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Progesterone and women's anxiety across the menstrual cycle

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ABSTRACT

Animal models and a few human investigations suggest progesterone may be associated with anxiety. Progesterone naturally fluctuates across the menstrual cycle, offering an opportunity to understand how within-person increases in progesterone and average progesterone levels across the cycle correspond to women's anxiety. Across two longitudinal studies, we simultaneously modeled the between- and within-person associations between progesterone and anxiety using multilevel modeling. In Study 1, 100 Polish women provided saliva samples and reported their anxiety at three phases of the menstrual cycle: follicular, peri-ovulatory, and luteal. A significant between-person effect emerged, revealing that women with higher average progesterone levels across their cycles reported higher levels of anxiety than women with lower progesterone cycles. This effect held controlling for estradiol. In Study 2, 61 American women provided saliva samples and reported their attachment anxiety during laboratory sessions during the same three cycle phases. A significant between-person and within-person association emerged: women with higher average progesterone levels reported higher levels of attachment anxiety, and as women's progesterone levels increased across their cycles, so too did their attachment anxiety. These effects held controlling for cortisol. In sum, both studies provide support for a link between menstrual cycle progesterone levels and subjective anxiety.

1. Introduction

Women's bodies undergo an important set of physiological changes over the course of each menstrual cycle. After ovulation, the ovaries release the steroid hormone progesterone to prepare the uterus for a possible pregnancy. Beyond causing physical changes across the menstrual cycle, progesterone has also been linked to changes in psychological states that may be functional in preparing for possible pregnancy. For example, research on both animals and humans is beginning to uncover a relation between progesterone and anxiety (Wirth, 2011), an emotion that functions to keep organisms vigilant to signs of danger (see Marks and Nesse, 1994). The current investigation examined how cyclical increases in progesterone across the menstrual cycle, as well as between-person differences in average cyclical progesterone levels, correspond to women's anxiety. We prospectively measured anxiety and progesterone three times over the course of one menstrual cycle in two samples of women. In Study 2, we extended our examination to a specific facet of anxiety—attachment anxiety.

1.1. Progesterone and anxiety

Research suggests a complex and potentially cyclic relationship between progesterone, stress, and anxiety (see Wirth, 2011 for a review). In rodents, for example, progesterone and its metabolites (e.g., allopregnanalone) rise in response to stressors, such as foot shock and swim stress (Barbaccia et al., 1996; Purdy et al., 1991). Progesterone's conversion to allopregnanalone, in turn, decreases anxiety and behaviors reflecting stress (Reddy et al., 2005; Rhodes and Frye, 2001). When progesterone is administered directly, rodents exhibit fewer anxiety behaviors (Toufexis et al., 2004).

Most investigations of humans have uncovered a positive association between progesterone and anxiety (but see Le Mellédo et al., 2001). Many of these studies reveal that progesterone increases in response to stressors (Childs et al., 2010; Droogleever Fortuyn et al., 2004; Maner et al., 2010; Roca et al., 2003; Seidel et al., 2013; Wirth and Schultheiss, 2006). For example, Wirth and Schultheiss (2006) demonstrated that fear of social rejection increased progesterone levels.

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Some research also suggests that directly administering progesterone predicts increased anxiety (Klatzkin et al., 2006; Roca et al., 2003; Van Wingen et al., 2007). For example, Roca et al. (2003) demonstrated that direct administration of progesterone augmented women's hormonal stress response to a treadmill stressor, suggesting that absolute levels of progesterone can increase the intensity of the stress response. This positive association between progesterone and anxiety may have implications for how anxiety fluctuates across the menstrual cycle.

Progesterone concentrations naturally shift across the menstrual cycle, and this cyclical pattern offers a unique opportunity to explore how changes in progesterone correspond to changes in anxiety. Indeed, several investigations have provided evidence suggestive of a positive association between cyclical increases in progesterone and anxiety (Gonda et al., 2008; Nillni et al., 2012). In one study, for example, women prone to experience anxiety (compared to women less prone) exhibited a stronger cognitive panic response during their luteal phase, when progesterone levels are elevated, compared to their follicular phase, when progesterone levels are lower (Nillni et al., 2012). A similar pattern appears to emerge among nonclinical populations. In another study, women reported higher anxiety and interpersonal sensitivity in the luteal phase compared to the follicular phase of their cycle (Gonda et al., 2008). Although such findings are suggestive, they are limited by their comparisons between cycle phases, rather than directly estimating the association between circulating progesterone levels and anxiety.

