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Negative affect is unrelated to fluctuations in hormone levels across the menstrual cycle: Evidence from a multisite observational study across two successive cycles

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ABSTRACT

Background: Female sex hormones may play a crucial role in the occurrence of cycle-related mood disorders. However, the literature is inconsistent and methodologically stringent observational studies on the relationship between sex hormones and negative affect are lacking.

Methods: In this longitudinal multisite study from Hannover, Germany, and Zurich, Switzerland, we examined oestrogen, progesterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone serum levels in association with negative affect as measured with the Positive and Negative Affect Schedule (PANAS). Negative affect and hormone assays were collected at four consecutive time points comprising menstrual, pre-ovulatory, mid-luteal and premenstrual phase across two cycles (n = 87 and n = 67 for the first and second cycles). The Beck Depression Inventory (BDI) was assessed once prior to the first cycle and included as a secondary measure.

Results: Mean negative affect scores did not significantly fluctuate across both cycles and there was in particular no symptom increase premenstrually. No sex hormone consistently related to repeated measures of negative affect across two consecutive cycles. The BDI sum-score assessed at baseline was not related to hormone levels across the first cycle.

Conclusions: This is the first multisite longitudinal study on the association between negative affect and sex hormone levels encompassing two consecutive menstrual cycles. Negative affect did not fluctuate across the cycle and there was no direct and uniform association between sex hormones and self-reported negative affect. These findings suggest that moderators such as personality traits and epigenetics should be considered in future research.

1. Introduction

A commonly purported belief is that fluctuations in sex hormones across the menstrual cycle contribute to women's emotion processing and experience of negative affect such as irritability, nervousness, anger, depression and anxiety [1–3]. Gender differences in psychopathology, characterised by an increased prevalence of externalising disorders in men and a higher prevalence of internalising disorders in women, originate simultaneously with hormonal changes during childhood/puberty [4]. However, the specific contribution of sex hormones to cycle-related negative affect is largely unknown [3,5]. Severe affective disorders related to the menstrual cycle that may require

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psychiatric treatment include premenstrual syndrome (PMS) and premenstrual dysphoric disorder (PMDD) [5,6]. Both conditions relate by definition to specific menstrual stages, but, most importantly, no consistent pattern emerged with respect to their association with sex hormone levels and hormone fluctuations across the cycle.

1.1. Negative affect related to hormone levels

An increased individual sensitivity to cyclic variations in ovarian hormones has been discussed as a trigger for mood symptoms across the menstrual cycle, but the efficacy of treatments with combined oral contraceptives for PMS/PMDD is inconclusive and at best modest [7–9]; see review by Halbreich [10]. Conversely, mood symptoms disappear in natural or medically induced anovulatory cycles [3]. Though a few small-sample studies reported associations of either oestrogen or progesterone with cycle-related negative affect [11], most studies, including adequately powered analyses with $n > 50$ women, did largely fail to replicate such associations [12–16]; for comprehensive reviews, see [3,5]. In addition to the premenstrual rise of progesterone, the period of the transition to menopause has also been suggested to increase the risk for depression [17]. However, findings are largely inconsistent and inconclusive [18,19], which is in part attributable to methodological biases and inadequate statistical modelling [20]. Accordingly, the literature indicates no clear association between sex hormones and the onset of depression during the transition to menopause [19,21].

1.2. Negative affect related to cycle phases

There is a long history of empirical work focused on mood alterations in healthy reproductive women [15,22,23], but these studies produced inconsistent associations with cycle phases. For instance, it has been shown that some mood symptoms fluctuate across the cycle, but others do not [24,25]. Very recently, a longitudinal study by Kiesner and colleagues [26] suggested that null-associations between menstrual cycle and mood symptoms in healthy women reported in earlier studies (e.g. [15]) could be due to inadequate statistical modelling of within-subject effects. Though the authors found modest variability in within-subject variance, they also reported significantly increased negative affect around menstruation [26]. Another longitudinal study likewise reported slight worsening of mood symptoms premenstrually in healthy women, but these changes were statistically not significant. There was neither an association between mood scores and length of the luteal phase [16]. Because both Harvey et al. [16] and Kiesner et al. [26] aggregated within-subject data from several cycles, here we will focus on two consecutive cycles separately, as fluctuations in negative affect could be variable.

1.3. Methodological issues

Most work aimed at associations between negative affect and sex hormones used dichotomous mood disorder categories. However, negative affect is a dimensional construct and even treatment-relevant disorders such as PMS and PMDD are simply extreme manifestations along this continuum [3]. Because mental disorders and most somatic diseases are dimensional traits rather than discrete taxa by nature [27,28], limiting research to dichotomous disorders with arbitrary diagnostic boundaries severely compromises the yield of psychopathological research and may even undermine the validity of research findings [29–31]. Therefore, in accordance with newly developed dimensional approaches to psychopathology [32,33], it is necessary to study dimensional behavioural phenotypes that cut through arbitrary diagnostic categories [27,29]. In addition, most previous studies relied on small samples of $n < 50$ women. Though it is well established that small-sample studies increase the rate of false-negative findings (i.e. β-errors), it is less known that these underpowered studies also produce inflated effect sizes and false-positive results (i.e. α-errors) [34]. That is, the smaller the sample size, the higher the probability that study results are flawed [34,35]. Observational studies with larger samples are therefore required to provide unbiased findings on associations between menstrual cycle phases, sex hormones, and negative affect.

