Published in final edited form as:

Psychoneuroendocrinology. 2020 November ; 121: 104827. doi:10.1016/j.psyneuen.2020.104827.

Longitudinal proneuroactive and neuroactive steroid profiles in medication-free women with, without and at-risk for perinatal depression: a liquid chromatography-tandem mass spectrometry analysis

Kristina M. Deligiannidis, MD^{a,b,c,d,*}, Aimee R. Kroll-Desrosiers, PhD^{e,f}, Yanglan Tan, PhD^g, Michelle L. Dubuke, PhD^{g,h}, Scott A. Shaffer, PhD^{g,h}

^aFeinstein Institutes for Medical Research, Northwell Health, Manhasset, NY 11030, USA;

^bDepartment of Psychiatry, Division of Psychiatry Research, Zucker Hillside Hospital, Northwell Health, Queens, NY, 11004, USA

^cZucker School of Medicine at Hofstra/Northwell, Hempstead, NY, 11549, USA

^dDepartment of Psychiatry, University of Massachusetts Medical School, Worcester, MA, 01655, USA

^eVA Central Western Massachusetts Healthcare System, Leeds, MA, 01053, USA;

^fDepartment of Population and Quantitative Health Sciences, University of Massachusetts Medical School, Worcester, MA, 01655, USA

^gMass Spectrometry Facility, University of Massachusetts Medical School, Shrewsbury, MA, 01545, USA;

^hDepartment of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, MA, 01655, USA

Abstract

Background: Neuroactive steroids (NAS) are derivatives of cholesterol or steroidal precursors made in the gonads, adrenal gland, placenta and brain. We characterized longitudinal plasma proneuroactive and NAS in healthy perinatal comparison women (HPCW), women at-risk for perinatal depression (AR-PND), and women with PND with/without comorbid anxiety. We hypothesized that AR-PND women who either did or did not go on to develop PND would have

* **Corresponding Author:** Kristina M. Deligiannidis, M.D., Director, Women's Behavioral Health, Zucker Hillside Hospital, Northwell Health, Associate Professor of Psychiatry and Obstetrics & Gynecology, Zucker School of Medicine at Hofstra/Northwell, Associate Professor, Feinstein Institutes for Medical Research, Adjunct Associate Professor of Psychiatry, University of Massachusetts Medical School, Glen Oaks, NY 11004, USA.; tel: 718-470-8184; kdeligian1@northwell.edu.

Publisher's Disclaimer: This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

SUPPLEMENTARY MATERIAL

Supplementary information accompanies this paper at <https://doi.org/-doi> to be determined.

elevated NAS concentrations as compared to HPCW and that NAS would be correlated to depressive and anxiety symptoms.

Methods: A prospective cohort study evaluated 75 medication-free perinatal women (HPCW, n=30; AR-PND, n=19; PND, n=26). Standardized depression and anxiety assessments and blood samples were completed across 5 visits. Structured Clinical Interviews for DSM-IV TR Disorders were administered at study entry and exit. Plasma pregnenolone, progesterone, 5 α - and 5 β -dihydroprogesterone, pregnanolone, allopregnanolone, deoxycorticosterone and tetrahydrodeoxycorticosterone were quantified by liquid chromatography-tandem mass spectrometry. Longitudinal relationships between risk-group, depression and anxiety symptoms, and NAS concentrations were analyzed using generalized estimating equations to control for repeated measures correlations.

Results: Perinatal 5 α -dihydroprogesterone, 5 β -dihydroprogesterone, allopregnanolone, deoxycorticosterone, and tetrahydrodeoxycorticosterone concentrations were higher in AR-PND and PND women compared to HPCW (β =3.57 $\pm$ 1.40 and β =2.11 $\pm$ 1.12, p =0.03; β =0.18 $\pm$ 0.06 and β =0.03 $\pm$ 0.05, p =0.02; β =1.06 $\pm$ 0.42 and β =1.19 $\pm$ 0.47, p =0.01; β =0.17 $\pm$ 0.07 and β =0.11 $\pm$ 0.06, p =0.05; β =0.03 $\pm$ 0.01 and β =0.03 $\pm$ 0.01, p =0.05, respectively). Perinatal allopregnanolone, 5 α -dihydroprogesterone and tetrahydrodeoxycorticosterone were positively associated with HAM-D₁₇ (all p <0.02). HAM-A was positively associated with 5 α - and 5 β -dihydroprogesterone, pregnanolone, allopregnanolone, deoxycorticosterone and tetrahydrodeoxycorticosterone (all p <0.05). A history of depression was associated with increased 5 α -dihydroprogesterone (2.20 $\pm$ 1.09, p =0.05), deoxycorticosterone (0.13 $\pm$ 0.06, p =0.03) and tetrahydrodeoxycorticosterone (0.03 $\pm$ 0.01, p =0.02).

Conclusion: To our knowledge, this study represents the largest prospective study of 5- α and 5- β reductase products of progesterone and deoxycorticosterone in HPCW and women AR-PND. Data suggest that PND is associated with both a reduction of progesterone to 5 β -dihydroprogesterone, 5 α -dihydroprogesterone, and allopregnanolone, and the 21-hydroxylation to deoxycorticosterone and tetrahydrodeoxycorticosterone. The shift towards 5 α -dihydroprogesterone, deoxycorticosterone and tetrahydrodeoxycorticosterone was associated with a history of depression, a significant risk factor for PND.

Keywords

neuroactive steroid; perinatal depression; mass spectrometry

1. INTRODUCTION

Perinatal depression (PND) afflicts approximately 1 in 9 women (Ko et al., 2017) and negatively impacts infant and child neurodevelopment and behavior. PND is frequently under-detected and under-treated (Cox et al., 2016), leaving women with prolonged symptoms and significant impairment (Hung et al., 2017). Maternal suicide due to psychiatric illness is the leading cause of direct deaths within a year after the end of pregnancy (Knight et al., 2019; Lindahl et al., 2005). Prenatal stress (Coburn et al., 2016), a prior depressive episode (Meltzer-Brody et al., 2013), a history of PND (Wisner et al., 2001)

and antepartum depressive/anxiety symptoms are significant risk factors for PND (Milgrom et al., 2008), which can onset either during pregnancy or after delivery.

The perinatal period is characterized by rapid and significant physiological change in brain and plasma levels of endocrine hormones, peptides and neuroactive steroids (NAS). NAS are derivatives of cholesterol or steroidal precursors made in the gonads, adrenal gland, placenta and brain, with plasma steroids largely derived from peripheral steroidogenic organs and brain steroids derived from both peripheral and central sources (Sze et al., 2018). NAS are rapid and potent allosteric modulators of γ -aminobutyric acid A receptor (GABA_AR) function (Paul and Purdy, 1992). Many NAS are positive allosteric modulators (PAMs) of the GABA_AR, binding at an allosteric site on the membrane-bound, ligand-gated ion channel receptor and facilitating negatively-charged chloride ion flow into the post-synaptic neuron (Paul and Purdy, 1992). Through their modulation of GABA_AR, NAS affect both tonic and phasic GABAergic neurotransmission, resulting in a change in neuronal activity (Stell et al., 2003). Pregnane NAS include allopregnanolone (3 α , 5 α -tetrahydroprogesterone; 3 α , 5 α -THP or ALLO), pregnanolone (3 α , 5 β -tetrahydroprogesterone; 3 α , 5 β -THP), and tetrahydrodeoxycorticosterone (3 α , 21-dihydroxy-5 α -pregnan-20-one, allotetrahydrocorticosterone or THDOC), while pregnenolone, progesterone, 5 α -dihydroprogesterone (5 α -DHP), 5 β -dihydroprogesterone (5 β -DHP) and deoxycorticosterone are pregnane steroid precursors which also exhibit neuroactive effects (Paul and Purdy, 1992; Rupprecht, 2003) (Supplementary Figure 1). NAS such as THDOC and ALLO are potent modulators of GABA_ARs, acting preferentially at extrasynaptic δ subunit-containing GABA_ARs that mediate tonic inhibition (Paul and Purdy, 1992).

NAS play a critical role in the regulation of the hypothalamic-pituitary-adrenal (HPA) axis during non-stress, acute and chronic stress conditions (Crowley and Girdler, 2014; Sarkar et al., 2011), with notable sex differences in rat brain (e.g. hippocampus (Hillerer et al., 2019)) and plasma NAS production following stress (Sze et al., 2018). For a comprehensive review of the preclinical NAS literature we refer the reader to a recent review by Giatti S et al. (Giatti et al., 2019). NAS modulate the extent and duration of preclinical stress-induced modulation of GABAergic transmission in the hypothalamic paraventricular nucleus (PVN), a region important in the initiation of the neuroendocrine and autonomic response to stress (Herman and Cullinan, 1997). NAS-sensitive δ subunit-containing GABA_ARs regulate corticotropin releasing hormone (CRH) neurons in the mouse PVN (Sarkar et al., 2011). In late pregnancy and lactation in humans, stress-induced activation of the HPA axis is normally suppressed (Brunton, 2016) and the inability to suppress the stress-induced activation of the HPA axis in the perinatal period has been proposed to be associated with PND (Melon et al., 2018).