Other research suggests a positive association between increases in progesterone across the cycle and anxiety using more socially relevant indicators (Conway et al., 2007; Derntl et al., 2013; Van Veen et al., 2009). For example, women diagnosed with generalized social anxiety disorder retrospectively reported that their social anxiety symptoms worsened over the course of the menstrual cycle, as progesterone levels typically increase (van Veen et al., 2009). In a non-clinical sample, women perceived fearful faces as particularly intense at times during their cycles when progesterone levels were heightened compared to when progesterone levels were lower (Conway et al., 2007). Relatedly, women's cyclical progesterone levels predicted their attention to social stimuli (Maner and Miller, 2014) and desire for social affiliation (Schultheiss et al., 2003). Taken together, these findings demonstrate that cyclical increases in progesterone correspond to heightened social sensitivity, which might reflect heightened levels of anxiety in social domains, such as interpersonal anxiety.

1.2. Current investigation

The current investigation tested whether within-person cyclical increases in progesterone were associated with anxiety by prospectively measuring women's progesterone and anxiety at three time points over the course of one menstrual cycle in two independent samples: community Polish women (Study 1) and American undergraduate women (Study 2). We examined these relations using both a measure of general anxiety (Study 1) and, given the studies finding an association between progesterone and social sensitivity, one specific, interpersonal form of anxiety: attachment anxiety (Study 2). Analyses also simultaneously estimated the between-person associations between average levels (across the cycle) of anxiety and progesterone.

Although previous research suggests a positive association between progesterone and anxiety, we are aware of no studies that have simultaneously examined the association between progesterone and anxiety both between women and within-women's menstrual cycles in nonclinical samples. Although progesterone rises over the course of each menstrual cycle, recent evidence suggests that average cyclical progesterone levels can vary from cycle to cycle within the same woman, suggesting both intra- and inter-cycle variation in progesterone (Eisenlohr-Moul and Owens, 2016; Jasienska et al., 2017; Jasienska and Jasienski, 2008). It is important then to understand how both sources of variance in progesterone –within cycle increases and average cycle levels –are associated with anxiety. The current investigation therefore employed multilevel modeling to examine whether within-person changes in progesterone *and* between-person differences in average progesterone levels are associated with anxiety. To better isolate the independent effects of progesterone, we also controlled for estradiol, another steroid hormone that varies across the menstrual cycle (Study 1), and cortisol, which is often associated with anxiety (Study 2; Dickerson and Kemeny, 2004; Mantella et al., 2008).

2. Study 1

Study 1 relied on a sample of community Polish women to examine whether within-person cyclical variation in progesterone and between-person average progesterone levels were associated with self-reported anxiety. During three lab sessions, occurring at the follicular, peri-ovulatory, and luteal phases of their menstrual cycles, participants provided saliva samples that were assayed for progesterone and estradiol, and reported their levels of anxiety. We controlled for women's estradiol levels because estradiol also varies across the menstrual cycle (Gilbert, 2000; Jasienska et al., 2017). Furthermore, some research indicates an association between estradiol and anxiety (e.g., Kajantie and Phillips, 2006), making it important to disentangle the association between progesterone and anxiety from any association with estradiol.

2.1. Method

2.1.1. Participants

Women were recruited through mailing lists, social media, and word of mouth in the Malopolska region of Poland. Women were eligible to participate if they had no history of diabetes, no diagnosed reproductive problems, regular menstrual cycles (consecutive cycles of similar length—within +/− 5 days), and had not been pregnant, breastfed, or used hormonal contraception within three months prior to participation. We recruited participants and collected data for 12 months. Of the 110 participants recruited, 102 attended all planned meetings. Because we could not assess hormone levels from any of the three samples of two women (one for progesterone and one for estradiol), our final sample included 100 women. Of the 300 possible assessments (100 participants × 3 assessments), we had corresponding assessments between progesterone and anxiety for 262 assessments and between progesterone, anxiety, and estradiol for 259 assessments. Participants were just under 30 years old on average (M_{age} = 28.82, SD = 4.6, age range: 18–37 years), all were Caucasian, and 71 (69.6%) were in long-term relationships (at least 6 months in length).