1.4. The present study

Because exploratory studies are particularly prone to false positives [36], we applied a stringent design that minimises the probability of false-positive findings. Firstly, we propose that statistical modelling should not merely rely on overall associations between negative affect and hormone levels across the cycle, but also specifically consider inter-individual differences in intra-individual change across the cycle [26]. Secondly, significant associations found across a first index cycle should be replicated using data from a second menstrual cycle. And thirdly, associations that replicated across both cycles should not only meet statistical significance, but also demonstrate practical significance, that is, an effect size that is sufficiently large to have implications for clinical practice [37,38]. Because this was an exploratory study at the outset we did not specify hypotheses prior to data analysis. However, data from the second cycle will be used to confirm possible associations explored in the first cycle. To the best of our knowledge, such a rigorous testing involving direct replication has not yet been applied to observational studies focusing on associations between negative affect and hormones. Specifically, we are not aware of any study that linked progesterone, oestrogen, LH, FSH and testosterone to negative affect at four consecutive measurements across two menstrual cycles.

2. Methods

2.1. Procedure and participants

The study was designed as a prospective observational study investigating serial measurements of hormonal and neurocognitive parameters in healthy women and women with endocrine disorders aged 18–40 years in up to two menstrual cycles. During a baseline visit women were interviewed to verify inclusion and exclusion criteria and a physical examination was performed to exclude medical conditions which might influence hormone levels or cognitive performance except for endometriosis, PCOS or hyperprolactinemia. Women were excluded if they were using oral contraceptives, had been pregnant or breastfeeding within the past six months, were using medication or had surgery which might interfere with endocrine parameters, had severe psychiatric or general somatic diseases (such as schizophrenia or cancer), worked irregular shifts, had menstrual or ovulation disorders except those investigated in the study (endometriosis, PCOS and hyperprolactinemia) and if they showed any additional abnormality in hormonal parameters (LH, FSH, estradiol, progesterone, testosterone, prolactin, fasting glucose, fasting insulin, and thyroid stimulating hormone) taken at cycle days 2–5 following the baseline examinations. Current and lifetime somatic and mental disorders were carefully evaluated at baseline examination within an anamnestic clinical interview based on ICD-10 criteria. Specifically, no women met diagnostic criteria for PMS/PMDD, although it is worth noting that some women reported subthreshold premenstrual complaints. Participants who were included in the study were then assessed four times across the menstrual cycle for two consecutive cycles. All hormone assays, questionnaires and other measures were taken at the same points in time (see Fig. 1). For every completed cycle, participants received 600 Swiss Francs (at Zurich study site) or 500 Euros (at Hannover study site). This study followed the guidelines of the World Medical Association Declaration of Helsinki 1964, updated in October 2013, and was conducted after approval by the Swiss and the Hannover Ethics Committee for investigations involving human subjects. All participants provided written informed consent. Women were compensated for their expenditures associated

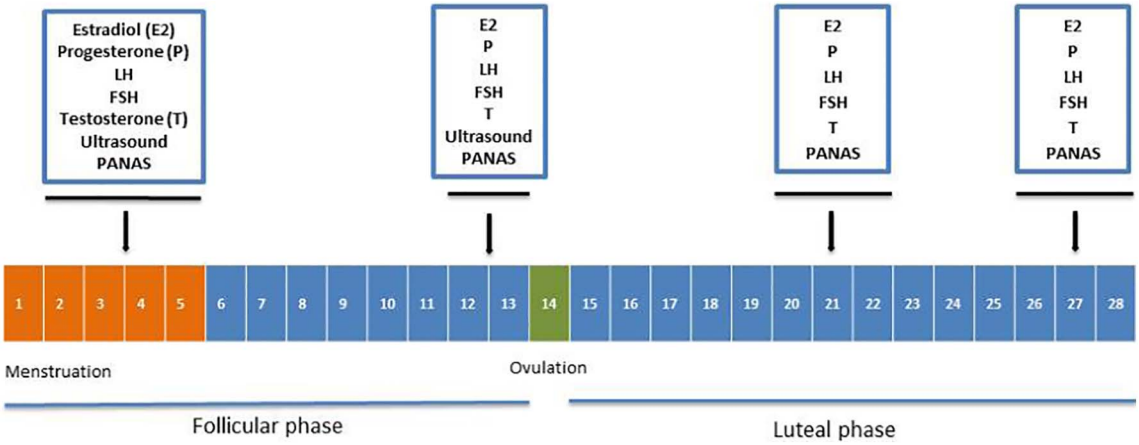

Fig. 1. Measurement occasions across the menstrual cycle.

with study participation. The study has been registered in clin.trial.gov (NCT02098668).

Data were collected from 87 menstruating women. Of those women, 58 presented no endocrinological pathology, 12 were diagnosed with endometriosis, 16 with polycystic ovary syndrome (PCOS) and one woman with hyperprolactinemia. Also, 12 women presented with obesity (defined as BMI > 30.0). Altogether 50 women were recruited in the Department of Psychiatry, Social Psychiatry and Psychotherapy, Medical School Hannover, Germany, and 37 women in the Clinic for Reproductive Endocrinology, University Hospital Zurich, Switzerland. All women with endometriosis, PCOS or hyperprolactinemia were recruited in Zurich. Word of mouth, direct invitation of eligible women in the consultations of the Clinic for Reproductive Endocrinology, University hospital Zurich, Switzerland and by gynecologists specialized in gynecological endocrinology as well as advertisement on the hospital and university boards were used for recruitment. A total of 67 women were re-assessed during a second menstrual cycle (Hannover: n = 47; Zurich: n = 20). The mean age at baseline visit was 30.2 years (SD = 5.5) and ranged from 20 to 40 years. The mean BMI was 25.0 (SD = 5.4) and ranged from 17.7 to 45.7. A total of n = 31 participants (35.6%) were married, n = 27 (31.0%) had children, and n = 27 (31.0%) had a university degree.

