Title

Effects of estrogen and progesterone on neuroactive steroids and cytokines in patients with suicidality.

Permalink

https://escholarship.org/uc/item/98t3h26q

Authors

Barone, Jordan
Wenzel, Elizabeth
Alluri, Viraja
et al.

Publication Date

DOI

Peer reviewed

Effects of estrogen and progesterone on neuroactive steroids and cytokines in patients with suicidality

J.C. Barone[a], **Elizabeth Wenzel**[a], **V. Alluri**[a], **D. Moriarity**[b,c], **G. Pinna**[a], **E. Walsh**[d], **D.R. Rubinow**[d], **A.L. Morrow**[d], **T.A. Eisenlohr-Moul**[a]

[a.] University of Illinois at Chicago, Dept of Psychiatry; 912 S Wood St, Chicago IL 60612

[b.] University of California, Los Angeles, Department of Psychiatry and Biobehavioral Sciences; 757 Westwood Plaza #4, Los Angeles CA 90095

[c.] Stanford University, Department of Genetics; 291 Campus Drive, Stanford CA 94305

[d.] University of North Carolina-Chapel Hill, Department of Psychiatry; 101 Manning Dr. #1, Chapel Hill NC, 27514

Abstract

Background: In ovulating psychiatric patients experiencing suicidality, suicidal ideation (SI) often peaks perimenstrually. Our recent double-blind, placebo-controlled, crossover randomized clinical trial (RCT; NCT03720847) showed that perimenstrual administration of estradiol and progesterone (EP) can prevent this peak in SI and depressed mood. In this pre-registered follow-up analysis, we studied how the menstrual cycle and experimental manipulation affected two neurobiological systems associated with the menstrual cycle and suicide risk: GABAergic neuroactive steroids (NAS) and peripheral cytokines.

Methods: In 26 psychiatric outpatients with natural menstrual cycles and past-month SI, we analyzed serum samples from three blood draws (midluteal, perimenstrual, midfollicular) per experimental condition (EP vs placebo) timed to a luteinizing hormone-surge ovulation test. Using gas chromatography/mass spectrometry (GC/MS), we measured the progesterone (P4)-derived pregnane NAS $(3\alpha,5\alpha)$-3-hydroxypregnan20-one $(3\alpha,5\alpha$-THP), $(3\alpha,5\beta)$-3-hydroxypregnan-20-one $(3\alpha,5\beta$-THP), $(3\alpha,5\alpha)$-3,21-dihydroxypregnan-20-one $(3\alpha,5\alpha$-THDOC), $(3\alpha,5\alpha)$-3-hydroxyandrostan-17-one $(3\alpha,5\alpha$-A), the androstane NAS $(3\alpha,5\beta)$-3-hydroxyandrostan-17-one $(3\alpha,5\beta$-A), $(3\alpha,5\alpha,17\beta)$-androstane-3,17-diol $(3\alpha,5\alpha$-A-

diol), (3α,5β,17β)-androstane-3,17-diol (3α,5β-A-diol), and their precursor pregnenolone. High sensitivity multiplex assay kits quantified peripheral cytokines IL-1β, IL-6, and TNF-α.

Results: P4-derived NAS fluctuated in parallel with P4 and increased with exogenous perimenstrual administration of EP. Conversely, androstane NAS either did not fluctuate or fluctuated inversely from P4, and these NAS decreased with exogenous EP. Peripheral cytokines did not show cyclical patterns, but each significantly predicted SI, depressed mood, or anxiousness. Concomitant SSRI medication use predicted lower androstane NAS.

Conclusions: While preliminary and exploratory, our findings provide critical descriptive context for future studies. Further, our work presents menstrual cycle-related patterns for ten frequently-studied biomarkers, allowing for improved quality of comparisons involving naturally-cycling populations in research.

1. Introduction

Many prior cross-sectional and observational studies of ovulating psychiatric outpatients with chronic suicidal ideation (SI) have shown that on average, SI and suicide attempts increase proximal to menses (Saunders & Hawton, 2006). Cyclical mood changes are associated with suicidality, with high rates of SI and attempts in patients with Premenstrual Dysphoric Disorder (PMDD; Eisenlohr-Moul et al., 2022), and high rates of premenstrual exacerbation (PME) of psychiatric symptoms among those with SI (Owens et al., 2023). Further, PME of psychiatric symptoms is transdiagnostic, such that exacerbation can occur for any symptom and has been observed in many psychiatric disorders (Kuehner & Nayman, 2021; Nolan & Hughes, 2022). SI is similarly transdiagnostic: while SI is most commonly linked to depressive disorders, disorders characterized by anxiety, agitation, impulse control, and substance use also contribute to suicide-related symptoms (Nock et al., 2010). Thus, both cyclical mood symptoms and SI are transdiagnostic phenomena, and recent work suggests that comorbidity of these outcomes with other psychiatric diagnoses is the norm. Our recent randomized controlled trial (RCT) aimed to identify if the abrupt withdrawal from estradiol (E2) and progesterone (P4) that precedes menses causes PME of SI in patients who are already experiencing chronic SI. Indeed, this trial showed that preventing this ovarian hormone withdrawal (via two weeks of stable, low-dose exogenous E2 and P4 starting one week post-ovulation) prevented PME of SI, while PME of SI was present during natural, unmanipulated cycles (Eisenlohr-Moul, Bowers, et al., 2022). We propose the menstrual cycle may contribute to PME of SI through two candidate neurobiological systems: (1) withdrawal from GABAergic neuroactive steroids (NAS), which are linked to reproductive state (Bäckström et al., 2011) and chronic affective symptoms (Locci & Pinna, 2017), and (2) increases in inflammatory cytokines, which are linked to depression and suicidality (Brundin et al., 2017). This paper describes pre-registered secondary analyses of serum from the parent RCT (NCT03720847; Eisenlohr-Moul et al., 2022) to explore

relationships between the menstrual cycle and these neurobiological systems that may underlie our clinical findings.

1.1. Relevant menstrual cycle physiology.

The prototypical 28-day menstrual cycle starts with menses (day 1), then is roughly divided into the follicular and luteal phases bisected by ovulation (~day 14). Follicular phase E2 starts low, steadily rises, then sharply peaks preceding ovulation, followed by a secondary, less abrupt midluteal peak. Follicular phase P4 is low and stable, then rises from ovulation until subsequent menses. The perimenstrual phase, defined as the days before and during menses (Schmalenberger et al., 2021), is characterized by acute withdrawal from E2 and P4 (see Supplementary Figure 1 for diagram). P4 metabolizes into NAS that modulate type A gamma-aminobutyric acid receptors (GABA$_A$R) (Nutt, 2006); see Supplementary Figure 2 for overview of metabolic pathway. Select NAS (i.e., 3α5α-tetrahydroprogesterone/3α5α-THP/allopregnanolone) parallel P4 flux, with a midluteal peak and abrupt perimenstrual withdrawal (Schmidt et al., 1994). Levels of hormones or NAS and their cyclical patterns have not been shown to differ between patients with cyclical mood changes (such as PMDD) and non-psychiatric controls (Nguyen et al., 2017; Thys-Jacobs et al., 2008). 3α5α-THP positively modulates the GABA$_A$R, augmenting inhibitory neurotransmission with sedative, anxiolytic, or analgesic effects (Bäckström et al., 2011). P4 has well-established anti-inflammatory properties (Zwahlen & Stute, 2023), while E2 is *potentially* anti-inflammatory (Gaskins et al., 2012); urinary inflammatory markers peak perimenstrually, alongside P4 and E2 withdrawal (Whitcomb et al., 2014).

1.2. The menstrual cycle and suicide risk: neuroactive steroids

GABAergic NAS play diverse roles in the pathophysiology of affective disorders (Eisenlohr-Moul & Barone, 2023) including Major Depressive Disorder (MDD) and PMDD; however, no work to date analyzes NAS in patients with PME, in which chronic psychiatric symptoms (e.g., depression) worsen cyclically alongside hormone fluctuations. Patients with "pure" PMDD (in which symptoms are confined to the luteal phase) do not demonstrate NAS deficits compared to controls (Nguyen et al., 2017; Schmidt et al., 1994). However, PMDD patients do appear to have a neurobiological sensitivity to normal NAS flux (see Hantsoo & Epperson, 2020) for review), as blocking the luteal phase increase in 3α5α-THP prevents symptom emergence (Bixo et al., 2017; Martinez et al., 2016). In contrast, between-person 3α5α-THP deficits compared to controls *do* appear in non-reproductive mood disorders with high suicide rates including MDD (Uzunova et al., 1998), Post-Traumatic Stress Disorder (Rasmusson et al., 2006), and anorexia nervosa (Dichtel et al., 2018). One study shows decreased NAS in post-mortem brain of individuals who died by suicide (Youssef et al., 2015); no studies to our knowledge examine *peripheral* NAS and suicidality.

Three primary gaps exist in this literature: (1) It is unknown if the proposed affective risk of decreased 3α5α-THP translates to other NAS with similar pharmacological properties. (2) We lack descriptive data relating NAS to suicidality. SI is a distinct, transdiagnostic entity with unique predictors than that of depression or anxiety alone, and therefore our data from a transdiagnostic sample provides rare opportunities to examine NAS longitudinally across the cycle in participants exhibiting SI. (3) While there is a substantial literature of NAS in

reproductive and chronic mood disorders (Locci & Pinna, 2017; Wenzel et al., 2021), it is unknown how these two bodies of work apply to PME of chronic psychiatric conditions, despite evidence that PME is up to ten times more common than PMDD (Hartlage et al., 2004). Overall, translational, transdiagnostic evidence suggests that NAS (particularly 3α5α-THP) relate to psychiatric symptoms that contribute to suicide risk, and that certain individuals are neurobiologically sensitive to normal NAS fluctuations across the cycle.