2.1.2. Procedure

Analyses focused on salivary progesterone and estradiol levels from the days women visited the lab, during which they also reported their level of anxiety (see below). These lab sessions were scheduled in the early follicular phase (between the 2nd and 8th day after menstrual onset), at peri-ovulatory phase [no later than 72 h after a luteinizing hormone (LH) surge or if the tests did not indicate an LH surge, on the 20th day of the cycle] and in mid-luteal phase (about one week after ovulation or participants' second session). To detect ovulation, participants were given LH ovulation kits, urine cups, and written instructions for conducting urine tests. LH typically surges one to two days prior to ovulation (Testart and Frydman, 1982) and luteinizing tests have been shown to be highly accurate in detecting ovulation (Guermandi et al., 2001). Women were asked to conduct LH tests from days 10–20 of their cycles or until a test indicated a positive result. Sixty-seven (65.7%) participants had a confirmed LH surge.

At each laboratory session, women completed a large survey packet presented in Polish. A section of the packet asked about their current mood. Particularly relevant to the investigation's primary hypothesis, one item asked women to indicate the extent to which they agreed with the statement “I feel anxious today” on a 7-point Likert scale

Table 1
Descriptive statistics for variables in Studies 1 and 2.

Measure	Follicular *M* (*SD*)	Ovulatory *M* (*SD*)	Luteal *M* (*SD*)	Cycle Average *M* (*SD*)
Study 1				
Progesterone (pg/mL)	51.70 (49.97)	94.37 (89.08)	108.88 (85.46)	84.04 (79.98)
Estradiol (pg/mL)	6.95 (6.87)	8.40 (7.67)	7.60 (6.85)	7.65 (7.15)
Anxiety	4.64 (1.90)	5.03 (1.74)	4.71 (1.90)	4.79 (1.85)
Study 2				
Progesterone (pg/mL)	16.74 (13.50)	38.21 (37.53)	79.40 (60.19)	44.82 (49.03)
Cortisol (ng/mL)	0.53 (0.30)	0.49 (0.30)	0.50 (0.21)	0.51 (0.27)
Attachment anxiety	54.92 (21.26)	55.0 (23.42)	51.78 (23.16)	53.91 (22.55)
Attachment avoidance	42.21 (20.77)	42.47 (20.64)	43.90 (22.45)	42.86 (21.19)

(1 = *Strongly Disagree* to 7 = *Strongly Agree*).

2.1.3. Hormone assays

Levels of 17-α-hydroxy-progesterone and 17-beta-estradiol were assessed from saliva samples. Women collected saliva samples after waking, at least 30 min after drinking, eating, or smoking. Women were instructed to freeze their saliva samples immediately after collection and to bring the frozen samples to the laboratory in portable freezers. Assays were conducted using commercially available hormonal assays of DRG International Inc. Elisa plates SLV3140 (sensitivity: 2.5 pg/mL; standard range: 10–5000 pg/mL). We conducted the assays in duplicate to enhance measurement validity. We controlled for the quality of hormonal measurements by including samples of known concentrations ("pools") of hormones in duplicates (19 control pools per plate). Inter-assay coefficients of variability (CV) were 14.1% for progesterone and 10.1% for estradiol, and intra-assay CVs were 4.9% for progesterone and 7.5% for estradiol (Schultheiss and Stanton, 2009).

2.2. Results

We examined between-phase differences in key variables using one-way ANOVA analyses. As expected, progesterone levels increased across menstrual cycle phases, $F(2,281) = 14.44$, $p < 0.001$, $\eta^2 = 0.09$, such that follicular levels were lower than both peri-ovulatory levels ($p < 0.001$) and luteal levels ($p < 0.001$). Peri-ovulatory and luteal phase levels did not differ significantly from each other ($p = 0.402$). Estradiol levels showed the expected trend across the cycle, but differences among phases were not statistically significant, $F(2,281) = 1.00$, $p = 0.369$, $\eta^2 = 0.01$. A lack of significant differences in average hormone values across phases may result from high intra-cycle and inter-individual variation in estradiol levels (Jasienska et al., 2017), or from problems with adequately assessing ovulation, especially for women that did not experience a confirmed LH surge. However, our hypotheses pertain to overall changes across the menstrual cycle, and do not hinge upon accurate timing of ovulation. For descriptive statistics, see Table 1.