2.2. Hormone measurements and assays

For each woman with a cycle length of 28 ± 4 days a series of 8 measurements of hormonal parameters was scheduled at predefined days of the cycle (at cycle days 4, 7, 9 or 10, 12, 13, 17, 21, 28). At the first measurement on cycle days 2–5 a first transvaginal ultrasound was performed to exclude any cysts interfering with the menstrual cycle. A second ultrasound was performed around cycle day 11 to measure follicular development in order to place the pre-ovulatory measurement as precise as possible. When no dominant follicle could be demonstrated in the second ultrasound control additional measurements were performed in 4–5 day intervals until follicular development could be confirmed or cycle day 30 was reached. Ovulation tests based on urine LH measurements (Evial Ovulations test Midstream, Inopharm GmbH, Muri, Switzerland and Clearblue digital Ovulations test, SPD Swiss Precision Diagnostics GmbH, Geneva, Switzerland) were used to confirm the day of ovulation. These tests were started either 5 days prior to the earliest ovulation based on the previous 6 cycles or when a 14 mm follicle was seen through transvaginal ultrasound. At each visit blood samples were collected for hormonal assessment between 7.00 and 10.00 am. At four time points, i.e. cycle days 2–5 (menstrual), pre-ovulatory, mid-luteal and premenstrual, the participants took neuropsychological tests and completed various questionnaires in addition to blood sampling.

In Hannover blood samples were initially frozen at − 30 °C and then stored at − 80 °C after few days. In Zurich blood samples were sent to the laboratory immediately after the sample was collected in the morning. To avoid bias due to different laboratory procedures and measurement methods, all samples were analyzed by the laboratory in Zürich. Estradiol was measured using electrochemiluminescence immunoassays ECLIA (Elecsys® Estradiol II) based on polyclonal antibody (Roche Diagnostics GmbH, Penzberg, Germany) with a functional assay sensitivity of 44 pmol/L and a coefficient of variation (CV%) of < 7.7%. From January 15th 2015, the ECLIA (Elecsys® Estradiol III) based on monoclonal antibody (Roche Diagnostics GmbH, Penzberg, Germany) with a functional assay sensitivity to 91.8 pmol/L (25 pg/mL) and CV% to < 3.36% was applied. The measurement of LH, FSH, progesterone, testosterone, TSH, and prolactin were performed using electrochemiluminescence immunoassays (ECLIA) applied on Cobas e-602 immunoassay autoanalyzer (Roche Diagnostics GmbH, Penzberg, Germany). The functional analytical assay sensitivity for LH, FSH, progesterone, testosterone, TSH, and prolactin was 0.1 IU/L, 0.1 mIU/L, 0.48 nmol/L, 0.416 nmol/L, 0.014 mIU/L, and 1.00 μIU/mL (0.047 ng/mL), respectively. Total imprecision (intra-assay and inter-assay) of each assay was assessed by measuring twenty replicates of quality control samples over 20 days. Total imprecision expressed as coefficient of variation (CV%) for LH, FSH, progesterone, testosterone, TSH, and prolactin was < 2.2, 2.1, 5.1, 3.9, 2.5, and 1.3 respectively. All analyses described in this section were performed at the Institute of Clinical Chemistry, University Hospital Zurich. For all methods, external quality controls were carried out at regular intervals by the society for promoting quality assurance in medical laboratories (INSTAND, Duesseldorf, Germany) and Reference Institute for Bioanalytics (RfB, Bonn, Germany).

2.3. Measures of negative affect

Negative affect was measured with the respective subscale of the Positive and Negative Affect Schedule (PANAS) [39]. That scale measures negative affect based on 5 items rated on a five-point Likert scale ranging from 1 “not at all” to 5 “very severe”. Both the original scale [39] and the German adaptation applied in the present study [40] demonstrated good validity and reliability. In the present study the internal consistency of the negative affect subscale was good (Cronbach's $\alpha = 0.84$). The PANAS was applied at four consecutive time points across the cycle concurrently with the hormone assays, that is, at menstrual (t1), pre-ovulatory (t2), mid-luteal (t3) and premenstrual (t4) phase. Finally, self-reported depression was assessed with the Beck Depression Inventory (BDI) [41], which has shown excellent psychometric properties [42] and which is the most widely applied self-report measure for depression worldwide. The sum-score of the BDI has a

possible range from 0 “no depressive symptoms at all” to 63 “extremely severe depression”. Scores between 11 and 17 are considered mild to moderate depression and scores of 18 and higher as clinically relevant depression. In the present study the internal consistency of the sum-score was good (Cronbach's α = 0.84). The BDI was applied only once prior to the first cycle. In the present study the BDI was therefore used as a secondary measure.