1.3. The menstrual cycle and suicide risk: inflammatory proteins

Longstanding evidence suggests inflammation relates to risk for MDD and suicidality. Meta-analytic work shows increased peripheral cytokines (e.g., IL-6, TNF-α) in patients with MDD versus controls (Köhler et al., 2017), and within-person changes in TNF-α have been shown to *predict* depressive symptoms (Moriarity et al., 2020). Positron Emission Tomography shows upregulation of translocator protein (TSPO; a spatial correlate of neuroinflammation) in brain regions of patients with SI (Holmes et al., 2018), while individuals with suicidal behaviors or attempts demonstrate increased IL-6, TNF-α, and IL-1β in peripheral blood (Janelidze et al., 2011; Vasupanrajit et al., 2022). No studies to date examine the role of the menstrual cycle in inflammation-mediated risk for suicidality, despite known or suspected anti-inflammatory properties of E2, P4, and NAS (Balan et al., 2022; Gaskins et al., 2012; Zwahlen & Stute, 2023).

1.4. Current Study

We present preregistered secondary analyses from NCT03720847, a double-blind, placebo-controlled, crossover RCT testing the hypothesis that perimenstrual withdrawal from E2 and P4 contributes to PME of SI in naturally cycling individuals with past-month SI. Primary results showed that acute perimenstrual administration of E2 and P4 ("EP") reduced PME of SI and depressed mood versus placebo ("PBO") (Eisenlohr-Moul, Bowers, et al., 2022). We now evaluate banked serum to explore relationships between the cycle and two candidate neurobiological pathways for suicidality (NAS, cytokines). We hypothesized that decreased GABAergic NAS (section 1.2) and increased inflammation-related cytokines (section 1.3) would each predict increased SI. Across the natural cycle, we hypothesized a midluteal peak in P4-derived GABAergic NAS (Nguyen et al., 2017; Schmidt et al., 1994), and a perimenstrual peak in inflammatory cytokines (Gaskins et al., 2012; Whitcomb et al., 2014). We explored the experimental effects of EP vs. placebo: we predicted that acute perimenstrual EP would increase NAS and decrease cytokines to match baseline midluteal levels, respectively. To our knowledge, we provide the first study of suicide-related candidate biomarkers across the cycle, and the first experimental data to show how perimenstrual EP administration alters NAS and cytokines in patients with suicidality.

2. Methods and Materials

2.1. Pre-registration

Hypotheses and analytic plan were pre-registered on the Open Science Framework (https://osf.io/crgaj).

2.2. Participants and Experimental Design.

Twenty-nine individuals were randomized in the parent placebo-controlled, triple-blind (investigator, participant, assessor), crossover RCT; experimental design can be found in Supplementary Figure 1 and is previously described in full with eligibility criteria (Eisenlohr-Moul, Bowers, et al., 2022). Briefly, participants attended an enrollment visit including a Structured Clinical Interview for DSM-5 (SCID-5; First et al., 2015), Columbia Suicide Severity Rating Scale (C-SSRS; Posner et al., 2011), eligibility review, and informed consent. Participants then completed a baseline cycle (28 days) of daily surveys measuring affective and suicide-related symptoms (sent at 5pm via text message). After the baseline cycle, participants were randomized to two subsequent experimental intervals of EP or PBO, bisected by a washout cycle (28 days), with each interval timed to days +7 to +20 following a positive 40mIU/ml urine luteinizing hormone (LH) test. The EP interval included transdermal E2 patches (Climara™; 0.1mg/day) and twice-daily oral micronized P4 (Prometrium™; 100mg per pill) to buffer the abrupt withdrawal from E2 and P4 that occurs just prior menses onset; the PBO interval included identical patches and pills. Blood was collected three times per condition: a midluteal visit (LH+7), a perimenstrual visit (LH+14), and a midfollicular visit (LH+22). Study protocol was approved by the University of North Carolina-Chapel Hill Institutional Review Board; all procedures were performed in accordance with relevant guidelines and regulations.

2.3. Daily symptoms.

Participants completed adapted versions of the Adult Suicidal Ideation Questionnaire (ASIQ; Reynolds, 1991) and the Daily Rating of Severity of Problems (DRSP; (Endicott et al., 2006). ASIQ and DRSP items were rated on 5- and 6-point Likert scales, respectively, ranging from "Not at All" to "Extremely" within the past 24 hours. Daily SI was an average of ideation-related ASIQ items (e.g., "I wished I were dead," "I felt that life was not worth living"). We used DRSP Item #1 for depressed mood ("I felt depressed, down, or blue") and DRSP Item #4 for anxiousness ("I felt anxious, keyed up, or on edge"). For each symptom (mean SI, depressed mood, anxiousness), a three-day composite score was calculated as that symptom's mean rating from the evening of the blood draw through the subsequent two days (e.g., symptom ratings 72 hours after blood collection).

2.4. Serum samples and assays.

Blood was sampled between 7:00AM and 8:00PM based on participant availability and varied between participant (consistent within participant). Thirty minutes after blood collection, serum was centrifuged, aliquoted, and stored at −80°C. 3500 μL of serum were available from each of six lab visits (three visits per condition). Using a highly sensitive and specific gas chromatography-mass spectrometry (GC/MS) method developed and validated by consultants (Porcu et al., 2009), we quantified (from 1500 μL banked serum) the following GABAergic NAS: 3α,5α-THP (allopregnanolone), (3α,5β)-3-hydroxypregnan-20-one (3α,5β-THP; pregnanolone), (3α,5α)-3,21-dihydroxypregnan-20-one (3α,5α-THDOC; THDOC), (3α,5α)-3-hydroxyandrostan-17-one (3α,5α-A; androsterone), (3α,5β)-3-hydroxyandrostan-17-one (3α,5β-A; etiocholanone), (3α,5α,17β)-androstane-3,17-diol (3α,5α-A-diol; androstanediol), (3α,5β,17β)-androstane-3,17-diol

(3α,5β-A-diol; etiocholanediol) and their precursor pregnenolone (see Supplementary Figure 2). Using Quantikine High Sensitivity ELISA assay kits for each cytokine (R&D Systems, Bio-Techne, Minneapolis, MN), we conducted assays (from 2000 μL serum) of IL-6, TNF-α, and IL-1β. Radioimmunoassay (RIA) was completed for E2 and P4 at each visit, which is used as reference to identify potentially anovulatory cycles.

2.5. Analytic plan.

General Approach.—Analyses were conducted in R (v4.0.2) using 'nlme' and 'lme4' for linear and logistic models, respectively (Bates et al., 2015; Pinheiro et al., 2021). Biomarkers were log-transformed to improve normality of residuals (White, 2019). Age and BMI were sample-standardized. All multilevel models predicting biomarkers from cycle phase covaried SSRI usage, age, and BMI, and included random intercepts (sample nested within person). Participants were asked to report each day if they experienced any new illnesses (e.g., upper respiratory infections, cold or flu symptoms), given that an acute infection could directly affect inflammatory pathways of interest. For NAS outcome models, we covaried self-reported illness on day of sample (0 = No Illness Reported, 1 = Illness) given the possible, hypothesized relationships between NAS and inflammation. For cytokine models, we omitted data from blood draws with simultaneous self-reported acute illness rather than covarying, since the cytokines of interest are directly upregulated by acute infection or illness (N=2 lab visits with symptoms consistent with cold, flu, or upper respiratory infection). Cycle phase is a dummy-coded predictor, and we ran each model twice to rotate the reference group and capture all pairwise comparisons (model 1: midluteal reference versus perimenstrual and midfollicular contrasts; model 2: perimenstrual reference versus midluteal and midfollicular contrasts). Because the present analyses are exploratory, pre-registered, and intended to serve as hypothesis-generation for future work, our analytic plan does not control for multiple comparisons due to our greater concern for control of Type II error (false negatives) in this context.

Exploratory and descriptive tests.—To determine potential covariates, we ran exploratory models to predict biomarkers from 1) time of day (linear and quadratic) and 2) SSRI usage. We first tested for relationships with time of day based on possible circadian effects on NAS or cytokines; time was rounded to the nearest hour. We next hypothesized SSRI usage would positively correlate to 3α-reduced NAS, as prior literature suggests SSRIs increase neurosteroidogenesis (Pinna et al., 2009). To test for associations between our biomarker systems, we ran individual linear multilevel models predicting IL-6, TNFα, and IL-1β, from 3α,5α-THP or 3α,5α-A, respectively. We hypothesized higher NAS would be associated with lower cytokines given prior evidence for anti-inflammatory effects of 3α,5α-THP (Balan et al., 2022). For NAS that were undetectable in at least 50% of samples (3α,5β-A, 3α,5β-A-diol), we explored likelihood of missingness (logistic) based on cycle phase, SSRI usage, BMI, and age. All exploratory and descriptive tests can be found in Section 3.2.

Primary Analyses.

Biomarkers and symptoms.—Within the placebo condition, between-person means (trait variance) for each biomarker were calculated as each person's mean across the

three placebo samples (e.g., unmedicated). Within-person deviations (state variance) for each placebo observation were calculated per sample by subtracting the person's placebo mean from that visit's value. To test hypotheses relating between- and within-person biomarker concentrations to symptoms in the natural menstrual cycle (placebo condition), we ran multilevel linear models where symptom was the outcome (SI, depressed mood, or anxiousness) and predictors included both between-person mean and within-person deviation of each biomarker. For example, we predicted SI, depressed mood, and anxiousness in separate models from the between-person mean and within-person deviations in 3α,5α-THP; this was repeated for each biomarker. Models were adjusted for SSRI use, cycle phase (midluteal, perimenstrual, midfollicular), age, and BMI. These between-person associations, without experimental EP, can be found in Section 3.3.