To control for the nested nature of the within-person data and simultaneously handle missing data, we estimated multilevel models using the HLM 7 computer program. In a 2-level model, we regressed anxiety scores onto *person-centered* levels of progesterone (within-person change) at Level 1 and women's mean progesterone levels across assessments (between-person differences) at Level 2. That is, the person-centered Level 1 progesterone variable represented the within-person effect—the extent to which each woman's progesterone at each assessment differed from her personal average. The grand-centered Level 2 progesterone variable represented the between-person effect—each woman's progesterone averaged across her three sessions. This is the recommended approach for simultaneously modeling both within- and between-person variance in multilevel modeling (see Raudenbush and Bryk, 2002, p. 139–141). Given that all participants completed assessments in the same order, yet assessments were not equally spaced, we controlled for order effects (see Bolger and Laurenceau, 2013) using two dummy codes, entered at Level 1. To help interpret the size of the primary effects, we report effect size *r*.[2]

Consistent with predictions, between-person differences in progesterone were positively associated with anxiety, $b = 0.008$, $SE = 0.002$, $t(95) = 3.35$, $p = 0.001$, $r = 0.33$, indicating that women who demonstrated higher levels of progesterone compared to other women in the sample also reported higher levels of anxiety. In contrast, within-person changes in progesterone were not associated with anxiety, $b = 0.001$, $SE = 0.002$, $t(161) = 0.49$, $p = 0.628$. The between-person effect of progesterone remained significant in a model that included person-centered estradiol at Level 1 and average estradiol at Level 2, $b = 0.01$, $SE = 0.003$, $t(94) = 3.62$, $p < 0.001$, $r = 0.35$. There was also a trend toward between-person variability in estradiol being negatively associated with anxiety, $b = -0.05$, $SE = 0.03$, $t(94) = -1.86$, $p = 0.066$, $r = 0.19$, but this trend did not reach traditional levels of significance. Between-person differences in estradiol were unassociated with anxiety in a model that did not control for progesterone, $b = 0.01$, $SE = 0.02$, $t(95) = 0.30$, $p = 0.764$. Within-person changes in estradiol were not associated with anxiety, $b = 0.02$, $SE = -0.02$, $t(163) = 0.97$, $p = 0.336$.

2.3. Discussion

Study 1 provided preliminary support for the hypothesis that progesterone is positively associated with anxiety. However, the link between progesterone and anxiety was observed only at a between-person level. Women with cycles characterized by higher average progesterone levels across the study also reported higher levels of anxiety. Greater within-person increases in progesterone across the menstrual cycle were not associated with greater increases in anxiety. However, because progesterone levels did not differ significantly between the second and third sessions, Study 1 may not have captured the full cyclical variability in progesterone levels.

Controlling for progesterone, we saw some weak evidence that women with higher average estradiol levels across their cycles reported lower levels of anxiety. However, the association between estradiol and anxiety should be interpreted with caution for two reasons. First, the association emerged only in a model that controlled for progesterone levels. Second, although estradiol levels were expected to be higher in the peri-ovulatory phase compared to the follicular and luteal phases, we did not observe statistically significant differences in estradiol concentrations across the three sessions. This pattern, along with the fact that progesterone levels did not increase from the peri-ovulatory to luteal assessments, casts some doubt on the timing of the peri-ovulatory session. In addition, recent research indicates that high variation in intra-cycle sex steroid levels (i.e. values from consecutive days often vary substantially, see Jasienska et al., 2017) creates a methodological challenge for studies investigating hormonal changes across the

[2] The effect size *r* reflects a correlation coefficient, and is calculated by taking the square root of the value obtained by dividing the squared *t* value by the value obtained by adding the squared *t* value and the *df* (see Rosenthal and Rosnow, 1991).

menstrual cycle, even when hormone levels are directly measured and ovulation is biochemically verified. Although our primary hypotheses do not hinge upon the timing of the peri-ovulatory session, the results should nonetheless be interpreted with caution.