Additionally, altered neurosteroidogenesis has been implicated in preclinical models of PND and in women (Deligiannidis et al., 2019; Deligiannidis et al., 2016; Hellgren et al., 2014; Maguire and Mody, 2008; Osborne et al., 2017) though data remain mixed likely due the clinical heterogeneity of the patient populations examined, the timing of perinatal samples, and the rigor of the NAS analytic methods. Low third trimester human ALLO serum levels have been associated with elevated third trimester depressive symptoms (Hellgren et al.,

2014), and low second trimester ALLO serum concentrations have been associated with the later development of PND (Osborne et al., 2017). 5 α -DHP, the precursor to ALLO, was elevated in women with antepartum major depressive disorder (Pearson Murphy et al., 2001). In our previous study in medication-free women at-risk for developing PND, perinatal plasma progesterone and 3 α , 5 β -THP (pregnanolone) concentrations were higher in women at-risk for PND compared to healthy perinatal comparison women (Deligiannidis et al., 2016). In some human studies a relationship between blood NAS concentrations and PND was not identified (Guintivano et al., 2018; Hellgren et al., 2017).

It has been hypothesized that between-group differences in perinatal plasma NAS concentrations could represent differential metabolism within the neurosteroidogenic pathway involved in the conversion of the GABAergic progesterone to ALLO and 3 α , 5 β -THP by either of the 5 α - or 5 β -reductases, respectively, and/or 3 α -hydroxysteroid dehydrogenase, or in the conversion of DOC to THDOC, either peripherally and/or in the brain (Giatti et al., 2019; Hellgren et al., 2017; Paul and Purdy, 1992). As reviewed earlier, NAS are important regulators of the stress response through their interactions with GABA and altered NAS concentrations could affect excitatory: inhibitory balance of the neurocircuit through their interactions with GABA. For example, in the current study's parent neuroimaging study, plasma postpartum ALLO concentrations were significantly correlated with postpartum intrinsic resting-state functional connectivity of neural networks important for self-appraisal and emotional perception in medication-free women with PND as compared to healthy comparison perinatal women (Deligiannidis et al., 2019). Alternatively, altered perinatal NAS concentrations could represent a trait marker of risk for the later development of PND.

The primary aim of this prospective observational study was to examine a panel of plasma proneuroactive and NAS in perinatal women at-risk for PND (AR-PND) compared to healthy perinatal comparison women (HPCW) and compared to AR-PND women who developed PND. We hypothesized that AR-PND women who either did or did not go on to develop PND would have elevated plasma concentrations of ALLO, 3 α , 5 β -THP, DOC and THDOC as compared to HPCW across the perinatal period and that the plasma panels would be correlated to current depressive and anxiety symptoms. We examined if a history of prior depression contributed to the NAS and steroid precursor panel results. In an exploratory analysis, we examined ratios of perinatal NAS to precursor steroids as an index of conversion of progesterone to NAS to examine neurosteroidogenesis across groups.

2. MATERIALS AND METHODS

2.1 Participants

741 English-speaking pregnant participants were consented and assessed for eligibility in medical center obstetric clinics using the Edinburgh Postnatal Depression Scale (EPDS) (Cox et al., 1987) at 22–38 weeks gestational age (as determined by first trimester ultrasound) to determine eligibility and interest. 88 eligible and interested women between 19–40 years old consented to the prospective study (Figure 1). Two groups enrolled during pregnancy: (1) 35 healthy HPCW and (2) 53 women AR-PND. Eight AR-PND women were removed from the analysis due to loss to follow-up (n=4) or withdrawal (n=4), resulting in

45 total women in the AR-PND group. Five HPCW women were removed from the analysis due to loss to follow-up (n=2) or withdrawal (n=3), resulting in 30 total women in the HPCW group.

Recruitment of both groups was balanced across the study. The HPCW group included women with an EPDS ≤ 5 and no current or past psychiatric diagnosis or family history of psychiatric illness, as ascertained by clinical and research interviews. The AR-PND group included women who either had an EPDS score ≤ 10 (indicating current depressive and/or anxiety symptomatology (Cox et al., 1987)) or, regardless of antepartum EPDS score at study entry, a personal history of PND or non-puerperal depression as determined by the Structured Clinical Interview for DSM-IV TR Disorders (SCID-IV), Patient Edition (First et al., 2001). As antenatal anxiety and depression symptoms are associated with, or may represent the early presentation of PND symptomatology, women who met criteria for an anxiety or depressive disorder not otherwise specified were included in the AR-PND group. For this manuscript, the use of “PND” refers to women who developed a new onset of adjustment disorder with depressed mood or depressive disorder (minor or major) during pregnancy or the postpartum period under study, resulting in three groups for our analysis: women who developed adjustment disorders/minor/major depression (PND, n=26); women who did not develop PND (AR-PND, n=19); and HPCW (n=30). Women who met SCID-IV criteria for a major depressive episode (MDE) at study entry were excluded as the study was designed to identify potential differences in NAS profiles between HPCW and AR-PND women who did or did not go on to develop PND.

Participants were excluded for multiple gestation pregnancy, lifetime history of manic episode or any psychotic disorder, elevated suicidal risk, and alcohol, nicotine or substance abuse/dependence in the 6 months prior to study entry or use during the study. Based on medical record review, subjects were excluded if they had any significant current medical illness. Available laboratory results including blood cell counts, oral glucose tolerance testing, chemistry panels, thyroid function tests, and viral serology conducted for routine care were reviewed and were within normal limits. Participants were medication-free except prenatal vitamins; as needed over-the-counter antacids, antihistamines and stool softeners were allowed. All participants provided written informed consent and each received monetary compensation. Institutional Review Boards approved the study which was conducted between April 2013 and February 2017. Study data were collected and managed using Research Electronic Data Capture (REDCap), hosted at Northwell Health (Harris et al., 2009).

2.2 Measures

2.2.1 Study design using clinical measures—Antepartum visits (visits 1 and 2) were completed in second and third trimesters. Postpartum visits were completed within one week (visit 3), 10 days to 10 weeks (visit 4), and 3–11 weeks postpartum (visit 5). The 11-week postpartum cut-off was based on literature suggesting a postpartum onset definition of up to 6–8 weeks of delivery as optimal (Forty et al., 2006), with the additional 3 weeks to allow research assessment completion for participants who were difficult to reach. Assessments done at visit 1 included the SCID-IV and demographics. The SCID-IV was

additionally completed at visits 4 or 5. Additional research assessments done at all visits included the EPDS, Structured Interview Guide for Hamilton Depression Rating Scale (HAM-D₁₇) (Williams, 1988), Structured Interview Guide for Hamilton Anxiety Scale (HAM-A) (Hamilton, 1959; Shear et al., 2001), Spielberger State-Trait Anxiety Inventory (STAI) (Spielberger et al., 1970), Pittsburgh Sleep Quality Index (PSQI) (Buysse et al., 1989), Sheehan Disability Scale (SDS) (Sheehan, 1983), and urine benzodiazepine tests. Additional assessments done at one of the postpartum visits included a labor, delivery and breastfeeding questionnaire. Blood samples for proneuroactive and neuroactive steroid analyses were obtained in the morning and collected into tubes containing EDTA. Plasma was stored at -80°C until analysis was completed by collaborators blind to the participant group assignment.

2.2.2 Neuroactive steroid quantification—Antenatal and postnatal plasma concentrations of proneuroactive steroids (pregnenolone, progesterone, and DOC and NAS (5 α DHP, 5 β -DHP, ALLO, pregnanolone and THDOC) were determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS) with a lower limit of quantification in human charcoal-stripped plasma of 0.008, 0.005, 0.004, 0.020, 0.008, 0.008, 0.020, and 0.008 ng/mL, respectively.

2.2.3 Standards—Progesterone (pregn-4-ene-3,20-dione), pregnenolone (3 β -hydroxypregn-5-en-20-one), deoxycorticosterone (11-deoxycorticosterone; DOC), 5 α -dihydroprogesterone (5 α -pregnan-3,20-dione; 5 α -DHP) and 5 β -dihydroprogesterone (5 β -pregnan-3,20-dione; 5 β -DHP) were purchased from Steraloids (Newport, RI). Allopregnanolone (3 α -hydroxy-5 α -pregnan-20-one; 3 α ,5 α -THP), pregnanolone (3 α -hydroxy-5 β -pregnan-20-one; 3 α ,5 β -THP), allotetrahydrodeoxycorticosterone (3 α , 21-dihydroxy-5 α -pregnan-20-one; 3 α ,5 α -THDOC), progesterone-d₉, pregnenolone-d₄ and 5 α -DHP-d₆ were synthesized and provided by Dr. Robert H. Purdy (in memoriam, Veterans Medical Research Foundation, San Diego, CA). DOC-d₈, and 3 α ,5 α -THP-d₄ were purchased from CDN Isotopes (Pointe-Claire, Quebec, Canada). 3 α ,5 α -THDOC-d₃ was purchased from Cambridge Isotope Laboratories (Tewksbury, MA).