2.4. Statistical analysis

The associations between repeated measures of PANAS negative affect and hormone levels were estimated using generalized estimating equations (GEE). These statistical models were introduced to fit regression analyses that account for within-subject correlation, which is an inherent part of longitudinal studies that rely on repeated outcome measures [43]. GEE are considered state of the art for longitudinal data analysis and superior to repeated measures ANOVA due to their psychometric properties [44,45]. GEE use all available data and impute missing values under the assumption of Missing Completely at Random (MCAR). Repeated measures of PANAS negative affect scores were successively entered as the outcome variables and the hormone measures separately as predictor variables. Because these measures were half-normally distributed, we fitted all models with inverse-Gauss (Wald) distribution and log link-function. The within-subject covariance was specified with the “unstructured” correlation type to avoid having any constraints on the covariance structure and a robust sandwich estimator was used to reduce the effects of outliers and influential observations. Because GEE pool together between-subject and within-subject effects, which limits the interpretation of results, we additionally computed a longitudinal intra-individual change model [46]. In such a model, only within-subject effects are considered by including relative change values between consecutive measurements of both the outcome variable and the predictor variable instead of absolute values for each time-point. Following Twisk [46], in these change models the covariance structure was specified as “independent”. Moreover, because change scores were approximately normally distributed, we fitted these models with normal distribution and identity link-function. Finally, because the BDI was assessed only once prior to the first cycle, we used this measure as an independent variable (predictor) and entered the hormone measures successively as the outcome (dependent variables). We fitted these models also with normal distributions and identity link-functions. Due to multiple testing (negative affect was successively regressed on 5 different hormones), we set the level of statistical significance at Bonferroni-corrected α = 0.01. All analyses were performed with SPSS 24 for Windows.

3. Results

Range, mean and standard deviation of all serum hormone levels are given in the Supplementary Table 1. A total of n = 4 and n = 3 women, respectively, had an anovulatory cycle at first and second menstrual cycle. Excluding these women did not alter the results reported below; therefore they were included in the analysis. Mean PANAS negative affect scores across cycles 1 and 2 are shown in Table 1. PANAS negative affect has a possible range from 1 to 5 and in the present study scores ranged from 1.0 to 4.2 (cycle 1) and from 1.0 to 3.9 (cycle 2). The estimated marginal means (95%-CI) across cycles 1 and 2, respectively, were 1.61 (1.51–1.72) and 1.51 (1.41–1.63). Mean scores at specific cycle phases remained highly stable across both cycles, with the largest standardized mean differences being very small (for cycles 1 and 2, respectively: Cohen's d < 0.11 and d < 0.26). In addition, there was no consistent pattern across cycles. While in cycle 1 mean scores remained almost perfectly stable, in cycle 2 they appeared to slightly decrease over the course of the cycle, though, noteworthy, that decline was marginally small and the sequentially Bonferroni-corrected linear polynomial contrast was statistically not significant (p = 0.262).

Table 1
PANAS negative affect scores across two menstrual cycles.

	Measurement occasion				Model effect
	T1 Mean (SE)	T2 Mean (SE)	T3 Mean (SE)	T4 Mean (SE)	p
Cycle 1 (n = 87)	1.61 (0.06)	1.64 (0.07)	1.62 (0.06)	1.58 (0.07)	0.772
Cycle 2 (n = 67)	1.60 (0.07)	1.53 (0.07)	1.46 (0.06)	1.48 (0.08)	0.091

Note.
T1: menstrual phase; T2: pre-ovulatory phase; T3: mid-luteal phase; T4: premenstrual phase.

Table 2
Mean intra-individual changes in PANAS negative affect scores across two menstrual cycles.

	Measurement occasion			Model effect
	T1 thru T2 Mean (SE)	T2 thru T3 Mean (SE)	T3 thru T4 Mean (SE)	p
Cycle 1 (n = 87)	0.01 (0.05)	0.02 (0.06)	− 0.03 (0.07)	0.887
Cycle 2 (n = 67)	− 0.08 (0.06)	− 0.07 (0.06)	0.08 (0.07)	0.127

Note.
T1: menstrual phase; T2: pre-ovulatory phase; T3: mid-luteal phase; T4: premenstrual phase.

In accordance with this evidence for overall stability across the cycle, intra-individual change remained almost unaltered across cycle 1 and substantially stable across cycle 2 (see Table 2). Again no statistically significant effects emerged. Adjusting the association between cycle phase and negative affect for obesity, endometriosis or PCOS did not alter the results. In addition, running the analyses for women with endocrinological disorders (including women with endometriosis, PCOS and hyperprolactinemia) separately did not produce divergent findings.

With respect to overall associations between PANAS negative affect and hormone levels across the cycle (between- and within-subject effects combined), no single effect reached statistical significance at Bonferroni corrected α = 0.01 (see Table 3). Adjusting for obesity and endocrinological disorders revealed that women with endometriosis had significantly higher overall mean scores in oestrogen, progesterone, LH and testosterone, but these effects did not alter the association between sex hormones and negative affect at specific cycle phases. In other words, the null-association between hormone levels and negative affect held when the significant main effect of endometriosis was statistically controlled for. With respect to associations between change scores (within-subject effects only) we found one significant association between change in PANAS negative affect and change in FSH from pre-ovulatory to mid-luteal phase (T2 thru T3) during the first cycle (see Table 4). That association corresponds to a medium effect size of r = − 0.30. However, the association between changes in FSH and negative affect did not remain statistically significant at Bonferroni-corrected significance level of α = 0.01 when adjusted for obesity (positive association with FSH) and endocrinological disorders (effect size: r = − 0.24, p = 0.035). Moreover, that unadjusted association did not replicate at Bonferroni-corrected significance level of α = 0.01 in the second cycle (r = − 0.16, p = 0.199; small effect).

Finally, we also regressed all repeated sex hormone levels on BDI scores obtained during baseline visit prior to the first cycle. In the present study the BDI ranged from 0 to 28 (mean = 5.01; SD = 5.00). According to established cut-offs, 9 women had mild to moderate depression and 2 had clinically relevant depression. BDI scores did not

Table 3
Associations between PANAS negative affect scores and hormone levels across two menstrual cycles.