One additional limitation of this study is that although we assessed and controlled for estradiol, we did not assess and control for cortisol, a hormone that is associated with stress and anxiety (Dickerson and Kemeny, 2004; Mantella et al., 2008). Study 2 addressed this limitation and also focused on a more specific form of anxiety.

3. Study 2

Study 2 advanced the investigation in two main ways. First, it examined whether the positive association between progesterone levels and anxiety extended to one specific facet of anxiety—attachment anxiety. Attachment anxiety has been conceptualized as a dispositional approach to relationships characterized by high need for closeness, fear of abandonment, and worry over rejection (Hazan and Shaver, 1987). Those higher in attachment anxiety worry about abandonment and monitor their relationships for signs of threat or waning investment (Simpson et al., 1999) and show heightened cortisol reactivity during relationship conflict discussions (Laurent and Powers, 2007; Powers et al., 2006). Thus, attachment anxiety can be thought of as a form of anxiety within an interpersonal context. Although often conceptualized as a stable dispositional trait, recent research suggests attachment anxiety varies within individuals and is responsive to cues of social inclusion and relationship distress (Girme et al., 2017; Haak et al., 2017). Study 2 therefore examined whether within-person shifts in attachment anxiety covary with women's cyclical progesterone levels. Second, Study 2 controlled for cortisol levels. Cortisol is associated with both stress and anxiety (Dickerson and Kemeny, 2004; Mantella et al., 2008), so it was important to evaluate whether the link between progesterone and anxiety emerged independent of the effects of cortisol.

American undergraduate women reported their levels of attachment anxiety and provided saliva samples at the follicular, peri-ovulatory, and luteal phases of their menstrual cycles. We assayed saliva samples for both progesterone and cortisol levels. This longitudinal design again permitted examinations of both the within- and between-person associations of hormone levels with attachment anxiety.

3.1. Method

3.1.1. Participants

We recruited 61 female university students over the course of 16 months through flyers and a mass screening survey. To be eligible, women were required to have (a) not used hormonal contraceptives within the past three months and (b) regular menstrual cycles (between 25 and 34 days). Participants were compensated with either course credit or $30. Participants were just over 21 years old on average (M_{age} = 21.7, SD = 3.44, age range: 18–42 years) and mostly White (57.4% Caucasian, 18.0% African American, 19.7% Hispanic, 3.3% Asian, 1.6% Middle Eastern). Fifty-three women (86.9%) were in relationships, lasting one year and four months on average (SD = 10.1 months).

3.1.2. Procedure

After researchers established eligibility through a phone interview, participants were asked to contact the lab at their next menstrual onset. We estimated women's full cycle length using this onset date and their last reported date of menstruation. This cycle length was then used to predict participants' next menstrual onset and date of ovulation (which was calculated by subtracting fifteen days from the menstrual onset estimate; Weinberg et al., 1994; Wilcox et al., 2000). Session 1 (which was always during the follicular phase) was scheduled four to seven days prior to expected ovulation. At session 1, after providing informed consent, women provided saliva through passive drool and completed computerized assessments that contained a measure of attachment anxiety. At this session, women also were given seven LH tests (ClearBlue ovulation) to be completed at home according to instructions. Women were instructed to begin the tests three days prior to their expected date of ovulation and to continue daily for seven days. If the test indicated an LH surge, participants were instructed to contact the lab to schedule their second session (peri-ovulatory). If the tests never indicated an LH surge, participants reported to the lab within one to two days of completing their LH tests. Forty-two (68.9%) participants had a confirmed LH surge. Session 3 (luteal) was scheduled 6–9 days after participants' LH surge. If a participant did not have an LH surge, her third session was scheduled 5–8 days after her second session.