2.2.4 Sample preparation—For “visit 1 and 2” study samples, standard solutions for calibration curves were prepared in methanol at 0.2–4000 ng/mL for progesterone and 0.04–800 ng/mL for all others. An internal standard solution of progesterone-d₉, pregnenolone-d₄, DOC-d₈, 5 α -DHP-d₆, 3 α ,5 α -THP-d₄ and 3 α ,5 α -THDOC-d₃ was prepared in methanol at 100 ng/mL each. Standard solutions for quality control benchmarks were prepared at low (5 ng/mL for progesterone and 1 ng/mL for all others), medium (50 ng/mL for progesterone and 10 ng/mL for all others) and high (500 ng/mL for progesterone and 100 ng/mL for all others) concentration. Standard calibrants and quality control benchmarks were prepared by spiking 20 μL of a standard solution and 20 μL of the internal standard mixture solution into 200 μL of human (male) charcoal-stripped plasma (BioreclamationIVT, Westbury, NY). Study samples were prepared in duplicate by spiking 20 μL of methanol and 20 μL of internal standard solution into 200 μL of plasma. Spiked plasma was mixed with 2 mL methyl tert-butyl ether (MTBE), followed by vortexing for 30 sec, shaking for 10 min and centrifugation for 10 min at 2000 rpm. The upper organic phase was then collected, dried

under nitrogen and reconstituted in 50 μ L of 50% methanol containing 2.0 mM lithium acetate and 0.1% (v/v) formic acid for LC-MS/MS analysis. For “visit 3, 4, and 5” study samples, standard solutions for calibration curves were prepared at 0.05–1000 ng/mL for progesterone, 0.01–200 ng/mL for all others, while the internal standard mixture solution was prepared at 10 ng/mL. All were followed by the same sample preparation procedures above.

2.2.5 LC-MS/MS analysis—Steroid analysis was performed on a NanoAcquity UPLC (Waters Corporation, Milford, MA) coupled with a Orbitrap Velos Pro (Thermo Scientific, Waltham, MA) mass spectrometer. Samples were loaded onto a custom-made fused-silica precolumn (15 cm long, 180 μ m I.D.) packed with 2 cm of 5 μ m (100 Å) Magic C18AQ particles (Bruker Daltonics, Billerica, MA). Steroids were separated on a custom-made fused-silica analytical column (100 μ m I.D. with gravity-pulled tip) packed with 20 cm of 3 μ m (100 Å) Magic C18AQ particles. The mobile phase consisted of (A) water containing 2 mM lithium acetate and 0.1% (v/v) formic acid and (B) methanol containing 2 mM lithium acetate and 0.1% (v/v) formic acid. Using a 2 μ L injection volume, samples were loaded at 5 μ L/min for 3 min using 40% B, then eluted (linear) at 0.6 μ L/min as follows: 0–2.0 min (40–85% B), 2.0–20 min (85% B), 20.0–20.1 min (85–40% B), 20.1–36.0 min (40% B). Electrospray ionization was carried out in the positive ion mode (1.4 kV) with an in-source collision induced dissociation energy of 35 V, an inlet capillary temperature of 350°C and an S-lens RF level of 60%. Tandem mass spectra were obtained in the parallel reaction monitoring mode using an HCD collision energy of 30 V and an isolation width of 2.0 Da for each $[M+Li]^+$ precursor ion as follows: progesterone (m/z 321.24), pregnenolone (m/z 323.26), 5 α - and 5 β -DHP (m/z 323.26), 3 α ,5 α - and 3 α ,5 β -THP (m/z 329.30), DOC (m/z 337.24), 3 α ,5 α -THDOC (m/z 344.29), progesterone- d_9 (m/z 330.30, used for quantitation of progesterone), pregnenolone- d_4 (m/z 327.28, used for quantitation of pregnenolone), 5 α -DHP- d_6 (m/z 329.29, used for quantitation of 5 α - and 5 β -DHP), 3 α ,5 α -THP- d_4 (m/z 329.29, used for quantitation of 3 α ,5 α - and 3 α ,5 β -THP), DOC- d_8 (m/z 345.29, used for quantitation of DOC), and 3 α ,5 α -THDOC- d_3 (m/z 344.29, used for quantitation of 3 α ,5 α -THDOC). All spectra were acquired by Orbitrap mass analyzer at resolution of 7500 over m/z 90–400 range. Product ions produced during HCD from the ion-molecule reaction $[M+Li]^+ > [M+Li+H_2O]^+$ were used as the basis of quantitation for all analytes using a 20 ppm mass tolerance. Calibration curves were constructed by plotting the peak area ratios (standard/internal standard) as a function of concentration, whereas the peak height ratios were used for 5 α -DHP and 5 β -DHP because they were not baseline separated. Calibration curve were fit by excluding the origin and using a 1/x axis weighting. All data was integrated and processed in Xcalibur (Version 2.2, Thermo Scientific).

2.2.6 Urinary benzodiazepine detection—To determine the presence or absence of benzodiazepine use which could confound plasma NAS measurements, a urine sample was obtained at the time of each blood draw. The urinary benzodiazepine drug test (Innovacon, Inc., San Diego, CA) is a lateral flow chromatographic immunoassay for the qualitative detection of oxazepam (major metabolite) with a cut-off concentration of 300ng/mL. Common benzodiazepines (e.g. alprazolam, clonazepam, diazepam, etc.) are detected with the assay.

2.2.7 Statistical analysis—Baseline and postpartum characteristics and perinatal depression and anxiety ratings were compared between PND, AR-PND, and HPCW groups using Fisher's exact tests for categorical variables and one-way ANOVA for continuous variables. Descriptive statistics (mean $\pm$ SD) were examined for NAS concentration, overall and by risk group. To assess the impact of outliers in our data, we observed outliers graphically, identified values outside of ± 3 standard deviations from the mean, and calculated Cook's D statistics. Results did not vary substantially from the final data reported here; therefore, we did not exclude any values from our analysis.

As we were interested in understanding longitudinal changes in NAS within each risk group (Hubbard et al., 2010), we used a generalized estimating equation (GEE) approach to control for the inherent correlation within participants across visits. This type of modeling correlates the data between the independent and dependent variables at each visit across the study to estimate an average response over time. All of our models were specified with an autoregressive covariance structure. We also measured the precursor progesterone, as differences in progesterone concentrations between groups could affect metabolite (i.e., ALLO and pregnanolone) concentrations. Main effects for risk group, HAM-D, and HAM-A were modeled separately, with individual mood scores as the independent variable and plasma concentrations as the dependent variable, as acute depression and its successful treatment has been associated with changes in plasma concentrations of NAS. Additionally, we ran main effects models with risk group (PND vs. HPCW and AR-PND vs. HPCW) as the independent variable and plasma concentrations as the dependent variable. As a sensitivity analysis, we ran models with each plasma concentrations as the dependent variables and included HAM-A scores and history of prior depression, to consider both depression history and anxiety severity in the same model.

To account for the timing during pregnancy or postpartum of the plasma measurement, we created a perinatal time variable, centering time around delivery date where timing prior to delivery (visits 1 and 2) was coded negatively and timing after delivery (visits 3, 4, and 5) was coded positively, as has been done in our previous work (Deligiannidis et al., 2019; Deligiannidis et al., 2016). All models were adjusted for this timing variable. For main effects models, *P* values are reported from a *z* test that a single regression coefficient was equal to 0 as well as from the overall Type 3 test of any difference among levels of a factor (such as risk group), adjusting for other variables in the model. All results are reported at a significance level of $\alpha=0.05$. As in a prior study (Deligiannidis et al., 2016), we did not adjust for multiple comparisons in our analysis to examine all potential significant associations that may inform future work, as well as to prevent an increase in the Type II error rate (Rothman, 1990). Analyses were conducted in SAS 9.4 (SAS Institute, Inc., Cary, North Carolina).

3. RESULTS

3.1 Study population characteristics

A total of 75 women participated. The average age was 29.2 years ($\pm$ standard deviation [SD] 4.8, 19.4–40.7) and the majority of subjects were white, non-Hispanic, employed and married. There were no between group differences in age, race, ethnicity, marital status,

education level, employment status, or number of children in household (Table 1). Overall, women were 31.2 weeks gestational age (± 3.8 weeks) at study entry. Over half of the women (53.3%) were nulliparous at study entry. While not statistically significant, a greater proportion of women in the AR-PND (21.1%) and PND (34.6%) groups had a Cesarean section compared to women in the HPCW group (16.7%; $p=0.30$).

Prior to study entry, a majority of AR-PND who did not develop and those who did develop PND had a prior depression diagnosis (68.4% and 84.6%, respectively); including 21.1% of AR-PND and 26.9% of PND with a prior MDE with perinatal onset, and 36.8% of AR-PND and 76.9% of PND had a prior anxiety diagnosis. At the final postpartum visit, 39.4% of the AR-PND participants developed a MDE with perinatal onset (i.e. PND group) and 77.1% had a SCID diagnosis of an anxiety disorder (data not shown). Of the AR-PND women who did not develop PND, 20.5% were diagnosed with an anxiety disorder at the final postpartum visit. No HPCW women developed anxiety or depressive disorders during the course of the study (Table 1).

3.2 Depression and anxiety ratings across perinatal period

PND women scored higher compared to HPCW and AR-PND women at each visit on HAM-D₁₇, HAM-A, EPDS, STAI-S, SDS, and PSQI perinatal scale scores (Table 2). AR-PND women had mean severity scores in between those seen in HPCW and PND women.

3.3 Primary analyses of perinatal plasma proneuroactive and neuroactive steroid by risk group and depressive/anxiety symptomatology

Descriptive statistics for perinatal plasma proneuroactive and NAS concentrations by risk group and perinatal time point are reported in Table 3 (and Supplementary Figure 2). This table depicts perinatal plasma proneuroactive and NAS concentrations (ng/mL) (mean $\pm$ SD) within each study visit.