Biomarkers across menstrual cycle.—Under placebo (natural cycle), multilevel models predicted each biomarker from cycle phase (midluteal, perimenstrual, midfollicular). We hypothesized that midluteal P4-derived NAS (3α,5α-THP, 3α,5β-THP, 3α,5α-THDOC) would be greater than perimenstrual or midfollicular (midluteal > perimenstrual = midfollicular). The relationships of biomarkers across menstrual cycle phase, without experimental EP, can be found in Section 3.4.

Biomarkers under experimental EP.—Finally, we tested how experimental EP altered cyclical trajectories described in the prior hypothesis. Multilevel linear models predicted each biomarker from condition (EP vs. placebo), cycle phase (midluteal, perimenstrual, midfollicular), and their interaction (condition X cycle phase). The results of the full crossover experiment can be found in Section 3.5.

3.   Results

3.1.   Participants and data.

Table 1 presents demographics. For any cycle where midluteal P4 < 3ng/ml (suggesting anovulation; (Speroff & Fritz, 2005), we removed that condition from analyses (N=1, placebo). Cytokine outliers were defined as greater than three standard deviations above the mean and winsorized (Ghosh & Vogt, 2012). Three participants were excluded in entirety: N=1 with inaccurate LH test; N=1 with anovulatory P4 levels in both conditions, despite positive LH test; and N=1 who completed the full experiment but refused blood draw. Our final dataset included 144 observations in 26 participants; the placebo-only dataset included 73 observations in 25 participants.

3.2.   Descriptive analyses: covariates, biomarker associations, detectability.

Our descriptive analyses probed for confounding variables that could account for effects in our primary models. In multilevel models predicting biomarker from time of day, there was no significant linear or quadratic effect of hourly time on any biomarker (all p's > 0.05; see Supplementary Table 1). We thus did not include time of day as a covariate. We tested SSRI usage (between-person) predicting biomarkers; we found that SSRI usage predicted lower 3α,5β-A-diol levels (*Est*=−0.64, *SE*= 0.24, *p*<0.05), and 3α,5β-A was marginally lower in participants taking SSRIs (*Est*=−0.34, *SE*= 0.20, *p*=0.1). SSRI relationships can

be found in Supplementary Figure 3. Based on this unexpected relationship and prior experimental work (Pinna et al., 2009), we included SSRI usage as a covariate. There was substantial missingness of the 3α,5β-reduced NAS, 3α,5β-A (48% undetectable) and 3α,5β-A-diol (57.8% undetectable). In individuals taking SSRIs, 3α,5β-A was undetectable in 70% of visits (vs 31% in those not taking SSRIs), and 3α,5β-A-diol was undetectable in 66% of visits (vs. 50% in those not taking SSRIs). A cross-tabulation of SSRI usage and detectability of 3α,5β-reduced NAS can be found in Supplementary Table 3.

We tested for associations between NAS and cytokines by predicting each inflammatory cytokine (TNF-α, IL-1β, and IL-6) from between- and within-person levels of one pregnane-derived NAS (3α,5α-THP) and one androstane-derived NAS (3α,5α-A), respectively. There were no significant associations of between- or within-person 3α,5α-THP or 3α,5α-A levels with TNF-α, IL-1β, or IL-6 (all p's > 0.05; see Supplementary Table 2).

3.3. Non-menstrual cycle associations between biomarkers and symptoms.

Results can be found in Table 2; Figure 1 depicts between-person associations, in the placebo (unmedicated) visits only (Level 1 N=73, Level 2 N=25). Counter to hypotheses, higher mean 3α,5α-THP predicted higher trait SI, and higher 3α,5β-THP at a given visit predicted higher depressed mood at that visit. We found no other significant relationships of NAS with symptoms. Supporting our hypotheses, higher mean IL-1β predicted greater mean SI and depressed mood, while higher mean IL-6 was associated with greater mean anxiousness. At the within person level, a visit's TNF-α level predicted depressed mood at that visit, while IL-1β predicted anxiousness at that visit.

3.4. Biomarkers across the cycle (placebo).

Results in Table 3 and Figure 2 (solid lines) depict within-person patterns of biomarker flux across the menstrual cycle in the placebo condition only (Level 1 N=73, Level 2 N=25). As expected, P4 was higher in the midluteal (LH +7) compared to perimenstrual (LH +14) or midfollicular (LH +22) phases; perimenstrual P4 was greater than midfollicular P4. Pregnane NAS 3α,5α-THP, 3α,5β-THP, and 3α,5α-THDOC fluctuated parallel to P4. Counter to hypotheses, androstane-derived 3α-reduced NAS also fluctuated across the cycle, albeit with a different pattern than P4. First, 3α,5α-A was higher in the midfollicular than midluteal phase. Second, 3α,5α-A-diol was lower in the midluteal versus perimenstrual and midfollicular phases. Third, there were no significant cyclical changes in 3α,5β-A or 3α,5β-A-diol. Age, BMI, and acute illness were not significant predictors of any biomarker. In models predicting both 3α-reduced diols (3α,5α-A-diol, 3α,5β-A-diol), SSRI medication usage significantly predicted lower levels of 3α,5α-A-diol and 3α,5β-A-diol (as seen in descriptive models, Section 3.2).

Counter to our hypotheses, we found no cyclical patterns in peripheral TNF-α, IL-1β, or IL-6 (Figure 2, green solid lines).

3.5. Biomarkers under exogenous EP administration (full crossover design).

We finally examined the effects of exogenous perimenstrual EP administration on each biomarker by adding an interaction term between condition and cycle phase to understand

how EP alters each biomarker trajectory across the cycle, where, in the EP condition, the midluteal visit (LH+7) was unmedicated, the perimenstrual visit (LH+14) was medicated, and the midfollicular visit (LH+22) took place during EP withdrawal (+1 day after completion of P4 pills/removal of E2 patch). Results are shown in Table 4 and Figure 2 (dashed lines; Level 1 N=144, Level 2 N=26). For P4 and pregnane NAS 3α,5α-THP, 3α,5β-THP, and 3α,5α-THDOC, there were significant interactions between condition and the midluteal-versus-perimenstrual and midluteal-versus-midfollicular contrasts. We found significant interactions between condition and the midfollicular vs perimenstrual contrast for 3α,5α-THP, 3α,5β-THP, and 3α,5α-THDOC, but this was non-significant for P4. For androstane-derived NAS, there was a significant main effect of condition (EP < PBO) for 3α,5β-A-diol; this was non-significant for 3α,5α-A, 3α,5α-A-diol, and 3α,5β-A. The nature of the interaction between condition and cycle phase differed by outcome: for 3α,5α-A, the interaction of condition with the perimenstrual vs midluteal contrast was significant, such that there were no significant effects in the PBO condition, but perimenstrual 3α,5α-A was significantly lower than midluteal 3α,5α-A in the EP condition. For 3α,5α-A-diol, condition interacted with cycle phase in all three contrasts: under PBO, 3α,5α-A-diol increased from the midluteal to perimenstrual phase then remained elevated in the midfollicular phase, while under EP, 3α,5α-A-diol decreased from the midluteal to perimenstrual phase, then increased from the perimenstrual to midfollicular phases. There were no significant interactions for 3α,5β-A. While 3α,5β-A-diol did not change across the cycle in the PBO condition, there was a significant decrease under EP from the midluteal to perimenstrual phase, then a significant increase under EP from the perimenstrual to midfollicular phase. Counter to hypotheses, condition did not interact with cycle phase predicting TNF-α or IL-1β. For IL-6, there was a significant interaction between condition and the perimenstrual vs midluteal contrast, such that IL-6 decreased from the midluteal to the perimenstrual phase in the EP condition only.

4. Discussion

Ovulating individuals between puberty and menopause are at notably greater risk than their male counterparts for SI or suicide attempt, especially during the perimenstrual frame. This is the first known study of GABAergic NAS and inflammatory cytokines – two systems of neurobiological relevance to suicide – across the menstrual cycle in patients with SI. Our novel descriptive study, using gold-standard LH surge testing to objectively confirm ovulation and GC/MS to assay NAS provides preliminary data with two major implications. First, our data guides future clinical research studying the pathophysiology of female suicide risk and PME of SI. Specifically, findings from the present manuscript drove our laboratory's subsequent two RCTs (NCT03498313: in preparation for publication; NCT04112368: currently active) utilizing similar study designs. Based on the present findings, our future work will test unique (rather than combined) experimental E2 and P4 effects on neurosteroidogenic enzymes, add measures of GABA$_A$R subunit expression, and compare group-level differences between those with PME of SI and non-psychiatric controls. Second, in line with the NIH commitment to inclusion of both male and female participants (sex as a biological variable) in clinical trials, our findings demonstrate menstrual cycle phase associations with frequently-studied NAS and inflammatory markers,

offering context for when the menstrual cycle may be a confounding variable in biological psychiatry research. We implore researchers studying GABAergic NAS to track menstrual cycle phase and/or reproductive status in female participants, and to covary cycle phase where possible, given our findings that P4-derived and androstane NAS fluctuate across the cycle (see Schmalenberger et al., 2021 for a practical guide).