	Hormones	Measurement occasion			
		T1 B (SE)	T2 B (SE)	T3 B (SE)	T4 B (SE)
Cycle 1 (n = 87)	Oestrogen	− 0.03 (0.04)	− 0.02 (0.04)	0.00 (0.05)	0.00 (0.02)
	Progesterone	0.05 (0.04)	0.03 (0.04)	0.04 (0.04)	0.02 (0.02)
	LH	0.00 (0.04)	− 0.02 (0.04)	0.07 (0.04)	− 0.03 (0.03)
	FSH	0.01 (0.06)	0.10 (0.05)	0.02 (0.06)	0.05 (0.03)
	Testosterone	0.07 (0.04)	0.08 (0.03)	0.03 (0.04)	0.04 (0.04)
Cycle 2 (n = 67)	Oestrogen	− 0.02 (0.03)	− 0.03 (0.03)	− 0.02 (0.02)	0.00 (0.04)
	Progesterone	− 0.01 (0.04)	0.00 (0.02)	0.00 (0.04)	− 0.02 (0.05)
	LH	0.02 (0.03)	− 0.02 (0.03)	− 0.01 (0.03)	− 0.07 (0.03)
	FSH	0.03 (0.03)	− 0.02 (0.03)	− 0.03 (0.03)	− 0.07 (0.04)
	Testosterone	− 0.02 (0.05)	0.04 (0.04)	− 0.03 (0.04)	− 0.05 (0.05)

Note.
T1: menstrual phase; T2: pre-ovulatory phase; T3: mid-luteal phase; T4: premenstrual phase.

Table 4
Associations between intra-individual changes in PANAS negative affect scores and changes in hormone levels across two menstrual cycles.

	Hormones	Measurement occasion		
		T1 thru T2 B (SE)	T2 thru T3 B (SE)	T3 thru T4 B (SE)
Cycle 1 (n = 87)	Oestrogen	− 0.02 (0.05)	0.05 (0.07)	− 0.07 (0.09)
	Progesterone	− 0.02 (0.05)	0.08 (0.06)	0.02 (0.07)
	LH	0.05 (0.05)	− 0.05 (0.06)	0.04 (0.05)
	FSH	0.01 (0.06)	− 0.13 (0.05)*	0.10 (0.12)
	Testosterone	0.01 (0.07)	0.04 (0.06)	0.03 (0.08)
Cycle 2 (n = 67)	Oestrogen	0.02 (0.04)	− 0.04 (0.06)	0.01 (0.06)
	Progesterone	− 0.05 (0.05)	− 0.01 (0.05)	− 0.04 (0.10)
	LH	0.00 (0.04)	− 0.10 (0.07)	− 0.05 (0.04)
	FSH	0.05 (0.03)	− 0.10 (0.08)	− 0.08 (0.06)
	Testosterone	0.01 (0.05)	− 0.06 (0.04)	− 0.02 (0.05)

Note.
T1: menstrual phase; T2: pre-ovulatory phase; T3: mid-luteal phase; T4: premenstrual phase.
* Statistically significant parameter at Bonferroni corrected $\alpha = 0.01$.

relate statistically significantly to hormone fluctuations across cycle 1. The effect sizes expressed as correlation coefficients were as follows. For oestrogen $r = 0.04$ ($p = 0.698$); for progesterone $r = -0.02$ ($p = 0.882$); for LH $r = 0.19$ ($p = 0.082$); for FSH $r = 0.02$ ($p = 0.871$); and for testosterone $r = 0.07$ ($p = 0.489$). That is, effect sizes were small (refers to LH) or close to zero (all other hormones), demonstrating that differences in BDI scores at baseline did not relate to variance in hormone levels across the first cycle.

4. Discussion

This study was designed to explore the association between sex hormones and negative affect across the menstrual cycle. Hormones, in particular oestrogen and progesterone, may be crucial in the aetiopathology of cycle-related mood disturbances, though the exact role that they play in the occurrence of negative affect and related disorders such as PMS and PMDD is mostly unclear [1–3]. In accord with some findings (e.g. [15]), but in contrast with others (e.g. [26]), we did not find that negative affect was increased premenstrually. As for associations with hormone levels, in the present study we found only one statistically significant effect during the first cycle, specifically, a significant negative association between changes in FSH and negative affect from pre-ovulatory to mid-luteal phase (within-subject effect). That association was of medium effect size ($r = -0.30$, $p < 0.01$), but did not replicate when adjusted for endocrinological disorders in the first cycle as well as unadjusted in the second cycle ($r = -0.16$, $p = 0.199$). With respect to the most researched sex hormones, that is, oestrogen and progesterone, we found no association with negative affect as assessed with the PANAS. Self-reported depression as obtained with the BDI prior to the first cycle did neither relate to hormone levels across the first cycle. Further note that this lack of statistically significant associations was not merely due to lack of power, as estimated effect sizes were marginally small and the achieved power to detect a medium effect size in the first cycle was > 0.80 even at Bonferroni-corrected significance level of $\alpha = 0.01$. Altogether these findings have important scientific and practical implications, as we will discuss in detail below.

The literature on fluctuations of mood symptoms across the cycle is largely inconsistent in women not using hormonal contraceptives [15,16,23–26]. Unfortunately, most of these studies, specifically [15,23–25], used small samples of women not taking hormonal contraceptives (all $n < 45$). As such underpowered studies are prone to inflated effect sizes and both false-positive and false-negative results [34], it remains open to debate whether negative affect fluctuates across the cycle. According to our data, based on two consecutive menstrual cycles and both within- and between-subject effects, an aggregate measure of mood symptoms comprising among others irritability, distress and nervousness remains highly stable across the cycle. In particular, there was no symptom increase premenstrually, suggesting that in women with and without endocrinological disorders, mood symptoms are not related to specific cycle phases.