Main effects models examining the relationship between depressive and anxiety symptoms and plasma concentrations across perinatal time points showed that perinatal 5 α -DHP, 5 β -DHP, ALLO, DOC, and THDOC concentrations were higher in AR-PND and PND women than HPCW ($\beta=3.57\pm 1.40$ and $\beta=2.11\pm 1.12$, $p=0.03$; $\beta=0.18\pm 0.06$ and $\beta=0.03\pm 0.05$, $p=0.02$; $\beta=1.06\pm 0.42$ and $\beta=1.19\pm 0.47$, $p=0.01$; $\beta=0.17\pm 0.07$ and $\beta=0.11\pm 0.06$, $p=0.05$; $\beta=0.03\pm 0.01$ and $\beta=0.03\pm 0.01$, $p=0.05$, respectively). No between-group differences in perinatal pregnenolone, progesterone or pregnanolone concentrations were identified. Perinatal ALLO, 5 α -DHP, and THDOC were positively associated with HAM-D₁₇ ($\beta=0.07\pm 0.03$, $p=0.01$; $\beta=0.19\pm 0.07$, $p=0.01$; $\beta=0.002\pm 0.001$, $p=0.02$, respectively) and EPDS ($\beta=0.06\pm 0.03$, $p=0.03$; $\beta=0.15\pm 0.08$, $p=0.04$; $\beta=0.002\pm 0.001$, $p=0.02$, respectively). Additionally, HAM-A was positively associated with 5 α -DHP, 3 α ,5 β -THP, 3 α ,5 α -THP, DOC, THDOC, and 5 β -DHP (all $p<0.05$; data not shown). STAI-S was positively associated with 5 α -DHP, 3 α ,5 α -THP, and DOC (all $p<0.05$; data not shown). There were no significant associations between SDS and perinatal pregnenolone, progesterone or pregnanolone concentrations. In an exploratory analysis, a history of depression in AR-PND and PND women was positively associated with increased DOC and THDOC (0.13 ± 0.06 , $p=0.03$; 0.03 ± 0.01 , $p=0.02$, respectively). In sensitivity models controlling for history of prior

depression, for each unit increase of $3\alpha,5\beta$ -THP, HAM-A increased by 0.03 ± 0.02 ($p=0.05$). For each unit increase of 5β -DHP, HAM-A increased by 0.01 ± 0.004 ($p=0.01$) (data not shown).

3.4 Exploratory analyses of antenatal and postpartum plasma neuroactive steroid to precursor ratios

In an exploratory analysis, eight NAS to precursor ratios were examined by risk group (Figure 2). During pregnancy (visits 1 and 2), GEE models revealed that progesterone: pregnenolone and DOC: progesterone increased significantly for all participants, while 5β -DHP: progesterone, ALLO: 5α -DHP, pregnanolone: progesterone decreased significantly over all participants ($p<0.05$ for all). During the postpartum (visits 3, 4, and 5), 5α -DHP: progesterone, 5β -DHP: progesterone, and ALLO: progesterone ratios increased significantly for all participants ($p<0.05$ for all).

Two NAS: precursor ratios differed between risk groups during pregnancy or the postpartum period. ALLO: 5α -DHP differed overall between risk groups during pregnancy ($p=0.009$) and postpartum ($p=0.04$). Additionally, pregnanolone: 5β -DHP differed significantly between risk groups during pregnancy overall (Type 3 $p=0.01$), where compared to HPCW, decreases were shown for both AR-PND (-2.7 ± 0.9) and PND (-0.2 ± 1.2). However, only the AR-PND group differed significantly from HPCW in this model ($p=0.004$ for AR-PND; $p=0.83$ for PND).

4. DISCUSSION

The primary aim of this prospective observational study was to examine a panel of plasma proneuroactive and NAS in perinatal women AR-PND compared to HPCW and compared to AR-PND women who developed PND. Our study yielded three main novel findings: (1) mean perinatal 5α -DHP, 5β -DHP, ALLO, DOC, and THDOC plasma concentrations were significantly higher in both AR-PND and PND women compared to HPCW, (2) no between-group differences in perinatal progesterone or pregnenolone plasma concentrations were identified, and (3) a history of depression in AR-PND and PND women was positively associated with significantly increased perinatal 5α -DHP, DOC and THDOC plasma concentrations. Our data suggest a shift towards reduction of progesterone to 5β -DHP, 5α -DHP, ALLO and 20-hydroxylation to DOC and THDOC in women AR-PND and with PND, where the shift towards 5α -DHP, DOC, and THDOC was associated with a history of depression, a significant risk factor for PND.

In our study we used repeated-measures to closely examine perinatal depression and anxiety across gestation and into the early postpartum period. AR-PND women had one or more risk factors for the development of PND, including a history of MDD or PND and/or current depressive and anxiety symptomatology in the mild range. Depressive and anxious symptomatology, as measured by the HAM-D, HAM-A, STAI-S and EPDS, was significantly greater in AR-PND compared to HPCW women at every perinatal time point. Reported sleep quality, as measured by the PSQI, and the SDS, a measure of functional impairment was also significantly worse in the AR-PND group as compared to HPCW

across all visits. Women who met a SCID diagnosis for PND reported similar overall symptoms but of greater severity than the AR-PND group.

The same NAS and pregnane steroid precursor profile across the perinatal period was identified in both AR-PND and PND women as compared to HPCW. This may be due to the fact that both AR-PND and PND groups experienced depressive and anxiety/stress symptoms, with AR-PND women experiencing lower severity of symptoms as compared to women with PND. Indeed both AR-PND and PND women had greater severity of depressive and anxiety/stress symptoms, as measured by the HAM-D, HAM-A and STAI-S, than HCW and several of the NAS were positively associated with depression/anxiety/stress measures. In sensitivity models controlling for history of prior depression, 5 β -DHP and 3 α 5 β -THP were positively associated with HAM-A. Thus it is possible that perinatal anxiety/stress in AR-PND and PND groups contributed to some of the shared NAS findings vs. HCW, consistent with preclinical data (Park et al., 2017). Our finding of elevated perinatal 5 α -DHP may also be consistent with a prior finding of elevated 5 α -DHP in antenatal major depression (Pearson Murphy et al., 2001). Our finding of elevated perinatal ALLO plasma concentrations in both AR-PND and PND women may be in contrast to studies that have reported a correlation between low ALLO and concurrent higher depression scores in the third trimester (Hellgren et al., 2014) and another human study that reported lower antenatal ALLO predicting the development of PND (Osborne et al., 2017). Outside of our previous study in at-risk women (Deligiannidis et al., 2016), we are not aware of other studies in depressed or at-risk perinatal women that examined DOC or THDOC plasma concentrations.

Although challenging to compare our studies to others which either did not use multiple perinatal time points or LC-MS/MS for analysis, we point out a few studies which help to integrate the current understanding of NAS biosynthesis and metabolism in perinatal women. One study examined the relationship between acute stressors in euthymic pregnant women and NAS profiles. It reported an inverse relationship between second trimester negative emotional symptoms, progesterone and a combined measure of ALLO plus pregnanolone (though neither ALLO nor pregnanolone were statistically significant alone; gas chromatography-MS was used for NAS analysis) (Crowley et al., 2016). In that study, an acute stressor during second trimester increased THDOC plasma concentrations but reduced progesterone and the combined measure of ALLO plus pregnanolone. The sample included a mixture of women with and without a history of depression (Crowley et al., 2016). In a separate study, lower concentrations of ALLO and pregnanolone have been reported in euthymic non-pregnant women with a history of depression (Girdler et al., 2012) both prior to and after an acute synthetic progesterone challenge. Pregnancy represents a natural 40-week chronic exposure to elevated concentrations of progesterone so we examined if a history of depression, which is a known risk factor for PND, explained some of our NAS profile findings in perinatal AR-PND women. A history of depression in AR-PND and PND women was positively associated with significantly increased perinatal 5 α -DHP, DOC and THDOC plasma concentrations. It is likely that the processes involved in the biosynthesis and metabolism of NAS function differently in active depression vs. in euthymic patients with a history of depression and we speculate that the NAS profile may look different depending upon the current NAS-state (e.g. acute experimental exposure vs. chronic natural exposure of pregnancy) in which it is examined. As future studies examine

neurosteroidogenesis in perinatal and non-perinatal women, it will be important that studies include larger panels of proneuroactive and neuroactive steroids, such as including DOC and THDOC, to better understand how the biosynthetic pathway may change in different steroid states, as most prior studies have restricted their study to ALLO. Also, given that a history of depression is associated with altered NAS and those alterations may differ due to current steroid-state, future studies should include examine the impact of a history of depression on their findings.

We did not replicate our earlier reported findings of elevated perinatal progesterone and pregnanolone in AR-PND women compared to HPCW (Deligiannidis et al., 2016). Both this study and our prior one recruited an AR-PND group and a HPCW group and both studies used LC-MS/MS for NAS steroid analysis, however AR-PND women in the prior study had higher rates of anxiety disorders than in this current study which could have impacted the results.

In exploratory analyses, we examined between group NAS to precursor ratios as an index of enzymatic conversion of pregnenolone to progesterone (via 3β -HSD), progesterone to DOC (via cytochrome P450 (CYP)21), progesterone to 5α -DHP and 5β -DHP (via 5α -reductase) and then ALLO or pregnanolone (via 3α -HSD). Experimental stress in mice is associated with changes in 5α -reductase expression (Agis-Balboa et al., 2007) and preliminary clinical data suggests that polymorphisms in 3α -HSD may be associated with plasma ALLO concentrations and antenatal depressive symptoms in women (Hellgren et al., 2017). ALLO: 5α -DHP differed significantly overall between risk groups during pregnancy and postpartum suggesting that 3α -HSD rate of conversion of 5α -DHP to ALLO may differ between risk groups. Additionally, pregnanolone: 5β -DHP differed significantly between risk groups during pregnancy overall, where compared to HPCW, statistically significant decreases in pregnanolone: 5β -DHP were seen for AR-PND but not PND women. This pattern of ratios, if additionally found statistically significant in a larger replication sample, could be a marker of mood susceptibility during times of physiologic changes in progesterone and be used to identify women at-risk who may benefit from preventative interventions.