At each session, participants provided saliva samples and completed the attachment anxiety measure. All sessions were scheduled to occur between 12 and 4 pm to control for diurnal fluctuations in hormones. To reduce error variance in the salivary assays, participants were instructed to abstain from exercising 12 h prior, smoking 6 h prior, eating 2 h prior, drinking caffeine 2 h prior, and having sex 3 h prior to their lab appointments. Adherence to these instructions was confirmed.

3.1.3. Measures

At each session, women completed a computer-based questionnaire about themselves and their relationships. We measured women's attachment anxiety using the Experiences in Close Relationships (Revised) questionnaire (Fraley et al., 2000). This is a 36-item measure, in which participants respond to items assessing both attachment anxiety (e.g., “I am afraid to lose my partner's love”) and attachment avoidance (e.g., “I prefer not to show a partner how I feel deep down”) on a 7-point Likert scale (1 = *Strongly Disagree* to 7 = *Strongly Agree*). To make the questionnaire relevant to both single and paired women, participants were instructed to indicate how they generally feel in romantic relationships. Because attachment anxiety is often correlated with attachment avoidance (e.g., Butzer and Campbell, 2008; Mickelson et al., 1997), we controlled for attachment avoidance in ancillary analyses.

3.1.4. Hormone assays

Saliva samples were frozen at −20 °C. To precipitate mucins, samples were thawed and centrifuged at 3000 rpm for 10 min. Supernatant was frozen in 250 μL aliquots until assayed. Hormone concentrations were measured by radioactive immunoassay (RIA) kits following the manufacturer's instructions (progesterone: Siemens Medical Solutions Diagnostics; cortisol: MP Biomedicals -LLC Diagnostics).

The antibody-bound ^{125}I hormone was then counted in a high-throughput, automated gamma counter. The lower limit of sensitivity of the RIA kits was 20 pg/mL for progesterone and 1.7 ng/mL for cortisol. Measurements were highly reliable between duplicates; coefficients of variation for progesterone and cortisol were below 6% and 7.5%, respectively. No data were excluded from analyses.

3.2. Results

Again, we examined between-phase differences in key variables using one-way ANOVA analyses. As expected, and as in Study 1, progesterone levels varied across menstrual cycle phases, $F(2,176) = 34.96$, $p < 0.001$, $\eta^2 = 0.28$, such that follicular levels were lower than both peri-ovulatory levels ($p = 0.015$) and luteal levels ($p < 0.001$), and peri-ovulatory levels were lower than luteal levels ($p < 0.001$). Attachment anxiety [$F(2,178) = 0.40$, $p = 0.674$], attachment avoidance [$F(2,178) = 0.11$, $p = 0.896$], and cortisol levels [$F(2,177) = 0.46$, $p = 0.630$] did not differ across cycle phases. For descriptive statistics, see Table 1.

As in Study 1, we used multilevel modeling to examine both the between-person and within-person associations between progesterone and attachment anxiety, again controlling for two session dummy codes to partial out any order effects. Conceptually replicating the pattern

from Study 1, between-person differences in progesterone were positively associated with attachment anxiety, $b = 0.20$, $SE = 0.09$, $t(59) = 2.25$, $p = 0.028$, $r = 0.29$, indicating that women who demonstrated higher levels of progesterone (on average across their sessions) compared to other women in the sample also reported higher levels of anxiety. Additionally, controlling this effect, within-cycle increases in progesterone were positively associated with increases in attachment anxiety, $b = 0.06$, $SE = 0.02$, $t(115) = 2.76$, $p = 0.007$, *effect size* $r = 0.25$, indicating that women who demonstrated greater increases in progesterone across the cycle also reported greater increases in anxiety.[3] Both associations remained significant controlling for between- and within-person differences in cortisol: for between-person progesterone levels, $b = 0.20$, $SE = 0.09$, $t(57) = 2.19$, $p = 0.033$, $r = 0.28$; for within-person changes in progesterone, $b = 0.06$, $SE = 0.02$, $t(112) = 2.61$, $p = 0.010$, $r = 0.24$. Associations also remained significant controlling for attachment avoidance: for between-person progesterone levels, $b = 0.20$, $SE = 0.08$, $t(57) = 2.50$, $p = 0.015$, $r = 0.31$; for within-person changes in progesterone, $b = 0.03$, $SE = 0.02$, $t(112) = 1.87$, $p = 0.064$, $r = 0.17$. Neither within-person changes in cortisol, $b = -1.19$, $SE = 3.57$, $t(112) = -0.33$, $p = 0.739$, nor between-person levels of cortisol, $b = 1.14$, $SE = 11.78$, $t(57) = 0.10$, $p = 0.923$, predicted attachment anxiety.