4.1. Do GABAergic NAS or inflammatory cytokines predict psychiatric symptoms?

Within this high-severity sample, we found few trait or state associations of GABAergic NAS with SI, depressed mood, or anxiousness. Although prior literature suggests low GABAergic NAS predict chronic psychiatric symptoms (Ströhle et al., 1999; Uzunova et al., 1998), we found that *higher* 3α,5α-THP (between-person) and *increased* 3α,5β-THP (within-person) predicted higher SI and depressed mood, respectively. This suggests that individuals with PME of SI, similar to PMDD, have a neurobiological sensitivity to GABAergic NAS flux (Hantsoo & Epperson, 2020). As sensitivity of the GABA$_A$R's response to NAS is determined by its subunit composition (Shen et al., 2007; Smith, 2013; Smith et al., 2007), future research should consider plasticity in the GABA$_A$R itself as a possible *transdiagnostic* mechanism underlying neuropsychiatric sensitivity to hormone flux (Hantsoo & Epperson, 2020; Locci & Pinna, 2017). We found no relationship between symptoms and between-person *or* within-person changes in 3α,5α-THDOC, 3α,5α-A, 3α,5α-A-diol, 3α,5β-A, or 3α,5β-A-diol. These null findings are notable, as many GABAergic NAS have similar structure and allosteric function at the GABA$_A$R, yet few studies examine other 3α-reduced GABAergic NAS beyond 3α,5α-THP. In sum, we provide preliminary evidence that hypothesized neurosteroidogenic deficits causing depressive symptoms may not translate to prediction of symptom severity in patients with suicidality, and our findings suggest little to no evidence for a relationship between peripheral GABAergic NAS and symptom severity in this high-risk sample.

We measured three inflammation-related cytokines in association with SI, depressed mood, and anxiousness. Between-person IL-1β predicted SI and depressed mood, and IL-6 predicted anxiousness, while higher within-person TNF-α and IL-1β were associated with increased depressed mood and anxiousness, respectively. This demonstrates consistent evidence of cytokines predicting psychiatric symptoms, although the between- or within-person patterns differed by immune protein. Prior mixed-design studies associate between-person IL-6 with depressed mood, and within-person IL-6 illness course severity in female but not male patients (Lamers et al., 2019). IL-6 predicted increased *anxiousness* in our sample, suggesting IL-6 may be a transdiagnostic marker of internalizing affective symptom severity. TNF-α predicted depressed mood within-person, replicating prior work in adolescents (Moriarity et al., 2020); interestingly, TNF-α is most often associated with depressed mood and suicide symptom severity *cross-sectionally* in meta-analyses (Vasupanrajit et al., 2022). A broad body of literature suggests inflammation is associated with psychiatric phenotypes; our work adds distinction between trait and state variance in these relationships, which can aid future work in understanding how overall levels versus acute changes in inflammation-related cytokines predict chronic or acute affective risk.

4.2. How do GABAergic NAS and inflammatory cytokines fluctuate across the menstrual cycle?

We characterized GABAergic NAS and inflammatory cytokines at three cycle phase timepoints, which has never been done in a sample with SI. We demonstrated that pregnane NAS (3α,5α-THP, 3α,5β-THP, and 3α,5α-THDOC) fluctuate cyclically with the precursor P4, and are supraphysiologically increased with exogenous perimenstrual EP. The increases in 3α,5α-THP under EP replicate work in PMDD showing the relationship between P4 and NAS (Klatzkin et al., 2006); we extend these known patterns by 1) showing a similar pattern of flux with 3α,5β-THP and 3α,5α-THDOC, less-studied NAS with similar structures and pharmacologic profiles, and 2) validating that the cyclical pattern of P4-derived NAS is not aberrant in individuals with PME of SI (suggesting that, similar to PMDD, symptoms arise due to an abnormal sensitivity to normal hormone flux). We did not expect androstane NAS to fluctuate cyclically as they are not direct metabolites of P4, which was supported by non-significant cycle phase differences for 3α,5β-A, and 3α,5β-A-diol; however, 3α,5α-A, 3α,5α-A-diol fluctuated *inversely* from P4, with midfollicular peaks and midluteal troughs. Surprisingly, exogenous perimenstrual EP *decreased* androstane NAS. To our knowledge, there are no studies characterizing androstane-derived NAS longitudinally across the menstrual cycle. One prior study showed that P4 administration decreases the sulfated form of DHEA, a precursor to many androstane NAS, in PMDD patients but not controls (Nguyen et al., 2017); our findings extend this work to a PME sample, showing that P4 decreases perimenstrual 3α,5α-A, 3α,5α-A-diol, 3α,5β-A, and 3α,5β-A-diol (downstream metabolites of DHEA). Together, there is evidence for an inverse relationship between P4 and DHEA-derived NAS in patients with cyclical mood changes. We raise the possibility that patients with cyclical changes in mood symptoms (PME or PMDD) could have a relative deficit in 3α-HSD, shunting 3α-HSD activity to favor P4 catabolism over testosterone or DHEA catabolism in the presence of enzyme saturation by P4. This hypothesis should be tested to identify the mechanistic pathway by which P4 fluctuations affect 3α-reduced NAS that are not direct metabolites of P4.

We demonstrated that peripheral TNF-α, IL-1β, and IL-6 do not fluctuate across the cycle; under EP, only IL-6 was affected, such that EP decreased perimenstrual IL-6. While prior studies showed that IL-6 and IL-1β *do* fluctuate across the menstrual cycle in urine (Whitcomb et al., 2014) samples, we did not observe these relationships in peripheral blood despite ovulation confirmation. We also demonstrate that preclinical NAS and inflammation relationships, such that 3α,5α-THP has anti-inflammatory properties (Balan et al., 2022), are not represented in human peripheral blood samples. Thus, despite the fact that menstruation is an inherently inflammatory local process (Evans & Salamonsen, 2012), this does not appear to be reflected in peripheral inflammation.

4.3. What is the role of potential confounding variables?

We showed that time of day does not predict peripheral GABAergic NAS or inflammatory cytokines, countering diurnal variations found in urinary steroid metabolites (Jerjes et al., 2006). It is crucial to replicate this null finding in larger samples. If replicated, this finding can increase inclusivity of future studies focused on peripheral biomarkers, as current best practice of scheduling participant blood draws at the same time of day is inherently biased

towards participants with similar (or highly flexible) schedules. We additionally showed that SSRI usage did not predict increased levels of 3α-reduced NAS, and in fact predicted lower 3α,5β-reduced NAS, which counters prior literature in samples with depression (Pinna et al., 2009; Uzunova et al., 1998).

4.4. Limitations

The present study has many strengths, including gold-standard LH-surge urine test for ovulation, daily symptoms, repeated blood sampling, a rarefied sample of outpatients with SI, a broad battery of NAS, and translation of basic science hypotheses into a clinical sample, but is not without limitations. First, peripheral blood does not necessarily correspond to CNS processes (Gigase et al., 2023), and we must therefore interpret findings with caution. Second, we cannot infer causality from any of our correlations between symptoms and biomarkers as we did not directly manipulate biomarkers of interest; future work should consider direct manipulation of NAS and/or cytokines to determine causal relationships. Third, SSRI usage was measured as a trait variable with no attention to dosage or specific medication. Fourth, while this sample is intentionally transdiagnostic to focus on the specific symptom construct of SI, we did not test if any of our findings differed between subsets of participants based on psychiatric diagnoses (e.g., Major Depressive Disorder only, anxiety disorders only, or specific combinations of comorbidities). Finally, we ran many exploratory tests in a small sample, which increases Type I error and necessitates that these findings serve as preliminary, hypothesis-driving data for future studies in larger samples. We urge caution before over-interpreting or generalizing these findings.

4.5. Conclusions

We present a pre-registered secondary analysis of GABAergic NAS and inflammatory cytokines across the menstrual cycle and under experimental EP from a parent RCT showing EP supplementation reduces PME of SI (Eisenlohr-Moul, Bowers, et al., 2022). We provide preliminary evidence that peripheral inflammatory biomarkers are positively correlated with psychiatric symptom severity, while peripheral GABAergic NAS are either unrelated or inversely related to symptom severity. We show that peripheral P4-derived GABAergic NAS fluctuate in parallel with P4 and increase with exogenous EP, while androstane-derived GABAergic NAS do not fluctuate or fluctuate inversely from P4 and decrease with exogenous EP. While preliminary, our findings serve as important foundation for future work into the pathophysiological mechanisms underlying suicide and perimenstrual suicide risk. We describe how ten frequently studied peripheral biomarkers in biological psychiatry do and do not fluctuate across the menstrual cycle, allowing for improved quality of comparisons involving naturally-cycling female participants in research. In sum, our work highlights relevance of the menstrual cycle to psychiatric biomarker research, and provides clues about possible mechanisms by which normal ovarian hormone fluctuations can contribute to perimenstrual suicide risk.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements:

References.

Bäckström T, Haage D, Löfgren M, Johansson IM, Strömberg J, Nyberg S, Andréen L, Ossewaarde L, van Wingen GA, Turkmen S, & Bengtsson SK (2011). Paradoxical effects of GABA-A modulators may explain sex steroid induced negative mood symptoms in some persons. Neuroscience, 191, 46–54. 10.1016/j.neuroscience.2011.03.061 [PubMed: 21600269]

Balan I, Aurelian L, Williams KS, Campbell B, Meeker RB, & Morrow AL (2022). Inhibition of human macrophage activation via pregnane neurosteroid interactions with toll-like receptors: Sex differences and structural requirements. Frontiers in Immunology, 13, 940095. 10.3389/fimmu.2022.940095 [PubMed: 35967446]

Bates D, Mächler M, Bolker B, & Walker S (2015). Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software, 67, 1–48. 10.18637/jss.v067.i01

Bixo M, Ekberg K, Poromaa IS, Hirschberg AL, Jonasson AF, Andréen L, Timby E, Wulff M, Ehrenborg A, & Bäckström T (2017). Treatment of premenstrual dysphoric disorder with the GABA(A) receptor modulating steroid antagonist Sepranolone (UC1010)-A randomized controlled trial. Psychoneuroendocrinology, 80, 46–55. 10.1016/j.psyneuen.2017.02.031 [PubMed: 28319848]

Brundin L, Bryleva EY, & Thirtamara Rajamani K (2017). Role of Inflammation in Suicide: From Mechanisms to Treatment. Neuropsychopharmacology, 42(1), 271–283. 10.1038/npp.2016.116 [PubMed: 27377015]

