., 2017). In our study the associations between maternal PDA and the two amniotic fluid glucocorticoids, 20 α -dihydrocortisol and 5 β -tetrahydrocortisol, showed no sex-specificity.

Whereas most studies on PDA and amniotic fluid steroid hormones limit the participants to those with cardio-metabolically healthy pregnancies (e.g., Baibazarova et al., 2013; Ghaemmaghami et al., 2014; Deuschle et al., 2018), we chose to include mothers with and without pregnancy complications, namely diabetes or hypertensive disorders. However, our sensitivity analyses excluding mothers with these conditions showed the same results.

The most notable strength of this study is the measurement of a comprehensive steroid hormone panel using the gold standard methodology of LC-MS/MS (Andrew and Homer, 2020). Many of the earlier studies on PDA and amniotic fluid steroid hormones (Glover et al., 2009;

Sarkar et al., 2008; Baibazarova et al., 2013) have used radioimmunoassay – a method more prone to cross-reactivity and over-estimation (Andrew and Homer, 2020). Panels of multiple steroid hormones have been used in steroid profiling for several purposes, including in mothers with healthy pregnancies (for example Hill et al., 2010; Colicino et al., 2023; Lahti-Pulkkinen et al., 2025), but not previously to study the associations of PDA with second trimester amniotic fluid steroid hormones. This approach yielded novel, in-depth information on how the steroid hormone pathway reacts to external stressors. Another strength is our comprehensive data on PDA: we were able to account not only for self-reported symptoms during pregnancy, but also for lifetime-to-date PDA diagnoses and medications. The definition and conceptualization of prenatal psychological distress varies widely in studies (Nast et al., 2013; Zijlmans et al., 2015), leading to results that are not easily comparable. This was also seen in our study, as the results would have been somewhat different had we chosen to look only at symptoms, diagnoses or medications. In our study, the response rate for symptom questionnaires filled in during pregnancy was lower for mothers undergoing amniocentesis (38.7 %) than in the rest of the cohort (70.4 %), likely mirroring the burdensome situation, but leading to possible bias in our self-report symptom data. The use of multiple validated sources for the definition of PDA yields more reliable results.

As for limitations, our sample size – although relatively large for a study with invasive biological sample collection – is quite small, limiting statistical power. The diagnostic and medication purchase data were collected from health registries and do not inform whether the women would have met diagnostic criteria or used medications at the time of amniocentesis. The low response rate for the symptom questionnaires may affect the results regarding self-reported symptoms, since it is possible that the mothers with PDA symptoms filled in the questionnaires less often. Furthermore, we were not able to control for the exact time of day of the amniocenteses. Since the levels of steroid hormones in plasma change throughout the day (Rácz et al., 2015), it is possible that the sampling time may also affect the levels of steroid hormones in amniotic fluid. As for the results concerning steroid-metabolizing enzymes, their activation is inferred from the steroid hormone ratios. It is, therefore, possible that other enzymes may also have been responsible for the changes in steroid hormones. It should also be considered that although the participants in our sample did not have fetal chromosomal anomalies, the sample was nonetheless a high-risk sample for these conditions and cannot be thought to represent normative pregnancy. We have previously reported a higher occurrence of congenital malformations and copy number variations in the children of this cohort with a positive fetal trisomy 21 screen result compared to those with a negative one (Czamara et al., 2022). However, it is unlikely that a similar study could be conducted with no clinical indication. Hence the representativeness limitation is an inherent limitation of human studies on prenatal amniotic fluid.

5. Conclusion

This study provided support for the prenatal programming hypothesis of PDA influencing fetal environment and showed that the levels of the amniotic fluid glucocorticoid metabolites, 20 α -dihydrocortisol and 5 β -tetrahydrocortisol, were lower in the fetuses of mothers with PDA. No steroid hormones, precursors or metabolites from the other steroid classes – progestogens, mineralocorticoids, androgens or estrogens – were associated with PDA. This study highlights the need to expand the view from a few steroid biomarkers to a more comprehensive panel to capture variation throughout the entire pathway and metabolism.

CRedit authorship contribution statement

Katri Räikkönen: Writing – review & editing, Supervision, Methodology, Funding acquisition, Data curation, Conceptualization. **Tiina Seikku:** Writing – original draft, Visualization, Methodology, Formal

analysis, Data curation. **Marius Lahti-Pulkkinen:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Data curation, Conceptualization. **Rebecca M Reynolds:** Writing – review & editing. **Homer Natalie Z M:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Formal analysis. **Margaux Billen:** Investigation. **Joanna P Simpson:** Writing – review & editing, Investigation. **Taru Tukiainen:** Writing – review & editing. **Ellie Phelan:** Validation, Investigation. **Verna Salo:** Writing – review & editing, Data curation. **Kati Heinonen:** Writing – review & editing, Methodology, Funding acquisition. **Polina Girchenko:** Writing – review & editing, Methodology.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.psyneuen.2026.107758](https://doi.org/10.1016/j.psyneuen.2026.107758).

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