The negative findings for oestrogen and progesterone found in the present study correspond with the null-results reported in various observational studies [12,14–16] as well as in randomised controlled trials [7,8,10,47] and suggest that a direct and uniform association between sex hormone serum levels and negative affect is unlikely. In agreement with these results, treating cycle-related depression and PMS/PMDD with hormonal contraceptives proves to be ineffective in most women [10]. Therefore, it is now supposed that sex hormones influence negative affect, PMS, PMDD and depression through their effects on the brain, where they modulate both the stress response and the serotonergic neurotransmitter systems [3,4,48]. Future studies should therefore not focus on hormone levels as the primary target for intervention, but rather on overarching complex neuroendocrinological circuits, neurotransmitter receptor profiles, second messenger systems, and (epi-)genetic factors [19]. Moreover, the very high stability of negative affect across the cycle reported in this study indicates that these mood states are manifestations of a stable underlying personality trait [49,50], here specifically neuroticism [51]. In phenotypic [52–54] and genetic research [55,56], the personality trait of neuroticism has shown to be the strongest extractable predictor of broad negative affect, including severe disorders of the internalising disease spectrum such as major depression and anxiety disorders (for

reviews, see [51,57,58]). Some research has further provided evidence that neuroticism predicts menopausal mood symptoms [20] and premenstrual negative affect ([59,60]; but see also [61]). Future studies on the neuroendocrinological substrates of negative affect, including PMS and PMDD, are therefore advised to account for neuroticism and to target the biological markers underlying this personality trait, which, not accidentally, involve both the stress response system and serotonergic pathways [62,63].

This said we also want to acknowledge the following four major limitations. Firstly, our measures of negative affect relied exclusively on self-report, which may possibly involve some bias. Secondly, though our sample was considerably larger than those commonly applied in neuroendocrinological research, a sample size of $n > 100$ would be preferable due to the substantial variance in hormone levels at given cycle phases [64]. Thirdly, the data from the second cycle that served as an external validation criterion consisted of a subset of women from the first cycle. Therefore, data from the two cycles were not independent. Fourthly, possible moderators such as neuroticism as well as genetic and neurobiological factors were not considered in this report. In conclusion, and despite these limitations, this work provides evidence that self-reported negative affect remains stable across the menstrual cycle and that it is not directly and uniformly related to fluctuating hormone levels. Future research should focus on neuroticism as a possible moderator of mood symptom fluctuations and also consider neurotransmitter profiles and (epi-)genetic factors.

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Conflicts of interest

All authors report none.