The inability to sufficiently adapt to chronic progesterone exposure of pregnancy may result in changes in the balance of neuronal excitability and inhibition and contribute to the development of PND (Deligiannidis et al., 2019; Maguire and Mody, 2009). The differences we report in perinatal NAS concentrations could represent differential metabolism within the progesterone-based biosynthetic pathway, either peripherally and/or in the brain (Paul and Purdy, 1992). Differences in NAS concentrations could affect further downstream interactions with glutamic acid decarboxylase (GAD) (Magnaghi et al., 2010) as changes in GAD mRNA expression are associated with changes in brain GABA and glutamate levels across the perinatal period (Zhao and Gammie, 2014). Additionally, there is increasing evidence that abnormalities in GABA_AR neuroplasticity, changes driven by chronic NAS exposure, alter the balance of neuronal excitability and inhibition (Maguire and Mody, 2008) in animal models. Altered NAS concentrations could affect downstream GABAergic inhibitory tone within the hypothalamus (Herman and Cullinan, 1997) affecting HPA axis function, evidenced by current depressive and anxiety symptomatology (Deligiannidis et al.,

2016). This is consistent with a growing literature, as reviewed by Crowley & Girdler (2014), supporting NAS, GABAergic and HPA axis dysregulation in human stress-related psychiatric disorders (Crowley and Girdler, 2014).

Strengths of this study include stringent inclusion/exclusion criteria and thorough research diagnostic and symptomatic evaluations. Study participants were free of psychotropic medications and hormonal contraceptive use which could confound NAS results. The use of a research diagnostic interview improved upon the rigor of prior studies that used the EPDS alone. We utilized both research-clinician administered instruments as well as self-report measures so that research findings could be easier translated to clinical practice, as the EPDS is used widely in this patient population.

A major strength is the use of an ultra-sensitive LC-MS/MS analytic method to quantify pregnane steroids and NAS in the same subjects repeatedly across time. There are now several published high performance liquid chromatography (Pearson Murphy and Allison, 2000), gas chromatography/MS (Hill et al., 2007; Martinez et al., 2016; Porcu et al., 2009) and LC-MS/MS methodologies (Deligiannidis et al., 2019; Deligiannidis et al., 2016; Martinez et al., 2016) used in pregnane steroid/NAS research across psychiatric disorders, yet much of the prior research in PND (Guintivano et al., 2018; Hellgren et al., 2017; Osborne et al., 2017) has used radioimmunoassay to measure NAS concentrations. Steroid immunoassays can suffer from problems with validity and are unable to examine snapshot profiling of the steroid pathway (Handelsman and Wartofsky, 2013). Gas chromatography/MS and LC-MS methods have been the gold standard of steroid measurement because of high selectivity and sensitivity for the analyte (Handelsman and Wartofsky, 2013). LC-MS methods are considered methodologically advantageous due to not requiring NAS derivatization prior to analysis, thus eliminating a source of assay variability. We additionally improved the LC/MS-MS analytic methods used in this study as compared to our earlier published study (Deligiannidis et al., 2016), to be able to fully resolve 5 α -DHP and 5 β -DHP chromatographically. We are not aware of any PND studies that have examined all of the 5- α and 5- β reductase products of progesterone and DOC, yet full examination of the biosynthetic pathway for the GABAergic NAS is needed for a greater understanding of the pathway, as compared to solely measuring ALLO. To our knowledge, this study represents the largest prospective study of 5- α and 5- β reductase products of progesterone and DOC in HPCW and women AR-PND and builds upon our prior work in this area.

There are limitations to this study. As the AR-PND group included a variety of clinical at-risk phenotypes, the underlying neuroendocrine mechanisms for each may differ, and inclusion as a group obscures the ability to identify potential differences in NAS profiles for individual clinical phenotypes (e.g. euthymic perinatal women with a history of PND vs. symptomatic perinatal women with no prior history of PND vs. symptomatic perinatal women with a history of MDD, etc.). Although we approached a diverse ethnic and racial pregnant population, women who ultimately were interested in and participated in the study were mainly white, non-Hispanic women, which could limit the generalizability of the results. Additionally, some women may have developed a MDE beyond the postpartum time frame under study which was restricted to when women could feasibly remain off of

psychotropic medications and hormonal contraceptives as their use would have made the NAS data challenging to interpret. Since the SCID was performed at study entry and exit, we cannot determine when women after study entry developed PND, either prior to or after delivery. Although we may have missed some women who went on to develop a PND episode, the lack of confounding medication use improved rigor. Additionally, our small sample size may have limited our ability to detect larger differences between groups.

Given recent clinical evidence that intravenous administration of synthetic ALLO (Meltzer-Brody et al., 2018) has antidepressant effects in postpartum depression, understanding neurosteroidogenesis in men, and in women across different reproductive phases (i.e. menstrual, perinatal, perimenopausal) each with differing pregnane steroid exposure, is critical to understanding the pathophysiology of depressive disorders and to identify novel therapeutic targets. This study builds upon limited pregnane steroid and NAS research in PND.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

We sincerely thank Drs. Anthony J. Rothschild and Peter J. Schmidt for their mentorship and gratefully thank the perinatal women who participated in this research. Results were previously presented in a poster at the American College of Neuropsychopharmacology (ACNP) 57th Annual Meeting, in Hollywood, FL in December, 2018 and in a poster at the 74th Annual Conference of the Society of Biological Psychiatry, in Chicago, IL in May, 2019.

ROLE OF THE FUNDING SOURCE

Research reported in this publication was supported by National Institutes of Health (NIH) Grants K23MH097794 and R01MH120313 (KMD) and 1S10RR027107 (SAS). The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. Dr. Deligiannidis currently receives research funding from the NIH, the Feinstein Institutes for Medical Research, Vorso Corporation and Sage Therapeutics. She also receives royalties from an NIH Employee Invention. Dr. Deligiannidis has served as a consultant to Sage Therapeutics. Dr. Kroll-Desrosiers receives funding from the Department of Veterans Affairs. Dr. Shaffer receives funding from the NIH. The authors declare that they have no conflict of interest.

REFERENCES

- Agis-Balboa RC, Pinna G, Pibiri F, Kadriu B, Costa E, Guidotti A, 2007 Down-regulation of neurosteroid biosynthesis in corticolimbic circuits mediates social isolation-induced behavior in mice. *Proc Natl Acad Sci U S A* 104, 18736–18741. 10.1073/pnas.0709419104. [PubMed: 18003893]
- Brunton PJ, 2016 Neuroactive steroids and stress axis regulation: Pregnancy and beyond. *J Steroid Biochem Mol Biol* 160, 160–168. 10.1016/j.jsbmb.2015.08.003. [PubMed: 26259885]
- Buyse DJ, Reynolds CF 3rd, Monk TH, Berman SR, Kupfer DJ, 1989 The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. *Psychiatry Res* 28, 193–213 [PubMed: 2748771]
- Coburn SS, Gonzales NA, Luecken LJ, Crnic KA, 2016 Multiple domains of stress predict postpartum depressive symptoms in low-income Mexican American women: the moderating effect of social support. *Arch Womens Ment Health* 19, 1009–1018. 10.1007/s00737-016-0649-x. [PubMed: 27329119]
- Cox EQ, Sowa NA, Meltzer-Brody SE, Gaynes BN, 2016 The Perinatal Depression Treatment Cascade: Baby Steps Toward Improving Outcomes. *J Clin Psychiatry* 77, 1189–1200. 10.4088/JCP.15r10174. [PubMed: 27780317]