3.3. Discussion

Extending the findings of Study 1, Study 2 demonstrated a positive association between women's cyclical progesterone and anxiety. The significant between-person effect revealed that women with cycles characterized by higher average levels of progesterone experienced greater attachment anxiety than women with cycles characterized by lower average progesterone levels. This pattern conceptually replicates that found in Study 1. The significant within-person effect revealed that as women's progesterone increased over the course of their menstrual cycles, so too did their attachment anxiety. These associations held controlling for both absolute levels and changes in cortisol and attachment avoidance. These results suggest that the association between cyclical progesterone and anxiety are robust and that they emerge in interpersonal contexts.

4. General discussion

Across two longitudinal studies using diverse samples of women, we found support for the predicted positive association between progesterone levels and anxiety. In Study 1, Polish women's average progesterone levels across their cycles (i.e., between-person differences) positively corresponded to their reports of anxiety. In Study 2, American women's between-person levels of progesterone and within-person cyclical increases in progesterone were positively associated with their attachment anxiety, a specific, interpersonal form of anxiety. Importantly, the association between progesterone and anxiety emerged controlling for effects of other steroid hormones: estradiol (Study 1) and cortisol (Study 2). Taken together, these findings reveal an independent positive relation between women's cyclical progesterone levels and their anxiety.

The methodological strengths of the current investigation enhance confidence in the associations that emerged. First, rather than relying on comparison of cycle phases, the current studies assessed progesterone directly. This measurement supports the contention that the relation is specific to progesterone levels, rather than the particular combination of hormonal levels that characterize each cycle phase. Second, by assessing progesterone and anxiety within women over time, we simultaneously estimated how two sources of variance in progesterone—within-person cyclical changes and between-person average differences—relate to anxiety. Indeed, this analytic approach indicated that women's average cycle progesterone levels, though rarely explored, are consequential for their subjective psychological states; women with cycles characterized by higher progesterone levels reported higher general anxiety and anxiety about their interpersonal relationships than women with cycles characterized by lower progesterone levels. Third, assessments of anxiety and progesterone were collected concurrently, avoiding limitations associated with retrospective reporting.

Furthermore, results of Study 2 indicate that this association extends to anxiety in interpersonal contexts. Changes in women's cyclical progesterone levels paralleled changes in their confidence about the stability of their romantic partnerships. This pattern suggests that cyclical progesterone levels may underlie women's interpersonal anxiety. The data presented here cannot attest, however, to whether this association is particular to interpersonal anxiety or rather, simply one manifestation of general anxiety. To disentangle these two possibilities, future research could explore whether the association between cyclical progesterone and interpersonal anxiety emerges controlling for the association between cyclical progesterone and women's general anxiety levels. Regardless of whether the association is uniquely related to interpersonal anxiety, Study 2's findings suggest the association between cyclical progesterone levels and anxiety extends to an interpersonal context. Whether cyclical increases in progesterone also underlie downstream changes in women's interpersonal behaviors is an open area for future investigation.

Because the rise in progesterone levels during the luteal phase prepares the female body for possible pregnancy, the positive correlation between progesterone increases and anxiety may suggest that progesterone underlies psychological processes relevant to preparing for a potential upcoming pregnancy. Pregnancy is a particularly vulnerable state for women as their mobility is limited and their caloric demand is heightened (Dufour and Sauther, 2002; Wall-Scheffler and Myers, 2013), thereby intensifying women's need to generate a safe and supportive environment. The positive association between progesterone and anxiety uncovered here suggests that rising cyclical progesterone may promote behaviors that create such an environment. That is, perhaps the same endocrinological mechanism that prepares the female uterine environment for pregnancy also motivates women to prepare an environment conducive for pregnancy and ultimately, motherhood. To investigate this possibility, future research might explore whether rising progesterone levels correspond to specific behaviors that may help generate an optimal environment for pregnancy and motherhood, such as avoidance of unfamiliar or threatening men, risk aversion, support seeking, or relationship maintenance.