Dichtel LE, Lawson EA, Schorr M, Meenaghan E, Paskal ML, Eddy KT, Pinna G, Nelson M, Rasmusson AM, Klibanski A, & Miller KK (2018). Neuroactive Steroids and Affective Symptoms in Women Across the Weight Spectrum. Neuropsychopharmacology, 43(6), 1436–1444. 10.1038/npp.2017.269 [PubMed: 29090684]

Eisenlohr-Moul TA, & Barone JC (2023). Optimizing Neuroactive Steroid Treatments for Stress-Related Affective Symptoms: Brief Reflections on Patient Population, Timing, and Access. Biological Psychiatry, 94(3), 191–193. 10.1016/j.biopsych.2023.05.015 [PubMed: 37437990]

Eisenlohr-Moul TA, Bowers SM, Prinstein MJ, Schmalenberger KM, Walsh EC, Young SL, Rubinow DR, & Girdler SS (2022). Effects of acute estradiol and progesterone on perimenstrual exacerbation of suicidal ideation and related symptoms: A crossover randomized controlled trial | Translational Psychiatry. Translational Psychiatry, 12(1), 528. 10.1038/s41398-022-02294-1 [PubMed: 36585408]

Eisenlohr-Moul TA, Divine M, Schmalenberger K, Murphy L, Buchert B, Wagner-Schuman M, Kania A, Raja S, Miller AB, Barone J, & Ross J (2022). Prevalence of lifetime self-injurious thoughts and behaviors in a global sample of 599 patients reporting prospectively confirmed diagnosis with premenstrual dysphoric disorder. BMC Psychiatry, 22(1), 199. 10.1186/s12888-022-03851-0 [PubMed: 35303811]

Endicott J, Nee J, & Harrison W (2006). Daily Record of Severity of Problems (DRSP): Reliability and validity. Archives of Women's Mental Health, 9(1), 41–49.

First MB, Williams JBW, Karg RS, & Spitzer RL (2015). Structured Clinical Interview for DSM-5—Research Version (SCID-5 for DSM-5, Research Version; SCID-5-RV). American Psychiatric Association, 2015.

Gaskins AJ, Wilchesky M, Mumford SL, Whitcomb BW, Browne RW, Wactawski-Wende J, Perkins NJ, & Schisterman EF (2012). Endogenous Reproductive Hormones and C-reactive Protein Across the Menstrual Cycle: The BioCycle Study. American Journal of Epidemiology, 175(5), 423–431. 10.1093/aje/kwr343 [PubMed: 22306563]

Ghosh D, & Vogt A. (2012). Outliers: An Evaluation of Methodologies.

Gigase FAJ, Smith E, Collins B, Moore K, Snijders GJLJ, Katz D, Bergink V, Perez-Rodriquez MM, & De Witte LD (2023). The association between inflammatory markers in blood and cerebrospinal fluid: A systematic review and meta-analysis. Molecular Psychiatry. 10.1038/s41380-023-01976-6

Hantsoo L, & Epperson CN (2020). Allopregnanolone in premenstrual dysphoric disorder (PMDD): Evidence for dysregulated sensitivity to GABA-A receptor modulating neuroactive steroids across the menstrual cycle. Neurobiology of Stress, 12, 100213. 10.1016/j.ynstr.2020.100213 [PubMed: 32435664]

Hartlage SA, Brandenburg DL, & Kravitz HM (2004). Premenstrual exacerbation of depressive disorders in a community-based sample in the United States. Psychosomatic Medicine, 66(5), 698–706. 10.1097/01.psy.0000138131.92408.b9 [PubMed: 15385694]

Holmes SE, Hinz R, Conen S, Gregory CJ, Matthews JC, Anton-Rodriguez JM, Gerhard A, & Talbot PS (2018). Elevated Translocator Protein in Anterior Cingulate in Major Depression and a Role for Inflammation in Suicidal Thinking: A Positron Emission Tomography Study. Biological Psychiatry, 83(1), 61–69. 10.1016/j.biopsych.2017.08.005 [PubMed: 28939116]

Janelidze S, Mattei D, Westrin Å, Träskman-Bendz L, & Brundin L (2011). Cytokine levels in the blood may distinguish suicide attempters from depressed patients. Brain, Behavior, and Immunity, 25(2), 335–339. 10.1016/j.bbi.2010.10.010 [PubMed: 20951793]

Jerjes WK, Cleare AJ, Peters TJ, & Taylor NF (2006). Circadian rhythm of urinary steroid metabolites. Annals of Clinical Biochemistry, 43(4), 287–294. 10.1258/000456306777695564 [PubMed: 16824279]

Klatzkin RR, Leslie Morrow A, Light KC, Pedersen CA, & Girdler SS (2006). Associations of histories of depression and PMDD diagnosis with allopregnanolone concentrations following the oral administration of micronized progesterone. Psychoneuroendocrinology, 31(10), 1208–1219. 10.1016/j.psyneuen.2006.09.002 [PubMed: 17046166]

Köhler CA, Freitas TH, Maes M, de Andrade NQ, Liu CS, Fernandes BS, Stubbs B, Solmi M, Veronese N, Herrmann N, Raison CL, Miller BJ, Lanctôt KL, & Carvalho AF (2017). Peripheral cytokine and chemokine alterations in depression: A meta-analysis of 82 studies. Acta Psychiatrica Scandinavica, 135(5), 373–387. 10.1111/acps.12698 [PubMed: 28122130]

Kuehner C, & Nayman S (2021). Premenstrual Exacerbations of Mood Disorders: Findings and Knowledge Gaps. Current Psychiatry Reports, 23(11), 78. 10.1007/s11920-021-01286-0 [PubMed: 34626258]

Lamers F, Milaneschi Y, Smit JH, Schoevers RA, Wittenberg G, & Penninx BWJH (2019). Longitudinal Association Between Depression and Inflammatory Markers: Results From the Netherlands Study of Depression and Anxiety. Biological Psychiatry, 85(10), 829–837. 10.1016/j.biopsych.2018.12.020 [PubMed: 30819515]

Locci A, & Pinna G (2017). Neurosteroid biosynthesis down-regulation and changes in GABAA receptor subunit composition: A biomarker axis in stress-induced cognitive and emotional impairment. British Journal of Pharmacology, 174(19), 3226–3241. 10.1111/bph.13843 [PubMed: 28456011]

Martinez PE, Rubinow DR, Nieman LK, Koziol DE, Morrow AL, Schiller CE, Cintron D, Thompson KD, Khine KK, & Schmidt PJ (2016). 5 α -Reductase Inhibition Prevents the Luteal Phase Increase in Plasma Allopregnanolone Levels and Mitigates Symptoms in Women with Premenstrual Dysphoric Disorder. Neuropsychopharmacology, 41(4), Article 4. 10.1038/npp.2015.246

Moriarity DP, Kautz MM, Giollabui NM, Klugman J, Coe CL, Ellman LM, Abramson LY, & Alloy LB (2020). Bidirectional Associations Between Inflammatory Biomarkers and Depressive Symptoms in Adolescents: Potential Causal Relationships. Clinical Psychological Science: A Journal of the Association for Psychological Science, 8(4), 690–703. 10.1177/2167702620917458 [PubMed: 32724728]

Nguyen TV, Reuter JM, Gaikwad NW, Rotroff DM, Kucera HR, Motsinger-Reif A, Smith CP, Nieman LK, Rubinow DR, Kaddurah-Daouk R, & Schmidt PJ (2017). The steroid metabolome in women with premenstrual dysphoric disorder during GnRH agonist-induced ovarian suppression: Effects of estradiol and progesterone addback. Translational Psychiatry, 7. 10.1038/tp.2017.146

Nock MK, Hwang I, Sampson NA, & Kessler RC (2010). Mental disorders, comorbidity and suicidal behavior: Results from the National Comorbidity Survey Replication. Molecular Psychiatry, 15(8), 868–876. 10.1038/mp.2009.29 [PubMed: 19337207]

Nolan LN, & Hughes L (2022). Premenstrual exacerbation of mental health disorders: A systematic review of prospective studies. Archives of Women's Mental Health. 10.1007/s00737-022-01246-4

Nutt D (2006). GABA$_A$ Receptors: Subtypes, Regional Distribution, and Function. Journal of Clinical Sleep Medicine, 02(02). 10.5664/jcsm.26525

Owens SA, Schmalenberger KM, Bowers S, Rubinow DR, Prinstein MJ, Girdler SS, & Eisenlohr-Moul TA (2023). Cyclical exacerbation of suicidal ideation in female outpatients: Prospective evidence from daily ratings in a transdiagnostic sample. Journal of Psychopathology and Clinical Science. 10.1037/abn0000838

Pinheiro J, Bates D, Debroy S, Sarkar D, & R Core Team. (2021). nlme: Linear and Nonlinear Mixed Effects Models. (3.1–153) [R]. https://CRAN.R-project.org/package=nlme

Pinna G, Costa E, & Guidotti A (2009). SSRIs act as selective brain steroidogenic stimulants (SBSSs) at low doses that are inactive on 5-HT reuptake. Current Opinion in Pharmacology, 9(1), 24–30. 10.1016/j.coph.2008.12.006 [PubMed: 19157982]

Porcu P, O'Buckley TK, Alward SE, Marx CE, Shampine LJ, Girdler SS, & Morrow AL (2009). Simultaneous quantification of GABAergic 3α,5α/3α,5β neuroactive steroids in human and rat serum. Steroids, 74(4–5), 463–473. [PubMed: 19171160]

Posner K, Brown GK, Stanley B, Brent DA, Yershova KV, Oquendo MA, Currier GW, Melvin GA, Greenhill L, Shen S, & Mann JJ (2011). The Columbia-Suicide Severity Rating Scale: Initial validity and internal consistency findings from three multisite studies with adolescents and adults. The American Journal of Psychiatry, 168(12), 1266–1277. 10.1176/appi.ajp.2011.10111704 [PubMed: 22193671]