- Cox JL, Holden JM, Sagovsky R, 1987 Detection of postnatal depression. Development of the 10-item Edinburgh Postnatal Depression Scale. *Br J Psychiatry* 150, 782–786 [PubMed: 3651732]
- Crowley SK, Girdler SS, 2014 Neurosteroid, GABAergic and hypothalamic pituitary adrenal (HPA) axis regulation: what is the current state of knowledge in humans? *Psychopharmacology (Berl)* 231, 3619–3634. 10.1007/s00213-014-3572-8. [PubMed: 24756763]
- Crowley SK, O'Buckley TK, Schiller CE, Stuebe A, Morrow AL, Girdler SS, 2016 Blunted neuroactive steroid and HPA axis responses to stress are associated with reduced sleep quality and negative affect in pregnancy: a pilot study. *Psychopharmacology (Berl)* 233, 1299–1310. 10.1007/s00213-016-4217-x. [PubMed: 26856852]
- Deligiannidis KM, Fales CL, Kroll-Desrosiers AR, Shaffer SA, Villamarin V, Tan Y, Hall JE, Frederick BB, Sikoglu EM, Edden RA, Rothschild AJ, Moore CM, 2019 Resting-state functional connectivity, cortical GABA, and neuroactive steroids in peripartum and peripartum depressed women: a functional magnetic resonance imaging and spectroscopy study. *Neuropsychopharmacology* 44, 546–554. 10.1038/s41386-018-0242-2. [PubMed: 30327498]
- Deligiannidis KM, Kroll-Desrosiers AR, Mo S, Nguyen HP, Svenson A, Jaitly N, Hall JE, Barton BA, Rothschild AJ, Shaffer SA, 2016 Peripartum neuroactive steroid and gamma-aminobutyric acid profiles in women at-risk for postpartum depression. *Psychoneuroendocrinology* 70, 98–107. 10.1016/j.psyneuen.2016.05.010. [PubMed: 27209438]
- First MB, Spitzer RL, Gibbon M, Williams JBW, 2001 Structured Clinical Interview for DSM-IV-TR Axis I Disorders - Patient Edition Biometrics Research Department, New York State Psychiatric Institute, New York, NY
- Forty L, Jones L, Macgregor S, Caesar S, Cooper C, Hough A, Dean L, Dave S, Farmer A, McGuffin P, Brewster S, Craddock N, Jones I, 2006 Familiality of postpartum depression in unipolar disorder: results of a family study. *Am J Psychiatry* 163, 1549–1553. 10.1176/appi.ajp.163.9.1549. [PubMed: 16946179]
- Giatti S, Garcia-Segura LM, Barreto GE, Melcangi RC, 2019 Neuroactive steroids, neurosteroidogenesis and sex. *Prog Neurobiol* 176, 1–17. 10.1016/j.pneurobio.2018.06.007. [PubMed: 29981391]
- Girdler SS, Lindgren M, Porcu P, Rubinow DR, Johnson JL, Morrow AL, 2012 A history of depression in women is associated with an altered GABAergic neuroactive steroid profile. *Psychoneuroendocrinology* 37, 543–553. 10.1016/j.psyneuen.2011.08.004. [PubMed: 21890277]
- Guintivano J, Sullivan PF, Stuebe AM, Penders T, Thorp J, Rubinow DR, Meltzer-Brody S, 2018 Adverse life events, psychiatric history, and biological predictors of postpartum depression in an ethnically diverse sample of postpartum women. *Psychol Med* 48, 1190–1200. 10.1017/S0033291717002641. [PubMed: 28950923]
- Hamilton M, 1959 The assessment of anxiety states by rating. *Br J Med Psychol* 32, 50–55 [PubMed: 13638508]
- Handelsman DJ, Wartofsky L, 2013 Requirement for mass spectrometry sex steroid assays in the Journal of Clinical Endocrinology and Metabolism. *J Clin Endocrinol Metab* 98, 3971–3973. 10.1210/jc.2013-3375. [PubMed: 24098015]
- Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG, 2009 Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. *Journal of biomedical informatics* 42, 377–381. 10.1016/j.jbi.2008.08.010. [PubMed: 18929686]
- Hellgren C, Akerud H, Skalkidou A, Backstrom T, Sundstrom-Poromaa I, 2014 Low serum allopregnanolone is associated with symptoms of depression in late pregnancy. *Neuropsychobiology* 69, 147–153. 10.1159/000358838. [PubMed: 24776841]
- Hellgren C, Comasco E, Skalkidou A, Sundstrom-Poromaa I, 2017 Allopregnanolone levels and depressive symptoms during pregnancy in relation to single nucleotide polymorphisms in the allopregnanolone synthesis pathway. *Horm Behav* 94, 106–113. 10.1016/j.yhbeh.2017.06.008. [PubMed: 28666923]
- Herman JP, Cullinan WE, 1997 Neurocircuitry of stress: central control of the hypothalamo-pituitary-adrenocortical axis. *Trends Neurosci* 20, 78–84 [PubMed: 9023876]

- Hill M, Cibula D, Havlikova H, Kancheva L, Fait T, Kancheva R, Parizek A, Starka L, 2007 Circulating levels of pregnanolone isomers during the third trimester of human pregnancy. *J Steroid Biochem Mol Biol* 105, 166–175. 10.1016/j.jsbmb.2006.10.010. [PubMed: 17583491]
- Hillner KM, Slattery DA, Pletzer B, 2019 Neurobiological mechanisms underlying sex-related differences in stress-related disorders: Effects of neuroactive steroids on the hippocampus. *Front Neuroendocrinol* 55, 100796 10.1016/j.yfrne.2019.100796. [PubMed: 31580837]
- Hubbard AE, Ahern J, Fleischer NL, Van der Laan M, Lippman SA, Jewell N, Bruckner T, Satariano WA, 2010 To GEE or not to GEE: comparing population average and mixed models for estimating the associations between neighborhood risk factors and health. *Epidemiology* 21, 467–474. 10.1097/EDE.0b013e3181caeb90. [PubMed: 20220526]
- Hung CI, Liu CY, Yang CH, 2017 Untreated duration predicted the severity of depression at the two-year follow-up point. *PLoS One* 12, e0185119 10.1371/journal.pone.0185119. [PubMed: 28934289]
- Knight M, K. B, D. T, J. S, R. K, S. K, Collaboration, K.J.J.o.b.o.t.M.-U., 2019 MBRRACE-UK Maternal, Newborn and Infant Clinical Outcome Review Programme, in: UK and Ireland Confidential Enquiries into Maternal Deaths and Morbidity, D.o. HS (Ed.). Maternal, Newborn and Infant Clinical Outcome Review Programme, University of Leicester, UK
- Ko JY, Rockhill KM, Tong VT, Morrow B, Farr SL, 2017 Trends in Postpartum Depressive Symptoms - 27 States, 2004, 2008 and 2012.. *MMWR Morb Mort Wkly Rep* 66, 153–158. 10.15585/mmwr.mm6606a1.
- Lindahl V, Pearson JL, Colpe L, 2005 Prevalence of suicidality during pregnancy and the postpartum. *Arch Womens Ment Health* 8, 77–87. 10.1007/s00737-005-0080-1. [PubMed: 15883651]
- Magnaghi V, Parducz A, Frasca A, Ballabio M, Procacci P, Racagni G, Bonanno G, Fumagalli F, 2010 GABA synthesis in Schwann cells is induced by the neuroactive steroid allopregnanolone. *J Neurochem* 112, 980–990. 10.1111/j.1471-4159.2009.06512.x. [PubMed: 19943853]
- Maguire J, Mody I, 2008 GABA(A)R plasticity during pregnancy: relevance to postpartum depression. *Neuron* 59, 207–213.S0896-6273(08)00537-0 [pii] 10.1016/j.neuron.2008.06.019. [PubMed: 18667149]
- Maguire J, Mody I, 2009 Steroid hormone fluctuations and GABA(A)R plasticity. *Psychoneuroendocrinology* 34 Suppl 1, S84–90. 10.1016/j.psyneuen.2009.06.019. [PubMed: 19632051]
- Martinez PE, Rubinow DR, Nieman LK, Koziol DE, Morrow AL, Schiller CE, Cintron D, Thompson KD, Khine KK, Schmidt PJ, 2016 5alpha-Reductase Inhibition Prevents the Luteal Phase Increase in Plasma Allopregnanolone Levels and Mitigates Symptoms in Women with Premenstrual Dysphoric Disorder. *Neuropsychopharmacology* 41, 1093–1102. 10.1038/npp.2015.246. [PubMed: 26272051]
- Melon LC, Hooper A, Yang X, Moss SJ, Maguire J, 2018 Inability to suppress the stress-induced activation of the HPA axis during the peripartum period engenders deficits in postpartum behaviors in mice. *Psychoneuroendocrinology* 90, 182–193. 10.1016/j.psyneuen.2017.12.003. [PubMed: 29274662]
- Meltzer-Brody S, Boschloo L, Jones I, Sullivan PF, Penninx BW, 2013 The EPDS-Lifetime: assessment of lifetime prevalence and risk factors for perinatal depression in a large cohort of depressed women. *Arch Womens Ment Health* 16, 465–473. 10.1007/s00737-013-0372-9. [PubMed: 23904137]
- Meltzer-Brody S, Colquhoun H, Riesenberger R, Epperson CN, Deligiannidis KM, Rubinow DR, Li H, Sankoh A, Clemson C, Jonas J, Kaness S, 2018 Efficacy and safety of brexanolone iv, a GABAA receptor modulator, in women with postpartum depression: results from two double-blind, randomised, placebo-controlled phase 3 studies.. *Lancet (London, England)*. 10.1016/S0140-6736(18)31551-4.
- Milgrom J, Gemmill AW, Bilszta JL, Hayes B, Barnett B, Brooks J, Ericksen J, Ellwood D, Buist A, 2008 Antenatal risk factors for postnatal depression: a large prospective study. *J Affect Disord* 108, 147–157. 10.1016/j.jad.2007.10.014. [PubMed: 18067974]
- Osborne LM, Gispén F, Sanyal A, Yenokyan G, Meilman S, Payne JL, 2017 Lower allopregnanolone during pregnancy predicts postpartum depression: An exploratory study. *Psychoneuroendocrinology* 79, 116–121. 10.1016/j.psyneuen.2017.02.012. [PubMed: 28278440]