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References

[1] M.A. Farage, T.W. Osborn, A.B. MacLean, Cognitive, sensory, and emotional changes associated with the menstrual cycle: a review, Arch. Gynecol. Obstet. 278 (4) (2008) 299–307.
[2] C.N. Soares, B. Zitek, Reproductive hormone sensitivity and risk for depression across the female life cycle: a continuum of vulnerability? J. Psychiatry Neurosci. 33 (4) (2008) 331–343.
[3] K.A. Yonkers, P.M. O'Brien, E. Eriksson, Premenstrual syndrome, Lancet 371 (9619) (2008) 1200–1210.
[4] C. Zahn-Waxler, E.A. Shirtcliff, K. Marceau, Disorders of childhood and adolescence: gender and psychopathology, Annu. Rev. Clin. Psychol. 4 (2008) 275–303.
[5] J. Cunningham, K.A. Yonkers, S. O'Brien, E. Eriksson, Update on research and treatment of premenstrual dysphoric disorder, Harv. Rev. Psychiatry. 17 (2) (2009) 120–137.
[6] A. Rapkin, A review of treatment of premenstrual syndrome and premenstrual dysphoric disorder, Psychoneuroendocrinology 28 (Suppl. 3) (2003) 39–53.
[7] C.A. Graham, B.B. Sherwin, A prospective treatment study of premenstrual symptoms using a triphasic oral contraceptive, J. Psychosom. Res. 36 (3) (1992) 257–266.
[8] H. Joffe, L.S. Cohen, B.L. Harlow, Impact of oral contraceptive pill use on premenstrual mood: predictors of improvement and deterioration, Am. J. Obstet. Gynecol. 189 (6) (2003) 1523–1530.
[9] K.A. Yonkers, C. Brown, T.B. Pearlstein, M. Foegh, C. Sampson-Landers, A. Rapkin, Efficacy of a new low-dose oral contraceptive with drospirenone in premenstrual dysphoric disorder, Obstet. Gynecol. 106 (3) (2005) 492–501.
[10] U. Halbreich, Selective serotonin reuptake inhibitors and initial oral contraceptives for the treatment of PMDD: effective but not enough, CNS Spectr. 13 (7) (2008) 566–572.
[11] M. Wang, L. Seippel, R.H. Purdy, T. Backstrom, Relationship between symptom severity and steroid variation in women with premenstrual syndrome: study on serum pregnenolone, pregnenolone sulfate, 5 alpha-pregnane-3,20-dione and 3 alpha-hydroxy-5 alpha-pregnan-20-one, J. Clin. Endocrinol. Metab. 81 (3) (1996) 1076–1082.
[12] A. Prasad, E.F. Schisterman, K.C. Schliep, K.A. Ahrens, L.A. Sjaarda, N.J. Perkins, et al., Depressive symptoms and their relationship with endogenous reproductive hormones and sporadic anovulation in premenopausal women, Ann. Epidemiol. 24 (12) (2014) 920–924.
[13] A.J. Rapkin, M. Morgan, L. Goldman, D.W. Brann, D. Simone, V.B. Mahesh, Progesterone metabolite allopregnanolone in women with premenstrual syndrome, Obstet. Gynecol. 90 (5) (1997) 709–714.
[14] D.R. Rubinow, M.C. Hoban, G.N. Grover, D.S. Galloway, P. Roy-Byrne, R. Andersen, et al., Changes in plasma hormones across the menstrual cycle in patients with menstrually related mood disorder and in control subjects, Am. J. Obstet. Gynecol. 158 (1) (1988) 5–11.
[15] R.G. Laessle, R.J. Tuschl, U. Schweiger, K.M. Pirke, Mood changes and physical complaints during the normal menstrual cycle in healthy young women, Psychoneuroendocrinology 15 (2) (1990) 131–138.
[16] A.T. Harvey, C.L. Hitchcock, J.C. Prior, Ovulation disturbances and mood across the menstrual cycles of healthy women, J. Psychosom. Obstet. Gynaecol. 30 (4) (2009) 207–214.
[17] E.W. Freeman, Associations of depression with the transition to menopause, Menopause 17 (4) (2010) 823–827.
[18] K.K. Vesco, E.M. Haney, L. Humphrey, R. Fu, H.D. Nelson, Influence of menopause on mood: a systematic review of cohort studies, Climacteric 10 (6) (2007) 448–465.
[19] M.P. Hengartner, Subtle scientific fallacies undermine the validity of neuroendocrinological research: do not draw premature conclusions on the role of female sex hormones, Front. Behav. Neurosci. 11 (2017) 3.
[20] W. Rossler, V. Ajdacic-Gross, A. Riecher-Rossler, J. Angst, M.P. Hengartner, Does menopausal transition really influence mental health? Findings from the prospective long-term Zurich study, World Psychiatry. 15 (2) (2016) 146–154.
[21] J. Vivian-Taylor, M. Hickey, Menopause and depression: is there a link? Maturitas 79 (2) (2014) 142–146.
[22] R.H. Moos, B.S. Kopell, F.T. Melges, I.D. Yalom, D.T. Lunde, R.B. Clayton, et al., Fluctuations in symptoms and moods during the menstrual cycle, J. Psychosom. Res. 13 (1) (1969) 37–44.
[23] L.A. Wilcoxon, S.L. Schrader, C.W. Sherif, Daily self-reports on activities, life events, moods, and somatic changes during the menstrual cycle, Psychosom. Med. 38 (6) (1976) 399–417.
[24] S. Abraham, G. Luscombe, I. Soo, Oral contraception and cyclic changes in premenstrual and menstrual experiences, J. Psychosom. Obstet. Gynaecol. 24 (3) (2003) 185–193.
[25] V. Natale, P. Albertazzi, Mood swings across the menstrual cycle: a comparison between oral contraceptive users and non-users, Biol. Rhythm. Res. 37 (6) (2006) 489–495.
[26] J. Kiesner, J. Mendle, T.A. Eisenlohr-Moul, M. Pastore, Cyclical symptom change across the menstrual cycle, Clin. Psychol. Sci. 4 (5) (2016) 882–894.
[27] M.P. Hengartner, S.N. Lehmann, Why psychiatric research must abandon traditional diagnostic classification and adopt a fully dimensional scope: two solutions to a persistent problem, Front. Psychiatry (2017) (n press).
[28] R. Plomin, C.M. Haworth, O.S. Davis, Common disorders are quantitative traits, Nat. Rev. Genet. 10 (12) (2009) 872–878.
[29] S.E. Hyman, The diagnosis of mental disorders: the problem of reification, Annu. Rev. Clin. Psychol. 6 (2010) 155–179.
[30] D. Watson, L.A. Clark, Clinical diagnosis at the crossroads, Clin. Psychol. Sci. Pr. 13 (3) (2006) 210–215.
[31] T.A. Widiger, D.B. Samuel, Diagnostic categories or dimensions? A question for the diagnostic and statistical manual of mental disorders—fifth edition, J. Abnorm. Psychol. 114 (4) (2005) 494–504.
[32] B.N. Cuthbert, T.R. Insel, Toward the future of psychiatric diagnosis: the seven pillars of RDoC, BMC Med. 11 (2013) 126.
[33] R. Kotov, R.F. Krueger, D. Watson, T.M. Achenbach, R.R. Althoff, R.M. Bagby, et al., The hierarchical taxonomy of psychopathology (HiTOP): a dimensional alternative to traditional nosologies, J. Abnorm. Psychol. (2017), http://dx.doi.org/10.1037/abn0000258.
[34] K.S. Button, J.P. Ioannidis, C. Mokrysz, B.A. Nosek, J. Flint, E.S. Robinson, et al., Power failure: why small sample size undermines the reliability of neuroscience, Nat. Rev. Neurosci. 14 (5) (2013) 365–376.
[35] M. Bakker, A. van Dijk, J.M. Wicherts, The rules of the game called psychological science, Perspect. Psychol. Sci. 7 (6) (2012) 543–554.
[36] E.J. Wagenmakers, R. Wetzels, D. Borsboom, H.L.J. van der Maas, R.A. Kievit, An agenda for purely confirmatory research, Perspect. Psychol. Sci. 7 (6) (2012) 632–638.
[37] M.P. Hengartner, Estrogen-based therapies and depression in women who naturally enter menopause before population average, JAMA Psychiatry 73 (8) (2016) 874.
[38] R.E. Kirk, Practical significance: a concept whose time has come, Educ. Psychol. Meas. 56 (5) (1996) 746–759.
[39] D. Watson, L.A. Clark, A. Tellegen, Development and validation of brief measures of positive and negative affect: the PANAS scales, J. Pers. Soc. Psychol. 54 (6) (1988) 1063–1070.
[40] H.W. Krohne, B. Egloff, C.-W. Kohlmann, A. Tausch, Investigations with a German version of the positive and negative affect schedule (PANAS), Diagnostica. 42 (2) (1996) 139–156.
[41] A.T. Beck, J. Erbaugh, C.H. Ward, J. Mock, M. Mendelsohn, An inventory for measuring depression, Arch. Gen. Psychiatry 4 (6) (1961) 561–571.
[42] A.T. Beck, R.A. Steer, M.G. Garbin, Psychometric properties of the Beck depression