Rasmusson AM, Pinna G, Paliwal P, Weisman D, Gottschalk C, Charney D, Krystal J, & Guidotti A (2006). Decreased Cerebrospinal Fluid Allopregnanolone Levels in Women with Posttraumatic Stress Disorder. Biological Psychiatry, 60(7), 704–713. 10.1016/j.biopsych.2006.03.026 [PubMed: 16934764]

Reynolds WM (1991). Psychometric Characteristics of the Adult Suicidal Ideation Questionnaire in College Students. Journal of Personality Assessment, 56(2), 289–307. 10.1207/s15327752jpa5602_9 [PubMed: 2056423]

Saunders KEA, & Hawton K (2006). Suicidal behaviour and the menstrual cycle. Psychological Medicine, 36(7), 901–912. 10.1017/S0033291706007392 [PubMed: 16573848]

Schmalenberger KM, Tauseef HA, Barone JC, Owens SA, Lieberman L, Jarczok MN, Girdler SS, Kiesner J, Ditzen B, & Eisenlohr-Moul TA (2021). How to study the menstrual cycle: Practical tools and recommendations. Psychoneuroendocrinology, 123, 104895. 10.1016/j.psyneuen.2020.104895 [PubMed: 33113391]

Schmidt PJ, Purdy RH, Moore PH, Paul SM, & Rubinow DR (1994). Circulating levels of anxiolytic steroids in the luteal phase in women with premenstrual syndrome and in control subjects. Journal of Clinical Endocrinology & Metabolism, 79(5), 1256–1260. [PubMed: 7962316]

Shen H, Gong QH, Aoki C, Yuan M, Ruderman Y, Dattilo M, Williams K, & Smith SS (2007). Reversal of neurosteroid effects at α4β2δ GABA A receptors triggers anxiety at puberty. Nature Neuroscience, 10(4), Article 4. 10.1038/nn1868

Smith SS (2013). The influence of stress at puberty on mood and learning: Role of the α4βδ GABAA receptor. Neuroscience, 249, 192–213. 10.1016/j.neuroscience.2012.09.065 [PubMed: 23079628]

Smith SS, Shen H, Gong QH, & Zhou X (2007). Neurosteroid regulation of GABAA receptors: Focus on the α4 and δ subunits. Pharmacology & Therapeutics, 116(1), 58–76. 10.1016/j.pharmthera.2007.03.008 [PubMed: 17512983]

Speroff L, & Fritz MA (2005). Clinical Gynecologic Endocrinology and Infertility. Lippincott Williams & Wilkins.

Ströhle A, Romeo E, Hermann B, Pasini A, Spalletta G, di Michele F, Holsboer F, & Rupprecht R (1999). Concentrations of 3α-reduced neuroactive steroids and their precursors in plasma of patients with major depression and after clinical recovery. Biological Psychiatry, 45(3), 274–277. 10.1016/S0006-3223(98)00328-X [PubMed: 10023501]

Thys-Jacobs S, McMahon D, & Bilezikian JP (2008). Differences in free estradiol and sex hormone-binding globulin in women with and without premenstrual dysphoric disorder. The Journal of Clinical Endocrinology and Metabolism, 93(1), 96–102. 10.1210/jc.2007-1726 [PubMed: 17956950]

Uzunova V, Sheline Y, Davis JM, Rasmusson A, Uzunov DP, Costa E, & Guidotti A (1998). Increase in the cerebrospinal fluid content of neurosteroids in patients with unipolar major depression who are receiving fluoxetine or fluvoxamine. Proceedings of the National Academy of Sciences of the United States of America, 95(6), 3239–3244. 10.1073/pnas.95.6.3239 [PubMed: 9501247]

Vasupanrajit A, Jirakran K, Tunvirachaisakul C, Solmi M, & Maes M (2022). Inflammation and nitro-oxidative stress in current suicidal attempts and current suicidal ideation: A systematic review and meta-analysis. Molecular Psychiatry, 1–12. 10.1038/s41380-021-01407-4

Wenzel ES, Pinna G, Eisenlohr-Moul T, Bernabe BP, Tallon RR, Nagelli U, Davis J, & Maki PM (2021). Neuroactive steroids and depression in early pregnancy. Psychoneuroendocrinology, 134, 105424. 10.1016/j.psyneuen.2021.105424 [PubMed: 34607173]

Whitcomb BW, Mumford SL, Perkins NJ, Wactawski-Wende J, Bertone-Johnson ER, Lynch KE, & Schisterman EF (2014). Urinary cytokine and chemokine profiles across the menstrual cycle in healthy reproductive-aged women. Fertility and Sterility, 101(5), 1383–1391. 10.1016/j.fertnstert.2014.01.027 [PubMed: 24581581]

White S (2019). Basic & Clinical Biostatistics (5th Ed). McGraw Hill Medical. https://accesspharmacy-mhmedical-com.proxy.cc.uic.edu/book.aspx?bookid=2724

Youssef NA, Bradford DW, Kilts JD, Szabo ST, Naylor JC, Allen TB, Strauss JL, Hamer RM, Brancu M, Brunca M, Shampine LJ, & Marx CE (2015). Exploratory investigation of biomarker candidates for suicide in schizophrenia and bipolar disorder. Crisis, 36(1), 46–54. 10.1027/0227-5910/a000280 [PubMed: 25410258]

Zwahlen M, & Stute P (2023). Impact of progesterone on the immune system in women: A systematic literature review. Archives of Gynecology and Obstetrics. 10.1007/s00404-023-06996-9

Highlights:

- There are no existing biomarkers for perimenstrual exacerbation of suicidality

- Neuroactive steroids do not correlate to suicidal ideation, depression, or anxiety

- Higher cytokines generally correlate with higher suicide-related symptoms

- Neuroactive steroids fluctuate across the menstrual cycle

- Estrogen and progesterone increase some neuroactive steroids, but decrease others
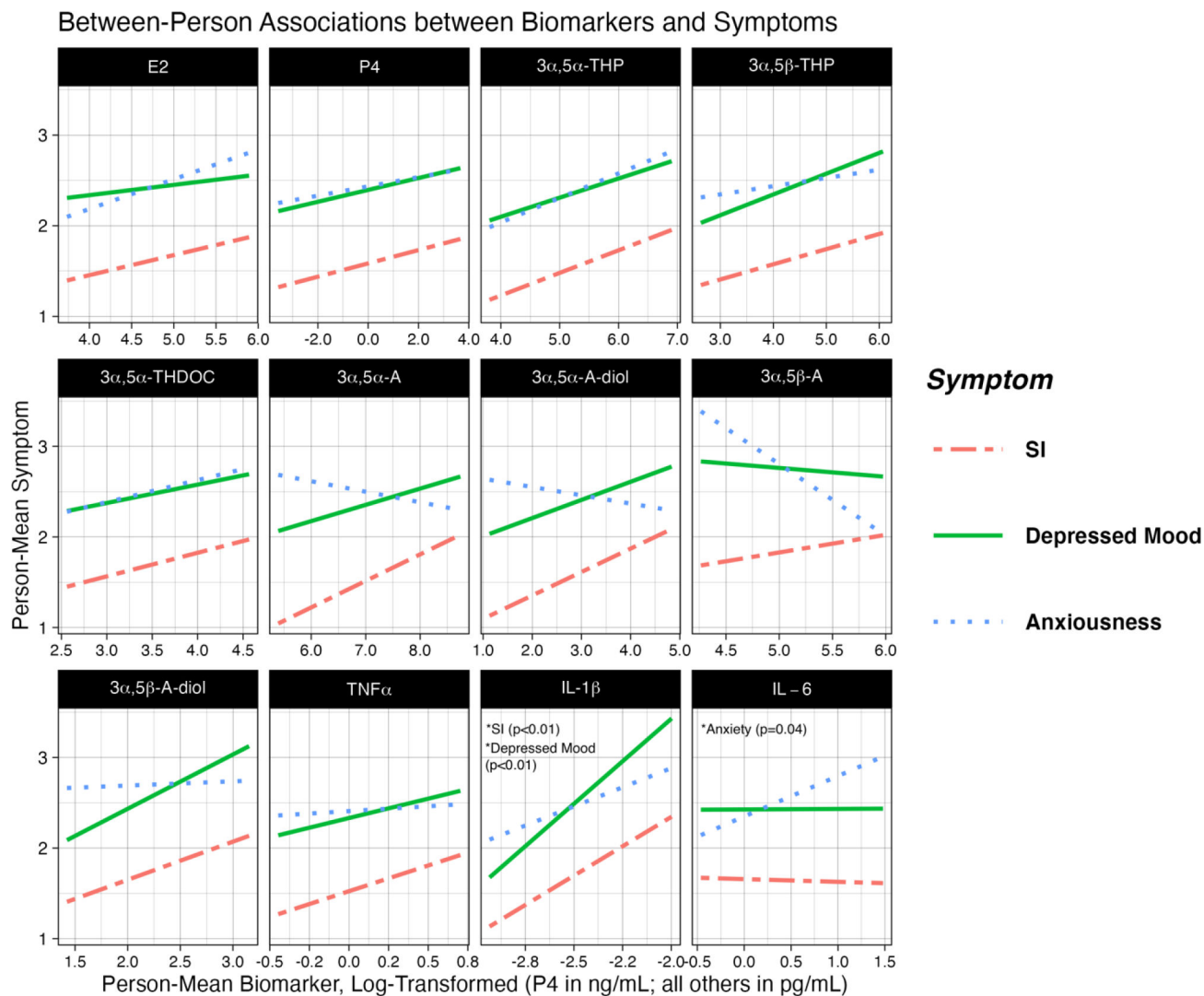
Figure 1.