- Park MH, Rehman SU, Kim IS, Choi MS, Yoo HH, 2017 Stress-induced changes of neurosteroid profiles in rat brain and plasma under immobilized condition. *Journal of pharmaceutical and biomedical analysis* 138, 92–99. 10.1016/j.jpba.2017.02.007. [PubMed: 28189891]
- Paul SM, Purdy RH, 1992 Neuroactive steroids. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* 6, 2311–2322 [PubMed: 1347506]
- Pearson Murphy BE, Allison CM, 2000 Determination of progesterone and some of its neuroactive ring A-reduced metabolites in human serum. *J Steroid Biochem Mol Biol* 74, 137–142. 10.1016/S0960-0760(00)00098-4. [PubMed: 11086232]
- Pearson Murphy BE, Steinberg SI, Hu FY, Allison CM, 2001 Neuroactive ring A-reduced metabolites of progesterone in human plasma during pregnancy: elevated levels of 5 alpha-dihydroprogesterone in depressed patients during the latter half of pregnancy. *J Clin Endocrinol Metab* 86, 5981–5987 [PubMed: 11739473]
- Porcu P, O'Buckley TK, Alward SE, Marx CE, Shampine LJ, Girdler SS, Morrow AL, 2009 Simultaneous quantification of GABAergic 3alpha,5alpha/3alpha,5beta neuroactive steroids in human and rat serum. *Steroids* 74, 463–473. 10.1016/j.steroids.2008.12.015. [PubMed: 19171160]
- Rothman KJ, 1990 No adjustments are needed for multiple comparisons. *Epidemiology* 1, 43–46 [PubMed: 2081237]
- Rupprecht R, 2003 Neuroactive steroids: mechanisms of action and neuropsychopharmacological properties. *Psychoneuroendocrinology* 28, 139–168. S0306453002000641 [pii]. [PubMed: 12510009]
- Sarkar J, Wakefield S, MacKenzie G, Moss SJ, Maguire J, 2011 Neurosteroidogenesis is required for the physiological response to stress: role of neurosteroid-sensitive GABAA receptors. *J Neurosci* 31, 18198–18210. 10.1523/jneurosci.2560-11.2011. [PubMed: 22171026]
- Shear MK, Vander Bilt J, Rucci P, Endicott J, Lydiard B, Otto MW, Pollack MH, Chandler L, Williams J, Ali A, Frank DM, 2001 Reliability and validity of a structured interview guide for the Hamilton Anxiety Rating Scale (SIGH-A). *Depress Anxiety* 13, 166–178 [PubMed: 11413563]
- Sheehan DV, 1983 *The Anxiety Disease*. Charles Scribner & Sons, New York.
- Spielberger CD, Gorsuch RL, Lushene R, Vagg PR, Jacobs GA, 1970 *State Trait Anxiety Inventory*. Mind Garden Inc.
- Stell BM, Brickley SG, Tang CY, Farrant M, Mody I, 2003 Neuroactive steroids reduce neuronal excitability by selectively enhancing tonic inhibition mediated by delta subunit-containing GABAA receptors. *Proc Natl Acad Sci U S A* 100, 14439–14444. 10.1073/pnas.24354571002435457100 [pii]. [PubMed: 14623958]
- Sze Y, Gill AC, Brunton PJ, 2018 Sex-dependent changes in neuroactive steroid concentrations in the rat brain following acute swim stress. *Journal of neuroendocrinology* 30, e12644 10.1111/jne.12644. [PubMed: 30194779]
- Williams JB, 1988 A structured interview guide for the Hamilton Depression Rating Scale. *Arch Gen Psychiatry* 45, 742–747 [PubMed: 3395203]
- Wisner KL, Perel JM, Peindl KS, Hanusa BH, Findling RL, Rapport D, 2001 Prevention of recurrent postpartum depression: a randomized clinical trial. *J Clin Psychiatry* 62, 82–86 [PubMed: 11247106]
- Zhao C, Gammie SC, 2014 Glutamate, GABA, and glutamine are synchronously upregulated in the mouse lateral septum during the postpartum period. *Brain Res* 1591, 53–62. 10.1016/j.brainres.2014.10.023. [PubMed: 25451092]

Highlights

- Perinatal neuroactive steroid concentrations increased in at-risk perinatal women
- Perinatal neuroactive steroids increased in perinatal depressed women
- A history of depression associated with increased deoxycorticosterone
- A history of depression associated with increased tetrahydrodeoxycorticosterone
- Liquid chromatography-tandem mass spectrometry of neuroactive steroids

Figure 1:

Participant Study Completion Flow Diagram * Based on medical record review, participants were excluded if they had any significant current medical illness. Available clinical laboratory results including blood cell counts, oral glucose tolerance testing, chemistry panels, thyroid function tests and viral serology conducted for routine care were reviewed and were within normal limits.

Figure 2:

Perinatal neuroactive steroid metabolite to precursor ratios Mean ratio values shown are from raw data values with standard deviation. The Y-axis is scaled logarithmically.

Abbreviations: V1: visit 1; V2: visit 2; V3: visit 3; V4: visit 4; V5: visit 5; AR-PND: at-risk perinatal depression; HPCW: healthy perinatal comparison woman; PND: perinatal depression; DHP: dihydroprogesterone

Table 1:

Participant Characteristics at Antenatal Study Entry and in the Postpartum

Variable ¹	HCPW (n=30)	AR-PND (n=19)	PND (n=26)	P-value ²
Antepartum Data				
Age in years, mean (SD)	29.3 (4.9)	29.9 (5.0)	28.5 (4.6)	0.64
Race, %				
Caucasian	63.3	78.9	76.9	
Black or African American	16.7	5.3	7.7	0.75
Asian	6.7	5.3	0.0	
Some other race	10.0	10.5	11.5	
Ethnicity, %				
Not Hispanic or Latino	73.3	84.2	84.6	0.86
Education level, %				
High school diploma or less	10.0	5.3	15.4	
Partial or completed college	60.0	73.7	73.1	0.47
Graduate/professional degree	30.0	21.1	11.5	
Marital status, %				
Never married	23.3	26.3	38.5	
Currently Married	76.7	68.4	46.2	0.08
Divorced/separated/widowed	0.0	5.3	15.4	
Employment status (currently employed), %	90.0	68.4	73.1	0.11
Number of children in household				
0	50.0	26.3	42.3	
1	43.3	57.9	42.3	0.52
2+	6.7	10.5	15.4	
Household income, %				
Below \$19,000	16.7	5.3	34.6	
\$19,000–\$2,000	26.7	31.6	34.6	0.20
\$53,000–\$111,000	26.7	36.8	19.2	
\$112,000 and above	30.0	21.1	11.5	
Receiving financial or housing assistance from government, %	10.0	5.3	23.1	0.25
Planned pregnancy, %	66.7	57.9	53.8	0.60
Parity, %				
Nulliparous	60.0	42.1	53.8	
Primiparous	36.7	47.4	34.6	0.61
Multiparous	3.3	10.5	11.5	
Thyroid Stimulating Hormone, mean (SD) in uIU/mL	2.1 (1.7)	1.4 (0.6)	1.7 (0.6)	0.28
Hamilton Depression Rating Scale (HAM-D ₁₇), mean (SD)	3.3 (2.7)	8.3 (2.7)	14.2 (6.0)	<.0001
Hamilton Anxiety Rating Scale (HAM-A), mean (SD)	5.8 (4.2)	11.9 (4.7)	18.7 (7.9)	<.0001

Variable ¹	HCPW (n=30)	AR-PND (n=19)	PND (n=26)	P-value ²
Edinburgh Postnatal Depression Scale (EPDS), mean (SD)	2.1 (2.5)	5.9 (3.7)	13.0 (3.8)	<.0001
Spielberger State-Trait Anxiety Inventory-State (STAI-S), mean (SD)	24.6 (7.8)	30.7 (7.8)	44.1 (9.8)	<.0001
Sheehan Disability Scale (SDS), mean (SD)	1.3 (3.3)	3.5 (3.3)	11.5 (5.9)	<.0001
SCID diagnosis at study entry, %				
Adjustment disorder with depressed mood	0.0	5.3	7.7	0.35
Depressive disorder, not otherwise specified	0.0	0.0	50.0	<.0001
Anxiety disorder	0.0	36.8	76.9	<.0001
Past depressive disorder	0.0	68.4	84.6	<.0001
Past postpartum depressive disorder	0.0	21.1	26.9	0.004
Postpartum Data³				
Gestational age at delivery (weeks), mean (SD)	39.4 (1.3)	39.2 (1.5)	39.2 (1.1)	0.83
Preterm delivery, %	3.3	5.3	3.8	0.99
Delivery method, %				
Vaginal	83.3	78.9	65.4	0.30
Cesarean section	16.7	21.1	34.6	
Labor induction, %	56.7	47.4	57.7	0.80
Infant weight (grams), mean (SD) at delivery	3282.8 (512.0)	3533.2 (413.5)	3585.0 (608.6)	0.09
Infant admitted to neonatal intensive care unit, %	6.7	10.5	3.8	0.72
Maternal complications during labor and delivery, %				
Gestational onset hypertension	3.3	5.6	3.8	0.99
Pre-eclampsia	6.7	5.6	11.5	0.76
Postpartum hemorrhage	0.0	0.0	0.0	-
Premature rupture of membranes	6.7	16.7	0.0	0.08
Breastfeeding status, %				
Full or partial	83.3	73.7	80.7	
Bottle-feeding	16.7	26.3	19.2	0.71
Thyroid Stimulating Hormone, mean (SD) in uIU/mL	1.6 (1.0)	1.0 (0.4)	1.2 (0.6)	0.07
Hamilton (Depression Rating Scale (HAM-D ₁₇), mean (SD)	4.0 (2.9)	6.2 (3.5)	17.1 (6.7)	<.0001
Hamilton (Anxiety Rating Scale (HAM-A), mean (SD)	4.5 (3.6)	7.9 (5.5)	19.0 (7.5)	<.0001
Edinburgh Postnatal Depression Scale, mean (SD)	2.4 (2.6)	4.1 (2.9)	14.7 (4.2)	<.0001
Spielberger State-Trait Anxiety Inventory-State (STAI-S), mean (SD)	26.4 (7.6)	29.5 (7.6)	50.1 (8.2)	<.0001
Sheehan Disability Scale (SDS), mean (SD)	1.3 (2.8)	2.5 (3.2)	12.8 (5.7)	<.0001
SCID diagnosis, %				
Major depression with peripartum onset	0.0	0.0	39.4	<.0001
Minor depression with peripartum onset	0.0	0.0	44.7	<.0001
Adjustment disorder with depressed mood	0.0	0.0	16.0	<.0001
Anxiety disorder	0.0	20.5	77.1	<.0001

¹Unless otherwise indicated, data are expressed as number (percentage) of participants. Percentages have been rounded and may not total 100.