Given that the current findings reveal that endogenous increases in progesterone across the cycle correspond to increases in anxiety, future research should investigate how larger increases in progesterone, such as the dramatic rise during pregnancy, correspond to anxiety. Although our results reveal a positive association between progesterone increases and anxiety, heightened anxiety during pregnancy (when progesterone levels are substantially elevated) is associated with poor fetal outcomes (e.g., Moutquin, 2003). Researchers have suggested that the reproductive costs of high anxiety should have favored a physiological mechanism to reduce stress during pregnancy (Kajantie and Phillips, 2006). Indeed, some findings suggest an inverse curvilinear relation between progesterone and anxiety (Andréen et al., 2009). That is, at lower concentrations (e.g., endogenous cyclical levels), progesterone may be related to elevated anxiety, whereas at higher concentrations (e.g., during pregnancy) progesterone may be related to reduced anxiety. Although our findings are congruent with the proposed positive association at lower endogenous concentrations during menstrual cycles, our data cannot speak to whether the association between progesterone and anxiety changes at higher progesterone concentrations.

[3] Notably, we also found a significant order effect, such that during the third session, compared to the first session, women reported lower levels of attachment anxiety, $b = -6.91$, $SE = 2.32$, $t(113) = -2.98$, $p = 0.004$, *effect size* $r = 0.27$.

Future research might therefore benefit from comparing the relation between women's progesterone and anxiety at both lower and higher concentrations, such as over the course of pregnancy.

Conclusions should be tempered by several limitations of our method and design. One limitation is that, within both studies, the order of participation across menstrual cycle phases remained constant: participants' first session occurred during the follicular, second during the peri-ovulatory, and third during the luteal phase. Although we attempted to address this limitation by controlling for session (and therefore order) within our models, we cannot rule out the possibility that this order somehow influenced our effects. A second limitation is that neither study examined progesterone, estradiol, and cortisol simultaneously. Although the relation between progesterone and anxiety emerged in each sample controlling for one of the additional hormones, assumptions that the emergent pattern is unique to progesterone should be interpreted with caution.

Additionally, both studies followed women over the course of only one menstrual cycle. Research suggests that there is substantial inter-cycle variability in women's progesterone levels (Eisenlohr-Moul and Owens, 2016; Jasienska et al., 2017; Jasienska and Jasienski, 2008). That is, women who have higher progesterone one cycle are not necessarily the same women who have higher progesterone the next cycle (Jasienska et al., 2017). Thus, the between-person differences in progesterone we observed may not reflect enduring dispositional differences between women but instead may suggest that women experience greater anxiety during higher progesterone cycles compared to lower progesterone cycles. Inter-cycle variation in sex hormone levels is influenced by genetic (Jasienska et al., 2006a, 2006b), developmental (Emaus et al., 2008; Jasienska et al., 2006b), and life style factors (Jasienska, 2003). However, the current findings suggest differences in progesterone levels across cycles could potentially also reflect changes in women's immediate environments, such as their risk of physical or social harm (e.g., likelihood of rejection). Future research may therefore benefit from assessing women across multiple menstrual cycles to better understand whether variation in average progesterone levels reflects input from women's immediate social and physical environments.

Last, although it is a strength of our study design that we followed women at three time points within a given menstrual cycle to examine the correlates of rising progesterone levels, any causal conclusions about the direction of the association should be drawn with caution. Cyclical increases in progesterone may promote greater anxiety, as our models suggest, but increased anxiety within a given cycle may also promote greater increases in progesterone. Future experimental designs could more clearly delineate the causal relationships between progesterone, situational factors, and affective states, such as anxiety.

5. Conclusion

The current findings add to a growing body of research implicating progesterone's role in psychological processes. Although increasing progesterone levels across the menstrual cycle serve primarily to prepare the female uterine environment for a possible pregnancy, the current findings suggest that changes in progesterone levels also correspond to changes in women's psychological vigilance. Moreover, our findings suggest that this association has consequences for women's perceptions of their immediate social relationships.

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