Symptoms (SI, Depressed Mood, Anxiousness) predicted by biomarkers at the between-person level (placebo condition only), with no attention to cycle phase. Y axis represents mean symptom score across placebo visits, per person. Depressed Mood and Anxiousness Likert scales ranged from 1–6 (Depressed Mood: *Sample Mean (M)=2.46, Sample Standard Deviation (SD)=0.90;* Anxiousness: *Sample M=2.47, Sample SD=0.74*), and SI Likert scale ranged from 1–5 (SI: *Sample M=1.62, Sample SD=0.72*). Lines represent regression between biomarker predictor (panel title) and respective symptom outcome (line type and color). Significant associations (p<0.05) are denoted with asterisks and text.
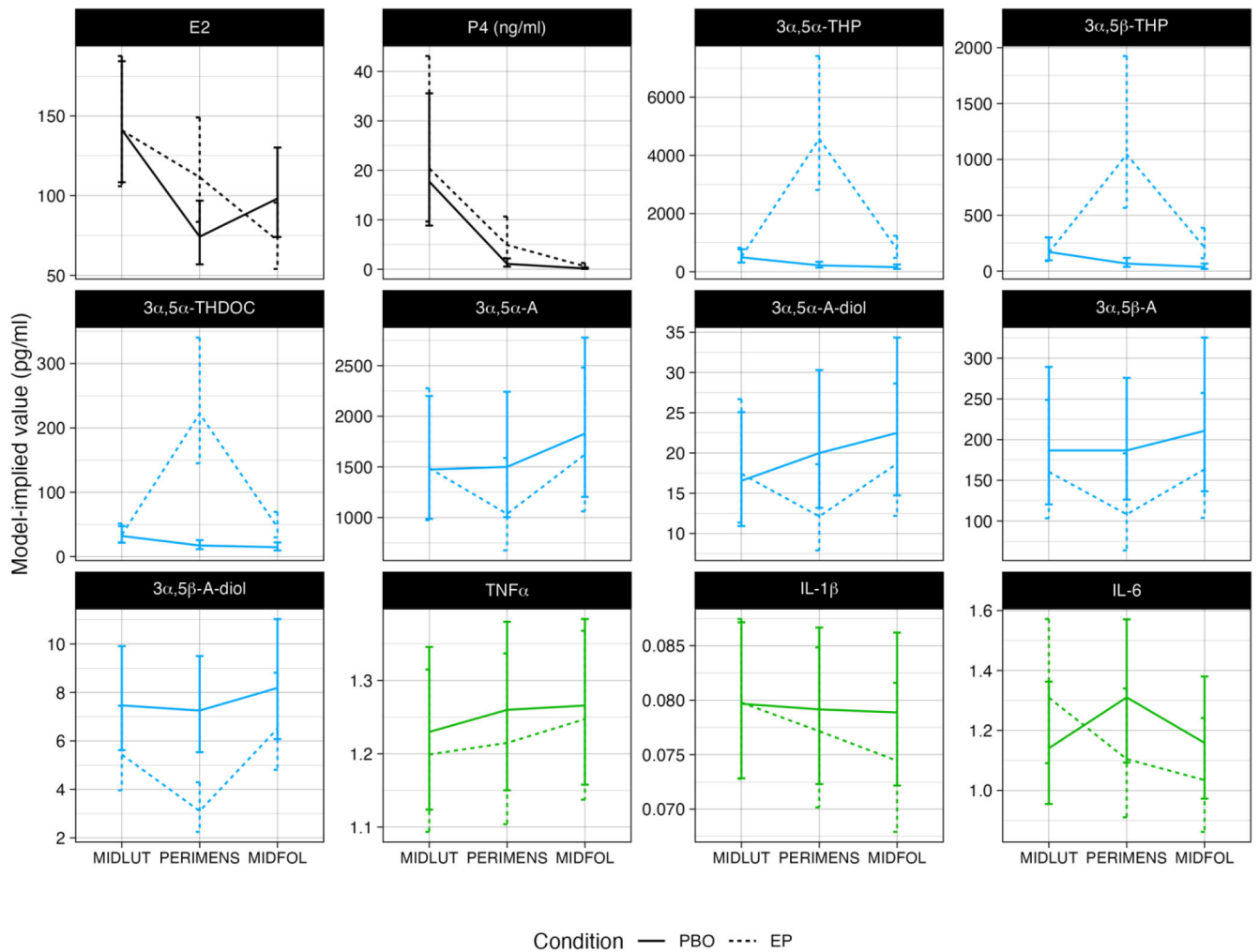
Figure 2.
Model-implied values of peripheral ovarian steroids E2 and P4 (black lines), GABAergic NAS (blue) and cytokines (green) at three timepoints across the menstrual cycle. Natural cycle (under placebo) shown with solid line; EP experimental condition shown with dotted line. Error bars represent 95% confidence interval. Values have been back-transformed from logarithmic scale (for model fitting) into raw scale for visualization. P4 measured in nanograms per milliliter (ng/ml); all others measured in picograms per milliliter (pg/ml). Abbreviations: MIDLUT = midluteal phase, PERIMENS = perimenstrual phase, MIDFOL = midfollicular phase.

Table 1.

Participant demographics and baseline clinical diagnoses, per SCID-5 interview.

	Total sample (N=26)	
	n	**%**
Age, years, mean (SD) BMI at recruitment, mean (SD)	28.7 (7.1) 26.5 (6.2)	
Race		
White/Caucasian	23	88.5
Asian	1	3.8
More than one race	1	3.8
Declined to answer	3	3.8
Education		
<4 yr college degree	4	15.4
4 yr college degree	13	50.0
Post-graduate degree	9	34.6
Household income		
Less than $25,000	7	26.9
$25,000 - $49,999	8	30.8
$50,000 - $99,999	7	26.9
$100,000 or more	4	15.4
Sexual orientation		
Heterosexual	10	38.5
Bisexual	7	26.9
Lesbian/Gay	3	11.5
Other Non-Heterosexual	6	23.1
Sex (Assigned Female at Birth)	26	100
Gender (Cisgender)	24	92.3
Cohabitation with Partner	18	69.2
Parity	9	34.6
Current SSRI use	11	42.3
Lifetime non-suicidal self-injury	15	57.7
Lifetime suicide attempt	15	57.7
Any Current Depressive Disorder	20	76.9
Any Current Anxiety Disorder	20	76.9
Any Current Substance Use Disorder	1	3.8
Any Current Obsessive-Compulsive Disorder	4	15.4
Any Current Eating Disorder	2	7.7
Current Attention Deficit/Hyperactivity Disorder	4	15.4
Current Posttraumatic Stress Disorder	7	26.7

Table 2.

Results from models predicting associations between biomarkers and symptoms in non-experimentally treated cycles (placebo; N=75 observations; N=25 participants). Models were adjusted for age, BMI, SSRI usage, and cycle phase (all covariate p's>0.05). Each biomarker was analyzed in three separate models to substitute outcomes (SI, depressed mood, anxiousness). Bolded estimates represent significant differences ($p < 0.05$).

		SI		Depressed Mood		Anxiousness	
		EST	*SE*	*EST*	*SE*	*EST*	*SE*
3α, 5α-THP	*Intercept*	−4.02	2.40	−2.03	3.35	−2.69	2.78
	Participant mean (*between*)	**1.09**	0.43	0.79	0.61	0.94	0.50
	Person-centered deviation (*within*)	−0.02	0.19	0.46	0.35	0.10	0.28
3α,5β-THP	*Intercept*	−0.02	1.55	0.05	1.97	1.74	1.73
	Participant mean (*between*)	0.42	0.35	0.47	0.44	0.15	0.38
	Person-centered deviation (*within*)	0.13	0.13	**0.59**	0.22	0.13	0.19
3α,5α-THDOC	*Intercept*	−0.33	2.12	−1.42	2.65	−1.28	2.21
	Participant mean (*between*)	0.66	0.63	1.18	0.79	1.16	0.65
	Person-centered deviation (*within*)	0.10	0.26	0.15	0.45	−0.27	0.36
3α,5α-A	*Intercept*	0.04	1.93	1.25	2.46	3.97	2.09
	Participant mean (*between*)	0.24	0.25	0.17	0.32	−0.19	0.27
	Person-centered deviation (*within*)	0.00	0.19	−0.25	0.34	0.11	0.27
3α,5α-A-diol	*Intercept*	1.21	0.70	**2.18**	0.87	**2.99**	0.75
	Participant mean (*between*)	0.20	0.18	0.14	0.23	−0.13	0.20
	Person-centered deviation (*within*)	0.05	0.29	0.45	0.50	0.35	0.39
3α,5β-A	*Intercept*	−0.77	3.12	2.62	4.20	**7.83**	3.10
	Participant mean (*between*)	0.51	0.58	0.02	0.78	−0.98	0.57
	Person-centered deviation (*within*)	−0.76	0.57	−0.81	0.83	0.45	0.69
3α,5β-A-diol	*Intercept*	1.31	2.30	0.21	2.50	1.28	1.30
	Participant mean (*between*)	0.18	0.88	0.88	0.96	0.42	0.47
	Person-centered deviation (*within*)	−0.88	0.42	−2.04	1.13	0.50	1.23
TNF-α	*Intercept*	**1.70**	0.31	**2.61**	0.39	**2.51**	0.33
	Participant mean (*between*)	1.08	0.89	−0.04	1.18	0.19	1.00
	Person-centered deviation (*within*)	0.29	0.50	**2.10**	0.85	0.33	0.63
IL-1β	*Intercept*	**7.60**	2.00	**9.73**	2.53	4.44	2.54
	Participant mean (*between*)	**2.28**	0.80	**2.86**	1.00	0.76	1.01
	Person-centered deviation (*within*)	0.04	0.41	0.58	0.74	**1.24**	0.47
IL-6	*Intercept*	**1.94**	0.28	**2.56**	0.35	**2.31**	0.26
	Participant mean (*between*)	−0.01	0.50	0.08	0.63	**1.04**	0.48
	Person-centered deviation (*within*)	0.31	0.19	0.25	0.33	0.19	0.23

Abbreviations: EST=estimate, SE=standard error.