inventory — 25 years of evaluation, Clin. Psychol. Rev. 8 (1) (1988) 77–100.
[43] S.L. Zeger, K.Y. Liang, P.S. Albert, Models for longitudinal data: a generalized estimating equation approach, Biometrics 44 (4) (1988) 1049–1060.
[44] G.A. Ballinger, Using generalized estimating equations for longitudinal data analysis, Organ. Res. Methods 7 (2) (2004) 127–150.
[45] R.D. Gibbons, D. Hedeker, S. DuToit, Advances in analysis of longitudinal data, Annu. Rev. Clin. Psychol. 6 (2010) 79–107.
[46] J.W.R. Twisk, Applied Longitudinal Data Analysis for Epidemiology: A Practical Guide, Cambridge University Press, Cambridge, 2003.
[47] K. Wyatt, P. Dimmock, P. Jones, M. Obhrai, S. O'Brien, Efficacy of progesterone and progestogens in management of premenstrual syndrome: systematic review, BMJ 323 (7316) (2001) 776–780.
[48] E.F. Naninck, P.J. Lucassen, J. Bakker, Sex differences in adolescent depression: do sex hormones determine vulnerability? J. Neuroendocrinol. 23 (5) (2011) 383–392.
[49] W. Fleeson, P. Gallagher, The implications of Big Five standing for the distribution of trait manifestation in behavior: fifteen experience-sampling studies and a meta-analysis, J. Pers. Soc. Psychol. 97 (6) (2009) 1097–1114.
[50] W. Fleeson, E. Jayawickreme, Whole trait theory, J. Res. Pers. 56 (2015) 82–92.
[51] B.B. Lahey, Public health significance of neuroticism, Am. Psychol. 64 (4) (2009) 241–256.
[52] J.W. Griffith, R.E. Zinbarg, M.G. Craske, S. Mineka, R.D. Rose, A.M. Waters, et al., Neuroticism as a common dimension in the internalizing disorders, Psychol. Med. 40 (7) (2010) 1125–1136.
[53] M.P. Hengartner, V. Ajdacic-Gross, C. Wyss, J. Angst, W. Rossler, Relationship between personality and psychopathology in a longitudinal community study: a test of the predisposition model, Psychol. Med. 46 (8) (2016) 1693–1705.
[54] M.P. Hengartner, W. Kawohl, H. Haker, W. Rossler, V. Ajdacic-Gross, Big Five personality traits may inform public health policy and preventive medicine: Evidence from a cross-sectional and a prospective longitudinal epidemiologic study in a Swiss community, J. Psychosom. Res. 84 (2016) 44–51.
[55] J.M. Hettema, M.C. Neale, J.M. Myers, C.A. Prescott, K.S. Kendler, A population-based twin study of the relationship between neuroticism and internalizing disorders, Am. J. Psychiatry 163 (5) (2006) 857–864.
[56] K.S. Kendler, J. Myers, The genetic and environmental relationship between major depression and the five-factor model of personality, Psychol. Med. 40 (5) (2010) 801–806.
[57] M.P. Hengartner, The detrimental impact of maladaptive personality on public mental health: a challenge for psychiatric practice, Front. Psychiatry 6 (2015) 87.
[58] J. Ormel, B.F. Jeronimus, R. Kotov, H. Riese, E.H. Bos, B. Hankin, et al., Neuroticism and common mental disorders: meaning and utility of a complex relationship, Clin. Psychol. Rev. 33 (5) (2013) 686–697.
[59] A. Miller, H. Vo, L. Huo, C. Roca, P.J. Schmidt, D.R. Rubinow, Estrogen receptor alpha (ESR-1) associations with psychological traits in women with PMDD and controls, J. Psychiatr. Res. 44 (12) (2010) 788–794.
[60] C. Ross, G. Coleman, C. Stojanovska, Relationship between the NEO personality inventory revised neuroticism scale and prospectively reported negative affect across the menstrual cycle, J. Psychosom. Obstet. Gynaecol. 22 (3) (2001) 165–176.
[61] E.W. Freeman, E. Schweizer, K. Rickels, Personality factors in women with premenstrual syndrome, Psychosom. Med. 57 (5) (1995) 453–459.
[62] J. Ormel, A. Bastiaansen, H. Riese, E.H. Bos, M. Servaas, M. Ellenbogen, et al., The biological and psychological basis of neuroticism: current status and future directions, Neurosci. Biobehav. Rev. 37 (1) (2013) 59–72.
[63] D.P. Soskin, J.R. Carl, J. Alpert, M. Fava, Antidepressant effects on emotional temperament: toward a biobehavioral research paradigm for major depressive disorder, CNS Neurosci. Ther. 18 (6) (2012) 441–451.
[64] I. Sundstrom Poromaa, M. Gingnell, Menstrual cycle influence on cognitive function and emotion processing-from a reproductive perspective, Front. Neurosci. 8 (2014) 380.