²P-values from Fisher's Exact Test for categorical variables and one-way ANOVA for continuous variables.

³Postpartum psychometric scale scores and Thyroid Stimulating Hormone results are presented as the subject's highest value taken from two postpartum visits. SD: Standard Deviation; PND: Perinatal depression; HCPW: Healthy comparison perinatal women; AR-PND: at-risk perinatal depression

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 2:Perinatal Psychometric Scale Total Scores (mean $\pm$ SD)

	N	HAM-D ₁₇	HAM-A	EPDS	STAI-S	SDS	PSQI
Antepartum							
HPCW	30	3.3 $\pm$	5.8 $\pm$	2.1 $\pm$	24.6 $\pm$	1.3 $\pm$	10.9 $\pm$
AR-	19	8.3 $\pm$	11.9 $\pm$	5.9 $\pm$	30.7 $\pm$	3.5 $\pm$	15.3 $\pm$
PND	26	14.2 $\pm$	18.7 $\pm$	13.0 $\pm$	44.1 $\pm$	11.5 $\pm$	23.8 $\pm$
Antepartum							
HPCW	27	3.6 $\pm$	5.3 $\pm$	2.3 $\pm$	24.6 $\pm$	1.4 $\pm$	11.2 $\pm$
AR-	18	9.1 $\pm$	11.3 $\pm$	5.7 $\pm$	32.8 $\pm$	3.13	20.9 $\pm$
PND	26	14.8 $\pm$	17.0 $\pm$	12.1 $\pm$	45.2 $\pm$	10.73	23.2 $\pm$
Postpartum							
HPCW	30	4.6 $\pm$	5.8 $\pm$	1.8 $\pm$	25.9 $\pm$	1.0 $\pm$	11.4 $\pm$
AR-	18	7.9 $\pm$	8.6 $\pm$	3.5 $\pm$	28.7 $\pm$	2.2 $\pm$	19.2 $\pm$
PND	26	13.7 $\pm$	16.0 $\pm$	11.4 $\pm$	42.3 $\pm$	8.0 $\pm$	23.6 $\pm$
Postpartum							
HPCW	30	3.6 $\pm$	3.8 $\pm$	2 $\pm$	25.6 $\pm$	1.1 $\pm$	10.0 $\pm$
AR-	19	5.7 $\pm$	6.9 $\pm$	3.3 $\pm$	27.1 $\pm$	1.8 $\pm$	14.9 $\pm$
PND	26	15.4 $\pm$	17.9 $\pm$	13.2 $\pm$	47.6 $\pm$	11.9 $\pm$	23.5 $\pm$
Postpartum							
HPCW	30	2.2 $\pm$	2.6 $\pm$	1.3 $\pm$	23.0 $\pm$	0.6 $\pm$	8.5 $\pm$
AR-	19	4.8 $\pm$	5.9 $\pm$	2.8 $\pm$	26.3 $\pm$	1.7 $\pm$	11.95
PND	24	15.1 $\pm$	15.5 $\pm$	12.9 $\pm$	45.7 $\pm$	9.1 $\pm$	19.46

Data was collected across perinatal time where Visit 1 occurred during 22–38 weeks gestational age, Visit 2 occurred during 30–39 weeks gestational age, Visit 3 occurred within 1 week postpartum, Visit 4 during 10 days to 10 weeks postpartum and Visit 5 during 3–11 weeks postpartum. Note, the sample size for each group may differ visit to visit if women declined the blood draw, the phlebotomist was unable to obtain a blood sample or the participant was unable to complete each study visit. Abbreviations: SD: Standard deviation; AR: at-risk; PND: Perinatal depression; HPCW: Healthy perinatal comparison women; HAM-D₁₇: Hamilton Depression Scale; HAM-A: Hamilton Anxiety Scale; EPDS: Edinburgh Postnatal Depression Scale; STAI-S: Spielberger State-Trait Anxiety Inventory; SDS: Sheehan Disability Scale; PSQI: Pittsburgh Sleep Quality Index

Table 3:
Perinatal Plasma Proneuroactive and Neuroactive Steroid Concentrations (ng/mL) (mean±SD)

	n	Pregnenolone	Progesterone	5α-Dihydroprogesterone	5β-Dihydroprogesterone	3α,5β-tetrahydroprogesterone (pregnanolone)	3α,5α-tetrahydroprogesterone (allopregnanolone)	Deoxycorticosterone	Tetrahydrodeoxycorticosterone
Antepartum Visit 1									
HPCW	30	2.5 ± 1.1	71.0 ± 27.1	13.3 ± 7.3	0.7 ± 0.4	4.1 ± 1.7	6.7 ± 2.8	0.8 ± 0.5	0.1 ± 0.1
AR-PND	18	2.8 ± 1.1	90.1 ± 39.9	19.9 ± 15.8	1.0 ± 0.8	4.0 ± 1.4	8.0 ± 3.0	1.2 ± 0.9	0.2 ± 0.1
PND	23	2.4 ± 1.0	86.3 ± 31.2	17.4 ± 9.5	0.7 ± 0.4	4.5 ± 2.0	9.1 ± 4.5	1.1 ± 0.7	0.2 ± 0.1
<i>p-value</i>		0.47	0.09	0.11	0.07	0.59	0.06	0.09	0.15
Antepartum Visit 2									
HPCW	23	2.5 ± 0.9	105.3 ± 40.3	18.4 ± 10.1	0.8 ± 0.4	4.0 ± 1.1	7.9 ± 3.2	1.3 ± 0.6	0.1 ± 0.1
AR-PND	17	2.5 ± 1.0	102.3 ± 45.9	21.0 ± 10.5	1.0 ± 0.5	3.9 ± 1.4	8.1 ± 2.9	1.2 ± 0.5	0.1 ± 0.1
PND	23	2.5 ± 0.8	108.9 ± 34.9	22.3 ± 9.9	0.8 ± 0.5	4.5 ± 1.9	10.3 ± 3.7	1.4 ± 0.5	0.2 ± 0.1
<i>p-value</i>		0.97	0.88	0.43	0.17	0.34	0.04	0.71	0.16
Postpartum Visit 3									
HPCW	21	0.4 ± 0.2	7.2 ± 5.2	2.8 ± 1.6	0.1 ± 0.3	1.3 ± 0.8	2.1 ± 1.0	0.2 ± 0.1	0.0 ± 0.0
AR-PND	15	0.4 ± 0.2	7.2 ± 4.7	3.4 ± 2.6	0.1 ± 0.1	1.0 ± 0.6	2.5 ± 1.5	0.2 ± 0.1	0.0 ± 0.0
PND	20	0.3 ± 0.2	5.4 ± 6.5	2.2 ± 1.7	0.0 ± 0.0	1.0 ± 1.0	2.2 ± 1.6	0.1 ± 0.1	0.0 ± 0.0
<i>p-value</i>		0.34	0.53	0.24	0.25	0.47	0.61	0.37	0.74
Postpartum Visit 4									
HPCW	27	0.4 ± 0.2	0.1 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
AR-PND	17	0.4 ± 0.4	0.2 ± 0.6	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.2	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
PND	14	0.5 ± 0.3	0.3 ± 0.9	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
<i>p-value</i>		0.54	0.45	0.23	0.14	0.69	0.38	0.28	0.88
Postpartum Visit 5									
HPCW	29	0.5 ± 0.2	0.5 ± 1.3	0.1 ± 0.2	0.0 ± 0.0	0.1 ± 0.2	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
AR-PND	18	0.4 ± 0.2	0.1 ± 0.3	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
PND	23	0.6 ± 0.3	0.7 ± 2.4	0.1 ± 0.2	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

n	Pregnenolone	Progesterone	5α-Dihydroprogesterone	5β-Dihydroprogesterone	3α,5β-tetrahydroprogesterone (pregnanolone)	3α,5α-tetrahydroprogesterone (allopregnanolone)	Deoxycorticosterone	Tetrahydrodeoxycorticosterone
	0.13	0.58	0.60	0.86	0.60	0.31	0.07	0.42
<i>p-value</i>								

Data was collected across perinatal time where Visit 1 occurred during 22–38 weeks gestational age, Visit 2 occurred during 30–39 weeks gestational age, Visit 3 occurred within 1 week postpartum, Visit 4 during 10 days to 10 weeks postpartum and Visit 5 during 3–11 weeks postpartum. Note, the sample size for each group may differ visit to visit if participants were unable to complete part of a study visit. Abbreviations: SD: standard deviation; AR: at-risk; PND: perinatal depression; HPCW: healthy perinatal comparison women.