Table 3.

Results from multilevel models predicting log-transformed biomarker levels from pairwise cycle phase contrasts in placebo (natural cycle) condition only. Bolded estimates represent significant differences between phases ($p < 0.05$).

	Progesterone		3α,5α-THP		3α,5β-THP		3α,5α-THDOC	
	EST	SE	EST	SE	EST	SE	EST	SE
Intercept (MIDLUT ref. group)	**2.94**	0.29	**6.15**	0.13	**5.23**	0.16	**3.81**	0.09
SSRI[*]	0.11	0.43	0.11	0.19	−0.01	0.24	−0.13	0.13
BMI[*]	−0.28	0.21	−0.04	0.09	−0.06	0.13	0.01	0.07
Age[*]	0.25	0.24	0.06	0.10	−0.02	0.13	−0.08	0.07
Illness[*]	0.12	0.62	0.30	0.27	−0.21	0.40	−0.31	0.21
PERIMENS vs MIDLUT	**−2.79**	0.22	**−0.81**	0.09	**−0.95**	0.14	**−0.62**	0.07
MIDFOL vs MIDLUT	**−4.64**	0.22	**−1.14**	0.09	**−1.49**	0.14	**−0.77**	0.07
Intercept (PERIMENS ref. group)	0.14	0.29	**5.34**	0.13	**4.28**	0.16	**3.19**	0.09
MIDFOL vs PERIMENS	**−1.84**	0.22	**−0.33**	0.10	**−0.55**	0.14	**−0.16**	0.08

	3α,5α-A		3α,5α-A-diol		3α,5β-A		3α,5β-A-diol	
	EST	SE	EST	SE	EST	SE	EST	SE
Intercept (MIDLUT ref. group)	**7.63**	0.21	**3.37**	0.27	**5.31**	0.15	**2.51**	0.16
SSRI	−0.66	0.33	**−0.94**	0.45	−0.30	0.24	**−0.88**	0.27
BMI	0.04	0.17	0.24	0.23	0.07	0.15	0.06	0.15
Age	−0.25	0.18	−0.44	0.25	0.05	0.13	−0.26	0.13
Illness	−0.07	0.29	−0.40	0.19	0.14	0.33	−0.13	0.16
PERIMENS vs MIDLUT	0.03	0.10	**0.19**	0.06	0.02	0.11	−0.04	0.08
MIDFOL vs MIDLUT	**0.21**	0.10	**0.30**	0.06	0.12	0.10	0.08	0.08
Intercept (PERIMENS ref. group)	**7.67**	0.21	**3.57**	0.27	**5.33**	0.15	**2.47**	0.15
MIDFOL vs PERIMENS	0.18	0.10	0.11	0.06	0.10	0.10	0.12	0.08

	TNF-α		IL-1β		IL-6			
	EST	SE	EST	SE	EST	SE		
Intercept (MIDLUT ref. group)	**0.19**	0.06	**−2.50**	0.06	0.19	0.12		
SSRI	0.03	0.09	−0.05	0.09	−0.09	0.17		
BMI	0.05	0.05	0.09	0.05	**0.22**	0.09		
Age	0.01	0.05	−0.06	0.05	−0.02	0.09		
PERIMENS vs MIDLUT	0.02	0.04	−0.02	0.05	0.13	0.11		
MIDFOL vs MIDLUT	0.03	0.04	−0.01	0.04	0.01	0.10		
Intercept (PERIMENS ref. group)	**0.21**	0.06	**−2.52**	0.06	**0.31**	0.12		
MIDFOL vs PERIMENS	0.01	0.04	0.01	0.05	−0.11	0.11		

Abbreviations: PERIMENS = perimenstrual phase, MIDLUT = midluteal phase, MIDFOL = midfollicular phase; EST= estimate, SE = standard error.

*Covariates reported from models with MIDLUT as reference group; there were no notable differences in effect size and direction of each covariate in PERIMENS as reference group model.

Table 4.

Results from multilevel models predicting log-transformed biomarker levels from pairwise cycle phase contrasts and interaction between pairwise cycle phase × condition (placebo or EP). Bolded estimates represent significance ($p < 0.05$).

	Progesterone		3α,5α-THP		3α,5β-THP		3α,5α-THDOC	
	EST	*SE*	*EST*	*SE*	*EST*	*SE*	*EST*	*SE*
Intercept (MIDLUT ref. group)	**2.95**	0.22	**6.08**	0.15	**5.22**	0.18	**3.81**	0.13
SSRI*	0.10	0.28	0.24	0.19	0.00	0.23	−0.10	0.17
BMI*	−0.13	0.15	−0.05	0.10	−0.01	0.13	0.04	0.10
Age*	0.13	0.15	0.09	0.10	0.02	0.13	−0.11	0.10
Illness*	−0.25	0.61	0.00	0.39	−0.15	0.49	−0.59	0.34
Condition	0.14	0.24	0.04	0.15	−0.04	0.19	0.05	0.13
PERIMENS vs MIDLUT	**−2.79**	0.23	**−0.82**	0.15	**−0.93**	0.18	**−0.62**	0.13
MIDFOL vs MIDLUT	**−4.65**	0.23	**−1.15**	0.15	**−1.53**	0.19	**−0.79**	0.12
Condition x (PERIMENS vs MIDLUT)	**1.36**	0.35	**3.00**	0.21	**2.78**	0.27	**2.51**	0.18
Condition x (MIDFOL vs MIDLUT)	**1.14**	0.33	**1.55**	0.21	**1.78**	0.26	**1.09**	0.18
Intercept (PERIMENS ref. group)	0.16	0.23	**5.26**	0.15	**4.29**	0.18	**3.19**	0.13
MIDFOL vs PERIMENS	**−1.86**	0.23	**−0.34**	0.15	**−0.60**	0.19	−0.17	0.13
Condition x (MIDFOL vs PERIMENS)	−0.22	0.35	**−1.45**	0.21	**−0.99**	0.27	**−1.42**	0.18

	3α,5α-A		3α,5α-A-diol		3α,5β-A		3α,5β-A-diol	
	EST	*SE*	*EST*	*SE*	*EST*	*SE*	*EST*	*SE*
Intercept (MIDLUT ref. group)	**7.55**	0.17	**3.12**	0.20	**5.31**	0.13	**2.32**	0.13
SSRI*	**−0.48**	0.22	**−0.40**	0.19	−0.27	0.16	**−0.42**	0.13
BMI*	0.01	0.15	0.12	0.21	−0.04	0.12	−0.05	0.14
Age*	−0.18	0.15	−0.24	0.21	0.13	0.10	−0.13	0.11
Illness*	−0.01	0.29	−0.22	0.20	0.14	0.35	−0.15	0.15
Condition	0.01	0.11	0.05	0.07	−0.15	0.13	**−0.32**	0.09
PERIMENS vs MIDLUT	0.02	0.11	**0.19**	0.07	0.00	0.13	−0.03	0.08
MIDFOL vs MIDLUT	**0.22**	0.11	**0.31**	0.07	0.12	0.12	0.09	0.08
Condition x (PERIMENS vs MIDLUT)	**−0.38**	0.16	**−0.55**	0.11	−0.39	0.22	**−0.53**	0.13
Condition x (MIDFOL vs MIDLUT)	**−0.13**	0.15	**−0.24**	0.10	**−0.10**	0.18	**0.09**	0.12
Intercept (PERIMENS ref. group)	**7.56**	0.17	**3.31**	0.20	**5.30**	0.13	**2.29**	0.12
MIDFOL vs PERIMENS	0.20	0.11	0.12	0.07	0.12	0.12	0.12	0.08
Condition x (MIDFOL vs PERIMENS)	0.25	0.16	**0.32**	0.11	0.29	0.23	**0.62**	0.13

	TNF-α		IL-1β		IL-6	
	EST	*SE*	*EST*	*SE*	*EST*	*SE*
Intercept (MIDLUT ref. group)	**0.19**	0.05	**−2.53**	0.05	0.12	0.10

	Progesterone		3α,5α-THP		3α,5β-THP		3α,5α-THDOC	
	EST	*SE*	*EST*	*SE*	*EST*	*SE*	*EST*	*SE*
SSRI*	0.04	0.07	0.00	0.07	0.03	0.13		
BMI*	0.04	0.04	0.07	0.04	**0.24**	0.07		
Age*	0.02	0.04	−0.05	0.04	0.02	0.07		
Condition	−0.03	0.04	0.00	0.04	0.14	0.10		
PERIMENS vs MIDLUT	0.02	0.04	−0.01	0.04	0.14	0.10		
MIDFOL vs MIDLUT	0.03	0.04	−0.01	0.04	0.02	0.10		
Condition x (PERIMENS vs MIDLUT)	−0.01	0.06	−0.03	0.06	**−0.31**	0.15		
Condition x (MIDFOL vs MIDLUT)	0.01	0.06	**−0.06**	0.05	−0.25	0.14		
Intercept (PERIMENS ref. group)	**0.21**	0.05	**−2.53**	0.05	**0.26**	0.10		
MIDFOL vs PERIMENS	0.00	0.04	0.00	0.04	−0.12	0.10		
Condition x (MIDFOL vs PERIMENS)	0.02	0.06	−0.03	0.06	0.06	0.15		

Abbreviations: PERIMENS = perimenstrual phase, MIDLUT = midluteal phase, MIDFOL = midfollicular phase, EST= estimate, SE = standard error.

*Covariates reported from models with MIDLUT as reference group; there were no notable differences in effect size and direction of each covariate in PERIMENS as reference group model.